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E. E. Ecker, … , T. F. Dozois, L. Barr

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Research Article

COMPLEMENT IN INFECTIOUS DISEASE IN MAN[1]

By E. E. ECKER, S. SEIFTER, T. F. DOZOIS, AND L. BARR

(From the Institute of Pathology, Western Reserve University and the University Hospitals, Cleveland)

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Since Ehrlich and Morgenroth (1) made the observation that antibody and complement are independent entities, and Wassermann (2) advanced the hypothesis that serum complement concentration serves as a measure of general resistance, many attempts have been made to elucidate the role of complement in acute and chronic diseases. Longcope (3) held that "terminal infection in chronic disease is probably the direct result of the diminished state of bacteriolytic complement."

Dick (4) noted that in cases of *pneumonia,* complement was low before crisis, and high on the second to third day following crisis. The recent work of Rutstein and Walker (5) also points to a diminished complement in patients with *pneumonia* at the time of their admission to the hospital. These authors further remarked that the sera of 7 of 12 patients with pneumonia, tested immediately after the administration of antipneumococcal serum, showed diminished complement titers. The sera of 6 of 11 patients with *serum disease* were also found by these authors to have decreased complement titers. Goussev (6) and Wendstrand (7) reported, however, an increase in complement in the sera of patients with pneumonia.

Gunn (8) found complement always present during the course of enteric fever, although in greater amount throughout the period of pyrexia than during convalescence. He remarked that a diminution of titer in favorable cases coincided with the production of antibody. According to Gunn, complement and antibody are not produced in any fixed ratio to one another.

In *erysipelas* Gunn (8) found complement present in greater amount during the acute stage of illness than during convalescence. Keefer and Spink (9) also noted greater fluctuations of complement titer in this disease than observed in controls without infections.

In cases of *tuberculosis* a low complement titer has been observed by Gaudeau (10), an elevation by Goussev (6), while Hanson (11) reported little or no change. Meersseman and Perrot (12), in their turn, claimed that a low complement titer in tuberculosis is an unfavorable sign.

Bertin (13) and Wendlberger and Volavsek (14) reported reduction of complement titer in some cases of *syphilis.*

In cases of *yellow fever,* da Costa Cruz (15) claimed that a low complement titer is of diagnostic significance. He based this conclusion on a study of 103 cases, 93.6 per cent of which he defined as being of low titer. During convalescence, previously diminished titers were found to increase rapidly toward normal.

Thompson (16) found a diminution of complement in the early stages of *Variola,* with a rapid return to normal in cases unattended by secondary infection. A failure of the titer to return to normal was observed in cases with secondary infection, and a progressive decrease of complement was also observed in cases complicated by terminal septicemia.

A reduction of complement titer during *malarial paroxysms* was reported by Cathoire (17), Vincent (18), and Wendlberger and Volavsek (14).

Hadjopoulos and Burbank (19) stated that the complement content of the serum of a patient with infectious disease shows considerable variation from the normal, and that "such variations have been demonstrated by us to have prognostic significance." They found that with the onset of infection a steady rise in the production of complement occurs; however, this stage passes rapidly into a so-called "negative alexic phase" which they consider to be the "serologic shock period." An abnormal fall of complement then ensues. Further, according to these workers, the decrease of complement is probably due to an increased destruction of complement, or to its adsorption by proteolytic by-products of known anticomple-

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mentary properties. During recovery and convalescence, "the ratio between complement production and destruction is reversed."

Others, notably Brinkmann (20) and Schuchardt (21), have failed to find sufficient changes of titer in pathological states to consider complement a measure of diagnostic or prognostic value.

With regard to laboratory animals, there is one instance in the literature which dramatically correlates complement titer with resistance to infection. In this instance, guinea pigs bred by Moore (22) were found to be deficient in complement and simultaneously highly susceptible to natural and experimental infections. This strain of guinea pigs, because of its innate lack of resistance to infection, is now extinct.

