COMPLEMENT AS A MEDIATOR OF INFLAMMATION

ENHANCEMENT OF VASCULAR PERMEABILITY BY PURIFIED HUMAN C'1 ESTERASE*

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A fundamental component of the inflammatory response is an increase in vascular permeability. The change in permeability is mediated in a number of different ways, depending at least partly upon the nature of the injurious stimulus. Several mechanisms have been suggested to explain the increased vascular permeability which accompanies antigen-antibody reactions, including the local release of histamine (1), the activation of a specific plasma permeability factor (2), and the evolution of one or more toxic agents, designated as anaphylatoxins (3), which in turn may effect a release of such pharmacologic mediators of inflammation as histamine and serotonin.

The participation of complement in the pathogenesis of inflammation has been suggested on the basis of studies of passive cutaneous anaphylaxis in the rat (4–6), but data inconsistent with this view have been published recently (6 *a*). The inflammatory response induced by the intraperitoneal injection of human plasma in rats also seems dependent upon the presence of complement (7). The precise manner in which complement may mediate an enhancement of vascular permeability is unknown, although it appears to be a requirement for generation of anaphylatoxin in certain experimental systems (6).

Considerable progress has been made in recent years in knowledge of the mechanism of action of complement in a variety of immune reactions. It is now well established that the interaction of antigen-antibody complexes with the first component of complement (C'1) results in activation of an enzyme,

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variously designated as C'1 esterase, activated C'1, converted C'1, or C'1a (8–13). The active enzyme, capable of hydrolyzing selected amino acid esters and of inactivating the fourth (C'4) and second (C'2) components of complement in solution, is a functional requirement for the interaction of antigen-antibody-C'1 complexes with C'4 and C'2 (14, 15). A naturally occurring inhibitor in human serum, a heat-labile α-globulin, has been purified and is capable under appropriate conditions of blocking both the esterolytic activity of C'1 esterase and its interaction with C'4 and C'2 (16–21). Human C'1 esterase has recently been highly purified (22) and shown to function as a component of complement in immune hemolysis (23).

Since the generation of C'1 esterase is an early event in the mechanism of action of complement, it would be predicted that if complement is a mediator of the inflammatory response induced by antigen-antibody reactions then purified C'1 esterase might be capable of inducing such a response. The present investigation was undertaken to test this hypothesis. The results to be presented demonstrate that enhancement of vascular permeability in guinea pig skin may in fact be effected by purified human C'1 esterase. This phenomenon is prevented by purified serum inhibitor of C'1 esterase and other agents or procedures which block or inactivate enzymatic activity. Although the observations to be reported are consistent with the possibility that histamine may be the final mediator of enhanced vascular permeability induced by C'1 esterase, direct evidence is not available and the mechanism of the reaction is not yet known.

Materials and Methods

Vascular permeability was tested using the technique of Miles and Wilhelm (24). Noninbred albino guinea pigs of either sex, usually weighing between 400 and 450 gm, were depilated and injected intravenously with 1.2 ml per kg of body weight of a 5 per cent solution of pontamine sky blue 6 X (E. I. DuPont de Nemours and Company, Wilmington) in 0.075 M pyrogen-free sodium chloride solution. The solutions to be tested were then injected intracutaneously into the dorsum of the guinea pig in a volume of 0.1 ml, using No. 26, ¼ inch steel hypodermic needles and silicone-coated tuberculin syringes. Fifteen minutes after the last injection, the size of the lesion was estimated by averaging the widest diameter and the diameter perpendicular to it. In most experiments, the results are expressed as the average of four guinea pigs. An occasional guinea pig reacted with increased vascular permeability to any solution containing human serum proteins. The values obtained in such guinea pigs were not included in the data to be presented.

Human C'1 esterase, an enzyme derived from the first component of complement, was prepared by column chromatography (22). A euglobulin fraction of fresh human serum was activated to C'1 esterase by incubation at 37°C for 15 minutes at pH 7.4, ionic strength of 0.15, applied to a DEAE cellulose column, and eluted with a sodium chloride gradient. The active enzyme, emerging at ionic strength 0.37, was concentrated and reapplied to a TEAE cellulose column at pH 9.0, 0.02 M glycine buffer and again eluted with a sodium chloride gradient. On some occasions, this second chromatographic step was repeated. The most highly purified preparations, purified 2400-fold with respect to serum and containing 400 units of enzyme per optical density unit at 280 mμ, were heterogenous by physicochemical and immunochemical

criteria. As was true of cruder preparations of C'1 esterase, purified preparations interacted with C'4 and C'2 in solution, and were functionally active in hemolytic systems requiring the first component of complement (23). Purified C'1 esterase lacked detectable activity attributable to Hageman factor, as measured in a specific test system (25).

