

Studies in vivo and in vitro on an abnormality in the metabolism of C3 in a patient with increased susceptibility to infection

Chester A. Alper, ... , James H. Jandl, Fred S. Rosen

J Clin Invest. 1970;49(11):1975-1985. <https://doi.org/10.1172/JCI106417>.

Research Article

In a patient with increased susceptibility to infection, lowered serum C3 concentration, and continuously circulating C3b, it was shown that purified ¹²⁵I-labeled C3 was converted to labeled C3b shortly after intravenous administration. The fractional catabolic rate of C3 was approximately five times normal at 10% of the plasma pool per hr. The synthesis rate and pool distribution of C3 were normal. Despite this evidence of C3 instability in vivo, no accelerated inactivation of C3 was found in vitro. Similarly, no free proteolytic activity could be detected in the patient's serum, and serum concentrations of known protease inhibitors were normal.

Complement-mediated functions, which were markedly deficient in the patient's serum, could be restored partially or completely by the addition of a 5-6S heat-labile beta pseudoglobulin from normal serum. The C3 proinactivator, which has these physicochemical characteristics, was also shown to be either absent or nonfunctional in the patient's serum. An unidentified 6S beta pseudoglobulin to which a monospecific antiserum was available was not detectable in the patient's serum. This last protein appeared not to be a complement component, nor was it the C3 inactivator or proinactivator. Finally, the substance or substances necessary for the conversion of C3b to C3c were missing from the patient's serum.

The administration of 500 ml of normal plasma to the patient corrected all of his abnormalities [...]

Find the latest version:

<https://jci.me/106417/pdf>



Studies In Vivo and In Vitro on an Abnormality in the Metabolism of C3 in a Patient with Increased Susceptibility to Infection

CHESTER A. ALPER, NEIL ABRAMSON, RICHARD B. JOHNSTON, JR.,
JAMES H. JANDL, and FRED S. ROSEN with the technical assistance of
LILLIAN WATSON

*From the Blood Grouping Laboratory and the Department of Medicine,
Children's Hospital Medical Center, Boston, Massachusetts 02115, the
Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical
Services, Boston City Hospital, Boston, Massachusetts 02118, and the
Departments of Pediatrics and Medicine, Harvard Medical School,
Boston, Massachusetts 02115*

ABSTRACT In a patient with increased susceptibility to infection, lowered serum C3 concentration, and continuously circulating C3b, it was shown that purified ¹²⁵I-labeled C3 was converted to labeled C3b shortly after intravenous administration. The fractional catabolic rate of C3 was approximately five times normal at 10% of the plasma pool per hr. The synthesis rate and pool distribution of C3 were normal. Despite this evidence of C3 instability in vivo, no accelerated inactivation of C3 was found in vitro. Similarly, no free proteolytic activity could be detected in the patient's serum, and serum concentrations of known protease inhibitors were normal.

Complement-mediated functions, which were markedly deficient in the patient's serum, could be restored partially or completely by the addition of a 5-6S heat-labile beta pseudoglobulin from normal serum. The C3 proinactivator, which has these physicochemical characteristics, was also shown to be either absent or nonfunctional in the patient's serum. An unidentified 6S beta pseudoglobulin to which a monospecific antiserum was available was not detectable in the patient's serum. This last protein appeared not to be a complement component, nor was it the C3 inactivator or proinactivator. Finally, the substance or substances necessary for the conversion of C3b to C3c were missing from the patient's serum.

The administration of 500 ml of normal plasma to the patient corrected all of his abnormalities partially or

completely for as long as 17 days. The changes in C3 were dramatic; serum concentration rose from 8 to 70 mg/100 ml, and C3b could no longer be detected. A second metabolic study during this normalization period showed a decrease in fractional catabolic rate toward normal.

The patient's histamine excretion was constantly elevated but increased further after a warm shower and after receiving normal plasma; at both times he had urticaria. These observations were consistent with the endogenous production of C3a and the resulting histamine release from mast cells. The inactivating mechanism for C3a was apparently intact in the patient's serum.

The difference in the electrophoretic mobilities of C3b and C3c was shown as well as the electrophoretic heterogeneity of C3c. Suggestive evidence was also presented that the form of C3 with an activated combining site for red cells, previously postulated by others, is a transient C3 conversion product with an electrophoretic mobility slower than that of C3 on agarose electrophoresis.

INTRODUCTION

In a previous publication (1), we described a young man with a lifelong history of infections, in whom all known aspects of host resistance were normal except those mediated by the complement system. Of the complement components, all appeared to be intact with the notable exception of C3. It was shown that the plasma concentration of this component was considerably re-

Received for publication 27 March 1970 and in revised form 18 June 1970.

duced by immunochemical assay but, more remarkably, that about three-quarters of the immunochemically reactive C3 was in the form of inactive conversion product.

When complement-mediated functions, such as bactericidal activity for smooth gram-negative organisms, chemotaxis for leukocytes, hemolysis of antibody-sensitized sheep erythrocytes, and the enhancement of the phagocytosis of pneumococci by normal leukocytes, were tested *in vitro*, they were all found to be grossly deficient. It was shown that these functions could not be significantly improved by the addition of purified C3 to his serum. However, small amounts of normal serum, insufficient in themselves to be effective in the assays, when added to the patient's serum, did produce significant improvement. It was therefore concluded that the patient lacked one or more substances, probably not complement components, necessary for full complement-mediated functions *in vitro*.

In the present report, further definition of the patient's defects, an analysis of the effects of an infusion of normal plasma, and some of the relationships of the patient's defects to normal mechanisms involving C3 are presented.

METHODS

Electrophoresis. Agarose gel electrophoresis was performed as described by Laurell and Niléhn (2). For C3 genetic typing and for the analysis of certain C3 conversion products, prolonged agarose electrophoresis was carried out on a modified apparatus, as described previously (3). To determine the position of protein-bound radioactivity in certain electrophoretic experiments, the agarose gel was immersed for 10 min in fixing solution (methanol:water:acetic acid, 45:45:10) containing sufficient amido black to stain the separated proteins faintly. The gel was then sliced into 2-mm segments from the origin through the α_1 region by means of 30 razor blades mounted in parallel in a plastic holder. The segments were transferred to counting tubes for measurement of radioactivity in a gamma scintillation counter. In other instances, the agarose gels after electrophoresis were fixed, dried, and exposed to Kodak No-Screen X-ray film for radiography. Agarose was substituted for agar in fibrin electrophoresis (4).

