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EDITED BY

FRANK J. DIXON
Scripps Clinic and Research Foundation
La Jolla, California

HENRY G. KUNKEL
The Rockefeller University
New York, New York

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LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

ROBERT E. ANDERSON, *Department of Pathology, The University of New Mexico, Albuquerque, New Mexico* (215)

HOWARD B. DICKLER, *Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland* (167)

O. GÖTZE, *Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California* (1)

H. J. MÜLLER-EBERHARD, *Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California* (1)

GEORGE F. SCHREINER, *Department of Pathology, Harvard Medical School, Boston, Massachusetts* (37)

EMIL R. UNANUE, *Department of Pathology, Harvard Medical School, Boston, Massachusetts* (37)

NOEL L. WARNER, *Genetics Unit, The Walter and Eliza Hall Institute, Melbourne, Australia* (215)

PREFACE

This volume contains four reviews that reflect both the broad spectrum and the interrelatedness of today's immunologic research. They range from the molecular characterization of the properdin system, to very critical analyses of some of the cell surface interactions which play important roles in the immune response and, finally, to a consideration of the vast amount of experimental data dealing with the effects of radiation on immunologic mechanisms. Today, the state of the immunologic jigsaw puzzle has progressed to the point where significant advances in one area almost immediately aid in the solution of problems in another, and the diverse reviews contained herein illustrate the cellular and molecular bases of this interrelationship.

Since its first description in 1954 the properdin system has been the center of much controversy. In the first article, Drs. Götze and Müller-Eberhard, leaders in the recent development of this field, define the properdin or alternative C pathway in molecular terms. The five plasma proteins constituting this pathway (initiating factor, proactivator, C3, proactivator convertase, and properdin) may be triggered by naturally occurring polysaccharides and lipopolysaccharides or by aggregates of IgA, after which they interact to form specific C3 and C5 convertases without participation of the classical components C1, C2, and C4. It now appears that properdin itself does not play the central role as was originally thought but rather is recruited late in the sequence and serves primarily to stabilize and protect the enzymes of the system against inactivators. The authors also review current knowledge of the biologically relevant interactions of this system, which apparently constitute a protective mechanism operative early during bacterial infection before effective antibody levels have been established.

Nowhere in the field of immunology has recent progress been greater than in our understanding of the events triggered in lymphocytes by ligand–surface receptor interactions. In the second review, Drs. Schreiner and Unanue draw on their own considerable experience in detailing and putting into broad biologic perspective the intricate sequence of events initiated in B cells by the interaction of their surface Ig with appropriate ligands. These events involving the cell surface, cytoplasm, and nucleus include redistribution of ligand-Ig complexes into polar caps, stimulation of cell movement with formation of uropods, endocytosis and elimination of ligand-Ig complexes and, finally, synthesis and replacement of Ig receptors, bringing the cell about full cycle. The free movement of recep-

tor–ligand complexes in the cell membrane, leading to the elimination of the ligand, has obvious implications for the immune response as does the stimulation of cell motion which would enhance any cell–cell interactions that might be involved. It already appears that failure of B cells to accomplish one or more of these responses may lead to tolerance rather than a response. Thus, these fundamental and probably relatively general cellular responses appear to be very integral elements of immunologic responsiveness.

Because the immune response involves a complex sequence of interactions among several different cell populations, the cell surface membranes via which these interactions are mediated are the object of much investigation. In the third article, Dr. Dickler, whose own research has made an important contribution to this field, discusses one of the most important elements of these cell surfaces, the Fc receptor, via which lymphoid cells can bind Ig and thus achieve a measure of immunologic specificity. Such Fc receptors are a property not only of a majority of B cells, but also of a substantial minority of T cells and many undefined lymphocytes. While the biological significance of such surface bound Ig is not as yet certain, it seems likely that it may play a role in antigen localization and is certainly required for antibody dependent cellular cytotoxicity.

