Elution of an Esterase from Antigen-Antibody Aggregates Treated with Human Complement.* (22401)

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It was reported recently(1) that partially purified first component of human complement (C'1) contains a pro-esterase. Adjustment of such a solution of purified C'1 to physiologic conditions of pH and ionic strength resulted in disappearance of the hemolytic activity of C'1 and appearance of anti-complementary and esterase activities. The complement-inactivating property was directed primarily toward the fourth component (C'4) and, to a lesser degree, toward the second component (C'2); esterase activity was observed with the synthetic substrates, p-toluenesulfonyl-L-arginine methyl ester (TAMe) and acetyl-L-tyrosine ethyl ester (AcTyEe) (2). A correlation existed between the time of disappearance of hemolytically active C'1 and the parallel appearance of both anti-complementary and esterase activities. In accordance with these observations and previous investigations(3-7), it was postulated that C'1 may be a pro-esterase.

It is the purpose of this paper to present further evidence for the pro-enzymatic role of C'1. Such evidence has been obtained by eluting from antigen-antibody aggregates, previously treated with fresh human serum, a fraction, representing less than 0.04% of the original serum nitrogen, which contains both esterase and anti-complementary activities. These properties are qualitatively similar to those obtained after "conversion" of purified C'1(1), and cannot be ascribed to any other known serum factor.

Materials and methods. Pre-formed *antigen-antibody aggregates*(6) were prepared by adding a solution of purified pneumococcal specific soluble substance, S-III† to purified antipneumococcal S-III rabbit serum† in the ratio of 1 mg S-III to 25 mg antibody N. The resulting suspension was brought to a final volume of 250 ml per mg S-III with 0.15 M NaCl. The mixture was stirred occasionally at 1° for 12-24 hrs. and then centrifuged at 4000 r.p.m. for 30 min. at 1°. The antigen-antibody precipitate was washed twice at 1° with 0.15 M NaCl of the same volume as the original suspension, stored at 1°, and used within 24 hrs. *Cholinesterase* activity of eluate preparations was determined by the method of Michel(8). *Hydrolysis of AcTyEe* and of acetyl-L-tryptophane ethyl ester (AcTrEe) was measured by the same technic used to measure *hydrolysis of TAMe*(1,9). *All other relevant materials and methods were presented previously*(1).

Results. 1. Preparation of Eluates. It was shown previously that antigen-antibody aggregates do not inactivate C'2 and C'4 in the absence of C'1(5,10). However, if antigen-antibody aggregates are first treated with serum reagents containing C'1 and washed free of occluded serum, they are then capable of inactivating C'2 and C'4 in the absence of additional C'1(6). This procedure of "activating" antigen-antibody aggregates by treatment with serum reagents containing C'1, designated the "Activation Stage"(6), is the basis for the elution experiments to be described. Fresh human serum was used as a source of C'1, and serum heated at 56° for 30 min. as a control reagent deficient in C'1.‡ Pre-formed antigen-antibody aggregates were treated with these serum reagents under con-

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‡ Serum heated at 56° for 30 min. is also deficient in C'2 and, to a lesser degree, C'3. However, these components are not involved in the "Activation Stage"(6).