The activity of C'1 esterase was measured *in vitro* by microformol titration of the acid liberated from *N*-acetyl-L-tyrosine ethyl ester during incubation under standardized conditions (16, 22). One unit of C'1 esterase is defined as that amount which liberates 0.5 micromole of H^+ in 15 minutes at 37°C under the conditions of this assay.

The *effect of heat* upon the esterolytic and permeability-increasing activities of C'1 esterase preparations was tested by incubating aliquots of enzyme at indicated temperatures for 30 minutes. The temperatures recorded are those of the surrounding bath. Similarly, the *effect of extremes of pH* was studied by titrating aliquots of C'1 esterase to the indicated pH values with hydrochloric acid or sodium hydroxide solutions, and allowing the mixtures to stand at room temperature for 30 minutes. Samples were then dialyzed against phosphate-buffered saline at 1°C before testing.

Subcomponents of C'1 were prepared as previously described (26). In essence, a euglobulin fraction of fresh human serum, applied to a DEAE cellulose column in the presence of trisodium ethylenediaminetetraacetic acid (EDTA), may be resolved into three activities, all of which are required for hemolytic C'1 activity and for generation of C'1 esterase. The three activities have been designated C'1q, C'1r, and C'1s. C'1q has been shown to be identical with the 11S component of Müller-Eberhard and Kunkel (27) and Taranta, Weiss, and Franklin (28). C'1r is a previously undescribed component, and C'1s is chromatographically and antigenically related to C'1 esterase (26, 29). Each of these fractions, or any combination of two of these fractions, is devoid of C'1 esterase activity, whereas combination of all three fractions results in generation of active enzyme.

Serum inhibitor of C'1 esterase (EI), a naturally occurring α-globulin in human serum capable of inhibiting the esterolytic and hemolytic activities of C'1 esterase stoichiometrically, was purified by column chromatography by published procedures (20, 21). One unit of inhibitor is defined as that amount which inhibits 10 units of C'1 esterase in the enzymatic assay described above (16). The specific activity of the preparation employed was 45 units per optical density unit at 280 mμ.

Diisopropylphosphofluoridate (DFP) (Sigma Chemical Company, St. Louis, Missouri) was diluted in isopropyl alcohol to a concentration of 2.5 M. Further dilutions were made in 0.12 M sodium bicarbonate solution to the desired concentrations and used at once (30). Solutions of DFP were incubated with C'1 esterase at 37°C for 30 minutes. Excess DFP was then removed by dialysis of the reaction mixture against 2 changes of 200 volumes of phosphate-buffered saline solution for 18 to 24 hours at 1°C.

Soy bean trypsin inhibitor (SBTI), 5 times crystalline (Nutritional Biochemicals Corp., Chagrin Falls, Ohio), was suspended in phosphate-buffered saline solution to a concentration of 200 micrograms per ml.

Histamine diphosphate (Sigma Chemical Company) was dissolved in phosphate-buffered saline solution.

The effect of *triprolidine hydrochloride* (actidil) (kindly donated by Burroughs Wellcome and Company, Tuckahoe, New York) upon the permeability-increasing properties of C'1 esterase was tested by the intravenous administration of 0.1 mg per kg of body weight of the test guinea pigs. The triprolidine hydrochloride, a known antihistaminic agent, was administered with the pontamine sky blue dye, before the intracutaneous injection of test substances.

Histologic sections were prepared from the freshly excised skin of a guinea pig which had been injected intracutaneously with 0.1 ml of C'1 esterase (20 units per ml), histamine diphosphate solution (20 micrograms per ml), and phosphate-buffered saline, but had not received an injection of pontamine sky blue dye. A series of intracutaneous injections was given

at various intervals of time before the animal was sacrificed. The skin was fixed in 10 per cent formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin.