Ionizing radiation has long been a potent tool in the study of immunologic responsiveness. However, much of this research was done prior to the development of our current understanding of cellular immunology and the recognition that subpopulations of lymphocytes exist and cooperate in immune responses. In the fourth article, Drs. Anderson and Warner review the critical earlier experiments dealing with the effects of radiation on immune responses and reexamine them in the light of our present knowledge of cellular immunology. They consider the effects of radiation on lymphoid cells *in situ* and *in vitro,* on antibody production, transplantation immunity and other forms of cellular immunity, and on tolerance, with the implied relationship to possible autoimmune responses. Finally, they outline ways in which radiation may be employed to study individual cellular components of the immune response by using the differential radiosensitivities of various lymphoid populations to help define their specific functions.

As always, the editors wish to thank the authors, who have given generously of their time and meticulous effort, and the publishers, who do much to ensure a volume of high quality.

FRANK J. DIXON
HENRY G. KUNKEL

CONTENTS

Ionizing Radiation and the Immune Response

ROBERT E. ANDERSON AND NOEL L. WARNER

The Alternative Pathway of Complement Activation[1]

O. GÖTZE[2] AND H. J. MÜLLER-EBERHARD[3]

Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California

I. Introduction

Two pathways for the recruitment of complement and its biological activities exist in plasma and serum. The first one, now termed the classi-

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[2] Recipient of an Established Investigatorship No. 73-144 from the American Heart Association.

[3] Cecil H. and Ida M. Green Investigator in Medical Research, Scripps Clinic and Research Foundation.

cal pathway, is triggered by contact of C1 with immune complexes containing antibodies of the IgG or IgM class. Recognition of immune complexes by C1 is followed by the assembly of the activation unit (C2, C3, C4) and the membrane attack unit (C5, C6, C7, C8, C9). The second pathway, the alternative or properdin pathway, is activated by naturally occurring polysaccharides and lipopolysaccharides and by aggregates of IgA. It consists of at least five distinct proteins: the initiating factor, proactivator, C3, proactivator convertase, and properdin. The two pathways lead to the formation of their respective C5-activating enzymes (convertases), and the action of either C5 convertase on C5 results in the self-assembly of the C5b-C9 complex. A requirement for C3 is shared by both pathways: Native C3 is required for the formation of the initial C3 convertase in the alternative pathway, and its major fragment, C3b, is an essential subunit of the C5 convertases of the classic as well as of the alternative pathways. The alternative pathway of complement activation may then be defined as comprising those plasma proteins that, after a triggering event, interact to form specific C3 and C5 convertases without the participation of the classical components C1, C2, and C4.

Three reviews of the properdin system have been published, the first one 2 years after the description of the system (*109*). An extensive review and compilation of the earlier literature by Schmidt appeared in 1959 (*218*). A comprehensive account of the more recent work was given by Osler and Sandberg in 1973 (*180*). The present chapter reviews the current knowledge of the proteins of the properdin system, their biologically relevant interactions, and the modulation and regulation of these interactions. Emphasis will be placed on a novel comprehension of the entire properdin pathway which grew out of the most recent work of this laboratory. In essence this concept envisages a single enzyme to fulfill the various functions of the pathway by being modulated sequentially by different factors.

II. History

Two different experimental approaches have been used independently in virtually all explorations of the properdin system. The observations on which these two approaches are based were made early this century by several of the first investigators of the complement system. The first set of observations that established the anticomplementary activity of cobra venom (*37, 69, 178, 204, 264*) led to the isolation of the active principle from cobra venom (cobra venom factor or CVF) and to an analysis of its action on the third component of complement (C3). The other series

of experiments that demonstrated the anticomplementary activity of yeast cells and certain bacteria (*37, 53, 269*) were the basis for the studies of Pillemer and his collaborators on the mechanism of action of these substances on C3. This work resulted in the formulation in 1954 of a new concept of natural resistance to infections and the first description of the properdin system (*196*). Only recently has it become clear that both active principles, CVF and yeast cells (zymosan), utilize the same zymogen in serum (proactivator or Factor B) to activate and consume C3.

