The Properdin System and Immunity: I. Demonstration and Isolation of a New Serum Protein, Properdin, and Its Role in Immune Phenomena

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ON the basis of epidemiological and experimental data, immunity has been divided into two general classes—acquired (or artificial) immunity and innate (or natural) immunity. The discovery in serum of specific protective substances, termed antibodies, has increased knowledge about the mechanism of acquired immunity. In conjunction with naturally occurring cellular and humoral constituents of blood, antibodies play a major role in specific acquired immunity. On the other hand, little is known concerning the factors or mechanisms involved in natural immunity. Landsteiner (*1*) states that substances, not identical with but somewhat analogous to specific acquired antibodies, are produced in certain animals without any relationship to prior antigenic stimuli. These normal substances are characterized by their low order of specificity and by their weak union with antigens. The role of complement, C′, and its four components C′1, C′2, C′3, and C′4 (*2*) in natural immunity reactions has also received considerable attention (*1–4*).

In an attempt to isolate one of the components (C′3) of complement, we encountered a new serum protein (*5*), which may be an important factor in natural immunity. This protein, tentatively named *properdin* (*6*), acts only in conjunction with complement and Mg^{++} and participates in such diverse activities as the destruction of bacteria, the neutralization of viruses, and the lysis of certain red cells. Properdin is a normal serum constituent and differs from antibody in many respects, particularly in its lack of specificity and in its exact requirements for its interactions. Investigators (*7*) from several departments of Western Reserve University have collaborated in studying certain activities of properdin. This report gives a brief summary of the various phases of this work (*8*), and more detailed accounts will follow soon.

Demonstration and Isolation of Properdin and Its Reaction with Zymosan

The insoluble residue from yeast cells that have been digested with trypsin and extracted with water and alcohol (*9*) is known as zymosan. Zymosan is composed mainly of carbohydrate and is derived from the yeast cell wall. Treatment of human serum with zymosan at 37°C inactivates selectively the third component (C′3) of complement. Originally, it was thought that this inactivation occurred by the adsorption of C′3 onto zymosan and that this might be a useful method for isolating C′3. However, all attempts to elute C′3 were unsuccessful (*10, 11*). Further studies (*10, 11*) disclosed that the inactivation of C′3 by zymosan was not a simple adsorption but had some of the characteristics of an enzymatic reaction. The loss of C′3 occurred only at temperatures above 20°C, at *p*H near 7, and in the presence of Mg^{++}. On the other hand, there was a stoichiometric relationship between the amount of zymosan added and the amount of C′3 activity that disappeared. The complexity of the system became even more apparent when the reaction was studied at 17°C. At this temperature, zymosan caused no significant inactivation of C′3 or of any other component of complement. Nevertheless, removal of the added zymosan left an altered serum, because incubation with fresh zymosan at 37°C failed to inactivate C′3. When the zymosan was not removed from the serum after the original incubation at 17°C, inactivation of C′3 took place if the temperature was raised to 37°C. The interpretation of these results is presented in Fig. 1.

Zymosan (Z) at 17°C combines with the new factor, properdin (P), to form an insoluble complex (PZ) that is capable of inactivating C′3 at 37°C but not at 17°C. The C′3 in serum that is deficient in properdin (RP) is not altered by zymosan at either temperature but is inactivated upon the addition of *both* properdin and zymosan at 37°C. This difference in the behavior of RP and normal serum is the basis for the present assay of properdin. Thus, one unit of properdin is defined as the smallest amount of sample that, in the presence of zymosan under controlled conditions, reduces the C′3 titer of an RP from an initial value of 120 units/ml to zero. Details of the assay are given in Appendix 1.

The combination of zymosan with properdin proceeds in stoichiometric proportions. It is retarded in a medium with an ionic strength greater than 0.2 and prevented if the ionic strength is raised to 0.4 or

higher. The PZ complex dissociates into its components, properdin and zymosan, in a medium of high ionic strength. The formation and dissociation of the PZ complex provide the basis for a 3000-fold purification of properdin (see Fig. 2 and Appendix 1).

