PROPERDIN FACTOR D: CHARACTERIZATION OF ITS ACTIVE SITE AND ISOLATION OF THE PRECURSOR FORM*

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Properdin factor D, a 23,500 mol wt euglobulin (6), interacts with properdin factor B and cobra venom factor (CoVF) to generate the CoVF-dependent C3 convertase which cleaves C3 (7) and activates the terminal complement components, C5–C9 (8). Fluid phase C3b, the major cleavage fragment of C3, interacts with factors B and D in a manner analogous to CoVF to form a fluid phase C3b-dependent C3 convertase (4). More recently, C3b bound to a sheep erythrocyte has been shown to react with factors B and D to generate a cell-bound C3b-dependent C3 convertase, EAC43B(D), which, like the classical cellular intermediate, EAC142, activates C3–C9 to bring the lytic sequence to completion (9). In the present study, factor D is shown to be inhibited by diisopropylphosphofluoridate (DFP) and derived from a previously unrecognized DFP-resistant precursor protein of similar molecular weight.

Materials and Methods

Crude, lyophilized cobra venom (Ross Allen’s Reptile Institute, Inc., Silver Springs, Fla.), hexadimethrine bromide and DFP (Aldrich Chemical Co., Inc., Milwaukee, Wis.), Enzodifusion fibrin plates and streptokinase (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.), tosyl-L-lysine chloromethyl ketone (TLCK) (Cyclo Chemical Div. of Travenol Laboratory, Costa Mesa, Calif.), crystalline trypsin and soybean trypsin inhibitor (SBTI)

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1 The nomenclature for 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, 2) and glycine-rich beta glycoprotein (3); factor D is synonymous with C3 proactivator convertase (4) and GBGase (5). The cellular intermediate bearing the C3b-dependent C3 convertase is represented by EAC43B(D); the parenthesis are placed about factor D to indicate that its presence in the intermediate is not established. Other abbreviations used in this paper: C-EDTA, rat serum diluted 1:15 in GVB-EDTA; CoVF, cobra venom factor; DFP, diisopropylphosphofluoridate; DGVB++, Veronal-buffered saline with dextrose; GVB-EDTA, Veronal-buffered saline containing 0.04M EDTA; QAE, quaternary aminoethyl; SBTI, soybean trypsin inhibitor; TLCK, tosyl-L-lysinechloromethyl ketone; Z, the average number of hemolytic sites/cell.

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(Worthington Biochemical Corp., Freehold, N. J.), UM-10 Diaflo ultrafiltration membranes (Amicon Corp., Lexington, Mass.), and Indubiose agarose for immunoelectrophoresis (Fisher Scientific Co., Pittsburgh, Pa.) were obtained as indicated.

Quaternary aminoethyl (QAE) A-50 Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) was equilibrated with buffer containing 0.0035 M PO₄, pH 8.0, and utilized for ion exchange chromatography. Conductance was measured at 0°C and expressed as millisiemens (mS). Gel filtration was performed with Sephadex G-75 using 0.05 M Veronal-buffered saline, pH 7.5, 7.5 mS, containing 5 × 10⁻⁴ M Mg⁺⁺, 1.5 × 10⁻⁴ M Ca⁺⁺, and 250 μg gelatin/ml. The elution volumes were calculated from the net weight of the fractions in preweighed tubes and the apparent mol wt was estimated using as standards ovalbumin, chymotrypsinogen, and ribonuclease A (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.).

Preparation of CoVF, Classical Complement Components, and Properdin Factors.—CoVF was prepared and assayed from crude cobra venom as previously described (6, 10). Properdin factors B and D were isolated from fresh frozen citrated human plasma (6). The factor B preparation exhibited a single line on alkaline disk gel electrophoresis, was free of C2 and other classical complement components by hemolytic assays, and was quantitated by radial immunodiffusion against monospecific antibody (11). The factor D preparation contained 86 μg protein/ml by Folin assay (12) with serum albumin as the standard, and was devoid of factor B and hemolytic C1 activity. The activated first component of guinea pig complement (13) and the human components, C2 (14) and C3 (15), were isolated and measured as described. The C3 preparation demonstrated a single line on alkaline disk gel electrophoresis. Fluid phase C3b, prepared by treatment of purified C3 with EAC14oxy2 (16), contained 400 μg/ml of C3b and no residual C3 hemolytic activity. Plasmin, obtained by streptokinase activation of plasminogen which had been isolated by affinity chromatography and Sephadex G-150 gel filtration, was assayed by its lytic activity in fibrin agarose plates relative to that of a standardized preparation (17, 18).