The findings of Whitehead *et al.* (*269*) on the action of yeast cell powder (zymin) on the heat-stable C3 were confirmed by Pillemer and Ecker (*190*) who prepared the active, insoluble, carbohydrate-rich material from yeast by tryptic digestion and extraction of the insoluble fraction with boiling water and ethyl alcohol. Studies on the action of this material, later termed *zymosan* by San Clemente and Ecker (*213*) to indicate its origin from yeast and its general carbohydrate character, led to the description by Pillemer of the new serum protein, *properdin*. The name is derived from the Latin and implies a capacity to destroy or eradicate. The properdin system was thought to be composed of properdin (P), complement, and magnesium ions (Mg^{2+}) (*189, 196*), because earlier investigations of the inactivation of C3 by zymosan had established a requirement for Mg^{2+} (*193*) as well as a hydrazine-sensitive and a heat-labile serum factor (*192*). The requirement for these serum factors was taken as an indication for the participation of C1, C2, and C4 (*189, 192*). The reaction between properdin and zymosan was shown to proceed at 15°–17°C in the presence of complement and Mg^{2+} and to result in the formation of a properdin–zymosan complex (PZ) that had the capacity to inactivate C3 when incubated with a source of this component at 37°C (*195*). Properdin could be recovered from the zymosan complex by elution with alkaline buffers of high ionic strength and could be further purified by precipitation at low pH and ionic strength (*195*). Properdin-depleted serum (RP) was found to be deficient in several biological activities, which could be restored upon addition of partially purified properdin. These activities included the killing of certain bacteria (*196*), the inactivation of susceptible viruses (*196*), the lysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH) (*196*), and the killing of the protozoan *Toxoplasma gondii* (*65, 94*). A large number of polysaccharides other than zymosan were reported to interact with the properdin system (*168, 194*) or to inhibit the formation of an active properdin–polysaccharide complex (*136*). Among these substances were cell wall constituents and endotoxins of *Escherichia coli*,

salmonellas, and pneumococci, certain glucans, dextrans, and levans as well as some preparations of gastric mucin. Strong inhibitory activity was found in several pyranosides but also in D-fructose and D-mannose.

The observations reported by Pillemer and his collaborators stimulated extensive investigations on the possible participation of properdin in various biological phenomena. Numerous publications dealt with the role of properdin in humoral defenses against bacterial and viral infections (*134, 179, 247, 259, 260, 262, 263*), lysis of PNH erythrocytes (*100, 101*), protection against the effects of ionizing radiation (*191, 205, 206*), sequelae of hemorrhagic and other types of shock (*68, 70, 139*), and resistance against cancer (*99, 208, 232*). The reports on the findings in these areas are too numerous and in part too uncritical to be all included in this review. Most of them are listed in Schmidt's monograph (*218*). We wish to emphasize, however, that the basic observations and conclusions of Pillemer and his collaborators published between 1954 and 1959, are born out by recent investigations. The flood of publications on the properdin system came to a virtual standstill in the years 1959–1960. This was due partly to the sudden death of Pillemer in August 1957 and partly to Nelson's proposal of an "alternative mechanism for the properdin system" in 1958 (*173*). Nelson's thesis was that the activities of the properdin system can be explained entirely by the presence in most sera of natural antibodies to zymosan that utilize C1, C2, and C4 for C3 fixation. This thesis was supported by the findings that (a) antigen–antibody complexes formed with limited amounts of antibody led to preferential fixation of C3 as did zymosan, (b) the rate of decay of PZ was similar to that of the EAC1,4,2 complex, (c) most sera, indeed, contained antibodies to zymosan, and (d) partially purified properdin, like antibody, agglutinated zymosan particles. Other investigators furnished additional evidence for the association of immunological specificity with the properdin system (*20, 45, 167, 179, 243, 261*). Today the controversy between Pillemer and Nelson is amply explained by the now established fact that, in human serum, both pathways are usually activated by zymosan (*231*).

More recently, a reevaluation of the concept of a second pathway of complement activation was undertaken. The presence of a second pathway of complement activation was firmly established by the work of (a) Gewurz *et al.* (*74*) who showed differential C3–C9 consumption by endotoxin and immune complexes, (b) Sandberg *et al.* (*214, 216*) who described the complement activating capacity of guinea pig γ_1 anti-DNP antibodies and their F(ab)'2 fragments, and (c) Frank and his collaborators (*55, 71*) who described a strain of genetically C4-deficient guinea pigs and the ability of C4-deficient serum to sustain complement activation. That complement-dependent reactions are not abrogated in C4-deficient guinea pig serum was consistent with results obtained by

Marcus *et al.* (*140*) indicating that the C3-cleaving activity found on lipopolysaccharide after interaction with guinea pig serum is not inhibited by an antiserum to C2.

