PROPERDIN FACTOR D

II. ACTIVATION TO $\bar{D}$ BY PROPERDIN*

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Activated properdin factor D ($\bar{D}$)¹ is a 23,500 mol wt euglobulin that acts on factor B in the presence of either cobra venom factor (CoVF)² (4) or C3b (5), the major cleavage product of C3, to form the CoVF-dependent and C3b-dependent [$\bar{C3B}(\bar{D})$] C3 convertases. These convertases are similar to the classical convertase, $C42$ (7), in their ability to cleave C3 and subsequently to activate the terminal complement sequence C5–C9. Furthermore, EAC43B($\bar{D}$), the cellular intermediate bearing the convertase, is hemolyzed by reaction with C3–C9 (8). The role of factor B during the decay and regeneration of $\bar{C3B}(\bar{D})$ is analogous to that of C2 in the reactions of $C42$ (8). $\bar{D}$ has been shown recently to be analogous to $\bar{C1}$: both cleave proteins that participate in the generation and decay of their respective C3 convertases, are susceptible to inhibition by diisopropylphosphofluoridate (DFP), and are derived from DFP-resistant precursor forms, D and C1, which may be activated by proteolytic treatment

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¹The nomenclature for the classical complement components conforms to that agreed upon under the auspices of the World Health Organization (1968, *Bull. W. H. O.* **39**:395). Properdin factor B is synonymous with C3 proactivator (1) and glycine-rich beta-glycoprotein (2); precursor factor D (3) is denoted by D; activated factor D (4) is represented as $\bar{D}$ and apparently is identical to C3 proactivator convertase (5) and GBGase (6). The cellular intermediate bearing the C3b-dependent C3 convertase is represented by EAC43B($\bar{D}$); the parentheses are placed about $\bar{D}$ to indicate that its presence in the intermediate is not established.

²Abbreviations used in this paper: CoVF, cobra venom factor; DFP, diisopropylphosphofluoridate; GVB-EDTA, VBS containing 0.1% gelatin and 0.04 M EDTA; mS, milliSiemens; QAE, quaternary aminoethyl; SP, sulphopropyl; TLCK, tosyl-L-lysine chloromethyl ketone; VBS, Veronal-buffered saline; Z, the average number of hemolytic sites/cell.

with trypsin (3, 9). Implicit in the identification of the precursor form of $\bar{D}$ is the existence of a plasma protein responsible for its physiologic activation. In the present study, an activated form of properdin (10), a 184,000 mol wt (11) gamma globulin, is shown to convert D to $\bar{D}$.

Materials and Methods

Hexadimethrine bromide and DFP (Aldrich Chemical Co., Inc., Milwaukee, Wisc.), tosyl-L-lysine chloromethyl ketone (TLCK) (Cyclo Chemical, Div. Travenol Laboratories, Inc., Los Angeles, Calif.), Indubiose agarose (Fisher Scientific Co., Pittsburgh, Pa.), rat serum (Pel-Freez Biologicals, Rodgers, Ark.), and zymosan lot. no. Y3660 (Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, N.Y.) were obtained as indicated.

The Preparation and Assay of Classical Complement Components and Properdin Factors.—Low ionic strength Veronal-buffered saline (VBS) with dextrose (DGVB⁺⁺), pH 7.5, 3.75 milliSiemens (mS), containing 5×10^{-4} M Mg⁺⁺, 1.5×10^{-4} M Ca⁺⁺ and 0.1% gelatin, and VBS containing 0.1% gelatin and 0.04 M EDTA (GVB-EDTA) were used as diluents in hemolytic assays. EAC43 were prepared as previously described (8) except that guinea pig C2, 200 effective molecules/cell, was used in place of human C2, and EAC423 were decayed at 37°C for 180 min to remove the C2. In the assay for $\bar{D}$ (3), 1×10^7 EAC43 and 6 ng of factor B in 0.1 ml DGVB⁺⁺ were incubated with the test samples in 0.1 ml DGVB⁺⁺ for 30 min at 30°C to generate the EAC43B($\bar{D}$) intermediate. 0.3 ml of rat serum diluted 1:15 in GVB-EDTA (C-EDTA) was added to the reaction mixture and incubation continued for 60 min at 37°C. The extent of hemolysis was determined and the average number of hemolytic sites/cell (Z) calculated.