RESULTS

Functional Characteristics of Properdin Factor D.—

Effective molecule titration: The quantitative relationship between the amount of factor D present and the number of effective hemolytic sites generated during incubation with EAC43 and factor B is shown in Fig. 1. Samples
FIG. 1. The average number of hemolytic sites/cell generated during incubation of EAC43 and factor B with variable amounts of factor D.

Inhibition: DFP and TLCK, inhibitors of enzymatic sites containing serine (20) and histidine (21) residues, respectively, were examined for their abilities to inhibit factor D. Three reaction mixtures, each containing 4.0 μg factor D in 2 ml DGVB++, were treated with either buffer, DFP (10⁻³ M), or TLCK (10⁻³ M) for 30 min at 30°C. After dialysis for 10 h against four changes of 500 ml DGVB++, each reaction mixture was assayed for residual factor D activity by effective molecule titrations. As shown in Fig. 2, DFP treatment of factor D irreversibly inhibited all functional activity, while exposure to TLCK had no effect. That the TLCK was active was demonstrated by its capacity to reduce by more than 95% the fibrinolytic activity of a sample of plasmin in a parallel experiment.

In contrast to factor D, the activities of factor B and cell-bound C3b in the formation of EAC43B(D), and that of EAC43B(D) in activating C3–C9 were not inhibited by treatment with DFP. Samples of 0.03 μg of factor B were exposed either to buffer or DFP, 10⁻³ M, for 30 min at 30°C, dialyzed, and assayed for residual hemolytic activity by effective molecule titration; no dif-
Fig. 2. The effects on factor D activity of DFP (●—●), TLCK (○—○), and buffer (▲—▲). Bar graphs indicate the fibrinolytic activity of plasmin treated similarly with TLCK or buffer.

The recognition that factor D was susceptible to inactivation by DFP led to an examination of human plasma for a DFP-resistant precursor form of the protein. Trypsin was selected as the activating agent because of its broad spectrum of activity in the proteolytic activation of proenzymes (22). A 25,000 mol wt fraction of serum proteins was used as the initial possible source of substrate because active factor D resides with molecules of this size.

Trypsin-inducible factor D activity: 1 ml of human serum was chromatographed on a 0.5 X 90 cm column containing Sephadex G-75, and the fractions eluting at a volume corresponding to 25,000 mol wt were pooled and concentrated by ultrafiltration to 1 ml. The pool was treated with DFP, 10^-4 M, for 30 min at 30°C to inactivate the factor D present, and then dialyzed to remove the DFP. 0.4-ml portions of 1:5 dilutions in DGVB'' of the DFP-treated 25,000 mol wt pool were exposed to trypsin in the final concentrations shown in Table I for 30 sec at 37°C in final reaction volumes of 0.45 ml DGVB''.

The reaction was stopped by the addition of 25 μg SBTI in 0.05 ml DGVB'',

TABLE I

Trypsin-Inducible Factor D Activity in a Pool of 25,000 Mol Wt Serum Proteins

| Trypsin added* | Factor D activity (U) |
|---------------|----------------------|
| µg/ml         | U/ml                 |
| 0             | 0.02                 |
| 1             | 0.21                 |
| 2             | 0.27                 |
| 4             | 0.32                 |
| 8             | 0.35                 |

* Each reaction mixture was neutralized with 50 µg/ml SBTI before assay for factor D activity. A reaction mixture containing only 10 µg/trypsin/ml and 50 µg SBTI/ml did not generate factor D activity.

and the reaction mixtures were assayed for factor D activity by means of the effective molecule titration. Trypsin treatment did uncover factor D hemolytic activity. In another experiment, the maximal effect was observed with 10 µg trypsin/ml and an interaction time with the pool of serum proteins of 30-120 s at 37°C.