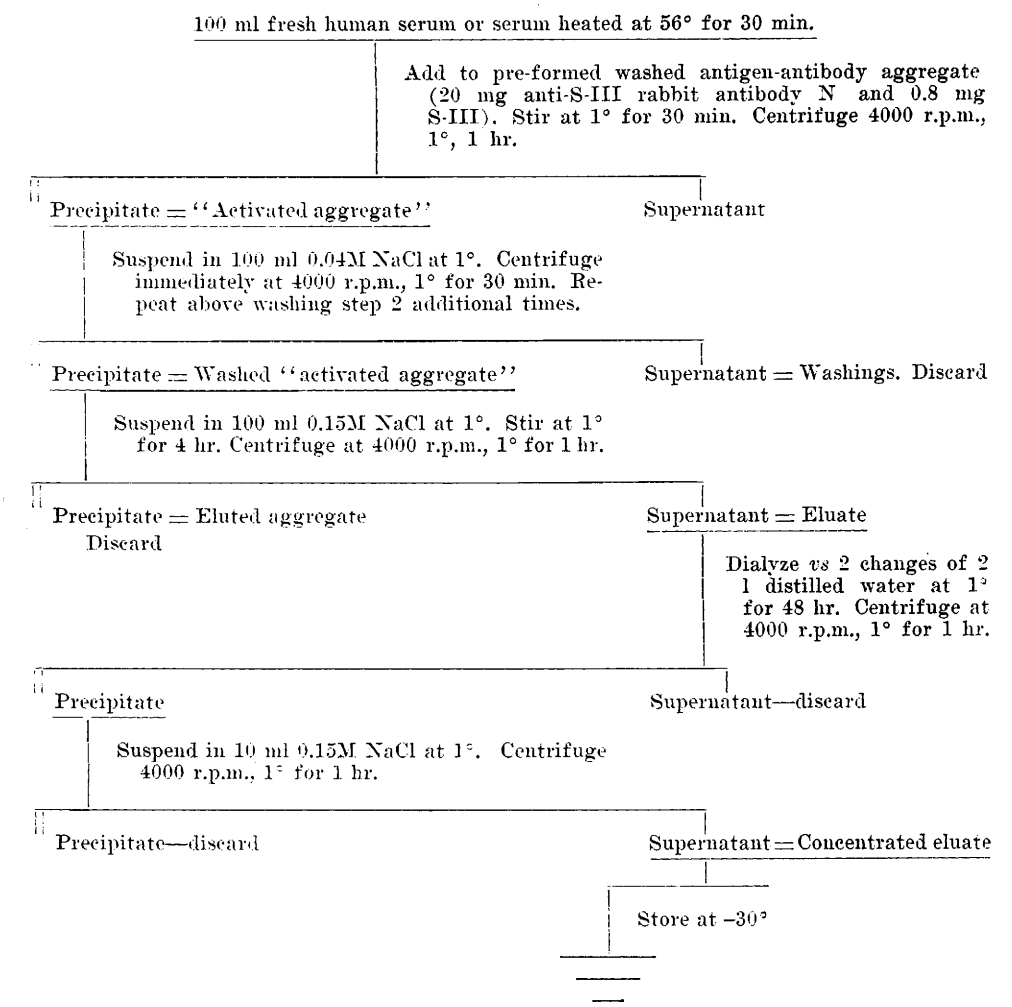

FIG. 1. Flow diagram for preparation of eluates from antigen-antibody aggregates treated with human serum reagents.

ditions identical to those described previously for the "Activation Stage"(6), washed with 0.04 M NaCl, and then eluted at 1° with 0.15 M NaCl. The eluates were dialyzed against distilled water, the small precipitates collected by centrifugation and dissolved in 0.15 M NaCl. A flow diagram of the entire procedure is shown in Fig. 1. The concentrated eluates did not contain measurable components of complement, properdin(11), acid or alkaline phosphatase,§ cholinesterase, prothrombin, proconvertin, proaccelerin, or thrombin. Spontaneous plasmin activity also could not be detected employing either fibrinogen or casein as substrates. Small amounts of Hageman factor(12) and of streptokinase-activable plasminogen were present. The latter could be demonstrated in the fibrinolytic assay; however, the amount was too small to be measured in the caseinolytic assay. Eluates prepared from fresh human serum and concentrated 10-fold over serum contained about 0.04 mg nitrogen/ml; with eluates prepared from serum heated at 56° for 30 min., the corresponding value was about 0.01 mg nitrogen/ml.

2. *Esterase and Complement-Inactivating Properties of Eluates.* The concentrated eluate derived from antigen-antibody aggregates

§ Kindly performed by Dr. J. W. Price.

TABLE I. Effect of Eluates AE and UE on Components of Complement in 0.75 ml R1.*

Eluate, ml	0.15M NaCl, ml	Inactivation of complement component† C'2	C'3	C'4
		%		
.01 AE	.24	0	0	60
.03 "	.22	35	0	80
.05 "	.20	35	0	95
.10 "	.15	50	0	100
.15 "	.10	70	0	100
.25 "	0	90	0	100
.25 UE	0	0	0	0

* R1 at pH 7.4, ionic strength 0.15, containing 2.5×10^{-3}M Ca^{++}, diluted 1/1.5 with respect to serum.

† Measured after incubation for 30 min. at 37°, based on complement component titers of R1 treated only with 0.25 ml of 0.15M NaCl.

treated with fresh human serum (designated AE) was highly anti-complementary because the preparation inactivated C'4 and, to a smaller degree, C'2. C'1 and C'3 were not significantly affected. The inactivation of C'2 and C'4 could be demonstrated by incubating AE with fresh serum or with R1 (the serum reagent deficient in C'1 but containing C'2, C'3 and C'4). Since plasmin does not inactivate C'2 and C'4 in R1(5), the use of this reagent minimized the possibility that the observed effects were due to activation of plasminogen to plasmin. In contrast to AE, the concentrated eluate derived from antigen-antibody aggregates treated with heated human serum (designated UE) did not inactivate complement or its components in serum or R1. A typical experiment demonstrating the effect of eluates AE and UE on components of complement in R1 at 37° is shown in Table I.