The inactivation of C'3 by zymosan occurs in two stages. The first stage is the combination of properdin with zymosan to give a PZ complex. This reaction proceeds only at temperatures above 10°C and goes to completion at or above 15°C. The *p*H optimum is close to 7. The complex fails to form in serum treated with cation exchange resin. The missing factor appears to be Mg^{++}, because an effective system can be reconstituted by the addition of Mg^{++} to 5×10^{-5} *M*. The formation of PZ also requires the presence of factors that resemble components of complement and that are probably identical with them. A variety of conditions known to destroy or to "fix" complement also prevent the formation of PZ. PZ is not formed when zymosan is added to serum fractions that lack C'1, C'2, or C'4 (*3, 12*), or to serums that have been treated with antigen-antibody aggregates (complement-fixation) or with trypsin, or to serums that have been heated at 50 to 52°C for 30 min. Addition of fresh complement or of those complement components that are destroyed by these procedures reconstitutes an active system. The formation of PZ does not significantly deplete any component of complement. The same term, *properdin,* has been employed for the factor in serum and also for the protein eluted from PZ. It has been assumed that the properdin molecule undergoes no changes during these reactions, but present evidence is not conclusive.

The second stage, namely the inactivation of C'3 by PZ, requires Mg^{++}, a temperature above 20°C, and a factor present in the serum proteins insoluble at *p*H 5.5 and ionic strength 0.02. The role of this factor and its relationship to C'1 is under investigation.

Our experiments have shown that properdin is not a specific antibody to zymosan. The combination of properdin and zymosan requires accessory factors that are not needed for the combination of a typical antibody with a typical antigen (Table 1). Moreover, when PZ is added to serum, the effects on complement are strikingly different from those seen after the addition of an antigen-antibody aggregate (complement-fixation) (Table 2). Whereas an antigen-antibody aggregate (for example, Anti-SIII–SIII) "fixes" human C'1, C'2, and C'4 at either 1°C or 37°C and does not significantly inactivate C'3, PZ inactivates C'3 specifically and only at a temperature greater than 20°C.

Table 1. A comparison of the factors and conditions required for the combination of (i) human properdin with zymosan and (ii) antibody with antigen.

Human properdin with zymosan requires	Antibody with antigen does not require
Mg^{++} ions	Mg^{++} ions
Complement	Complement
Temp. $>$ 10°C	Temp. $>$ 10°C
*p*H 6.5 to 8.2	*p*H 6.5 to 8.2
$\mu = < 0.4$	$\mu = < 0.4$

Present evidence suggests that C'3 is an enzyme that either combines irreversibly with its substrate, PZ, or is destroyed in the reaction. Thus, C'3 may play the same role in the properdin system as it does in a specific immune system (*13*). In each case, after the "preparation" of the antigen, either by properdin or by antibody and complement, C'3 may exert its lytic or lethal action. The interaction of properdin with bacteria, red cells, and perhaps even with viruses is probably analogous to the interaction of properdin with zymosan. Experiments to test this hypothesis are in progress.

Properdin Content of Serums from Various Species and Human Body Fluids

Table 3 shows that there are differences in the properdin content of serums from different animal