It was at this time that the C3 proactivator had been isolated from human serum by us and characterized as the precursor of a C3-cleaving enzyme (*85, 86*). The discovery of the proactivator (PA) was a direct consequence of the study of the mechanism of action of CVF, which had previously been isolated and used to generate anaphylatoxin *in vitro* (*112*), to remove anaphylatoxinogen *in vivo* (*249*), and to inactivate C3 *in vitro* and *in vivo* (*38, 123, 174, 227, 265*). That the action of CVF on complement was not restricted to C3 but did result in the activation of the terminal complement components was subsequently reported by several laboratories (*14, 17, 84, 188*). It appears that the requirement of a heat-labile cofactor for the effect of cobra venom and bacteria on the third, heat-stable complement component was first pointed out by Nathan in 1917 (*171*). This report seems to have been forgotten. Fifty years later, it was found that highly purified CVF, a glycoprotein with a molecular weight of 140,000 daltons, does not affect isolated C3 but functions together with at least one normal serum protein, C3 proactivator, with which it forms an enzymatically active complex (*18, 157, 159, 166*).

Two modes of interaction of CVF with PA became apparent. The CVF and PA interact reversibly in the presence of Mg^{2+} to form a complex that has some C3- activating capacity (*41, 85*). The formation of a highly active stable complex is dependent on the proteolytic activation of PA by PA convertase (Factor D) (*41, 105, 251*). The recognition of PA as the precursor of a CVF-dependent C3 convertase prompted an exploration of its physiological role. It was found that inulin, a polyfructose that was used instead of zymosan (*148*) and that was known to generate anaphylatoxin in serum without significant consumption of early acting complement components (*182*), generated PA-cleaving activity in serum. The enzyme responsible for PA cleavage was termed C3PA convertase (PAse). This work led to the concept of the "C3 activator system," which included a heat-labile (PA) and a hydrazine-sensitive factor as well as PAse (*85*). Subsequent work resulted in the identification of C3 as the hydrazine-sensitive factor and in the description of the C3b-dependent feedback mechanism of the alternative pathway (*160*). Because of various similarities, a relationship of the C3 activator system to the properdin system appeared probable and was confirmed by showing the requirement of chromatographically isolated, highly purified properdin for full expression of the C3 activator system (*89*). The two previously recognized cofactors of properdin, the hydrazine-sensitive Factor A (*183*) and the heat-labile Factor B (*21*) could be equated with C3 (*78, 160*) and PA (*77, 85*), respectively. Finally, the very recent evi-

dence that CVF is, indeed, cobra C3b and is immunochemically related to human C3 (5) unifies the two described experimental approaches.

III. Nomenclature

The nomenclature of the properdin system has unfortunately been confusing because of the many synonyms used by different workers for the same activities or factors. Again, this is, in most part, due to the different approaches to the study of the problem, but it may also be attributed to the preference of some investigators to use historical symbols rather than terms reflecting the known function of the proteins involved. To date, a definitive nomenclature has not been adopted, although a provisional agreement has been reached to use, interchangeably, capital-letter symbols and descriptive, functional terms (Table I) (Resolution of the Complement Nomenclature Committee at the Second International Congress of Immunology, 1974, Brighton, U.K.).

IV. Proteins of the Alternative Pathway

A. Initiating Factor

Initiating factor (IF) is a heat-stable, 7 S pseudoglobulin that appears to consist of two identical polypeptide chains of 80,000–90,000 daltons each (222). It behaves very similarly to C3b inactivator on ion exchange and molecular sieve chromatography and electrophoresis. However, the two entities are physically distinct as evidenced by their different mobilities upon alkaline polyacrylamide gel electrophoresis. To date, the

TABLE I
Nomenclature of the Properdin System Proteins[a]

Component	Symbol	Scripps Clinic	Others
Initiating factor	IF	IF	—
Third component of complement	C3	C3	A
Proactivator	B	PA	GBG
Proactivator convertase	D	PAse	GBGase
Properdin	P	P	—
C3b inactivator	C3b INA	KAF	—
C3b inactivator accelerator	A-C3b INA	β_{1H}	—

[a] Activated components are generally denoted by the use of an overbar, e.g., $\bar{B}$ or $\bar{P}$ for activated Factor B or activated properdin.

final purification of IF has not been achieved. By physical and immunochemical properties, IF is distinct from immunoglobulins, from the C3b inactivator, and from other known proteins of normal serum.