In a separate experiment, two guinea pigs were injected intravenously with 0.8 ml of saccharated iron oxide (proferrin, Merck, Sharpe and Dohme, West Point, Pennsylvania), a dose equivalent to 16 mg of available iron. Within 2 minutes, the animals were injected intracutaneously into separate sites with 0.1 ml of C'1 esterase (40 units per ml), histamine diphosphate (40 micrograms per ml), or buffer. Ten minutes later, the guinea pigs were sacrificed and the injected skin sites excised. Part of the tissue was fixed in 10 per cent formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin or Turnbull blue–iron stain. Remaining portions of tissue were fixed in Caulfield's solution, embedded in methacrylate, sectioned on a Porter-Blum microtome, and observed in an RCA electron microscope.

Phosphate-buffered saline (Dulbecco A) (31) at pH 7.2–7.4 was used as diluent unless otherwise noted.

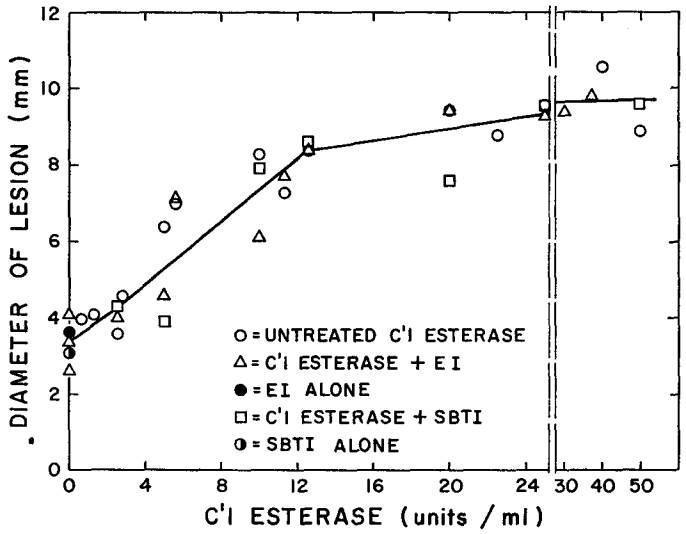
RESULTS

1. The Permeability-Increasing Activity of Purified C'1 Esterase.—The intracutaneous injection of 0.1 ml of solutions of human C'1 esterase at concentrations of 5 units per ml or more increased vascular permeability in the guinea pig, as indicated by the increased diameter of the blue area surrounding the point of injection. The appearance of the injected site was strikingly similar to that which followed the intracutaneous injection of histamine diphosphate solutions. At threshold concentrations, the lesions were pale and often showed central pallor, while at higher concentration, the intensity of the blue color was deeper and central pallor was often absent. Typical dose-response curves are shown in Text-figs. 1 to 4; a comparison with the dose-response curve for histamine is shown in Text-fig. 4.

C'1 esterase preparations at various stages of purification were tested. These samples ranged in specific activity (expressed as units of C'1 esterase per optical density unit at 280 mμ) from 16 to 400. All preparations tested increased vascular permeability. Cruder preparations, however, were more active per unit of C'1 esterase than purer preparations, although the purest fraction tested had significant activity at a level of 1 microgram of protein in the injection site. Two interpretations of these observations were possible: either the permeability-increasing activity of purified C'1 esterase was due entirely to a contaminating permeability factor or extraneous permeability factors were present in the cruder preparations, enhancing the effect of C'1 esterase itself. Experiments to differentiate these possibilities form the substance of this paper.

2. The Effects of Serum Inhibitor of C'1 Esterase and Soy Bean Trypsin Inhibitor.—Purified C'1 esterase was incubated at 37°C for 10 minutes with amounts of purified serum inhibitor of C'1 esterase (EI) in such concentration as to inhibit 25, 50, 75, and 100 per cent of the available enzyme. When various dilutions of these mixtures were injected intracutaneously into guinea pigs, their permeability-increasing activities were not related to their *original*

content of C'1 esterase but rather to the *residual* concentration of free enzyme (Text-fig. 1). For example, if 50 units of C'1 esterase were treated with 5 units of serum inhibitor of C'1 esterase (stoichiometric equivalence), neither free enzyme nor permeability-increasing activity remained. At lesser concentrations of inhibitor, the permeability-increasing property of the mixtures was similar to that of untreated preparations of C'1 esterase of comparable esterolytic