Antigen-antibody crossed electrophoresis (5) and immunofixation electrophoresis (6) were performed with rabbit antiserum specific for C3 prepared by immunization with purified C3 (7). Preparative electrophoresis was carried out in Pevikon (8).

Other protein separation techniques. Euglobulin and pseudoglobulin were separated by overnight dialysis of serum at 4°C against phosphate or EDTA buffer, pH 5.4, ionic strength 0.02, followed by centrifugation. Gel filtration was performed at 4°C through columns of Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). Preparative ultracentrifugation (9) was carried out with sucrose density gradients of 10–30%.

Metabolic studies with ^{125}I -labeled C3. Studies of the behavior *in vivo* of isotopically labeled C3 were performed as described previously (10).

Other labeled proteins. The purified anticomplementary protein (CoF) from *Naja naja* venom (11) was labeled

with ^{125}I by the iodine monochloride technique (12). Purified ^{125}I -labeled C3 anaphylatoxin (C3a), prepared by the method of Dias da Silva, Eisele, and Lepow (13), was kindly provided by Dr. Irwin Lepow.

Stability of C3 *in vitro*. Whole clotted blood, whole blood taken into EDTA (9 mg of disodium EDTA to 7 ml of blood), and serum from the patient and two normal subjects were incubated at 37°C. Aliquots were removed at hourly intervals, were frozen at -80°C, and later were thawed and examined in antigen-antibody crossed electrophoresis with antiserum to C3. Serum samples were also tested for hemolytic complement. The areas under the C3 and conversion product peaks were determined by weighing (10), and the area under the C3 peak was expressed as a percentage of the total area for each pattern. The patterns of the patient's serum or plasma were corrected for the conversion product present in the sample prior to incubation. The per cent C3 for each experiment was plotted on a semilogarithmic scale versus time of incubation. The points obtained by this analysis were fitted to a straight line in each instance. The slopes of these lines were taken as the conversion rate of C3 *in vitro* as a first order process.

Complement-mediated functions. Hemolytic complement, bactericidal activity, chemotaxis, and enhancement of phagocytosis were measured as described previously (1).

Serum protease and protease inhibitors. The patient's fresh serum and normal serum and a solution of trypsin (100 mg/100 ml) as a positive control were subjected to agarose electrophoresis. The gel containing the separated proteins was transferred to light-exposed and developed X-ray film and incubated in a moist chamber for 1 hr. On removal of the gel, the film was examined for evidence of digestion. The patient's serum was tested for esterolytic activity with *N*-acetyl-L-tyrosine ethyl ester and *p*-toluenesulfonyl-L-arginine methyl ester in a pH Stat (Radiometer Co., Copenhagen, Denmark) and with benzoyl-DL-arginine nitroanilide HCl by a colorimetric method (14). Total trypsin-inhibiting capacity (15) and α_2 -macroglobulin (16) were assayed by trypsin-binding methods, and the C1 esterase inhibitor was measured by an esterolytic technique (17). In addition, the concentrations of α_1 -antitrypsin, α_2 -macroglobulin, C1 esterase inhibitor, α_1 -antichymotrypsin, and the inter- α trypsin inhibitor in the patient's serum were estimated immunochemically (18) with monospecific antisera.

Urinary histamine. Urinary histamine excretion (19) was measured in the patient on several occasions and in normal subjects.

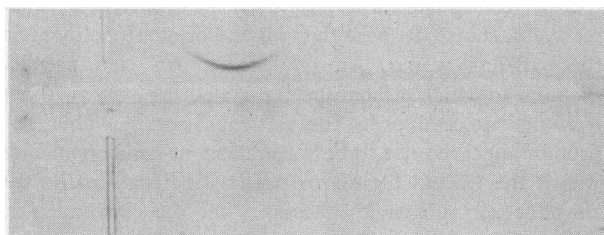


FIGURE 1 Immunoelectrophoresis in agarose gel. The anode is at the right, the origins at the left. The top antigen trough contained normal serum; and the bottom antigen trough contained the patient's serum. The antibody trough contained antiserum to the unidentified beta globulin. No precipitin arc is seen with the patient's serum.

Studies of an unidentified beta globulin. An antiserum prepared in a rabbit against a purified 4.3S gamma glycoprotein (20) reacted with an unidentified beta globulin in fresh normal serum. The antiserum had previously been shown not to react with C2, C3, C4, C5, C9, or a variety of other known proteins. The precipitin arc of this protein was absent from the patient's serum on immunoelectrophoresis (Fig. 1). As judged by gel filtration through Sephadex G-200, the antigen in normal serum was approximately 6S, and it did not become 9S on treatment of serum with *Naja naja* venom in the presence of Mg^{++} . The antiserum was found by Dr. Peter J. Lachmann of Cambridge, England, not to react with purified C3 inactivator.

RESULTS

Metabolic studies with ^{125}I -labeled C3. Metabolic studies with intravenously administered ^{125}I -labeled C3 SS revealed a normal synthesis rate of C3 of 1.2 mg/kg per hr and a markedly increased fractional catabolic rate of 10.1% of the plasma pool per hr (Patient, I, in Fig. 2 and Table I). As in previous studies (10), the same labeled C3 preparation was given to a normal control subject and yielded values for metabolism within the previously determined normal range. Plasma samples obtained 10 min and 2 hr after injection were analyzed for protein-bound radioactivity after agarose electrophoresis, as shown in Fig. 3. Whereas the protein-bound radioactivity had the electrophoretic mobility of C3 in the 10-min and 2-hr samples from the normal subject and in the 10-min sample from the patient, approximately 40% had the mobility of C3b or C3c in the 2-hr sample from the patient. Thus, there was substantial conversion of the administered, labeled C3 by 2 hr in vivo.

