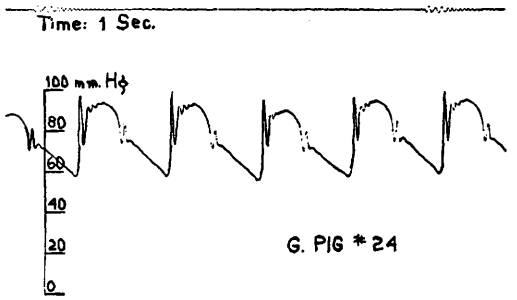

FIG. 1. Record of carotid pressures of a Hartley strain guinea pig weighing about 1000 g under procaine anesthesia, conscious and turned to the prone position 2 min. previously. Mean blood pressure = 68 mm Hg, systolic 88-94, diastolic 55-58, pulse rate 260/min., respiration 64/min., cardiac cycle = 0.232 sec., diastole = 0.118 sec., systole = 49% of cycle, late diastole rate of descent = 179 mm/sec.

taken up by systole (defined as the time from the first upswing of pressure to the bottom of the incisura) was greater than the physiological limit (40-45%) placed by these authors. A retouched record from a conscious prone animal illustrating these points is shown in Fig. 1. The guinea pig heart is therefore capable of faster filling and coronary outflow than was described for other small mammals.

Summary. In 8 guinea pigs under local or general anesthesia, systolic and diastolic end pressures in the carotid artery averaged 77 and 47 mm Hg respectively. The calculated mean pressure averaged 57 mm Hg. The portion of the cardiac cycle taken up by systole was greater than that described for other small mammals in about 40% of the measurements.

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Observations on a Pro-esterase Associated with Partially Purified First Component of Human Complement (C'1).* (22376)

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Investigations on the mechanism of inactivation of human complement by plasmin and by antigen-antibody aggregates(1-5) indicated that the first component of complement (C'1) may exist in serum as enzyme precursor. A mechanism of complement-"fixation" was proposed(3-5) in which it was postulated that antigen-antibody aggregates convert C'1 to active enzyme ("activated C'1"), which in turn inactivates the second and fourth components of complement (C'2 and C'4). Although available experimental data are compatible with this hypothesis, direct evidence has awaited studies with purified systems. Our experiments demonstrate that a partially purified preparation of C'1 converts, under certain conditions of pH, ionic strength and temperature, to material which has lost its hemolytic C'1 activity but

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has gained ability to inactivate complement and to hydrolyze the synthetic ester, p-toluene-sulfonyl-L-arginine methyl ester (TAMe). Disappearance of hemolytically active C'1 occurs simultaneously with appearance of both complement-inactivating and esterase activities. The esterase appears to be distinct from plasmin, thrombin, and acid and alkaline phosphatase.