Guinea pig C1 (12) and C2 (12) and human C3 were isolated and quantitated as previously described (13, 14). Properdin factor D was isolated from plasma collected in hexadimethrine and EDTA by quaternary aminoethyl (QAE) Sephadex (Pharmacia Fine Chemicals, Piscataway, N. J.) chromatography and two filtrations through Sephadex G-75 (3). $\bar{D}$ was prepared from a pH 6.0 euglobulin precipitate of fresh frozen citrated plasma by identical chromatographic procedures. The preparation of $\bar{D}$ exhibited a single band on alkaline disc gel electrophoresis (Canalco Inc., Rockville, Md.) which corresponded to the position in a replicate gel from which activity was eluted. Protein concentrations in D and $\bar{D}$ preparations were measured by the Folin assay (15) with serum albumin as the standard. Properdin factor B, isolated and quantitated as previously described (4, 16) was homogeneous on alkaline disc gel electrophoresis and was devoid of C2 by hemolytic assay (17).

Chromatography of and Immunochemical Assay for Properdin.—Anion exchange chromatography was performed with QAE Sephadex A-50 equilibrated in 0.0035 M PO₄, 0.002 M EDTA, pH 8.0. Sulphopropyl (SP) Sephadex C-25 was equilibrated with 0.0035 M PO₄, 0.002 M EDTA, pH 7.5, and utilized for cation exchange chromatography. Conductance was measured at 0°C and expressed as mS. Sephadex G-200 Superfine gel filtration was carried out on a 1.5 x 60 cm column by upward flow with VBS containing 0.002 M EDTA. The elution volumes were calculated from the net weight of the fractions in pre-weighed plastic tubes. The column was calibrated by the elution volumes of C4, IgG, transferrin, and albumin contained in a serum sample and detected by radial immunodiffusion. Acid disc gel electrophoresis of properdin was performed in 7.5% gels according to instructions supplied by the manufacturer (Canalco).

Properdin was detected immunochemically in column fractions by radial immunodiffusion using monospecific rabbit antihuman properdin. The antiserum, prepared by immunization with zymosan-eluted properdin (18) and rendered monospecific by adsorption with serum depleted of properdin (18), demonstrated the same specificity as that provided by Dr. I. H. Lepow (19). The immunodiffusion gels, 2 mm thick, were prepared after diluting the anti-

serum 1:275 in VBS containing 0.005 M EDTA and 1.5% agarose. Wells 3 mm in diameter were used for screening the QAE Sephadex column, and 2 mm in diameter for the SP Sephadex and Sephadex G-200 columns. After allowing diffusion of the samples for 24 h at 37°C, the precipitin rings were developed with 1% tannic acid in water (20), and the diameters measured with a calibrated eyepiece. In plates containing serum samples or the final purified preparations, wells 2 mm in diameter were used, the samples allowed to diffuse for 72 h at 25°C, and the plates washed overnight in 0.075 M NaCl before staining with tannic acid. Properdin concentrations were calculated from a standard serum which had been calibrated against 50 normal sera whose mean properdin concentration was taken as 25 $\mu\text{g}/\text{ml}$ (19).

RESULTS

Since neither C3b nor factor B, alone or in combination, activated D(3), the initial search for a factor with this capacity focused on the other known protein of the properdin pathway, properdin itself. In order to minimize alterations in native properdin, it was isolated directly from plasma, rather than by elution from zymosan or euglobulin precipitation.

Conversion of Factor D to $\bar{D}$ by Activated Properdin.—80 ml of fresh plasma obtained from blood drawn into plastic tubes containing hexadimethrine (3.6 mg/10 ml blood) and EDTA (10 mg/10 ml blood) (21) were dialyzed at 4°C against the QAE Sephadex starting buffer. After centrifugation, the resulting supernate was applied to a 5×90 cm column containing QAE Sephadex. Fractions containing properdin, as assessed by radial immunodiffusion, appeared in the early effluent, just before the initial protein peak. These fractions were pooled and concentrated by ultrafiltration to 5 ml, dialyzed at 4°C against the SP Sephadex starting buffer, and applied to a 0.9×30 cm column containing SP Sephadex. Elution with a linear salt gradient and assay by radial immunodiffusion revealed two distinct peaks of properdin, at 6.5 mS (peak I) and 7.2 mS (peak II) (Fig. 1). The fractions were examined also for their ability to activate D. 50 μl of 1:20 dilutions in DGVB⁺⁺ of the column fractions were added to 50 ng D in 50 μl DGVB⁺⁺, and the reaction mixtures were assessed for $\bar{D}$ by their capacity to form EAC43B($\bar{D}$). Fractions were examined also for their inherent content of $\bar{D}$ activity by omission of D from the assay. Fractions in peak I demonstrated minimal capacity to form EAC43B($\bar{D}$) and had no $\bar{D}$ activity by themselves (Fig. 1). The chromatographic fractions containing peak II were capable of generating $\bar{D}$ from its precursor and exhibited some activity in the absence of added D. The fractions containing properdin were assessed also for prekallikrein, plasminogen proactivator, and preplasma thromboplastin antecedent (21) and were found to be devoid of activities associated with these proteins. Although some trailing of the ability to activate D was observed, attention was focused on peak II.