Studies of C3 stability in vitro. In view of the apparent marked instability of C3 in vivo in the patient, evidence for similar instability in his blood in vitro was sought. The conversion rates of C3 on incubation of whole clotted blood or serum at 37°C were similar for the patient and for the normal controls and were approximately 20% of native C3 per hr. The rates in the presence of EDTA were 1.6% per hr for the patient

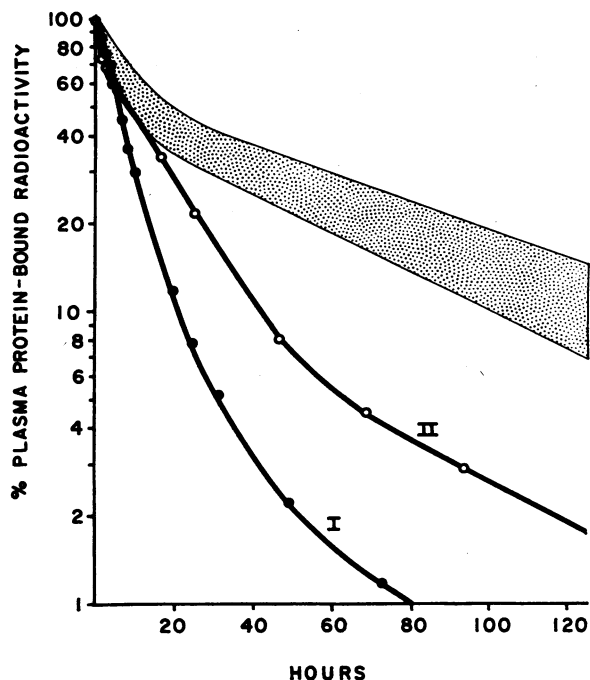


FIGURE 2 Plasma disappearance curves of ^{125}I -labeled C3 in 11 normal subjects (shaded area) and in the patient under base line conditions (I) and after the infusion of 500 ml of normal plasma (II).

and 0.9% per hr for the control. Hemolytic complement decayed at approximately 2.6% per hr in both the patient's serum and in the normal control serum.

The nature of the circulating C3 conversion product. On prolonged agarose gel electrophoresis and immunofixation with anti-C3, the conversion product in the patient's fresh plasma formed a single band (Fig. 4). C3c produced by incubation of normal C3 SS serum for 2 days at 37°C consisted of multiple bands which were mostly of more anodal mobility than the conversion product in the patient's plasma. The mobility of the patient's conversion product was the same as that of

TABLE I
Metabolism of ^{125}I -labeled C3

Subject	C3 concentration*	Catabolic rate	Synthesis rate	Extravascular pool to plasma ratio
	mg/100 ml	% plasma pool per hr	mg/kg per hr	
Patient, I (base line)	30	10.08	1.2	0.92
Patient, II (after infusion of normal plasma)	65	6.47	1.6	1.12
Mean of normals ± 2 SD†	148.4 ± 35.7	2.35 ± 1.05	1.38 ± 0.51	0.90 ± 0.41

* Total immunochemically determined C3.

† From reference (10).

C3b produced by brief treatment of purified C3 with trypsin or by incubation of the patient's serum (to which C3 had been added to normal concentration) with antigen-antibody precipitate. The immunochemically reactive C3 in the patient's serum formed a single peak of approximately 9S on both gel filtration through Sephadex G-200 (Fig. 5) and sucrose density gradient ultracentrifugation. Thus, the conversion product had physicochemical characteristics similar to those of C3b SS (21).

Absence of C3b to C3c conversion in the patient's serum. After incubation at 37°C for 2-7 days, the electrophoretic and gel filtration characteristics of the immunochemically reactive C3 were exclusively those of C3b, as seen in Figs. 4 and 5. Thus, a mediator of the conversion of C3b to C3c appeared to be absent from the patient's serum.

Characterization of the substance in normal serum capable of restoring complement-mediated functions to

the patient's serum in vitro. Normal serum was subjected to various treatments and fractionation procedures. As can be seen in Table II and Figs. 6 and 7, the restoring substance was a 5-6S heat-labile beta pseudoglobulin present in "complement-fixed" serum. Since it was known from previous studies that C1, C4, C2, C5, and C6 were normal in concentration and that "C3" was essentially normal by reagent titration (implying that C7, C8, and C9 were normal in concentration), it seemed unlikely that the substance deficient in the patient's serum was a complement component. The present studies provide further support for this conclusion in that C2 deficient human serum, serum from a patient with hereditary angioneurotic edema (deficient in C4 and C2), C5 deficient mouse serum, and C6 deficient rabbit serum were as effective as normal serum in restoring complement-mediated functions to the patient's serum.