As pointed out above in the discussion of the work of Rutstein and Walker, complement titer may decrease as a result of the administration of antiserum. Thomas and Dingle (23) found this to be true also in the case of rabbits injected with a concentrated antimeningococcus serum, the result of which was the disappearance of hemolytic complement for as long as 24 hours. Meningococcal bacteremia, they found, persisted for a longer time in rabbits so treated than in animals receiving no antiserum. According to these authors, the disappearance of complement activity is due to the prozone effect, as well as to the anticomplementary action of the antiserum.

From a different viewpoint, the role of complement in infectious disease has been emphasized by the recommendations of numerous authors that serum containing complement be administered together with antiserum, as suggested, for instance, by Fairley and Stewart (24) and Kolmer (25) in cases of meningococcal infection.

This summary of complement in infectious diseases may be concluded with the statement of Osborn (26) that, "Very much more work is required before the clinical significance of complement estimations can be assessed, and it will require much investigation, which some might regard as of an academic nature, before this section of immunological chemistry can obtain any wide application in the diagnosis, prognosis, or treatment of disease."

It might be added that many of the apparent contraindications and inconsistencies encountered in the literature surveyed can be attributed to the lack of a uniform technique of complement titration, and to an inadequate definition of the limits of "normal" and "abnormal" complement titers.

The present paper is concerned with a detailed study of complement titers in 278 cases of various infectious diseases. This study is unique, in contrast to earlier work, in that it includes not only the determination of over-all complement titers, but the approximation of the titers of the individual complement components as well. Further, a beginning is made in the correlation of titer with serum protein concentrations, leukocyte count, and body temperature.

METHODS

The subjects of this study were patients in the Cleveland City Hospital, Contagious Division, or in the University Hospitals. In the majority of cases serial studies were made, with at least 2 blood samples being obtained from a patient during the course of hospitalization. In most cases the first blood sample was obtained on the day of admission prior to the use of therapeutic measures. This precaution was observed because of the fact that administered antiserum may depress the complement titer, although the authors, in control experiments, found that the highest blood levels of sulfonamide drugs and penicillin encountered in this study were without significant effect on the complement titer.

(*a*) *Collection of serum.* Blood was obtained by venous puncture, allowed to clot for several hours at 3 to 4° C., centrifuged, and the serum decanted and frozen at − 35° C. until used. The greatest number of specimens were titrated within a day of collection, others were titrated after storage for several days. Repeated trials showed that storage for this time at − 35° C. did not appreciably affect the complement titer.

(*b*) *Complement titration.* The overall complement titer, expressed in units per ml. of serum, is given in the summary tables, the unit being the minimum amount of serum which hemolyzes completely a standard dose of sensitized sheep red cells. The red cell substrate is made up as follows: about 5 ml. of defibrinated sheep blood is washed 4 times with 40 ml. portions of 0.9 per cent saline. Packed cells are then added to 100 ml. of saline so that a 1:20 dilution of the suspension gives a reading of 280 in a Klett-Summerson colorimeter. One hundred ml. of saline is now prepared so as to contain 20 units per ml. of hemolysin. This sensitizing solution is mixed rapidly with 100 ml. of the cell suspension; a 1:20 dilution of this mixture in saline should now give a reading of 140 in the colorimeter. Adjustments to obtain this reading are made either by addition of packed cells or by dilution with saline.

One volume of the test serum is now diluted with 4 volumes of saline (1:5). To 18 serological tubes are added amounts of the 1:5 serum dilution ranging from

0.01 ml. to 0.45 ml. with a 0.2 ml. Kahn pipette. Saline is added to bring the volume to 0.5 ml., and 0.5 ml. of the cell substrate is added with rotation of the tubes. The tubes are then incubated in a water bath for 30 minutes at 37° C. After incubation they are centrifuged at 4° C. for 5 minutes and the supernates compared visually with a previously prepared set of color standards.