Eluate AE not only inactivated C'4 and C'2 but also had esterase activity against TAMe (Table II) and AcTyEe. No activity could be demonstrated against L-lysine ethyl ester (LEe) or AcTrEe. Eluate UE was inactive as an esterase.

Discussion. It has been shown that an eluate derived from antigen-antibody aggregates treated with fresh human serum (eluate AE) was capable both of inactivating C'4 and, to a lesser degree, C'2, and of hydrolyzing the synthetic esters, TAMe and AcTyEe. However, when antigen-antibody aggregates were treated with human serum which had been previously heated at 56° for 30 min., the corresponding eluate (UE) contained none of these activities. Thus, the factor which was adsorbed by aggregates is heat-labile. Two independent lines of evidence suggest the conclusion that this heat-labile factor is C'1.

First, previous investigations(5,6) showed that antigen-antibody aggregates could inactivate C'2 and C'4 in R1 only when the aggregates were treated with serum reagents rich in C'1. In the experiments described here, aggregates were treated with fresh serum (rich in C'1) or with heated serum (deficient in C'1) under conditions identical to those employed in a previous study(6). Since eluate AE inactivated C'2 and C'4 and eluate UE did not, it is concluded that C'1 must have been a factor adsorbed from unheated serum. Since C'1 activity is not demonstrable in the eluate, one inference is that C'1 is altered in some manner during adsorption by and elution from antigen-antibody aggregates.

Second, recent studies with partially purified human C'1(1) showed that a purified preparation of C'1 maintained its hemolytic activity as long as it was kept at pH 5.5, ionic strength 0.30. However, when the solution was brought to pH 7.4, ionic strength 0.15, it lost its C'1 activity and simultaneously acquired both anti-complementary and esterase activities. This preparation was designated "converted C'1" and its activities were strikingly similar to those of the active eluate (AE) described above. Similarly, purified C'1 maintained at pH 5.5, ionic

TABLE II. Esterase Activity of Eluates AE and UE. 1 ml TAMe,* 2.5 ml buffer.

Eluate, ml	0.15M NaCl, ml	Esterase activity,† .05N NaOH, ml
—	1.5	.027
.5 AE	1.0	.046
1.0 "	.5	.108
1.5 "	0	.152
1.5 UE	0	.011

* 0.4M TAMe dissolved in pH 7.4 phosphate buffer, ionic strength 0.15.

† Net micro-titration after 60 min. at 37°.

strength 0.30 appeared to be identical to the factor in fresh serum which was adsorbed by antigen-antibody aggregates.

Since "converted C'1" would be formed spontaneously under the conditions of adsorption and elution, a direct experiment employing antigen-antibody aggregates and purified C'1 is not as yet feasible. However, "converted C'1" and the active eluate have the following properties in common: esterase activity against TAMe and AcTyEe (2), but not against LEe or AcTrEe; ability to inactivate C'4 and, to a lesser extent, C'2; anti-complementary activity demonstrable both at 1° and 37°; esterase activity not demonstrable at 1°; absence of components of complement; activity independent of conversion of plasminogen to plasmin or of the presence or absence of properdin(11), prothrombin, proconvertin, proaccelerin, thrombin, or Hageman factor(12); absence of spontaneous plasmin activity, of cholinesterase, and of acid or alkaline phosphatase.

A detailed kinetic study of the esterase and complement-inactivating properties of "converted C'1" and of the active eluate is in progress. However, on the basis of evidence now available, the conclusion is warranted that C'1 probably exists in serum as a proenzyme which is activated by antigen-antibody aggregates. The immediate significance of this conclusion is two-fold: a long-standing postulate(13,14) that antigen-antibody aggregates activate one or more proenzymes in serum is substantiated, and the nature of one enzyme system is indicated; the mechanism of complement "fixation" by antigen-antibody aggregates is clarified. The significance of these observations in immunity and hypersensitivity remains to be determined.

Summary. An eluate (AE) prepared from antigen-antibody aggregates previously treated with fresh human serum contains esterase and anti-complementary activities. A corresponding eluate (UE) prepared from aggregates treated with serum heated at 56° for 30 min. does not contain these activities. The anti-complementary activity of AE is directed primarily against C'4; the esterase activity is demonstrable using TAMe and AcTyEe as substrates. Antigen-antibody aggregates adsorb C'1 or a factor closely resembling it, which upon elution appears to be identical to a previously described esterase derived from partially purified C'1. These results indicate that C'1 exists in serum as a proenzyme which is converted to an active esterase by antigen-antibody aggregates.

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