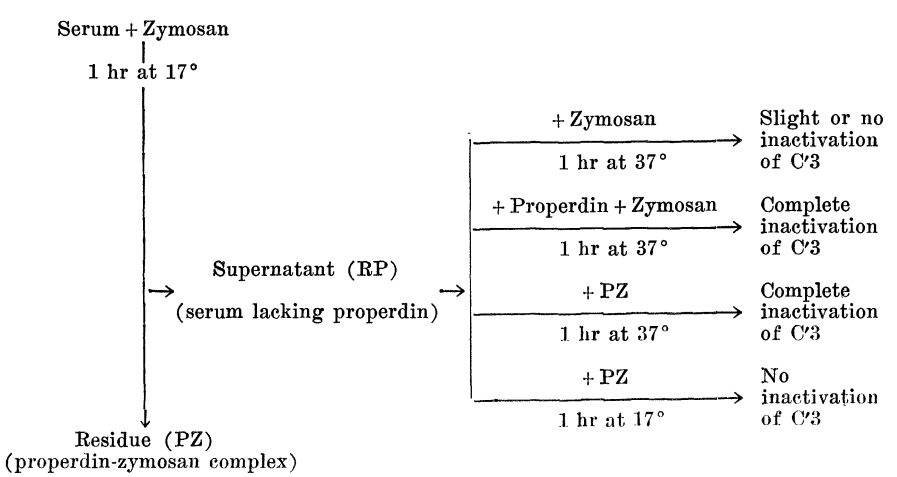

Fig. 1. The combination of zymosan with a substance (properdin) in human serum that is necessary for the inactivation of C'3 by zymosan at 37°C.

Table 2. A comparison of the effect of (i) properdin-zymosan complex (PZ) and (ii) antibody-antigen aggregates (Anti-SIII-SIII) on human complement components.

Human serum and RP treated with	Effect on complement components
PZ for 2 hr at 37°C	Complete loss of C'3; no loss of C'1, C'2, C'4
Anti-SIII-SIII for 2 hr at 37°C	Slight loss of C'3; almost complete loss of C'1, C'2, C'4
PZ for 18 hr at 1°C	No loss of any of the components
Anti-SIII-SIII for 18 hr at 1°C	Slight loss of C'3; almost complete loss of C'1, C'2, C'4

Table 3. Properdin titers in the serums of various animals.

Species	Properdin (units/ml serum)
Rat	25–50
Mouse	10–20
Cow	10–20
Hog	8–12
Human	4– 8
Rabbit	4– 8
Sheep	2– 4
Guinea pig	1– 2

species. Of the warm-blooded animals tested, the rat has the highest titer and the guinea pig has the lowest, while the human is intermediate. These results suggest that properdin may be important in natural immunity, for it is well known that the rat is very resistant to infection and the guinea pig is quite susceptible. Similarly, both the serum properdin level and the natural resistance of the cow and the hog are greater than that of the sheep.

Although normal human serum contains between 4 and 8 units of properdin per milliliter, no properdin is demonstrable in human spinal fluid, ascitic fluid, pleural fluid, colostrum, milk, and extracts of white cells and platelets.

Properties of Human Properdin

Human properdin is a euglobulin with a molecular weight at least 8 times that of gamma globulin. It represents not more than 0.03 percent of the total serum proteins. It is found in serum Fraction III separated by the method of Deutsch *et al.* (*14*). It is precipitated quantitatively with many other euglobulins by dialysis at *p*H 5.5 and low ionic strength. Properdin in serum is stable to heating at 48°C; its activity is slowly destroyed at 50°C, while complete inactivation occurs in 30 min at 56°C. Purified properdin, on the other hand, is stable to heating at 66°C for 30 min (Table 4), but is rapidly inactivated at 100°C. In this respect, properdin resembles certain typical antibodies (*15, 16*) that are more resistant to heat inactivation in the purified state than in serum or on admixture with albumin. Properdin is stable between *p*H 4.8 and 8.4 at ionic strength 0.15 and is not destroyed by freezing and thawing or by prolonged storage at 0°C or −20°C.

Properdin in serum is not destroyed by hydrazine or trypsin or by removal of divalent cations. It is not inactivated or removed by antigen-antibody aggregates. Properdin is not a component of either the clotting or plasmin systems of the blood. Oscar D. Ratnoff (personal communication) found that purified properdin was distinct from fibrinogen, fibrin, prothrombin, thrombin accelerator globulin, convertin, and antihemophilic substance, and from plasminogen, plasmin, and plasmin inhibitors. Properdin is not a component of hemolytic complement and is not necessary in specific immune systems. However, properdin has been found to participate in the bactericidal, virus-neutralizing, and hemolytic activities of serum in the absence of specific antibody but in the presence of complement and Mg^{++}.