In order to demonstrate that the trypsin-inducible activity represented a single protein, plasma was subjected to QAE Sephadex anion exchange chromatography and all fractions were screened for trypsin-inducible factor D activity. 80 ml of plasma obtained from freshly drawn blood collected into hexadimethrine (3.6 mg/10 ml blood) and EDTA (10 mg/10 ml blood) were dialyzed against the 0.0035 M PO₄ starting buffer at 4°C. After centrifugation, the resulting supernate was applied to a 5-x 90-cm column containing QAE Sephadex in 0.0035 M PO₄, and the protein eluted with a gradient of increasing NaCl concentration. The column fractions were assayed for factor B in microtiter plates, for factor D by effective molecule titrations, and for trypsin-inducible factor D activity. 0.4-ml portions of the 15-ml fractions were treated with trypsin, 10 µg/ml, in a final reaction volume of 0.5 ml for 30 sec at 37°C, and the reaction was stopped by the addition of 50 µg SBTI in 0.1 ml DGVB++. 0.5 ml of this reaction mixture was added to 5 x 10⁷ EAC43 and 0.03 µg factor B in 0.5 ml DGVB++, and the effective molecule titration of factor D performed as described. Trypsin-inducible factor D activity eluted at 7.5 mS, just preceding factor D which eluted at 8.7 mS (Fig. 3). The peaks of factor D and trypsin-induced factor D activity were distinct from and free of factor B activity which eluted at 10.3 mS.

The fractions containing the trypsin-inducible factor D activity were pooled, concentrated to 5 ml by ultrafiltration, and applied to a 2.6-x 90-cm column containing Sephadex G-75. The trypsin-inducible factor D activity was determined as during the screening of the QAE Sephadex column; the active fractions were pooled, concentrated to 1 ml, and applied to a 0.5-x 90-cm column containing Sephadex G-75 which had been calibrated with ovalbumin (45,000
mol wt), chymotrypsinogen (25,000 mol wt), and ribonuclease A (13,700 mol wt). Trypsin-inducible factor D activity filtered at a volume corresponding to a mol wt of 25,000, and no factor D activity independent of trypsin treatment was present (Fig. 4). Because of its function and size, this trypsin-inducible factor D activity was designated “precursor factor D.”

Participation of precursor factor D in the generation of the CoVF-dependent C3 convertase and in cleavage of factor B: Because factor D was originally
described as being required for the generation of the CoVF-dependent C3 convertase (6) and for the fluid phase C3b-dependent cleavage of factor B (4), the ability of trypsin-activated precursor factor D to fulfill the factor D requirements in these reactions was examined. 4 μg of the precursor factor D preparation, isolated by QAE Sephadex chromatography and Sephadex G-75 gel filtration with recycling and quantitated by Folin assay, were treated with buffer or 0.45 μg trypsin for 30 s at 37°C in a final reaction volume of 0.04 ml DGVB++; the reaction was stopped by the addition of 4.5 μg SBTI in 0.01 ml DGVB++. 0.5 ml DGVB++ containing 5 μg CoVF and 10 μg factor B was added to this reaction mixture, and incubation was carried out for 30 min at 37°C. The generation of the CoVF-dependent C3 convertase was measured by the addition of 5 X 10⁶ unsensitized sheep erythrocytes in 0.1 ml GVB-EDTA. 0.4 ml guinea pig serum was diluted 1:4 in GVB-EDTA, followed by incubation for 60 min at 37°C. The complete reaction mixture generated 0.39 hemolytic sites/cell, whereas no sites were generated when the precursor factor D was not trypsin-activated, nor when precursor factor D was omitted from the otherwise complete reaction mixture (Table II). 1.0 μg factor D, when assayed simultaneously, yielded 0.86 hemolytic sites/cell.