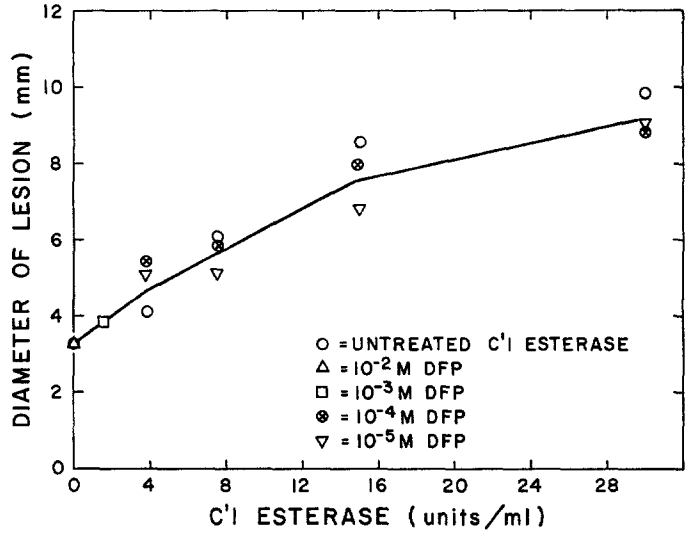


TEXT-FIG. 1. Inhibition of C'1 esterase by serum inhibitor of C'1 esterase, and lack of inhibition by soy bean trypsin inhibitor: Correlation between residual esterolytic activity and permeability-enhancing activity. The abscissa represents the actual concentrations of free C'1 esterase in the test solutions. Various amounts of serum inhibitor of C'1 esterase (EI) were incubated with a fixed amount of enzyme to achieve various levels of inhibition. The curve demonstrates the permeability-increasing activity of the mixtures as a function of the *residual free* esterase in the inhibited mixtures. Various amounts of C'1 esterase were incubated with soy bean trypsin inhibitor (SBTI) at a concentration of 100 μg per ml. No inhibition of esterolytic or permeability-increasing activities occurred. The curve demonstrates this correlation.

activity. The figure depicts the correlation between *residual* C'1 esterase activity and permeability-increasing activity.

Similar experiments were performed with soy bean trypsin inhibitor (SBTI), an agent without effect on the esterolytic property of C'1 esterase (10) but known to inhibit the permeability-increasing properties of diluted human plasma (PF/Dil) (32) and of activated Hageman factor (33). Soy bean trypsin inhibitor at a concentration of 100 micrograms per ml had no detectable inhibitory effect either on the esterolytic or permeability-increasing effects of preparations of C'1 esterase. The correlation between these activities in the presence of SBTI is also shown in Text-fig. 1.

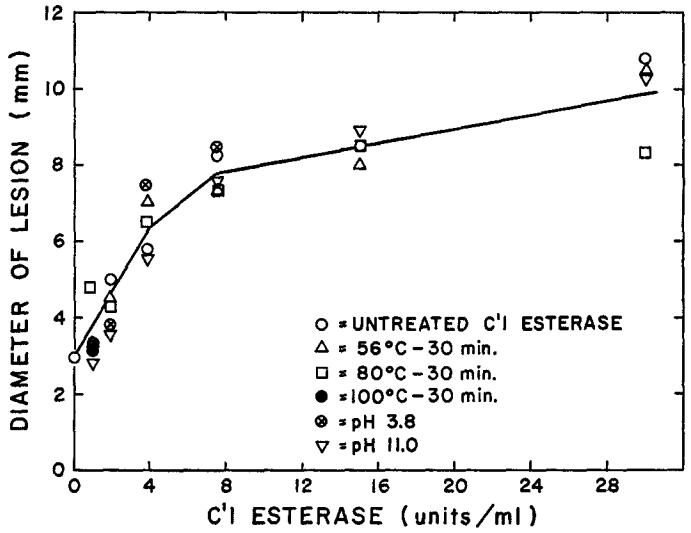
3. The Effect of Diisopropylphosphofluoridate.—Purified C'1 esterase was treated with DFP at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} M, resulting in 100, 97, 30, and 11 per cent inhibition of esterolytic activity, respectively. DFP inhibited the permeability-enhancing activity of C'1 esterase as well, the extent of inhibition being proportional to the loss of esterolytic activity (Text-fig. 2). Thus, the permeability-enhancing activity after removal of excess DFP was equivalent to that of untreated enzyme of comparable esterolytic activity.