The eluate from *bovine* properdin-zymosan complex exhibits properdin and conglutinin (*17, 18*) activities. *Human* properdin, however, has no conglutinating activity. Experiments are in progress to determine whether the conglutinin activity in bovine properdin preparations is due to properdin itself or to an impurity.

Activities of Serum Deficient in Properdin (RP)

Serum deficient in properdin after zymosan treatment (RP) was compared with normal serum in several of the physiological activities of blood. Table 5 shows that the removal of properdin by zymosan at 17°C is a very selective process. RP contains most of the complement and complement component activities of normal serum. The preparation of RP does not destroy factors associated with the clotting or plasmin systems. In fact, RP resembles normal serum in all respects except that (i) it is poorly, or not at all, bactericidal against a variety of organisms, (ii) it has lost its heat-labile virus-neutralizing properties, and (iii) it is not hemolytic against certain red cells.

Table 4. Properties of purified human properdin.

1) Euglobulin with minimum solubility between *p*H 4.8 and 6.5
2) Sedimentation constant about 27 S
3) Represents not more than 2 to 4 μg of protein nitrogen per milliliter of serum, or about 0.03 percent of the serum proteins
4) Stable to heating at 66°C for 30 min
5) Destroyed at 100°C in 5 min
6) Not a component of the blood-clotting, plasmin, or hemolytic complement systems
7) Participates in bactericidal, virus-neutralizing, and hemolytic reactions in the presence of complement and Mg^{++}.

Table 5. Comparison of the factors and activities of untreated human serum and properdin-free serum (RP).

Factor or activity	In untreated serum	In properdin-free serum (RP)
Hemolytic activity for sensitized sheep cells	Present	Present
Individual complement components	Present	Present
Susceptibility to complement fixation	Present	Present
Plasminogen	Present	Present
Plasmin inhibitors	Present	Present
Clotting factors	Present	Present
Hemagglutinin	Present	Present
Susceptibility of C'3 to inactivation by zymosan	Present	Absent
Heat-labile bactericidal activity	Present	Absent
Heat-labile virus-neutralizing activity	Present	Absent
Hemolytic activity for unsensitized cells	Present	Absent

Moreover, as is shown in the following section, the addition of purified properdin to RP restores these activities.

Properdin and Bactericidal Activity

Most of the experiments on the bactericidal activity of human serums were made by using a strain of dysentery bacillus (*Shigella dysenteriae*) as the test organism. The tests were made by adding a known number of viable bacteria, in the form of a washed suspension, to the serum, incubating the mixture for a standard time, and determining the residual number of viable bacteria by serial dilution and plating out. All normal human serums so far tested have shown high bactericidal activity, for they reduced the concentration of viable bacteria by a factor of about 10^5 during 2 to 4 hr incubation at 37°C. There was little variation among individual serums or pools of serums. Control experiments in which bacteria were incubated with buffered albumin solutions showed no reduction in count. In contrast to normal serum, serum from which the properdin had been completely removed (RP) was nonbactericidal, as was properdin by itself. However, RP to which sufficient properdin had been added to give the concentration present in serum had a bactericidal activity almost as high as normal serum. This indicated that properdin, acting in conjunction with factors present in RP, was involved in the bactericidal activity of normal serum against *Sh. dysenteriae*. Present evidence suggests that components of complement are among the necessary factors present in RP. Serum can be rendered nonbactericidal by treatment with reagents known to inactivate complement; for example, antigen-antibody aggregates, low concentrations of hydrazine, and zymosan at 37°C. A systematic study is under way to determine the susceptibility of other bacteria to the properdin system. Preliminary tests with other organisms have also shown that RP is less bactericidal than normal serum.