Reactivation. The smallest amount of test serum found to yield barely a trace of hemolysis is employed for reactivation. Usually, normal sera require 0.02 ml. of a 1:30 dilution, but in many instances where the overall titer is low, as occurs in disease, higher quantities are required. Thus, in the case of a normal serum 0.02 ml. of the 1:30 dilution is pipetted into each of 5 tubes. The tubes then receive the previously prepared complement reagents in amounts adequate for reactivation, but avoiding anticomplementary effects. To the first tube is adde the "pH 5.4 $-\mu$ 0.02" supernate lacking C'1; the second receives the "pH 5.4 $-\mu$ 0.02" precipitate lacking C'2; the third receives Zymosan-treated serum which lacks C'3; and the fourth, NH_4OH treated serum lacking C'4. Generally, 0.3 ml. of each previously tested component is employed. The complement reagents were prepared from pooled sera which had an overall titer of 0.15 of 1:5 dilution. Therefore, in order to obtain at least 2 units of the reagents, they were diluted so as to correspond to a 1:5 dilution of the original serum. The fifth tube reacts as a control, and receives no complement reagent. Each tube is made up to 0.5 ml. volume with saline, and incubated for 30 minutes at 37° C. in a water bath.

In normal human serum the first component to disappear upon dilution is C'3, followed closely by C'2. At the point where these components disappear the serum still contains sufficient concentrations of C'1 and C'4 to produce 80 to 90 per cent hemolysis of the standard unit of red cell substrate. Accordingly, C'1 and C'4 may be said to be present in 8 to 9 times the effective concentration of C'2 and C'3.

(*c*) *Determination of serum protein.* If sufficient serum was available, protein determinations were performed by the refractometric method using a dip refractometer, the scale readings of which had previously been correlated with human serum protein concentration as determined by micro-Pregl nitrogen analysis. From time to time values were obtained for the same sera by nitrogen analysis, and always were found to be in good agreement.

(*d*) *Clinical data.* These were obtained from the hospital case records at the close of the laboratory work. During the course of the investigation none of the laboratory workers was familiar with clinical diagnosis, treatment, condition, etc., of the patient.

In all cases in which they were available for the day on which a blood sample for complement titration was taken, the total white blood cell count and the maximum body temperature of the patient were abstracted from the records.

RESULTS

a. The distribution of overall complement titers. Table I compares the distribution among 3 categories of complement titers obtained in a study of 248 cases of infectious disease and in 40 normals. From the distribution among this series of normals, as well as among previous series (28), complement titers of 25 to 50 units may be considered as "normal," titers below 25 units as low, and those above 50 units as high. Considered in these categories of titer, the distribution of titers in the diseased series is different from that in the normal series. Although the deviation from the normal of those sera showing a very low titer is more striking than the amount of deviation showed by those with high titers, there is enough departure from the normal in those with high titers to make the observation significant. The tendency toward low titers in the diseased series is even more significant, and the table shows that 30.6 per cent of the patients exhibited diminished complement titers. It may be further pointed out that about ⅓ of these, or 9.6 per cent of the total cases, showed titers less than 13 units per ml. of serum.

Among the separate diseases shown in the

TABLE I

Distribution of complement titers in 248 cases of infectious disease and in 40 normal sera

Disease	No. of cases	Lowest titer observed in course of disease: Percentage of cases showing the following titers		
		*over 50 units**	*25 to 50 units**	*less than 25 units**
Scarlet fever	73	17.8	53.4	28.8
Epidemic meningitis	38	7.9	42.1	50.0
Measles	37	10.8	54.1	35.1
Pneumococcal infections of all types	36	19.4	61.1	19.5
Encephalitis (including all etiological types)	11	9.1	72.7	18.2
Typhoid fever	10	0.0	83.3	16.7
Erysipelas	7	42.9	42.9	14.2
Chicken pox	6	0.0	83.3	16.7
Subacute bacterial endocarditis	5	0.0	60.0	40.0
Miscellaneous†	25	12.0	60.0	28.0
All cases	248	13.7	55.7	30.6
Normals	40	5.0	95.0	0.0

* Units per ml. of whole serum.

† Includes cases of rheumatic fever, gonococcal arthritis, naso-pharyngitis, peritonsillar abscess, influenza, and other upper respiratory infections.

table, cases of epidemic (meningococcal) meningitis present the most marked tendency toward a low complement titer, and cases of erysipelas the greatest tendency toward high titers. The distribution of titers in pneumococcal infections tends to be symmetrical about the normal range.