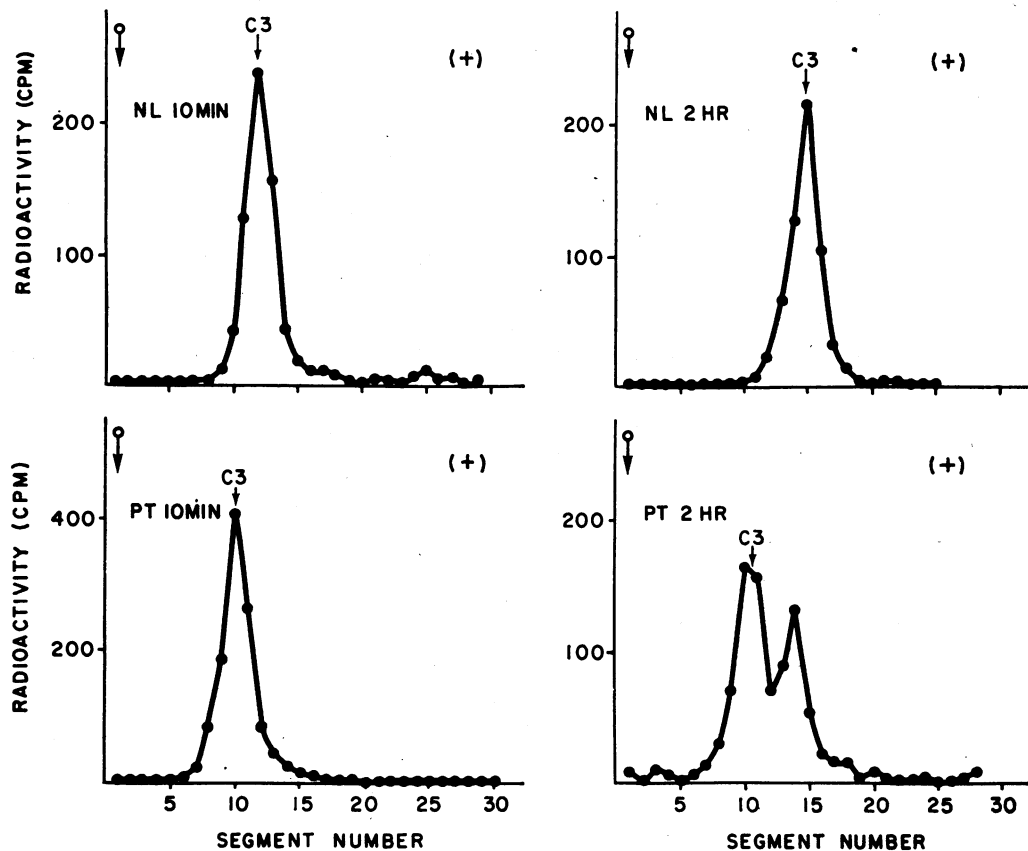


FIGURE 3 Electrophoretic distribution of plasma radioactivity after intravenous administration of purified ^{125}I -labeled C3. The upper patterns are from a normal subject 10 min (NL 10 MIN) and 2 hr (NL 2 HR) after administration. The lower patterns are from the patient (PT 10 MIN and PT 2 HR). The positions of C3 are indicated for each experiment, as well as those of the origins (arrows on open circles) and anodes (+). The extra anodal radioactive peak in the 2-hr sample from the patient corresponded in electrophoretic mobility to C3b or C3c.

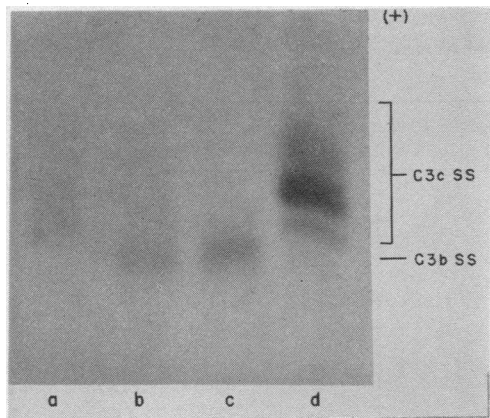


FIGURE 4 Prolonged agarose gel electrophoresis and immunofixation with anti-C3. The anode is at the top and only the β_1 - α_2 region is shown. The conversion product in the patient's fresh serum (c) had the mobility of C3b SS and was essentially unchanged after 2 days of storage at 37°C (b). However, a sample obtained 2 days after the infusion of normal plasma (a) and stored for 2 days at 37°C showed the pattern of C3c SS similar to that produced on storage of normal C3 SS serum under the same conditions (d).

Studies of the proinactivator of C3. Because of the physicochemical similarities of the restoring substance to those of the C3 proinactivator characterized by Müller-Eberhard and associates (11), evidence for the presence or absence of this protein in the patient's serum was sought. On addition of ^{125}I -labeled C3 and of purified CoF to normal serum and to the patient's serum, incubation for 30 min at 37°C, agarose electrophoresis, and radioautography, it was evident that, whereas the CoF induced complete conversion of the labeled C3 in normal serum, the labeled C3 in the patient's serum underwent only slightly more conversion than did the labeled C3 in buffer control (Fig. 8). Furthermore, ^{125}I -labeled CoF when added to the patient's serum or to the buffer control emerged entirely as a 7S protein from a Sephadex G-200 column, whereas there was a 9S peak when the labeled CoF was added to normal serum. This indicated that the normal complex between CoF and proinactivator (11) could not be generated in the patient's serum, and that therefore, the patient's serum lacked functional proinactivator.

Serum proteases and protease inhibitors. Neither the patient's whole serum nor his serum proteins separated in agarose electrophoresis showed digestion of gelatin on an exposed, developed X-ray film. Similarly, there was no evidence in his serum of abnormal esterolytic activity for *N*-acetyl-L-tyrosine ethyl ester, *p*-toluenesulfonyl-L-arginine methyl ester, nor for benzoyl-DL-arginine nitroanilide HCl. The concentrations of major protease inhibitors were normal as shown in Table III,

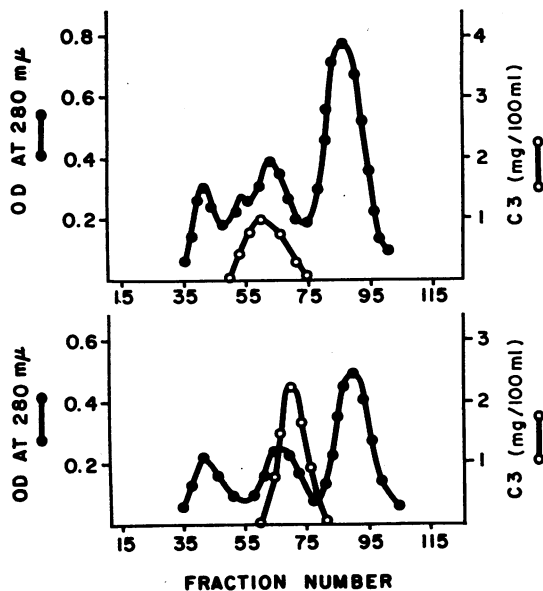


FIGURE 5 Gel filtration on Sephadex G-200 of the patient's serum stored for 2 days at 37°C (upper pattern), and his serum obtained 2 days after the infusion of normal plasma and stored for 2 days at 37°C (lower pattern). The closed circles represent protein concentration and the open circles C3 concentration.

as was the pattern of trypsin inhibitors in fibrin agarose electrophoresis.

The effects of infusion of normal plasma. Approximately 500 ml of plasma from a healthy donor judged to be free of hepatitis were infused intravenously over a 45-min period. Within a few minutes of the completion of the infusion, successive crops of nonpruritic urticaria developed over the patient's face, trunk, and proximal

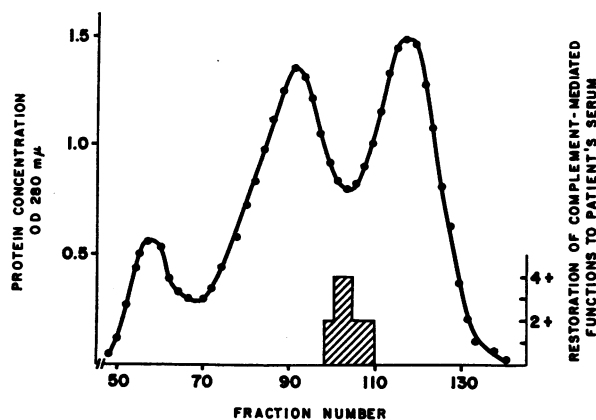


FIGURE 6 Gel filtration on Sephadex G-200 of normal serum. Only the fractions shown in the hatched area at the bottom of the figure restored complement-mediated functions (bactericidal activity, enhancement of phagocytosis, and chemotaxis) to the patient's serum.