In order to analyze further the association between properdin antigen contained in peak II with activation of D, the second peak was pooled, concentrated to 0.75 ml, and filtered on Sephadex G-200. Fractions were screened for properdin by radial immunodiffusion, and for the capacity to activate D by hemolytic assay. The peak representing properdin antigenicity corresponded

to the fractions containing the ability to activate D and to those exhibiting activity in the absence of D (Fig. 2).

To assess the relationship between protein content and the ability of the properdin obtained by Sephadex G-200 gel filtration to activate D, analytical disc gel electrophoresis was performed. Fractions containing the peak of properdin eluted from Sephadex G-200 were pooled and concentrated to 1 ml.

FIG. 1. SP Sephadex chromatography of the QAE Sephadex pool containing properdin. The elution positions of properdin (●—●) detected by radial immunodiffusion (ring diameter squared, D^2) are shown in the upper panel. The capacity of column fractions to react with EAC43 and factor B to generate hemolytically active sites (Z) in the presence (○—○) and absence (▲—▲) of D, is shown in the lower panel. The ionicity (mS) of the salt gradient is shown (---).

FIG. 2. Sephadex G-200 gel filtration of the concentrated SP Sephadex pool containing the second properdin peak (peak II). The elution position of properdin detected by radial immunodiffusion (○—○), and the capacity of fractions to react with EAC43 and factor B to generate hemolytically active sites (Z) in the presence (●—●) and absence (▲—▲) of D are shown. The void volume and elution volumes of marker proteins also are indicated.

Two 50- μ l samples, each containing 4 μ g properdin, were applied to acid disc gels, and electrophoresis was carried out until the buffer fronts had traversed the gel lengths. One gel was stained with Coomassie blue, demonstrating a single band, and the paired gel was sliced into 2-mm segments which were eluted overnight at 4°C in 0.1 ml of 0.05 M Tris, pH 8.5, 0.3 M NaCl. The gel eluates were assessed for their capacity to activate D by addition of 50 μ l of 1:5 dilutions in DGVB⁺⁺ of the eluates to 50 ng D in 50 μ l of DGVB⁺⁺, followed by the hemolytic assay for D. Eluates capable of activating D were obtained from gel slices corresponding to the single band of protein observed in the replicate, stained gel (Fig. 3). Alkaline disc gel electrophoresis of 50 μ l

FIG. 3. Acid disc electrophoresis of purified properdin. Beneath is shown the amount of hemolytically active $\bar{D}$ (Z) generated by interaction of purified D with eluates of each slice of the replicate, unstained gel.

of the properdin preparation revealed no protein bands and no functional D activating activity in gel eluates, indicating that neither properdin nor other contaminating proteins entered the gel at an alkaline pH. Thus, properdin antigenicity and the ability to activate D corresponded through ion exchange and gel filtration chromatography, procedures which had purified properdin to homogeneity, as demonstrated by disc gel electrophoresis. The purity of the properdin preparation was demonstrated also by immunoelectrophoresis in which no precipitin bands were observed with rabbit antinormal human serum, while a single precipitin arc in the gamma region was developed with mono-specific rabbit antihuman properdin.

To examine further the interaction between properdin and D, the purity of the latter was assessed by alkaline disc gel electrophoresis. Samples containing 5 μ g D, which had been purified by QAE Sephadex and Sephadex G-75 chromatography and employed in the above experiments, were applied to alkaline disc gels. After electrophoresis, one gel was stained with Coomassie blue, yielding a single band, and the other was sliced into 2-mm segments which were eluted

at 4°C for 2 h in 0.1 ml GVB⁺⁺. To assay for D, 50 μ l of 1:5 dilutions of the eluates in DGVB⁺⁺ were added to 15 ng properdin in 50 μ l DGVB⁺⁺, followed by the hemolytic assay for $\bar{D}$. The same eluates were assayed for $\bar{D}$ activity in the absence of properdin. The protein band in the stained gel corresponded to the position in the paired gel yielding eluates capable of generating $\bar{D}$ when interacted with properdin (Fig. 4). A small amount of properdin-independent $\bar{D}$ activity was found in the same region.