In analyzing the relationship of component titers to these changes in overall titer, it was found that in practically all cases diminution of complement titer was due primarity to decrease of C'4, and secondarily to decrease of C'2 and C'1. C'3 was apparently decreased only very slightly or not at all. On the other hand, all 4 components apparently participated in the shift toward increased titers.

b. Cases exhibiting very low complement titers. As seen in Table II, 12, or 4.3 per cent, of a total of 278 cases of infectious disease exhibited titers equal to or less than 10 units per ml. of serum. The most striking aspect of these complements, as seen in the table, was their almost complete loss of C'4 titer. C'2 titers were considerably reduced or absent in at least 7 of these instances, while C'1 titers were diminished, though not so sharply, in at least 4 cases. Very little change was encountered in the C'3 titer of any of these cases.

Of possible significance is the fact that 4 of these patients died, and that their lowest levels of complement titer occurred on the day or days preceding death. On the other hand, the low titers exhibited by patients who subsequently recovered were coincident with serious phases in the course of the disease, and recovery was accompanied by a rapid return to a normal complement titer and to normal component titers. Such a course is exemplified in Case No. 1 shown in Table II. This patient, suffering with epidemic meningitis, was admitted to the hospital in a comatose state, and was found to have a titer of 10 units of complement per ml. of serum. Three days later, after the institution of treatment, the patient continued in a serious condition although she began to regain consciousness; nevertheless the complement titer had completely disappeared. Three days later the patient was fully on the road to recovery, and the complement titer had returned to a normal value of 36 units per ml. of serum.

c. Complement titers in fatal cases. Fourteen, or 5.0 per cent, of the total of 278 cases investigated died during the course of the study; of these, 8, or 2.9 per cent of the total number of cases, died without complications such as cancer, coronary disease, or other serious non-infectious diseases. Of the 8 having "uncomplicated" deaths, 4, or 50 per cent, had extremely low complement titers. Of the 6 dying with complications, none had low titers.

Table III shows the disposition of complement titers in 9 fatal cases of infectious disease. Besides the tendency toward low titers already noted, the high titers found in the 2 cases of Type III pneumococcal meningitis are noteworthy. The participation of the individual components in all of

TABLE II

Cases having 10 or less units of complement

Case no.	Diagnosis	Lowest C' titer in course of study	C'1	C'2	C'3	C'4	Remarks
		units per ml. serum					
1	Epid. men.	0		L		0	Recov. titer
3	Staph. men.	0		0		0	Died
38	Chronic fever of unknown etiol.	2				0	Recov. titer
86a	Epid. men.	0	N	N	N	0	Recov. titer
93	Pertussis, pn.	10	L	N	N	Tr	Died
117f	Strep., pn.	0	N	L	N	0	Recov. titer
191	Epid. men.	10	SL	0	N	0	Recov. titer
216	Post-measles enceph.	0	L	L	N	Tr	Died
240*	Epid. men.	0	N	N	N	Tr–0	Recov. titer
268c	Post-measles enceph., pn.	10	N	L	N	SL	Died
271a*	Epid. men., serum sickness	0	SL	Tr	SL	0	Recov. titer
276a*	Epid. men. later serum sickness	9	N	L	N	L	Recov. titer

* Cells agglutinated.
N = normal
L = low
SL = slightly low
0 = zero
Tr = trace

TABLE III

Complement titers in fatal cases

Case no.	Age	Diagnosis	Last C′ titer	Date of last C′ titration	Date of death	Remarks
	years		*units per ml. serum*			
3	29	Staph. men.	0	1-20	1-20	No C′2 and C′4
39	30	Type XII Pn. men.	31	2-8	2-10	
61	51	Epid. men.; uremia	23	2-16	2-23	Low C′4
64	30	Pn. Type III men.	83	2-16	2-18	All components higher
74	40	Pn. Type III men.	83	2-15	2-15	All components higher, particularly C′3
93	16 mon.	Pertussis pneum.; nephrotic synd.	10	2-20	2-23	C′1 low; C′4 trace
140	35	Lobar pneumonia	50	2-26	2-29	
216	31	Post-measles enceph.	0	3-7	3-8	C′1, C′2 low; no C′4
268	6	Post-measles enceph.	10	4-27	5-21	C′1 low; C′4 trace

these deviations of titer is the same as discussed in previous sections of this paper.