TABLE II
Restoration by Serum and Serum Fractions of Complement-Mediated Functions
to the Patient's Serum

Test sample	Bactericidal activity	Chemotactic activity	Enhancement of phagocytosis
Euglobulin from normal serum	—	—	—
Pseudoglobulin from normal serum	+	+	—*
Normal serum heated at 52°C for ½ hr	—	—	—
Normal serum incubated with antigen-antibody precipitate at 37°C for 1 hr	+	+	+
C2-deficient human serum	+	+	+
Serum from a patient with hereditary angioneurotic edema	+	+	+
C5-deficient mouse serum	+	+	+
C6-deficient rabbit serum	+	+	+

* Pseudoglobulin from normal serum resulted in enhancement of phagocytosis if purified C3 was added.

extremities. These lesions came and went over the next 5 or 6 hr only to return transiently for several hours 17 days later. They were identical with those observed after he took a shower (1).

The changes that occurred in C3 are shown in Figs. 9 and 10 and consisted of the following: (a) the transient appearance over the first hour following the completion of the infusion of a conversion product slower in electrophoretic mobility (γ) than C3; (b) anodal broadening of the C3b area with the appearance of several minor peaks; on prolonged agarose immunofixation electrophoresis, it could be seen that material with the appearance and mobility of C3c had emerged; (c) a gradual decrease of protein in the area of C3b-C3c over a 6 hr period after the infusion so that by 16 hr, conversion products were no longer visible; (d) a gradual rise in the concentration of C3 from the initial level of

about 8 mg/100 ml to 70 mg/100 ml by the 7th day after the normal plasma infusion; and (e) persistence of the elevation in C3 concentration and absence of detectable conversion products until 17 days after the infusion, at which time C3 began to fall and C3b reappeared. By 29 days the initial C3 constellation had been reestablished.

As is apparent from Fig. 10, restoration of hemolytic complement paralleled the changes in C3, as did the restoration of bactericidal activity, chemotaxis, and enhancement of phagocytosis, although these functions deteriorated somewhat more rapidly than did C3. Although ¹²⁵I-labeled C3 was promptly converted to C3b or C3c in the patient's serum during the restoration period on the addition of CoF in vitro, no radioactive 9S peak could be demonstrated on gel filtration after the addition of ¹²⁵I-labeled CoF.

Following the infusion of normal plasma, the unidentified beta globulin became detectable at 5.6 hr, reached a peak concentration of 8% of that in normal

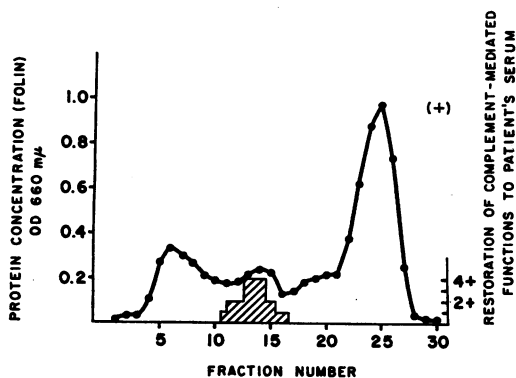


FIGURE 7 Preparative electrophoresis on Pevikon of normal serum. The anode was at the right. The fractions that restored complement-mediated functions to the patient's serum are indicated by the hatched area at the bottom of the figure.

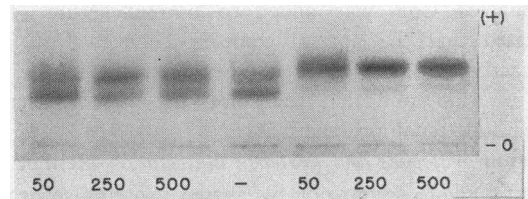


FIGURE 8 Radioautograph of agarose gel electrophoresis. ¹²⁵I-labeled C3 was added to the patient's serum (three left-hand patterns), 0.15 M phosphate buffered saline, pH 7.4 (center pattern), and normal serum (three right-hand patterns). These mixtures were then incubated with purified CoF (anticomplementary protein from *Naja naja* venom) for 30 min at 37°C and subjected to electrophoresis. The amounts in μ g of CoF added per ml are shown at the bottom of the figure.

TABLE III
Serum Concentrations of Protease Inhibitors

	Immunochemical assay					Enzymatic assay		
	α_1 -Anti-trypsin	α_1 -Anti-chymotrypsin	Inter- α trypsin inhibitor	α_2 -Macroglobulin*	CI inhibitor	Total anti-trypsin activity	α_2 -Macroglobulin	CI inhibitor
	% normal pool†	% normal pool	% normal pool	% normal pool	% normal pool	mg trypsin inhibited per ml	% normal pool	U/ml
Patient	111	129	160	92	88	0.83	91	7.8
Normal	68-138§	64-151	75-138	39-154	44-170	0.6-1.6	47-153	4-7

* Uncorrected for age or sex.

† The normal pool was composed of equal parts of serum from 24 healthy young adults.

§ Mean \pm 2 SD.

pooled serum at 16 hr, and was again no longer detectable after 25 hr.

Storage, at 37°C for 2 days, of a serum sample obtained 48 hr after the normal plasma infusion resulted

in conversion of the patient's C3 to material with the characteristics of C3c SS (Figs. 4 and 5) (22).