The requirement for IF in the properdin system was recognized through work on an unusual serum protein that was found in the circulation of some patients with chronic hypocomplementemic glomerulonephritis (*235*), originally designated persistent hypocomplementemic glomerulonephritis (*266*) or progressive glomerulonephritis in the infant (*79*). This protein, termed C3 nephritic factor (NF), was shown to generate, in the presence of serum cofactors, a Mg^{2+}-dependent C3 convertase that, once formed, acted on C3 in the presence of ethylenediaminetetraacetic acid (EDTA) (*235, 245*). One of the cofactors, a heat-labile pseudoglobulin, was subsequently identified as PA (*212*), the others as native C3 and PAse (*224*). Initially it was suggested that NF might be related to IgG3 (*241, 242*). When NF was purified it was found to be a 7 S γ-globulin with a molecular weight of 150,000 daltons, being distinct from IgG (*246*). Nephritic factor is a glycoprotein with an isoelectric point of 8.75–8.95 (*47*). It is composed of two disulfide-linked polypeptide chains of 85,000 daltons each (*220*). An antiserum to NF was found to remove a factor from normal human serum that is essential for activation of the properdin system by zymosan, inulin (*152, 221, 222*), or rabbit erythrocytes (*197, 220*). This factor, which was termed IF, can be recovered in precursor form from the anti-NF immune absorbent column by elution with 0.2 *M* glycine–HCl, pH 2.2 (*221, 222*).

B. Proactivator

Proactivator was the first component of the properdin system to be recognized as an enzyme or, more specifically, the zymogen of a proteinase (*85, 86, 160, 166*). Proactivator is a β_1-glycoprotein with an isoelectric point of 6.6 (*59*). It consists of a single polypeptide chain of 93,000 daltons (*80, 81, 273*). The total carbohydrate content of PA was reported to be 10.6%, and the sedimentation coefficient $s_{20,w}$, 6.2 S (*24*). By sucrose density ultracentrifugation, its *s* rate was found to be 5.7–6 S (*41*). The protein exhibits genetic polymorphism with two common alleles, Bf F, (Gb^F) and Bf S (Gb^S) (*6, 7*), and it is genetically linked to the major histocompatibility locus in man (*3*) and monkey (*272*). The observed polymorphism was thought to be due to a tetrameric structure of PA (*4, 7*). The single polypeptide nature of the molecule necessitates revision of this concept.

Proteolytic activation of the molecule results in its cleavage into two fragments, Ba and Bb. The Bb fragment, previously called C3 activator (C3A) because it carries the active site of the enzyme (*85*), has a

molecular weight of 63,000 daltons, (*81*) and migrates as a γ-globulin (*24, 85*). The Ba fragment, which is inert, has a molecular weight of 30,000 daltons (*81*) and migrates as an α_2-globulin (*24, 85, 89*). The enzyme cleaves *N*-acetylglycyl-L-lysine methyl ester (AGLME) which is also a substrate for C2, the classical pathway counterpart of PA (*40, 42*). Diisopropylfluorphosphate (DFP) at a concentration of 10^{-3} to 10^{-2} *M* (*62, 81*), *p*-nitrophenyl *p'*-guanidine benzoate, an active site titrant for trypsin (*35*), and the trypsin inhibitors of bovine lung (Trasylol®), soybean, and lima bean as well as ovomucoid do not inhibit the action of the enzyme on C3 (O. Götze, unpublished). Recently, however, it was possible to demonstrate under modified conditions DFP inactivation of, and incorporation into, the zymogen and the activated form of Factor B and to establish that the enzyme constitutes a serine protease (*151a*).

A protein, apparently identical to Bb, was isolated from human plasma utilizng dextran sulfate and termed β_2-glycoprotein II by Haupt and Heide (*98*). These authors also observed electrophoretic heterogeneity of the isolated glycoprotein that could not be explained by differences in the neuraminic acid content of the two observed major bands. Genetic polymorphism as an explanation for these observations was, therefore, considered. Boenisch and Alper (*23*) purified the same protein, which they termed glycine-rich γ-glycoprotein (GGG) on the basis of its glycine content (8.35% of the peptide moiety). They observed an immunochemical relationship of GGG to a β-globulin that they subsequently purified and termed glycine-rich β-globulin (GBG) (*24*). Later, the identity of GBG with PA was established (*85*).