d. Serum protein concentration and complement titer. Because complementary activity is associated with serum proteins, an attempt was made to determine whether a relationship exists between complement titer and serum protein concentration. Table IV summarizes the relationship of 190 complement titers to 3 arbitrarily conceived categories of serum protein concentration. While it is difficult to define a "normal" range of serum protein concentration, the range of 6.5 to 7.4 per cent as given in the table has been found to be fairly normal with the refractometric method. In any event, the intent of the analysis is not so much to relate complement titers with so-called normal or abnormal serum protein concentrations, as to show the general distribution of titers over the encountered range of protein concentrations. The table reveals that serum protein concentration, in a general way, is a contributing factor in the determination of overall complement titer. A serum with low protein concentration tends in the direction of low titer, while a serum of high protein content tends toward high titer as opposed to low. Again, it is emphasized that these tendencies are modified by more significant factors which determine titer, inasmuch as complement components constitute only a very small fraction of the total serum proteins.

e. White blood cell count and complement titer. Table V shows the distribution of 181 complement titers among 4 arbitrarily defined categories of total leukocyte counts. It is apparent that no general relationship exists between cell count and titer, and that even a limited tendency is expressed in only one instance. This tendency is represented by the fact that 17.0 per cent of the sera showing less than 25 units of complement per ml. were obtained from patients having cell counts greater than 24,000 per cu. mm. This figure is in contrast to the extremely low percentages occurring otherwise in this range of leukocyte count. However, the occurrence of high white cell counts is not general for all infectious diseases, and this must be considered in evaluating the aforementioned

TABLE IV

Serum protein concentration and complement titer

Per cent protein	Sera having complement titers of:*						Total of all titers	
	Over 50 units		25 to 30 units		Less than 25 units			
	no.	*per cent*	*no.*	*per cent*	*no.*	*per cent*	*no.*	*per cent*
Less than 6.5	6	20.0	38	34.9	22	45.8	66	34.7
6.5 to 7.4	19	63.3	59	52.7	24	50.0	102	53.7
More than 7.4	5	16.7	15	13.4	2	5.2	22	11.6
Total of	30	100.0	112	100.0	48	100.0	190	100.0

* Units per ml. serum.

TABLE V

White blood cell count and complement titer

White cells per cu. mm.	Sera having complement titers of:*						Total of all titers	
	Over 50 units		25 to 50 units		Less than 25 units			
	no.	*per cent*	*no.*	*per cent*	*no.*	*per cent*	*no.*	*per cent*
12,000 and less	14	42.4	53	49.5	17	41.5	84	46.4
12,000 to 24,000	18	54.5	52	48.6	17	41.5	87	48.1
24,000 to 36,000	1	3.1	0	0.0	3	7.3	4	2.2
Over 36,000	0	0.0	2	1.9	4	9.7	6	3.3
Total of all cell counts	33	100.0	107	100.0	41	100.0	181	100.0

* Units per ml. serum.

tendency. As a matter of fact, almost all of the bloods having low complement titers and simultaneously high cell counts were obtained from patients with epidemic meningitis. On the other hand, in 2 cases of typhoid fever both the complement titer and white cell count were low. In other instances, including cases of scarlet fever, measles, and chicken pox, there was no relationship between titer and cell count.

f. Body temperature and complement titer. Since fever is a common phenomenon in infection, an attempt was made to correlate temperature and complement titer. Table VI relates 244 titers with the maximum body temperature of the patient on the day the blood sample was taken for complement titration. It is seen that there is the same distribution of temperatures for each category of complement titer, so that it may be said that there is no apparent relationship between body temperature and titer.