The results of a second metabolic study with 125 I-labeled C3 (Patient, II) performed after the plasma

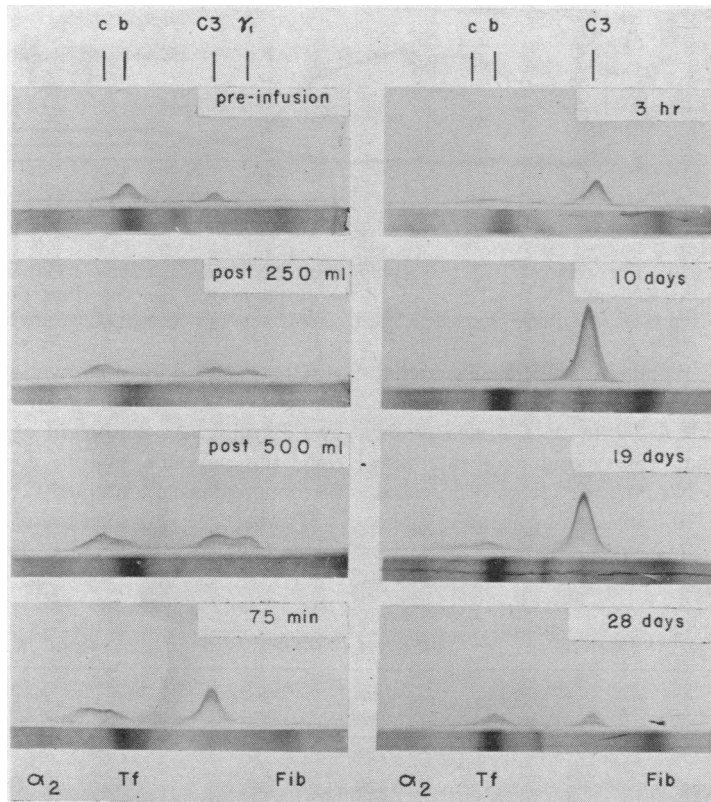


FIGURE 9 Antigen-antibody crossed electrophoresis of the patient's serum in relation to the infusion of normal plasma. The anode for the first electrophoresis was at the left, and for the second dimension, it was at the top. The positions of C3, C3b (b), C3c (c), and the γ_1 conversion product are indicated at the top, and the positions of the α_2 area, transferrin (Tf), and fibrinogen (Fib) on the stained strips below each pattern are shown at the bottom.

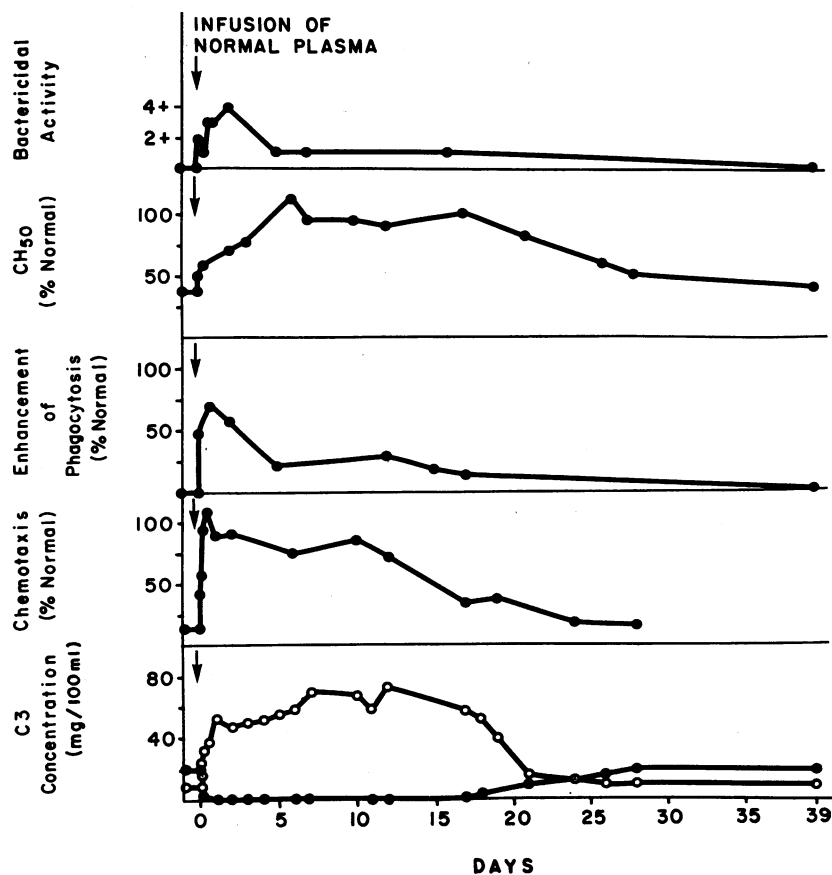


FIGURE 10 The patient's C3 and complement-mediated functions following the infusion of 500 ml of normal plasma. In the graph for C3 concentration, the open circles represent C3 itself and the closed circles represent conversion products.

infusion are shown in Fig. 2 and Table I. The fractional catabolic rate fell to approximately 6.5% of the plasma pool per hr, and the synthesis rate of C3 was again normal and similar to that observed during the base line study of this patient. No labeled C3b or C3c could be detected in either the 10-min or 2-hr samples. The conclusion that the rise in C3 concentration was the

result of the patient's own synthesis and not of the infusion of C3 in the donor plasma was substantiated by C3 typing. At the height of the rise in C3 concentration, the patient's plasma contained C3 SS, whereas the donor plasma contained C3 FS.

Histamine excretion studies. Urinary excretion of histamine was significantly elevated at all times in this

TABLE IV
Histamine Excretion

Subject	Urine volume	Collection period	Total histamine	Urine histamine concentration
	<i>ml</i>	<i>hr</i>	μg	$\mu\text{g/ml}$
Patient, resting	1420	24	407	0.287
Patient, 4 hr after a warm shower	255	4	814	3.192
Patient, 12 hr after plasma infusion	385	5	458	1.190
Normal subjects*		24	5-58	(0.003-0.072)†

* From reference (19).

† An estimate based on total histamine and assuming a range of normal 24 hr urine volumes of 800-2000 ml.

patient, even at times when he refrained from bathing (Table IV). Following a warm shower, histamine excretion increased markedly, as it also did following the plasma infusion after he exhibited urticaria (Table IV).