Nomenclature, materials and methods. Nomenclature, and reagents and methods employed for titration of human *complement* and its components are summarized elsewhere(3, 6-8). Partially purified C'1 loses hemolytic and gains esterase and complement-inactivating activities when adjusted to physiologic pH and ionic strength in the absence of inhibitors; this new product is designated "*converted* C'1" without implying that the esterase and complement-inactivating activities are necessarily derived from hemolytically active C'1. R1 (serum fraction soluble at pH 5.5, ionic strength 0.02, which lacks C'1 but contains C'2, C'3 and C'4) contains inhibitors preventing this apparent inactivation of C'1 and was therefore added in equal amount to the initial dilution in titration of C'1 in purified fractions. Five units of R1 and 1 ml sensitized sheep erythrocytes were then added to 0.2 ml of each dilution and the mixtures incubated at 37°C for 30 minutes(3). *Spontaneous fibrinolytic activity* of preparations of C'1 was measured by a method employing bovine fibrinogen‡ and thrombin(1). To inhibit "conversion" of C'1, the ionic strength of reaction mixtures was adjusted to 0.3. The effect of streptokinase (SK) on fibrinolytic activity was tested by addition of SK to "converted C'1" at ionic strength 0.10 or 0.15 prior to addition of fibrinogen and thrombin; SK was effective in final concentrations of 9 to 500 Christensen(9) units/ml. *Caseinolytic activity.* In quadruplicate, 0.3 ml "converted C'1" was mixed with 0.2 ml barbital buffer (0.025 M barbital and 0.125 M sodium chloride at pH 7.5) or of streptokinase (Varidase§) dissolved in buffer in concentration of 10,000 units/ml. These mixtures were incubated at 37°C, 60 minutes. Five-tenths ml 5% casein ("Hammersten quality," Nutritional Biochemicals Co.) was added to 2 tubes at start and to other tubes at end of incubation period. Two ml 0.3 N trichloracetic acid were then added to each tube and the mixture centrifuged. Tyrosine-like activity of the supernatant solution was tested by minor modification of the method of Wu(10). *Digestion of TAMe.* Ability of preparations of C'1 or "converted C'1" to digest p-toluenesulfonyl-L-arginine methyl ester (TAMe) was tested by the method of Troll, Sherry and Wachman(11), modified so that substrate was 0.4 M TAMe dissolved in sodium phosphate buffer (pH 7.5, ionic strength, 0.15). Ionic strength was controlled by altering the concentration of sodium chloride in final mixture. Titration of acid liberated was measured with 1% alcoholic phenolphthalein as indicator. *Digestion of LEe.* Ability of solutions of "converted C'1" to digest L-lysine ethyl ester (LEe) was determined by Sherry's(12) modification of the method of Hestrin(13). Final concentration of LEe in the enzyme-substrate mixture was 0.04 M. Alkaline phosphatase and acid phosphatase activities of 1 ml aliquots of "converted C'1" were determined by the method of Shinowara(14). Thrombic activity was determined at 37° by addition of 0.1 ml of either C'1 or "converted C'1" to 0.3 ml of fibrinogen‖ (300 mg/100 ml of 0.15 M sodium chloride) or to mixture of 0.2 ml fibrinogen and 0.1 ml 0.05 N calcium chloride(15). Ionic strength of the mixtures was controlled by addition of 0.1 ml of sodium chloride in suitable concentration.

Results. 1. *Purification of Human C'1.* A procedure for purification of human C'1 has been reported previously(16). An independent method has been developed which takes advantage of the influence of ionic strength on solubility of C'1 at pH 5.5. The entire fractionation is carried out at pH 5.5 at low temperatures, and C'1 is in the soluble phase only at ionic strength 0.20 or

‡ Kindly provided by Dr. W. H. Seegers.

§ Kindly provided by the Lederle Laboratories Division, American Cyanamid Co.

‖ Kindly provided by the Warner-Chilcott Laboratories.

greater. The importance of this choice of conditions for fractionation will be apparent later. Either fresh human serum or RP may be employed as starting material. RP is serum rendered deficient in properdin by treatment with zymosan at 17° for one hour (17). It offers advantages of being readily available in this laboratory and of greatly reducing the properdin content of partially purified fraction of C'1. However, the method is applicable either to fresh human serum or RP, and the presence or absence of properdin in the final product does not influence reactions to be described.

100 ml serum or RP is dialyzed against pH 5.5 acetate buffer, $\mu = 0.02$, at 1° for 36-48 hrs. The precipitated R2 is recovered by centrifugation at 1°, washed twice with 100 ml of dialysate, and extracted at 1° for 1 hr with 200 ml pH 5.5 acetate buffer, $\mu = 0.12$. The remaining precipitate is re-extracted at 1° for 1 hr with 100 ml pH 5.5 acetate buffer, $\mu = 0.50$. This extract is dialyzed overnight against 2 liter pH 5.5 acetate buffer, $\mu = 0.20$, at 1°. The supernate after centrifugation is brought to 10% methanol at 0° to −2°, and the resulting precipitate suspended in 0.3 M NaCl to a final volume of 5 ml and centrifuged in the Spinco preparative ultracentrifuge at 1°, 100,000 g for 1 hr. The slightly opalescent supernatant, at pH 5.5, $\mu = 0.30$, is stored at −30°.