g. Examples of particular cases. Tables VII to IX summarize the data obtained in 3 cases of infectious disease, and serve to illustrate some of the changes in titer encountered. For the most part these tables are self-explanatory, although Case 117, the most fully studied case, deserves some comment. On admission, this patient was in serious condition and simultaneously his complement titer was also diminishing; in the course of the next few days he became comatose and at the same time his titer continued to decline, particularly the C'4 titer. In addition to penicillin, the patient received several blood transfusions and the complement titer increased somewhat; however the condition of the patient did not improve markedly. On 3-16-44, with the patient continuing in a dangerous state, his titer became zero with a simultaneous disappearance of C'4 titer. From that time on the patient began to improve until the time he was discharged. Three days before discharge his complement titer was fully restored concomitant with a restoration of C'4 titer.

TABLE VI

Maximum body temperature and complement titer

Max. temp. on day of blood sample	Sera having complement titers of:*						Total of all titers	
	Over 50 units		25 to 50 units		Less than 25 units			
°C.	*no.*	*per cent*	*no.*	*per cent*	*no.*	*per cent*	*no.*	*per cent*
36 to 38	13	38.2	45	32.2	25	35.7	83	34.0
38 to 39	9	26.5	41	29.3	18	25.7	68	28.0
39 to 40	5	14.7	28	20.0	13	18.6	46	18.8
40 to 41	7	20.6	25	17.8	14	20.0	46	18.8
Over 41	0	0.0	1	0.7	0	0.0	1	0.4
Total of all temperatures	34	100.0	140	100.0	70	100.0	244	100.0

* Units per ml. serum.

TABLE VII

J. R. Bronchopneumonia. Streptococcal Septicemia

Date*	Titer: complete hemolysis	C'1	C'2	C'3	C'4	Serum protein	Remarks
	units per ml. serum					*grams per cent*	
2-24-44	23					6.3	
2-29-44	13	SL	SL	N	N	6.4	25,000 units penicillin daily until 3-9-44
3-2-44	6	L	SL	N	L	7.8	500 ml. whole blood given
3-3-44	13	SL	SL	N	L	7.5	
3-6-44	12	SL	SL	N	L	6.8	500 ml. whole blood given on 3-7-44
3-8-44	12	SL	SL	SL	L	6.8	Sulfadiazine given from 3-11-44 to 3-16-44
3-16-44	0	SL	L	SL	0	5.7	Sulfadiazine level in blood: 3.6 mgm. per cent
4-1-44	31	N	N	N	N	7.0	

* Admitted 2-24-44—discharged, 4-4-44.
Key: N = normal titer
SL = slightly diminished
L = low, greatly diminished
0 = no titer

TABLE VIII

J. H. Scarlet fever. Measles

Date*	Titer: complete hemolysis	C'1	C'2	C'3	C'4	Serum protein	Remarks
	units per ml. serum					*grams per cent*	
3-8-44	56	SH	SH	SH	N	5.8	From 3-8-44 to 3-13, 18.5 grams sulfadiazine
3-16-44	56	SH	SH	SH	SH	7.1	From 3-19-44 to 3-23, 17 grams sulfadiazine
3-22-44	56	N	SH	SH	N	6.7	

* Admitted, 3-8-44; discharged, 3-25-44.
Key: N = normal titer
SH = slightly high titer, somewhat increased

TABLE IX

D.J. Epidemic meningitis, treated with antitoxin and sulfa drugs

Date*	Titer: complete hemolysis	C'1	C'2	C'3	C'4
	units per ml. serum				
2-22-44	2	L	L	SH	Tr to 0
2-24-44	0	SL	L	SH	0
2-26-44	5	N	L	H	Tr to 0
3-7-44	23	N	N	SH	N

* Admitted, 2-22-44; discharged, 3-7-44.
Key: N = normal titer
SH = slightly high
H = markedly increased or high
SL = slightly low or diminished
L = very low, or greatly diminished
Tr = trace
0 = no titer

SUMMARY AND CONCLUSIONS

Without going into detail about the fate of complement in separate and particular diseases, a subject reserved for future papers, the following general conclusions may be drawn from this study of 278 cases of infectious disease.

1. While overall complement titer often changes in the course of infectious disease, the extent and changes are by no means always similar in different diseases, nor even in individual instances of the same disease.