C. Components C3 and C3b

Component C3, a β_2-glycoprotein (*161, 163*), consists of two disulfide-linked polypeptide chains of 110,000 daltons (α-chain) and 70,000 daltons (β-chain) (*25, 177*). Proteolytic activation results in cleavage of the α-chain and the release of a basic 9000-dalton fragment, the anaphylatoxin C3a (*25, 26, 51, 102, 103*). The major fragment, C3b, is more negatively charged than its precursor and migrates as an α_2-globulin. Concurrent with the loss of C3a, the b fragment acquires a transiently available binding site for suitable acceptors (*162*). A second, stable binding site allows the bound or fluid phase molecule to interact with the immune adherence receptor (C3 receptor) on various mammalian cells (*54, 135, 172*). Component C3b is degraded by a serum enzyme, C3b inactivator (C3b INA), also called conglutinogen-activating factor (KAF), which cleaves C3b into C3c (140,000 daltons) and C3d (30,000 daltons) (*75, 209*). The C3b also contains a site with which it interacts with PA to form a reversible complex in the presence of Mg^{2+} (*149, 151, 175, 250,*

253). This complex, C3b,B is converted to a C3-converting enzyme ($\overline{C3b,B}$) upon proteolytic activation of PA by PA convertase (*149, 151, 160, 175, 250, 253*). Moreover, C3b also exhibits a binding site for activated properdin (*58, 224*).

D. Proactivator Convertase

Human PAse, the second recognized proteinase of the properdin system (*85, 160*), is a 2.5–3 S α-globulin with a molecular weight of 24,000 daltons consisting of a single polypeptide chain (*81*). Its isoelectric point as determined by isoelectric focusing was found to be 7.4 (*59*). The enzyme has also been purified from guinea pig serum (*29, 52*) and was found to have a molecular weight of 22,000 daltons, an *s* rate of 2.6 S, and an isoelectric point of 9.5 or 9.35, respectively. An antigenic relationship between guinea pig and human PAse has been demonstrated (*29b*). The reported isoelectric properties appear incompatible with the electrophortic behavior of PAse in fresh serum (*160*).

Human PAse was reported to exist both in an active and a zymogen form in fresh serum. The active enzyme can be generated by treatment of the precursor with trypsin (*62*). The reported effect of activated properdin on the zymogen (*63*) has been reinterpreted (*58*). Treatment with 5×10^{-3} *M* DFP completely inactivates the enzyme, whereas tosyl-L-lysine chloromethylketone (TLCK) at a concentration of 10^{-2} *M* has no effect (*62*). In another report 10^{-2} *M* DFP resulted in only partial inactivation of PAse. (*81*). The trypsin inhibitors from bovine lung (Trasylol®), soybean, lima bean, and ovomucoid have no effect on the active enzyme (O. Götze, unpublished). Further evidence for the serine esterase nature of PAse was obtained by showing that cyclohexylbutylphosphonofluoridate inhibits the enzyme noncompetitively at a concentration of 5×10^{-5} *M* and that *p*-tosyl-L-arginine methyl ester (TAME) inhibits it in a competitive fashion (*57*). Proactivator convertase does not act on casein, hemoglobin, fibrin, or elastin (*82*).

E. Properdin

Activated properdin ($\overline{P}$) is one of the most cationic proteins of human serum, having an isoelectric point of greater than 9.5 (*89*). It was first obtained in highly purified form by Pensky *et al.* (*184*) who determined its molecular weight (223,000 ± 7000 daltons) and sedimentation coefficient ($s_{20,w}$, 5.1–5.3). Minta and Lepow (*155*) analyzed the protein again and found its molecular weight to be 184,000 ± 12,000 daltons. The carbohydrate moiety (9.8%) of the protein is composed of hexose (including fucose), hexosamine, and sialic acid (*155*). Properdin is a tetramer that is dissociated into subunits of similar or identical molecular