Inactivation of C3a. Upon the addition of ^{125}I -labeled C3a to the patient's base line serum and to normal serum and subsequent agarose electrophoresis and radioautography, it was found that the same change in the mobility of C3a from mid-gamma to rapid beta occurred in both specimens, suggesting the presence of C3a inactivation in both the normal serum and serum from the patient (Fig. 11). In addition, although an incubated mixture of purified $\text{C}\bar{1}_s$, C4, C2, and C3 resulted in anaphylatoxin generation as judged by contraction of guinea pig ileum (23), the same mixture (with $\text{C}\bar{1}_s$ in excess of the C1 esterase inhibitor in the patient's serum) in the presence of the patient's serum failed to produce such contraction.¹

DISCUSSION

The observations in this report shed light not only on the molecular pathophysiology in an unusual patient but also on the normal physiology of complement. The primary defect in this patient remains to be defined. As a working hypothesis, it seems reasonable to assume that the patient lacks an inhibitor of some protease in analogy to the absence of the inhibitor of $\text{C}\bar{1}$ in patients with hereditary angioneurotic edema (24). Our failure thus far to demonstrate uninhibited protease activity either as esterolytic activity or as an accelerated conversion of C3 in vitro in the patient's serum or plasma does not exclude this hypothesis. Patients with hereditary angioneurotic edema do not have free $\text{C}\bar{1}$ in their serum except during attacks, although they lack the $\text{C}\bar{1}$ inhibitor at all times (25). If the patient's primary defect is the lack of an inhibitor of a protease with C3 as a substrate, thus explaining the continuous conversion of C3 to C3b, other proteins might also be lowered in concentration if they, too, are substrates of the postulated enzyme. Alternatively, the continuous attack on C3 with the consequent production of large amounts of conversion products might result in exhaustion of substances which bind these products, much as haptoglobin is lowered when hemoglobin is released from red cells in vivo. However, the inactivating mechanism for C3a, at least, appears to be intact in our patient, as shown by the normal change in the mobility of ^{125}I -labeled C3a from gamma to beta on addition to his serum (26) and functional inactivation of C3a.

If the protein capable of restoring complement-mediated functions to the patient's serum in vitro is the sub-

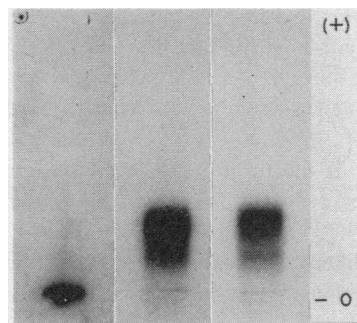


FIGURE 11 Radioautograph of agarose gel electrophoresis. The left-hand pattern is of ^{125}I -labeled C3a in buffer with 1% human albumin; the middle pattern is of labeled C3a added to the patient's serum; and the right-hand pattern is of labeled C3a added to normal serum.

stance primarily defective, the possibility that it may be the same as the proinactivator is intriguing but puzzling. Although the physicochemical characteristics of the two proteins are similar, and it has been shown that the patient's serum is devoid of active proinactivator, it is difficult to understand why a protein known to be able to induce conversion and inactivation of C3 in conjunction with CoF (or a presumed human analogue of CoF) should normalize complement-mediated functions. The rise in concentration of the proinactivator following the plasma infusion was only partial, since, although CoF induced C3 conversion, no 9S inactivator complex could be detected.

It may well be that the protein capable of restoring complement-mediated functions is the same as that shown to be necessary for full enhancement of phagocytosis of pneumococci by normal leukocytes in the presence of antibody, the first four components of complement, and a dialyzable cofactor (27). If this is so, and this is the same protein responsible for the observed decrease in conversion of C3 to C3b in vivo following the plasma infusion, it may be thought of as a C3 stabilizer in vitro as well as in vivo, on the cell as well as in the fluid phase. Work to obtain evidence on this point is currently in progress.

The restoration, complete or partial, of all measured defective functions in the patient by an infusion of normal plasma strongly supports our original hypothesis that he has an inborn error (1). The surprising length of time for which this restoration persisted (17 days) suggests either that the substance which he primarily lacks is degraded very slowly or that it is extraordinarily potent or both.

The transient appearance of the gamma conversion product in the patient's plasma is of interest, particularly since it immediately followed the plasma infusion and coincided with the onset of his urticaria. We have noted earlier (6) that this slow conversion product,

¹ Performed in collaboration with Dr. Irwin H. Lepow.

which is of identical mobility in agarose electrophoresis as the slow hydrazine product described by Lundh (28), is seen during storage of whole serum, particularly in the cold, and transiently on incubation of serum with zymosan and $\overline{C1}$ s in excess of the $\overline{C1}$ inhibitor. It is possible that this is the form of C3 with the activated combining site for red cells postulated by Müller-Eberhard and coworkers (11).

The most likely explanation for the patient's elevated urinary excretion of histamine at all times is that with constant conversion of C3, some C3a is constantly generated, and C3a is known to be capable of releasing histamine from rat (23) and human (29) mast cells. The urticaria following the plasma infusion might then have resulted from the sudden influx of C3 to serve as substrate for the generation of more C3a with the consequent further rise in the patient's urinary histamine. Similarly, as the conversion mechanism reaccelerated at about 17 days, the concentration of C3 was high, and the urticaria observed at that time might be explained in similar fashion. We have no explanation for the patient's urticaria following exposure to water.

The nature of the beta globulin antigen remains obscure. It appears to be one of the proteins secondarily missing from the patient's serum, and the evidence indicates that it is not the C3 inactivator or proinactivator.

The effects on C3 of the infusion of normal plasma are strongly reminiscent of the behavior of Factor VIII in the plasma of patients with von Willebrand's disease (30, 31) after receiving Fraction I-0 or plasma from patients with hemophilia A or normal subjects. Whereas the effects of such infusions have been interpreted as an induction of Factor VIII synthesis, a mechanism similar to that observed for C3 in the present patient may be operating.

The role of the C3 inactivator (32) or KAF (33) in the conversion of C3b to C3c, evidence that the patient's serum also lacks this protein, and the relationship of this protein to the C3 coating of the patient's red cells (34, 35) will be the subject of another report.

ACKNOWLEDGMENTS

We are grateful to Dr. Virginia Donaldson, Cincinnati, Ohio, for performing the C1 inhibitor esterolytic assay and assays for esterolytic activity for *N*-acetyl-L-tyrosine ethyl ester and *p*-toluenesulfonyl-L-arginine methyl ester. Dr. Carlos Biro of Mexico, D.F., kindly supplied C6 deficient rabbit serum, and Dr. Henry Winn of Boston donated C5 deficient mouse serum.

This work was aided by Grants HD-02723, AM-13855, HE-07652, TO1-AM-05391, FR-76, 1-K3-AM-19650, AI-05877 and TO1-A1-00366 from the United States Public Health Service.