Properdin and the Heat-Labile Inhibitor of Viral Hemagglutination from Human Serum

Previous investigations (*19*) have demonstrated that human and other serums contain a heat-labile factor that combines with and inactivates viruses. This factor not only inhibits hemagglutination by influenza, mumps, and Newcastle disease viruses but also prevents infection by these agents. Ginsberg and Horsfall (*19*) made a detailed study on this factor in guinea pig serum and showed that hemolytic complement alone was not responsible for such antiviral activities of serum. The present study has been concerned with human serum, but work is in progress on the identification of antiviral factors in other serums, especially guinea pig serum.

In collaboration with Harold Ginsberg, the role of the properdin system in the heat-labile antiviral activity of human serum was investigated. These studies reveal that properdin and serum factors resembling, or identical with, components of complement, especially C'3 and C'4, are necessary for inhibition of viral hemagglutination. The inactivation or removal of any

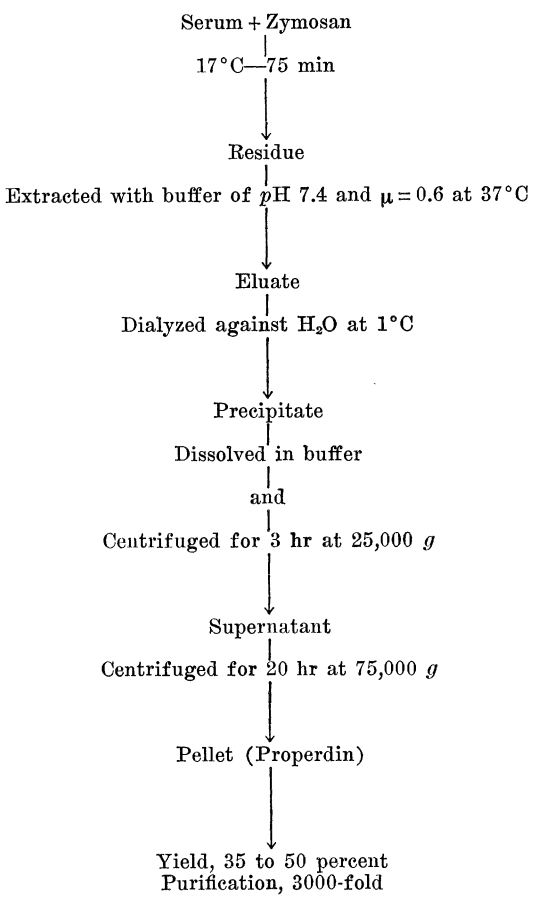

Fig. 2. Flow diagram for the isolation of human or bovine properdin.

Table 6. The properdin and complement titers in rats subjected to total body irradiation of 500 r.

Post-radiation (days)	Properdin (units/ml)	C′ 100% hemolytic units (ml)	Percentage of normal serum values			
			C′1	C′2	C′3	C′4
0	25–35	30–40	100	100	100	100
2	4– 6	50	100	100	100	200
7	< 1	50	100	135	200	200
13	< 1	60	100	200	300	200

of these factors inhibits antiviral activity. The addition of human properdin to serum deficient in properdin (RP) restores its antiviral activity. Studies are in progress to determine the exact role of the properdin system in the neutralization of viruses.

The Properdin System and Total Body Irradiation

Severe bacteremia is commonly encountered following total body irradiation of animals (*20, 21*). Antibiotics given early in the postradiation phase are moderately effective (*21–23*) in reducing the mortality and morbidity and in prolonging the survival time.

As stated previously, rats have a high content of properdin in their serums and are also very resistant to bacterial infections. The susceptibility of irradiated rats to severe bacteremia of enteric origin suggested that the properdin system might be destroyed by total body irradiation. Experiments were conducted in collaboration with A. R. Moritz to test this possibility. Large groups of animals were subjected to total body irradiation of 500 r. Blood was collected 2, 7, and 13 days after irradiation, and the serums of each group were pooled. The properdin and complement titers of these serums were compared with pooled serum from untreated animals. Table 6 shows that properdin falls markedly during the early postradiation period. It is noteworthy that complement and complement-component titers increased after irradiation. The low properdin levels reached between 2 to 7 days suggest a causal relationship between the destruction of properdin and the onset of severe bacteremia with subsequent death. While the serums from untreated animals had bactericidal and virus-neutralizing properties, the serums from irradiated rats had lowered bactericidal activity and had little or no virus-neutralizing power.