Stoichiometry of the Interaction Between Factor D and Properdin.—Reaction mixtures containing variable amounts of properdin (Fig. 3) and 0.1 μ g D (Fig.

FIG. 4. Alkaline disc gel electrophoresis of purified D. Beneath is shown the amount of $\bar{D}$ hemolytic activity (Z) generated by the interaction of purified properdin (Fig. 3) with eluates of each slice of the replicate, unstained gel (O—O). Also shown is properdin-independent $\bar{D}$ activity present in eluates (●—●).

4) in 0.1 ml DGVB⁺⁺ were assayed hemolytically for $\bar{D}$. A linear relationship existed between the input of properdin and the amount of $\bar{D}$ generated, with 25 ng properdin/ 10^8 EAC43 activating sufficient D for production of one hemolytic site/cell (Fig. 5). Treatment of properdin with 5×10^{-3} M DFP or 10^{-2} M TLCK for 60 min at 37°C, followed by extensive dialysis, resulted in no decrease in the capacity of properdin to activate D.

For comparison, the capacity of properdin to interact with EAC43 and factor B in the absence of D, noted in Figs. 1 and 2, was examined quantitatively. Variable amounts of properdin in 0.1 ml DGVB⁺⁺ were reacted with EAC43 and factor B, and the hemolytic site developed with C-EDTA. A linear response was obtained, with 1 μ g properdin/ 10^8 EAC43 capable of generating one hemolytic site/cell (Fig. 5). This amount of properdin is 40 times that giving equivalent hemolytic activity in the presence of D. To distinguish this activity of properdin apparent at high concentrations from that of $\bar{D}$, properdin was treated with 5×10^{-3} M DFP for 60 min at 37°C, dialyzed to remove the DFP, and hemolytically assayed with EAC43 and factor B. No impairment of

FIG. 5. Hemolytic activity (Z) generated during incubation of EAC43, factor B, and variable amounts of properdin, with (○—○) and without (●—●) addition of D. The upper and lower scales represent the amounts of properdin incubated in the presence and absence of D, respectively.

its activity was seen, although, under the same conditions, the activity of $\bar{D}$ was greatly reduced. To determine whether properdin acted by enhancing small amounts of pre-existing $\bar{D}$ that might be present in the D preparations, the effect of properdin on purified $\bar{D}$ was examined. Mixtures containing variable amounts of $\bar{D}$, with and without addition of 25 ng properdin/ 10^8 EAC43, were assessed for hemolytic activity; essentially identical results were obtained in the presence and absence of properdin.

To assess quantitatively the generation of $\bar{D}$ from limited D by excess properdin, variable amounts of D were added to 15 ng properdin in 0.1 ml DGVB⁺⁺, and the mixtures were assayed for $\bar{D}$ activity. The relationship between amount of $\bar{D}$ generated and the input of its precursor was linear, and 175 ng D/ 10^8 EAC43 were sufficient to form one hemolytic site/cell (Fig. 6). A reagent blank containing only properdin yielded 0.17 Z , and was subtracted from the other reagent mixtures.

DISCUSSION

The essential role for $\bar{D}$ in the generation of the C3b-dependent C3 convertase (5, 8), and the recognition of the precursor form D (3), indicate the existence of a protein in the properdin pathway that is responsible for conversion of D to $\bar{D}$. Although C3b is necessary for cleavage of factor B by $\bar{D}$, activation of D does not occur with C3b, either in the fluid phase or bound to an erythrocyte (3). Since purified, activated properdin, the first factor in this pathway identified (22) and isolated (10), induces cleavage of factor B and C3 when

FIG. 6. Hemolytic activity (Z) generated during incubation of EAC43, factor B, and properdin with variable amounts of D.

added to serum (23), the search for a protein capable of activating D focused on properdin.