The final fraction contains 17-25% of the C'1 in the original serum or RP, with a purification of 30-50 fold. Thus, it represents about 0.6% of the original serum protein, which fortuitously coincides with the value found earlier(16). The preparation is heterogeneous in the ultracentrifuge, showing at least 3 components at pH 5.5, ionic strength 0.30.¶

This partially purified fraction of C'1, unlike the previous preparation(16), is not anticomplementary when tested by methods here described. It contains no measurable C'2 or C'4 and only trace amounts of C'3. In preparations concentrated 20 fold over the starting material, the properdin content(17) is about 8 units/ml when prepared from serum and 1 unit/ml or less when prepared from RP. The fraction contains appreciable amounts of streptokinase-activable plasminogen, and the plasmin so activated is fibrinolytic, caseinolytic, and capable of hydrolyzing TAMe and LEe. However, in absence of streptokinase and maintaining the fraction at pH 5.5, ionic strength 0.30, significant spontaneous fibrinolytic, caseinolytic, and esterase activities are *not* demonstrable. Trace amounts of thrombin and perhaps prothrombin are detectable in some preparations. The recently described clotting factor, Hageman factor(18), is a constant contaminant.

2. Activation of esterase and complement-inactivating properties of partially purified C'1. The motivation for our experiments was provided by the observation that a solution of partially purified C'1 rapidly loses its hemolytic C'1 activity, when adjusted to pH

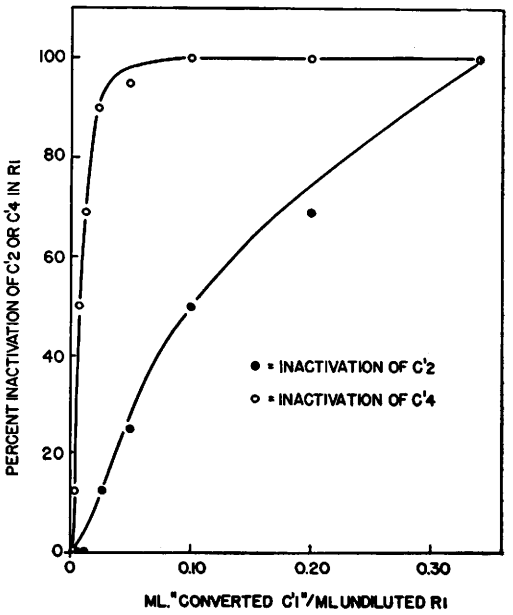

FIG. 1. Inactivation of complement components in human R1 by "converted C'1." "Converted C'1" prepared by diluting partially purified C'1 (5120 units/ml) with equal vol of water to bring to ionic strength 0.15, adjusting the pH to 7.4, and incubating at 37° for 15 min. 0.1 ml of double dilutions of "converted C'1" and 0.15 ml of pH 7.4 barbital-Ca^{++}-Mg^{++} buffer were added to 0.75 ml of 1/1.5 R1 at pH 7.4, ionic strength 0.15, containing 2.5×10^{-3} M Ca^{++}. Samples were incubated at 37° for 30 min. and titrated for complement components. C'3 activity was unaffected and is not shown.

¶ Kindly performed by Dr. M. D. Schoenberg.

TABLE I. Inactivation of Complement-Components in Whole Serum by "Converted C'1."

Serum	"Converted C'1"*	Buffer	Inactivation of complement component† C'1	C'2	C'3	C'4
	ml		%			
1	.02	.48	0	0	0	50
1	.05	.45	0	33	0	95
1	.20	.30	0	50	33	100
1	.50	—	0	67	33	100

* Partially purified C'1 (12,800 units/ml) was diluted with equal vol of water to bring to ionic strength 0.15, adjusted to pH 7.0, and incubated at 37° for 15 min.

† Measured after incubation for 30 min. at 1°, based on complement component titers of serum treated only with 0.5 ml buffer (pH 7.4, barbital-Ca^{++}-Mg^{++}).

7.4, ionic strength 0.15. Concurrently with disappearance of C'1, the preparation becomes highly anticomplementary. This anticomplementary activity of "converted C'1" is a reflection of the ability of the preparation to inactivate C'4 and, to a much smaller degree, C'2. C'3 is not significantly affected. Since plasmin does not inactivate C'2 and C'4 in R1(3), R1 was used to avoid any inactivation of complement which might be due to activation of plasminogen. A typical experiment employing R1 is shown in Fig. 1, in which the reactions were performed at 37°. However, the complement-inactivating property of "converted C'1" is also demonstrable both at 1° and in whole serum (Table I). It will be noted that again C'4 is much more susceptible to inactivation than C'2, and that C'3 is affected little or not at all. It will also be noted that C'1 in serum is not inactivated. The pattern of events is also qualitatively the same when whole serum is incubated at 37° for 30 minutes with "converted C'1."