To examine fluid phase cleavage of factor B, two 0.8-μg samples of the precursor factor D preparation, with and without activation by 10 μg trypsin/ml followed by neutralization with 100 μg/ml SBTI, were interacted with 17 μg factor B and 2 μg C3b for 60 min at 37°C in a final reaction volume of 0.03 ml DGVB++. Immunoelectrophoretic analysis (Fig. 5) revealed cleavage of factor B, with the appearance of a fragment of γ-mobility, occurring only when factor B was interacted with C3b and trypsin-activated precursor factor D. No cleavage of factor B occurred in reaction mixtures that included: C3b alone, C3b and buffer containing trypsin and SBTI, precursor factor D not activated with trypsin, or trypsin-activated precursor factor D in the absence of C3b.

Effect of DFP on precursor factor D before and after activation by trypsin: Two reaction mixtures, each containing 2.5 μg of the precursor factor D preparation, were exposed either to buffer or DFP, 10⁻³ M, for 30 min at 30°C in a final volume of 2 ml DGVB++. After dialysis to remove the DFP, 0.4-ml por-

| Additions to CoVF and factor B | Indirect lysis activity (Z) |
|-------------------------------|-----------------------------|
| Precursor factor D, trypsin-treated | 0.39 |
| Precursor factor D | 0.01 |
| Trypsin, SBTI | 0.00 |
| Factor D | 0.86 |
Fig. 5. Immunoelectrophoretic analysis of the capacity of trypsin-activated precursor factor D to induce cleavage of factor B in the presence of C3b.

DISCUSSION

The demonstration that DFP inhibits properdin factor D activity indicates that a serine esterase is essential for the generation of the alternate pathway C3 convertases, a situation analogous to the role of C1 (20) in the formation of the classical C3 convertase, C42. The nature of the active site of factor D was established by utilizing the generation of the hemolytically active cellular intermediate, EAC43B(D), bearing the C3b-dependent C3 convertase (Fig. 1). The intermediate could not be formed when factor D had been pretreated with DFP (Fig. 2). This evidence of the enzymatic nature of factor D is consistent with previous studies revealing that factor D activity is undiminished, subsequent to the formation and decay of the CoVF-dependent C3 convertase (23) and that less than equimolar amounts are sufficient for maximal generation of this convertase (24).

The definition of properdin factor D as a serine esterase prompted a search for its parent proenzyme form. The physiologic activator of the precursor form of factor D was unknown, and trypsin was employed for this purpose because of its broad proteolytic activity and known ability to activate other proen-
enzymes, including Cls (22, 25). The first indication of the existence of trypsin-inducible factor D activity was provided by analysis of the pool of 25,000 molwt serum proteins which had been pretreated with DFP (Table I); the generalization of new factor D activity was dependent upon the interaction of the pool with trypsin. Analysis of plasma fractionated by QAE Sephadex anion exchange chromatography (Fig. 3) revealed a single peak of trypsin-inducible factor D activity just preceding and discrete from factor D, and independent of factor B. The failure of trypsin to elaborate factor D activity in other fractions is consistent with the activity being derived from a single plasma protein. Filtration of the pool obtained from the QAE Sephadex chromatography on Sephadex G-75 further purified this protein, and refiltration with appropriate molecular markers established a molwt of 25,000 for this protein (Fig. 4), similar to that previously reported for factor D (26).

That this plasma protein was indeed the precursor of factor D was established by the criteria which had originally defined factor D: the ability to interact with CoVF and factor B to generate the CoVF-dependent C3 convertase (6) (Table II) and the capacity to induce cleavage of factor B in the presence of C3b (4, 26) (Fig. 5). In both reactions, the trypsin-treated protein exhibited factor D-like activity, whereas the untreated protein was inactive. Accordingly, this protein from which trypsin elaborated factor D activity, as assessed by the formation of the cell-bound C3b-dependent C3 convertase, the generation of the CoVF-dependent C3 convertase, and the cleavage of factor B, was designated “precursor factor D.” The additional finding of the DFP resistance of the protein in its precursor state, and the DFP sensitivity of its trypsin-activated state (Table III) completed the analogy to the known plasma serine esterases, each of which is derived from a DFP-resistant proenzyme.