Stroud and Brues (*24*) have recently reported the partial protection of irradiated mice by the injection of fractions of serum, especially Fraction III (Cohn). Since practically all the properdin of serum is recovered in Fraction III, properdin may be the agent responsible for protection. Indeed, in a preliminary experiment, rats subjected to total body irradiation of 660 r (LD_{90} in 30 days) and injected intravenously with 250 units of purified cow properdin 2, 4, and 7 days after irradiation were partially protected. A control group of irradiated rats injected with saline buffer were not protected.

Further studies are under way to elucidate the mechanism of the loss of properdin in irradiation injury and the role of properdin in irradiation illness. Large amounts of human and bovine properdin are being prepared and will be tested for their efficacy in protecting animals against bacteremia following irradiation.

Role of the Properdin System in Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disease in which the patient's erythrocytes are lysed both *in vivo* and *in vitro* by his own serum or by the serum of compatible donors (*25, 26*). No specific hemolysin or antibody against PNH cells has been detected in serum (*25, 26*). Thus, the disease appears to be caused by a defect in the PNH erythrocytes.

Marked similarities were noted earlier (*11*) between the requirements for the inactivation of C′3 by zymosan and for the hemolysis of PNH erythrocytes. Both reactions require Mg^{++} and serum components resembling C′, and both proceed optimally at *p*H 7. Neither system requires a specific antibody. Stroma prepared from PNH red cells inactivate C′3 at 37°C in a manner analogous to that of zymosan.

Experiments conducted recently with C. F. Hinz, Jr., and W. S. Jordan, Jr., reveal that the inactivation or removal of any factor of the properdin system results in an inhibition of lysis of PNH cells. Serum lacking or deficient in properdin is not lytic for PNH cells, although it contains adequate amounts of all the components of C′ and all measurable elements of the clotting and plasmin systems. The addition of purified human, cow, or hog properdin to serums lacking or deficient in properdin restores the lytic activity of the serum. Properdin alone in high concentrations does not lyse PNH cells. It is also relevant that guinea pig serum, naturally rich in C′, poor in properdin, and inactive in the PNH system, hemolyzes PNH cells after the addition of human or cow properdin or of small amounts of rat serum, which is extremely rich in properdin. These results suggest that the properdin system may be involved in hemolytic phenomena in which a specific hemolysin cannot be demonstrated. E. R. Arquilla (personal communication) has found that the factors in guinea pig serum that are responsible for the lysis of unsensitized sheep cells resemble those of the properdin system. The addition of human or cow properdin to guinea pig serum that has been depleted of properdin restores its full lytic activity.

Appendix 1

Preparation of Reagents for the Titration of Properdin

Serum free of C′3 and properdin (R3). Methods for the preparation of R3 have been described previously (*10, 11*). Special precautions are taken to discard any R3 which, when tested at the two-unit level, is either hemolytic alone or anticomplementary, as judged by failure to give complete hemolysis with one-

fourth of a 100-percent hemolytic unit of C′. An R3 should be capable of measuring 120 C′3 units/ml of RP.

Serum lacking or deficient in properdin (RP). The same amount of zymosan that is required to prepare an R3 is employed to prepare RP. RP is prepared only from serum from which suitable R3 could also be prepared.

Between 2 and 4 mg of zymosan per milliliter of serum is suspended evenly in serum at 15°C to 18°C for 75 min. The control of temperature is important. Temperatures below 15°C result in an incomplete removal of properdin, while temperatures above 18°C result in a loss of C′3 activity in RP. After incubation, the mixture is centrifuged at 4000 rev/min for 15 min at 0°C. The supernantant (RP) is decanted and tested for properdin and for C′ and C′-component activities. If satisfactory, the RP is dispensed in test tubes and maintained at −20°C until needed. In this state, it remains stable for at least 6 wk.