"Conversion" of C'1 is dependent on time, pH, ionic strength, and temperature. When partially purified C'1 is incubated at 37° for 15 min. at pH 7.4, "conversion" is complete at ionic strength 0.15, only 50% complete at ionic strength 0.20, and is entirely prevented at ionic strength 0.25. At a single ionic strength, 0.15, and at pH 7.4, "conversion" is essentially instantaneous at 37° but requires about one hour at 10° and several hours at 1°. The kinetics of this reaction and of the complement-inactivating properties of "converted C'1" will be presented elsewhere. Use of R1 as test reagent for the assay of complement-inactivating properties of "converted C'1" indirectly precludes the role of plasmin in these reactions(3). The finding that fibrinolytic and caseinolytic activities are absent both before and after the "conversion" reaction directly precludes this possibility. Furthermore, the same amount of streptokinase-activable plasminogen is present before and after "conversion." "Conversion" of C'1 is attended not only by appearance of

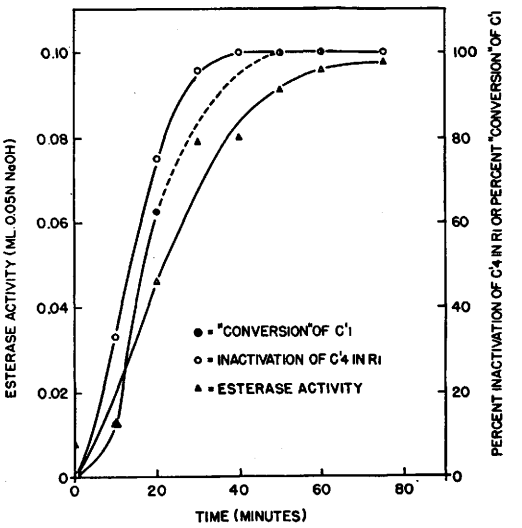

FIG. 2. Disappearance of hemolytic C'1 activity during "conversion" correlated with appearance of esterase and C'4-inactivating properties. Partially purified C'1 (7680 units/ml) was diluted with equal vol of water at 1° to bring to ionic strength 0.15. pH was adjusted rapidly to 7.1 (pH measured at 25° on a separate aliquot) and the sample placed immediately in 10° bath. Aliquots were withdrawn at indicated time intervals and assayed immediately. Hemolytic C'1 was determined as described in this report. The broken portion of the curve represents probable course of reaction, as estimated from the titrations. Marked anticomplementary activity in this region did not permit recording of actual C'1 titers. Esterase activity was determined by adding 0.25 ml of C'1 aliquots to 0.5 ml of 0.4 M TAMe, 1.25 ml of phosphate buffer, and 0.5 ml of 0.8 M sodium chloride. The reaction mixtures, at pH 7.4 and ionic strength 0.30, were incubated at 37° for 1 hr and titrated in usual manner. C'4-inactivating activity was determined by adding 0.01 ml of C'1 aliquots to 0.75 ml of 1/1.5 R1 at pH 7.4, ionic strength 0.15, containing 2.5×10^{-3} M Ca^{++}, and 0.24 ml of pH 7.4 barbital-Ca^{++}-Mg^{++} buffer. Samples were incubated at 1° for 30 min. and C'4 titrated in the usual manner.

complement-inactivating activity but also of esterase activity. The ability to hydrolyze the synthetic ester, p-toluenesulfonyl-L-arginine methyl ester (TAMe), is acquired during the "conversion" reaction. Within limits of experimental error, appearance of esterase activity parallels appearance of complement-inactivating activity and can be further correlated with disappearance of hemolytic C'1 activity. These relationships are shown in Fig. 2.

The esterase is not appreciably active against L-lysine ethyl ester (LEe). In this respect, it again differs from plasmin, which hydrolyzes both TAMe and LEe(11), but resembles thrombin, which hydrolyzes TAMe but not LEe(19). However, the esterase activity of "converted C'1" is apparently not ascribable to thrombin. Thrombic activity is either undetectable or present in only trace amounts in purified preparations of C'1 and no increase is observed following "conversion." Furthermore, purified bovine thrombin‡ which has been diluted to contain the same clotting activity as may be present in partially purified C'1 is inactive against TAMe.