The hemolytic assay for $\bar{D}$ involving formation of EAC43B($\bar{D}$) (3), the cellular intermediate bearing the C3b-dependent convertase, was used to assess quantitatively the ability of chromatographic fractions to activate purified D. Radial immunodiffusion with monospecific antihuman properdin antiserum was employed to identify fractions containing properdin, and allowed determination of the correspondence between properdin protein and the capacity to generate $\bar{D}$ from D during purification procedures. Indeed, during sequential chromatography of a QAE Sephadex effluent on SP Sephadex (Fig. 1) and Sephadex G-200 (Fig. 2), fractions containing properdin antigenically also were capable of activating D. Furthermore, properdin purified in this manner was homogeneous by acid disc gel electrophoretic analysis, with the band of protein staining corresponding to the position in a replicate gel of eluates capable of activating D (Fig. 3). The purity of D was demonstrated by alkaline disc gel electrophoresis in a similar manner (Fig. 4), supporting the contention that direct interaction between properdin and D generates $\bar{D}$. Assessment of the hemolytic activity resulting from interaction between properdin and D revealed linear stoichiometric titrations of each factor with properdin effective in nanogram amounts (Figs. 5 and 6). The concordance on ion exchange and gel filtration chromatography between properdin protein and the capacity to activate D, the purity of the preparations used, and the linear stoichiometry of their hemolytic titrations indicate that direct interaction between properdin and D

results in generation of $\bar{D}$. In addition, the inability of DFP and TLCK to inhibit the capacity of purified properdin to convert D to $\bar{D}$ excluded activation of D by potentially trace-contaminating proteolytic enzymes, such as kallikrein, plasminogen activator, and plasma thromboplastin antecedent.

Properdin alone, in much larger amounts than required for hemolytic activity in the presence of D, was capable of reacting with EAC43 and factor B to yield an intermediate susceptible to lysis by C3-C9 (Fig. 5). The insensitivity of this function to inactivation by DFP excluded the possibility of action by contaminating $\bar{D}$, which is DFP-sensitive. The finding that relatively large amounts of properdin circumvented a need for D is reminiscent of the observations that large amounts of factor B can obviate the requirement for $\bar{D}$ in the formation of the CoVF-dependent (24) and C3b-dependent C3 convertases (25).

Other investigators have obtained properdin in its activated state (23) as indicated by its ability to induce cleavage of C3 and factor B in serum without the addition of complex polysaccharides. In this study, using plasma as starting material and avoiding chromatography with acidic buffers, two forms of properdin were recognized, distinguishable by their differing effects on D and elution characteristics on SP Sephadex chromatography. Properdin purified from peak II was apparently in its activated state as indicated by its ability to generate $\bar{D}$ from D and to induce cleavage of C3 when added to serum depleted of properdin by prior adsorption with zymosan (unpublished results). However, properdin contained in peak I was unable to activate D (Fig. 1) and may represent isolation of a precursor form of properdin which will be useful in the delineation of the early steps in the properdin pathway. That this form of properdin is indeed potentially active is revealed by the development of D activating activity after repeated freezing and thawing.

SUMMARY

The protein in the properdin pathway responsible for conversion of precursor factor D to $\bar{D}$ has been isolated and found to be identical with properdin. Sequential ion exchange and gel filtration chromatography demonstrated identity between properdin protein, measured by radial immunodiffusion, and the capacity to activate D to $\bar{D}$, assessed by formation of the intermediate, EAC43B($\bar{D}$). Properdin, purified in this manner, was homogeneous on acid polyacrylamide disc gel electrophoretic analysis, with the band of protein corresponding to the position of eluates in the replicate gel capable of activating highly purified D. Demonstration of the homogeneity of purified D by alkaline disc gel electrophoresis, coupled with the linear stoichiometric hemolytic titrations of each factor, indicates that direct interaction between properdin and D generates $\bar{D}$. Thus, activation of D by properdin represents a mechanism in the properdin pathway by which $\bar{D}$ becomes available for formation of the C3b-dependent C3 convertase.