2. Insofar as they were studied serially, 248 cases of infectious diseases showed a distribution of complement titers significantly different from that in a series of 40 normals; 30.6 per cent of the 248 patients exhibited complement titers less than 25 units per ml. of serum, the lower limit of "normal" titer. On the other hand, a minimum of 13.7 per cent of the patients showed titers over 50 units per ml. of serum, the upper limit of "normal" titer. In the control group of normal individuals, only 5.0 per cent showed over 50 units of complement. Since titers were not determined every day for a given patient, it is possible that additional low levels of titer were not observed,

so that the figure of 30.6 per cent must be taken as a minimum.

3. In 12 cases, or 4.3 per cent of a total of 278 patients, the complement titer was found to be as low or lower than 10 units per ml. of serum. The low titer usually coincided with a serious phase of the patient's condition; with the improvement of this condition the titer would tend to return to the normal value. However, of these 12 patients, 4 died, giving a fatality rate for this group of 33.3 per cent. This is a striking rate when compared with the figure of 5.0 per cent fatality for the entire group of 278 patients.

4. In those cases showing a decrease in overall complement titer there was a marked decrease or disappearance of C'4; in other cases, besides the diminution of C'4 titer, a decrease of C'2 and C'1 titers was encountered. C'3 titer did not diminish at all, except in one case in which it changed slightly. All of the cases exhibiting no complement titer whatsoever also showed no C'4 titer, a fact which is even more striking when it is recalled that this component is present in abundance (*i.e.,* its titer is considerably higher than the titers of C'2 and C'3) in normal human complement.

When diminished titers improved there was a concomitant increase in the titers of the diminished components.

5. Among the separate diseases, the occurrence of a low titer was most frequent in cases of epidemic (meningococcal) meningitis, in which 19 of 38 patients studied were shown to have diminished titers. Among patients with subacute bacterial endocarditis, 2 of 5 cases studied exhibited low titers.

6. High titers, *i.e.,* titers over 50 units per ml. of serum, were most frequently observed in cases of erysipelas. However, significant numbers of patients with scarlet fever and of those with pneumococcal infections showed increased titers. All 4 components of complement apparently participated in this increase.

7. As yet the authors can see no *general* prognostic value in complement titer, although it may be said that in those relatively few cases in which the titer diminished to very low levels, *e.g.,* 10 units per ml. of serum, the prognosis is grave. While experience has shown that patients with low titer survived in 2 out of 3 instances, it has also shown that those in whom low titer *persists,* and particularly low C'4 titer, succumbed.

It must be further remarked that not all patients with infectious disease who succumbed exhibited low titers immediately preceding their death. As a matter of fact, 2 patients with pneumococcus Type III meningitis exhibited extraordinarily high titers of 86 units per ml. in the days preceding death.

The question of the prognostic value of complement titer, however, is not a closed issue.

8. Observing all of the precautions counseled in the main body of this paper, serum protein concentration appears to bear relationship to complement titer. Modified by more significant factors which determine titer, a low protein concentration disposes a serum toward low titer, and a high protein content toward high titer.

9. In general, there is no apparent relationship between a patient's total white blood cell count and complement titer. In cases of meningococcus meningitis, however, the coincidence of low titer and high cell count may be significant. Whether these are independent events in the course of this particular disease, or are related through the phenomena of opsonification and phagocytosis, is not known.

10. No apparent relationship between body temperature and complement titer was found.

11. These studies have been carried out over a period of several years, in which time some progress has been made in the titration of the complement complex. Stimulated by the fact that a more reliable method of complement titration is necessary to clarify the problem of complement in disease, such a method has been developed in this laboratory eliminating most of the errors found in previous methods, and will be the subject of a future paper.

12. Keeping in mind the dictum of Osborn quoted in the introduction to this paper, the present authors deem it premature to speculate on the full meaning of these changes of titer in infectious disease. Their future work will be aimed at determining the mechanisms of diminution or increase of titer in particular diseases, and the role of complement fixation, opsonification, anticomplementary substances, etc., in this respect.

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