No measurable acid or alkaline phosphatase** activities are present in preparations of "converted C'1".

Discussion. It has been shown with a partially purified preparation of human C'1 that a correlation exists between disappearance of hemolytic C'1 activity, which takes place under certain conditions of pH, ionic strength, and temperature, and appearance of esterase and complement-inactivating properties. The esterase appears to be distinct from plasmin, thrombin, and acid and alkaline phosphatases.

The complement-inactivating activity is directed primarily against C'4. C'1 and C'3 are not appreciably affected. It is not clear whether C'2 is actually inactivated. Disappearance of this component may be an artefact of the method of assay since it takes place only in the presence of very large amounts of "converted C'1". Under these conditions, "converted C'1" may vitiate the assay of C'2 by destroying sufficient *C'4* in the test mixture to prevent immune hemolysis. Additional investigation of this possibility is required.

Esterase activity of "converted C'1" has thus far been observed only with the substrate, TAMe. Studies with other synthetic esters, in addition to LEe which is not appreciably hydrolyzed, are of obvious interest and are in progress.

The earlier proposal that C'1 may be a pro-enzyme(3-5) serves as one possible interpretation of the observations reported. The pro-esterase here described would then be identical with hemolytically active C'1, and the esterase would be identical with the complement-inactivating activity of "converted C'1". However, evidence for such an explanation is still indirect: 1) a good correlation exists between C'1 disappearance and the nearly-simultaneous appearance of both esterase and complement-inactivating activities, as shown in Figure 2; 2) the disappearance of C'1 in partially purified C'1 is probably not due to digestion by the esterase, since the esterase is inert toward C'1 in whole serum (Table I); 3) the known esterase-inhibitor, diisopropylfluorophosphate (DFP), has been reported to inhibit immune hemolysis by guinea pig complement(20,21). The precise relationship of C'1 to the pro-esterase described above remains to be determined by direct experimentation.

Summary. 1. A procedure for partial purification of human C'1 is described. 2. Under certain conditions of pH, ionic strength, and temperature, partially purified C'1 loses its hemolytic activity and gains complement-inactivating and esterase properties. This preparation is designated "converted C'1". 3. Complement - inactivating activity of "converted C'1" is directed primarily against C'4; esterase activity is demonstrable using TAMe as substrate. 4. A correlation exists between disappearance of hemolytically active C'1 during "conversion" reaction and the parallel appearance of complement-inactivating and esterase properties. 5. The possible role of C'1 as a pro-esterase is discussed.

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

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Polioviruses in Tissue Cultures of Human Conjunctiva and Kidney Cells.* (22377)

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(With the technical assistance of Walter B. Flagg, Salina B. Dwight, and S. Robert Wilson.)

South Jersey Medical Research Foundation and Camden Municipal Hospital, N. J.

In the search for a stable strain of tissue culture cells which could be propagated serially on glass, and which could be substituted for monkey kidney cells in mass production of polioviruses, the presumably normal human cell lines established by Chang(1,2) were studied.

To determine if 2 of Chang's cell lines derived from conjunctiva and kidney could support the multiplication of the polioviruses, growth curve experiments were carried out in bottle cultures of these cells and compared to control growth curves in similar cultures of first generation rhesus monkey kidney cells.

Materials and methods. Technics used in this laboratory for the serial subcultivation of conjunctiva and kidney cells have been reported(3). The Mahoney, MEF-1 and Saukett strains of poliovirus were received from the Connaught Laboratories, Toronto, Canada, and were the 3 monkey kidney tissue culture adapted strains, Types 1, 2 and 3, used in the preparation of Salk vaccine. They were the strain pools 54, 55 and 56 distributed to each of the 28 laboratories participating in the 1954 poliomyelitis vaccine field trial. The Parker strain of Type 1 virus was obtained from Dr. David Bodian(4) in the 52nd monkey kidney tissue culture passage. It received 4 additional rhesus monkey kidney passages in our laboratory. The Cleveland 80-4 strain of Type 1 virus, obtained from Dr. Albert Sabin(5), was isolated in HeLa

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