We have established the following criterions for RP. (i) It must have a C′ titer and C′-component titers of not less than 75 percent of the parent serum. (ii) Upon treatment with zymosan at 37°C for 1 hr, its C′3 titer should not decrease more than 25 percent. (iii) It should not be anticomplementary when combined with R3. (iv) It should contain not less than 90 or more than 180 C′3 units/ml when tested against 2 units of R3.

A single treatment of serum with zymosan at 15°C to 18°C is often insufficient to remove all the properdin. Such an RP may be used for the assay of properdin, but it is not always suitable for bactericidal and virus-neutralizing studies. A second treatment of RP with zymosan at 37°C for 15 to 30 min removes all the remaining properdin with only a slight loss of C′ or C′-component activities. This treatment is generally employed for bactericidal and virus-neutralizing studies.

Titration of Properdin

One-half millimeter of RP is added to each of a series of test tubes containing 3 mg of zymosan. Varying amounts of the material to be tested are then added. If necessary, sufficient $MgCl_2$ is added to yield a final Mg^{++} concentration of $5 \times 10^{-5} M$. Each is made up to a final volume of 1.5 ml with *p*H 7.4 barbital buffer containing $5 \times 10^{-5} M$ Mg^{++} and is incubated with occasional mixing at 37°C for 1 hr. The mixtures are then centrifuged, and the supernatants are tested for C′3 activity. A unit of properdin is defined here as the smallest amount of serum, body fluid, serum fraction, and so forth, that will result in the loss of 120 units (50 percent end-point), of C′3 from 1 ml of RP in this test system.

A typical protocol for a properdin titration is given in Table 7. The C′3 titers are calculated on the basis that the mixtures of RP, test serum, buffer, and zymosan are at a dilution of 1 : 3. Sample 9 is a routine C′3 titer on the test serum. Sample 8 is an anticomplementary control. The presence of the test serum should not depress the C′3 titer of the RP; in most instances, it should enhance it. Sample 10 verifies the test and shows that the RP contains all the accessory factors necessary for the inactivation of C′3.

Table 7. A typical protocol for a properdin titration on a normal human serum. The properdin titer is 4 to 8 units/ml of test serum.

Tube No.	RP (ml)	Test serum (ml)	Buffer (ml)	Zymosan (mg)	C′3 titer (units/ml)	
1	0.5	0.50	0.50	3	0	
2	.5	.25	.75	3	0	
3	.5	.12	.88	3	0	end-point
4	.5	.06	.94	3	tr	end-point
5	.5	.03	.97	3	30	
6	.5		1.0	3	120	
7	.5		1.0		120	
8	.5	.50	0.5		180	
9		.50	1.0		240	
10	.5	1 standard unit properdin in 0.1 ml buffer	0.9	3	0	

With the present method it is difficult to determine properdin concentrations lower than 1 unit/ml of sample. An assay is being developed to determine the presence of minute amounts of properdin that will involve both this method and a bactericidal test. Methods for the titration of complement and its components have been described elsewhere (*10, 12*).

Appendix 2

Purification of Properdin

Properdin has been isolated in good yield from human and bovine serums by a process that makes use of the specific combination of properdin with zymosan and subsequent dissociation of the properdin-zymosan complex (Fig. 2). The properdin-zymosan complex is prepared by suspending zymosan in serum for 75 min at 17°C, centrifuging the complex, and washing it three times with physiological saline at 1°C. The residue is then dissociated by stirring with a slightly alkaline buffer of ionic strength 0.6 at 37°C; the zymosan is centrifuged down, leaving properdin in the supernatant. The properdin is precipitated from the supernatant by dialysis against distilled water at *p*H 5.8. The precipitate is dissolved in buffer at *p*H 7.5, ionic strength 0.15 and freed of suspended impurities by centrifugation at 25,000 *g*. The supernatant is then centrifuged at 75,000 *g* to yield properdin as a pellet. This process affords a 3000-fold purification of properdin compared with the original protein content of serum on a protein nitrogen basis. An over-all yield of 35 to 50 percent has been consistently obtained.