Although there are compelling analogies between factor D and Cl as indicated by their corresponding enzymatic roles in the generation of the alternate and classical pathway C3 convertases, respectively, there are significant distinctions between the two proteins. The molwt of factor D is less than that of the smallest known subunit of Cl, the 36,000 molwt light chain of Cls (27) and Cl is unable to substitute for factor D in its reactions. However, it is not inconceivable that these proteins have a common phylogenetic ancestor.

The appearance of active factor D in the chromatographic separation of

### Table III

**Effect of DFP on Precursor Factor D before and after Activation by Trypsin**

| Sequence of treatment of precursor factor D | Factor D activity (U/ml) |
|-------------------------------------------|--------------------------|
| Buffer, trypsin-SBTI                        | 0.52                     |
| DFP, trypsin-SBTI                          | 0.48                     |
| Trypsin-SBTI, buffer                       | 0.50                     |
| Trypsin-SBTI, DFP                          | 0.00                     |
plasma, despite avoidance of activation of both Hageman factor and its substrates and divalent cation-dependent enzymes, raises the question of whether a portion of factor D exists in an active state in plasma. This possibility is supported by the ability of CoVF to deplete C3 in vivo, despite the inability of CoVF to interact with precursor factor D to generate the CoVF-dependent convertase in vitro as indicated by this study (Table II). Furthermore, cleavage of factor B and C3 have been reported for a patient genetically deficient in the C3b inactivator (28), although precursor factor D is inactive in the cleavage of factor B in the presence of C3b in vitro (Fig. 5). Whether naturally occurring plasma protein inhibitors control active factor D in a manner analogous to the effect of the C1 inhibitor on C1 is yet to be examined.

Implicit in the identification of a precursor form of factor D is the existence of one or more plasma proteins responsible for its physiologic activation. That the nonphysiologic activation of precursor factor D by trypsin was inefficient was indicated by the generation with this reaction of only one-eighth the hemolytic activity available in a similar amount of factor D isolated from plasma in its activated state (Table II). The inefficiency may be explained by the capacity of trypsin to inactivate factor D: incubation of 10 μg trypsin/ml with active factor D preparations results in loss of factor D hemolytic activity with a half-life of approximately 1 min at 37°C. Properdin, the 186,000 mol wt gamma globulin required for zymosan-induced formation of the C3b-dependent C3 convertase (29, 30) has been isolated in an apparent activated state, so that its introduction into serum resulted in cleavage of factor B and C3. Treatment of purified C3 and factor D with this form of properdin did not lead to cleavage of either factor, indicating that the primary effect of properdin was on plasma proteins other than C3 (31). Properdin, partially purified from an eluate of zymosan that had been incubated in serum (32) has recently been observed to participate in the activation of precursor factor D.

**SUMMARY**

The activity of properdin factor D was measured by the generation of the hemolytically active cellular intermediate, EAC43B(D), bearing the C3b-dependent alternate pathway C3 convertase. Treatment of factor D with DFP prevented formation of EAC43B(D); thus, a serine esterase is essential for the generation of the alternate pathway C3 convertase, a situation analogous to the role of C1 in the formation of the classical C3 convertase, C42. The definition of factor D as a serine esterase prompted a search for its proenzyme form, and resulted in the chromatographic isolation from plasma of a single peak of trypsin-inducible factor D activity, distinct from activated factor D. Analytical gel filtration indicated an apparent mol wt of 25,000. This protein from which trypsin elaborated factor D activity, as assessed by the formation of EAC43B(D), the generation of the CoVF-dependent C3 convertase, and the cleavage of factor B in the presence of C3b, was designated “precursor
factor D.” The DFP resistance of precursor factor D, and the susceptibility of its trypsin-activated form to inactivation by DFP is analogous to the behavior of other plasma serine esterases, including C1.