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REFERENCES

1. Götze, O., and H. J. Müller-Eberhard. 1971. The C3-activator system: an alternate pathway of complement activation. *J. Exp. Med.* **134**(Suppl.):90.
2. Alper, C. A., I. Goodkofsky, and I. H. Lepow. 1973. The relationship of glycine-rich beta glycoprotein to Factor B in the properdin system and to the cobra venom factor-binding protein of human serum. *J. Exp. Med.* **137**:424.
3. Fearon, D. T., K. F. Austen, and S. Ruddy. 1974. Properdin factor D: characterization of its active site and isolation of the precursor form. *J. Exp. Med.* **139**:355.
4. Hunsicker, L. G., S. Ruddy, and K. F. Austen. 1973. Alternate complement pathway: factors involved in cobra venom factor (CoVF) activation of the third component of complement (C3). *J. Immunol.* **110**:128.
5. Müller-Eberhard, H. J., and O. Götze. 1972. C3 proactivator convertase and its mode of action. *J. Exp. Med.* **135**:1003.
6. Rosen, F. S., and C. A. Alper. 1972. An enzyme in the alternate pathway to C3 activation and its activation by a protein in normal serum. *J. Clin. Invest.* **51**:80(abstr.).
7. Müller-Eberhard, H. J., M. J. Polley, and M. A. Calcott. 1967. Formation and functional significance of a molecular complex derived from the second and fourth components of human complement. *J. Exp. Med.* **125**:359.
8. Fearon, D. T., K. F. Austen, and S. Ruddy. 1973. Formation of a hemolytically active cellular intermediate by the interaction between properdin factors B and D and the activated third component of complement. *J. Exp. Med.* **138**:1305.
9. Fearon, D. T., S. Ruddy, J. D. Knostman, C. B. Carpenter, and K. F. Austen. The functional significance of complement. In *Advances in Nephrology*. J. Hamburger, J. Crosnier, and J. L. Funck-Brentano, editors. Yearbook Medical Publishers, Chicago. In press.
10. Pensky, J., C. F. Hinz, E. W. Todd, R. J. Wedgwood, J. T. Boyer, and I. H. Lepow. 1968. Properties of highly purified human properdin. *J. Immunol.* **100**:142.
11. Minta, J. O., and I. H. Lepow. 1973. Physical and chemical studies on human properdin purified by elution from zymosan and by affinity chromatography. *J. Immunol.* **111**:286(Abstr.).
12. Nelson, R. A., J. Jensen, I. Gigli, and N. Tamura. 1966. Methods for the separation, purification and measurement of the nine components of hemolytic complement in guinea pig serum. *Immunochemistry*. **3**:11.
13. Nilsson, I. R., and H. J. Müller-Eberhard. 1965. Isolation of β 1F-globulin from human serum and its characterization as the fifth component of complement. *J. Exp. Med.* **122**:277.
14. Ruddy, S., and K. F. Austen. 1969. C3b inactivator of man. I. Hemolytic measurement of inactivation of cell-bound C3. *J. Immunol.* **102**:533.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.
16. Hunsicker, L. G., S. Ruddy, C. B. Carpenter, P. H. Schur, J. P. Merrill, H. J.

- Müller-Eberhard, and K. F. Austen. 1972. Metabolism of third complement component (C3) in nephritis. Involvement of the classic and alternate (properdin) pathways for complement activation. *N. Eng. J. Med.* **287**:835.
17. Rapp, H. J., and T. Borsos. 1970. The Molecular Basis of Complement Action. Appleton-Century-Crofts Inc., New York.
18. Todd, E. W., L. Pillemer, and I. H. Lepow. 1959. The properdin system and immunity. IX. Studies on the purification of human properdin. *J. Immunol.* **83**:418.
19. Minta, J. O., I. Goodkofsky, and I. H. Lepow. 1973. Solid phase radioimmunoassay of properdin. *Immunochemistry.* **10**:341.
20. Alpert, E., Monroe, M., and Schur, P. H. 1970. A method for increasing the sensitivity for radial immunodiffusion assay. *Lancet.* **1**:1120.
21. Kaplan, A. P., and K. F. Austen. 1972. The fibrinolytic pathway of human plasma. Isolation and characterization of the plasminogen proactivator. *J. Exp. Med.* **136**:1378.
22. Pillemer, L., L. Blum, I. H. Lepow, O. A. Ross, E. W. Todd, and A. C. Wardlaw. 1954. The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science (Wash. D. C.)*. **120**:279.
23. Götze, O., and H. J. Müller-Eberhard. 1974. The role of properdin in the alternate pathway of complement activation. *J. Exp. Med.* **139**:44.
24. Cooper, N. R. 1973. Formation and function of a complex of the C3 proactivator with a protein from cobra venom. *J. Exp. Med.* **137**:451.
25. Fearon, D. T., K. F. Austen, and S. Ruddy. The properdin pathway. Mechanisms of complement activation and analogies to the classical pathway. *In* Proceedings of the International Symposium on Immunological Aspects of Rheumatoid Arthritis. J. Clot and J. Sany, editors. S. Karger, AG, Basel. In press.