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7. Detailed reports will be published later on the role of the properdin system (i) in total body irradiation with A. R. Moritz; (ii) in infectious and noninfectious diseases with W. S. Jordan, Jr., Harold Ginsberg, and R. H. Seibert; (iii) in virus neutralization with Harold Ginsberg and R. H. Seibert; (iv) in paroxysmal hemoglobinuria with C. F. Hinz, Jr., and W. S. Jordan, Jr.; (v) in clotting and plasmin systems and in hepatic disease with O. D. Ratnoff. Physical characterizations of properdin are being conducted with M. D. Schoenberg.
Thanks are due to Leona Wurz and Jack Pensky for valuable technical assistance.
8. This investigation was supported by a grant from Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y. Zymosan was kindly supplied by Fleischmann Laboratories, Standard Brands, Inc., Stamford, Conn.
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Edward Monroe Freeman, Pioneer Plant Pathologist

EDWARD MONROE FREEMAN, pioneer plant pathologist and Dean Emeritus of the College of Agriculture, Forestry and Home Economics of the University of Minnesota, died at his home near the St. Paul Campus of the University on 5 February 1954. Dr. Freeman was born in St. Paul 12 February 1875, attended public schools in his native city, and obtained the B.S., M.S., and Ph.D. degrees from the University of Minnesota in 1898, 1899, and 1905, respectively. Except for 1 year at Cambridge University, England, in 1901–02, and 2 years in the United States Department of Agriculture, 1905–07, Dr. Freeman's professional life was spent at the University of Minnesota, from which he retired to emeritus status in 1943.

Not only was Dr. Freeman one of the most eminent of the pioneers in the development of scientific plant pathology in the United States, but he also contributed significantly to the development of a broader and deeper scientific basis for education and research in the general field of agriculture. He was especially well qualified for his extraordinary contributions by exceptional native ability, a liberal education, and wide experience.

Freeman's education was unusually extensive and intensive. He did not have formal education in agriculture; he studied in the College of Science, Literature and the Arts of the University. And he actually studied sciences, literature, and the arts. Everything interested him but especially the sciences. For a time he studied mathematics, astronomy, physics, chemistry, geology, zoology, and botany with approximately equal avidity. Gradually, however, he developed special interest in zoology and botany and finally chose botany as his major in graduate work

It was characteristic of Freeman that he tried to learn everything possible about everything that he decided to study. It is natural, then, that he acquired unusually extensive knowledge about the plant kingdom—slime molds, algae, fungi, liverworts, mosses, ferns, and seed plants. He became an exceptionally good general botanist. But the fungi finally captured his special interest, partly because they were so interesting in themselves and partly because they were so important as parasites on other plants. Freeman never lost interest in the evolution and nature of parasitism, but he also developed a very deep interest in preventing the devastating effects of fungus parasites of economic plants. He became a plant pathologist.

As a plant pathologist, Freeman developed concepts and charted fields of investigation that contributed richly to the understanding and control of plant diseases. His concepts were compounded of shrewd speculation, tempered by thorough scientific scholarship, and buttressed by results of keen observation and persistent experimentation. He was essentially curious, imaginative, and speculative, but he was practical also. He had a passionate interest in the facts and theories of science, but he also had an impelling desire to learn their practical values. As a graduate assistant, he taught pharmacognosy; he went to Cambridge to study plant parasite-host relationships in the laboratory of the eminent Marshall Ward; as assistant professor of botany in an academic department, he gave courses in economic botany and plant pathology and in 1905 he published the 400-page book, *Minnesota Plant Disease.*

In the preface to his book, Freeman emphasizes the