REFERENCES

1. Goodkofsky, I., and I. H. Lepow. 1971. The functional relationship of factor B in the properdin system to C3 proactivator of human serum. J. Immunol. 107:1200.
2. Götze, O., and H. J. Müller-Eberhard, 1971. The C3-activator system: an alternate pathway of complement activation. J. Exp. Med. 134(Suppl.):90.
3. 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.
4. Müller-Eberhard, H. J., and O. Götze. 1972. C3 proactivator convertase and its mode of action. J. Exp. Med. 135:1003.
5. 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. 51:80a. (Abstr.)
6. 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.
7. Nelson, R. A., Jr. 1966. A new concept in immunosuppression in hypersensitivity reactions and in transplantation immunity. Surv. Ophthal. 11:498.
8. Pickering, R. S., M. L. Wolfson, R. A. Good, and H. Gewurz. 1969. Hemolysis induced by cobra venom factor activation of the terminal complement components in guinea pig serum. Fed. Proc. 28:818.
9. 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.
10. Ballow, M., and C. G. Cochrane. 1969. Two anticomplementary factors in cobra venom: hemolysis of guinea pig erythrocytes by one of them. J. Immunol. 103:944.
11. 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. Engl. J. Med. 287:835.
12. Lowry, O. H., N. J. Roseborough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 183:265.
13. 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.
14. Ruddy, S., and K. F. Austen. 1967. A stoichiometric assay for the fourth component of complement in whole human serum using EAC’16p and functionally pure human second component. J. Immunol. 99:1162.
15. Nilsson, U. R., and H. J. Müller-Eberhard. 1965. Isolation of β1F-globulin from human serum and its characterization as the fifth component of complement. J. Exp. Med. 122:277.
16. Ruddy, S., L. G. Hunsicker, and K. F. Austen. 1972. C3b inactivator of man.
III. Further purification and production of antibody to C3b INA. *J. Immunol.* **108**:657.

17. Deutsch, D. G., and E. T. Mertz. 1970. Plasminogen purification from plasma by affinity chromatography. *Science (Wash. D. C.)*. **170**:1095.

18. 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.

19. Borsos, T., and H. J. Rapp. 1967. Immune hemolysis: a simplified method for the preparation of EAC4 with guinea pig or with human complement. *J. Immunol.* **99**:263.

20. Becker, E. L. 1965. Small molecular weight inhibitors of complement action. *Complement, Ciba Found. Symp.* 58.

21. Shaw, E. 1967. Site specific reagents for chymotrypsin and trypsin. *Methods Enzymol.* **11**:677.

22. Ratnoff, O. D., and G. B. Nafl. 1967. The conversion of C'1s to C'1 esterase by plasmin and trypsin. *J. Exp. Med.* **126**:377.

23. Fearon, D. T., S. Ruddy, and K. F. Austen. 1973. Serum proteins involved in decay and regeneration of cobra venom factor-dependent complement activation. *J. Immunol.* **111**:1730.

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. Valet, G., and N. R. Cooper. 1973. Isolation of the proenzyme forms of C1r and C1s from human serum. *J. Immunol.* **111**:292. (Abstr.)

26. Ruddy, S., D. T. Fearon, and K. F. Austen. 1973. Participation of factors B, D, E, and C3b inactivator in cobra venom (CVF)-induced activation of the alternate C pathway. *J. Immunol.* **111**:289. (Abstr.)

27. Sakai, K., and R. M. Stroud. 1973. Purification, molecular properties, and activation of C1 proesterase. *J. Immunol.* **110**:1010.

28. Abramson, N., C. A. Alper, P. J. Lachman, F. S. Rosen, and J. H. Jandll. 1971. Deficiency of C3 inactivator in man. *J. Immunol.* **107**:19.

29. 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.

30. 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.

31. Götze, O., and H. J. Müller-Eberhard. 1973. The role of properdin in the alternate pathway of complement activation. *J. Immunol.* **111**:288. (Abstr.)

32. Koethe, S. M., K. F. Austen, and I. Gigli. 1973. Requirements for the early components of complement and Hageman factor for the activation of the alternate pathway by zymosan. *Fed. Proc.* **32**:960. (Abstr.)