REFERENCES

- Alper, C. A., N. Abramson, R. B. Johnston, Jr., J. H. Jandl, and F. S. Rosen. 1970. Increased susceptibility to infection associated with abnormalities of complement-mediated functions and of the third component of complement (C3). *N. Engl. J. Med.* **282**: 349.
- Laurell, C.-B., and J.-E. Niléhn. 1966. A new type of inherited serum albumin anomaly. *J. Clin. Invest.* **45**: 1935.
- Alper, C. A., and R. P. Propp. 1968. Genetic polymorphism of the third component of human complement (C3). *J. Clin. Invest.* **47**: 2181.
- Heimbürger, N., and G. Schwick. 1962. Die Fibrinagar-Electrophorese. 1. Mitteilung: Beschreibung der Methode. *Thromb. Diath. Haemorrh.* **7**: 433.
- Laurell, C.-B. 1965. Antigen-antibody crossed electrophoresis. *Anal. Biochem.* **10**: 358.
- Alper, C. A., and A. M. Johnson. 1969. Immunofixation electrophoresis: a technique for the study of protein polymorphism. *Vox Sang.* **17**: 445.
- Nilsson, U. R., and H. J. Müller-Eberhard. 1965. Isolation of β_{10} -globulin from human serum and its characterization as the fifth component of complement. *J. Exp. Med.* **122**: 277.
- Müller-Eberhard, H. J. 1960. A new supporting medium for preparative electrophoresis. *Scand. J. Clin. Lab. Invest.* **12**: 33.
- Kunkel, H. G. 1960. Macroglobulins and high molecular weight antibodies. In *The Plasma Proteins*. F. W. Putnam, editor. Academic Press, Inc., New York. **1**: 279.
- Alper, C. A., and F. S. Rosen. 1967. Studies of the in vivo behavior of human C3 in normal subjects and patients. *J. Clin. Invest.* **46**: 2021.
- Müller-Eberhard, H. J., U. R. Nilsson, A. P. Dalmasso, M. J. Polley, and M. A. Calcott. 1966. A molecular concept of immune cytotoxicity. *Arch. Pathol.* **82**: 205.
- McFarlane, A. S. 1958. Efficient trace-labelling of protein with iodine. *Nature (London)*. **182**: 53.
- Dias da Silva, W., J. W. Eisele, and I. H. Lepow. 1967. Complement as a mediator of inflammation. III. Purification of the activity with anaphylatoxin properties generated by interaction of the first four components of complement and its identification as a cleavage product of C3. *J. Exp. Med.* **126**: 1027.
- Erlanger, B. F., N. Kokowsky, and W. Cohen. 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem.* **95**: 271.
- Eriksson, S. 1965. Studies in alpha₁-antitrypsin deficiency. *Acta Med. Scand.* **177** (Suppl.): 1.
- Ganrot, P. O. 1966. Determination of α_2 -macroglobulin as trypsin-protein esterase. *Clin. Chim. Acta.* **14**: 493.
- Levy, L. R., and I. H. Lepow. 1959. Assay and properties of serum inhibitor of C1 esterase. *Proc. Soc. Exp. Biol. Med.* **101**: 608.
- Laurell, C.-B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15**: 45.
- Huff, J. A., V. E. Davis, and H. Brown. 1966. A simplified technique for the estimation of histamine in blood and urine. *J. Lab. Clin. Med.* **67**: 1044.
- Boenisch, T., and C. A. Alper. 1970. Isolation and properties of a glycine-rich gamma glycoprotein of human serum. *Biochim. Biophys. Acta.* **214**: 135.
- Müller-Eberhard, H. J., A. P. Dalmasso, and M. A. Calcott. 1966. The reaction mechanism of β_{10} -globulin (C3) in immune hemolysis. *J. Exp. Med.* **123**: 33.

22. Lundh, B., and C.-B. Laurell. 1967. Charge and size of the conversion products of serum β_{1c} -globulin. *Immunology*. 13: 649.
23. Dias da Silva, W., and I. H. Lepow. 1967. Complement as a mediator of inflammation. II. Biological properties of anaphylatoxin prepared with purified components of human complement. *J. Exp. Med.* 125: 921.
24. Donaldson, V. H., and R. R. Evans. 1963. A biochemical abnormality in hereditary angioneurotic edema: absence of serum inhibitor of C'1-esterase. *Amer. J. Med.* 35: 37.
25. Donaldson, V. H., and F. S. Rosen. 1964. Action of complement in hereditary angioneurotic edema: the role of C'1 esterase. *J. Clin. Invest.* 43: 2204.
26. Lepow, I. H., W. Dias da Silva, and R. A. Patrick. 1969. Biologically active cleavage products of components of complement. In *Cellular and Humoral Mechanisms in Anaphylaxis and Allergy*. H. Z. Movat, editor. S. Karger, New York. 247.
27. Johnston, R. B., Jr., M. R. Klemperer, C. A. Alper, and F. S. Rosen. 1969. The enhancement of bacterial phagocytosis by serum. The role of complement components and two cofactors. *J. Exp. Med.* 129: 1275.
28. Lundh, B. 1965. The effect of incubation with hydra-
zine and immunoprecipitate on β_{1c} -globulin. *Acta Pathol. Microbiol. Scand.* 63: 266.
29. Lepow, I. H., K. Willms-Kretschmer, R. A. Patrick, and F. S. Rosen. Gross and ultrastructural observations on lesions produced by intradermal injection of human C3a in man. *Amer. J. Pathol.* In press.
30. Nilsson, I. M., M. Blombäck, and B. Blombäck. 1959. v. Willebrand's disease in Sweden. Its pathogenesis and treatment. *Acta Med. Scand.* 164: 263.
31. Blombäck, M., J. E. Jorpes, and I. M. Nilsson. 1963. von Willebrand's disease. *Amer. J. Med.* 34: 236.
32. Tamura, N., and R. A. Nelson, Jr. 1967. Three naturally-occurring inhibitors of components of complement in guinea pig and rabbit serum. *J. Immunol.* 99: 582.
33. Lachmann, P. J., and H. J. Müller-Eberhard. 1968. The demonstration in human serum of "conglutininogen-activating factor" and its effect on the third component of complement. *J. Immunol.* 100: 691.
34. Abramson, N., C. A. Alper, F. S. Rosen, and J. H. Jandl. 1968. C'3 coating of red cells without hemolytic anemia. *Proc. 12th Cong. Int. Soc. Hematol.* 122 (FF-1). (Abstr.)
35. Abramson, N., C. A. Alper, F. S. Rosen, and J. H. Jandl. 1969. A normal serum protein that removes C'3 from red cells. *Clin. Res.* 17: 318. (Abstr.)