TEXT-FIG. 2. Inhibition of C'1 esterase by diisopropylphosphofluoridate (DFP): Correlation between residual esterolytic activity and permeability-enhancing activity. A solution of C'1 esterase containing 55 units/ml was incubated with various concentrations of DFP for 30 minutes and then dialyzed. The curve demonstrates the permeability-increasing activity of DFP-treated C'1 esterase as a function of residual active enzyme, each preparation being tested at several dilutions. The permeability-increasing activity is proportional to the esterolytic activity remaining after DFP treatment.

4. The Effect of Heat and pH.—Purified C'1 esterase is relatively more stable at elevated temperatures than cruder preparations (22). Thus, incubation of a highly purified preparation for 30 minutes at 56°C resulted in 67 per cent inactivation; at 80°C, in 61 per cent inactivation; and at 100°C, in 99 per cent inactivation. Again, the permeability-increasing activities of the heated preparations were proportional to residual enzyme concentration and equivalent to that of untreated enzyme tested at the same concentrations (Text-fig. 3). Similarly 92 per cent of the esterolytic activity was inactivated by treatment of C'1 esterase at pH 3.8, and 36 per cent was inactivated at pH 11.0; these procedures also failed to dissociate esterolytic and permeability-enhancing activities (Text-fig. 3).

5. Generation of C'1 Esterase and Permeability-Enhancing Activities from Subcomponents of C'1.—Partially purified preparations of C'1q, C'1r, and C'1s were tested individually, and in combinations of two or all three for esterolytic and permeability-increasing activities. As reported previously, only the combination of C'1q, C'1r, and C'1s resulted in generation of C'1 esterase (26). Moreover, in contrast to other mixtures, this combination possessed marked permeability-increasing activity (Table I). The permeability-increasing property could be inhibited by a stoichiometric amount of purified serum



TEXT-FIG. 3. The effect of heat and pH upon the esterolytic and vascular permeability increasing properties of C'1 esterase. As in the previous figures, the permeability-increasing property of C'1 esterase is plotted as a function of the residual C'1 esterase activity after treatment with heat or extremes of pH.

inhibitor of C'1 esterase. The activity generated was similar to that achieved by comparable concentrations of purified C'1 esterase. C'1q and C'1r each had slight permeability-increasing activity, but only when tested at concentrations greater than in the mixture of C'1q, C'1r, and C'1s.

6. The Effect of Intravenously Injected Triprolidine upon the Permeability-Enhancing Property of C'1 Esterase.—One way in which C'1 esterase might increase vascular permeability might be to induce the local release of histamine or a histamine-like substance. The gross appearance of the area of blueing provoked by C'1 esterase was similar to that which followed the injection of histamine. It was therefore of interest to test the effect of a known antihistaminic agent, triprolidine hydrochloride. The intravenous injection of 0.1 mg of this substance per kg of body weight inhibited the vascular

permeability-increasing property of C'1 esterase and of histamine diphosphate (Text-fig. 4 and Fig. 1). This effect was highly significant, the extent of inhibition of the permeability-increasing activity of C'1 esterase by triprolidine being 16-fold. *In vitro*, however, triprolidine was without effect on the esterolytic activity of C'1 esterase, even when tested at a concentration of 2 mg per ml.

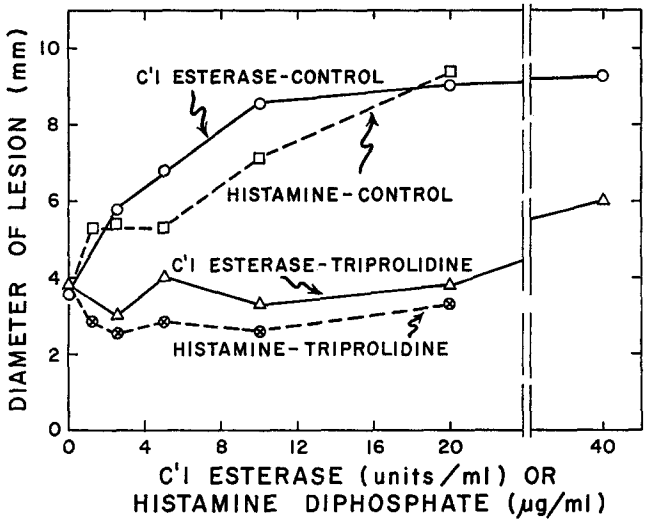
TABLE I

Generation of C'1 Esterase and Permeability-Enhancing Activity from Subcomponents of C'1

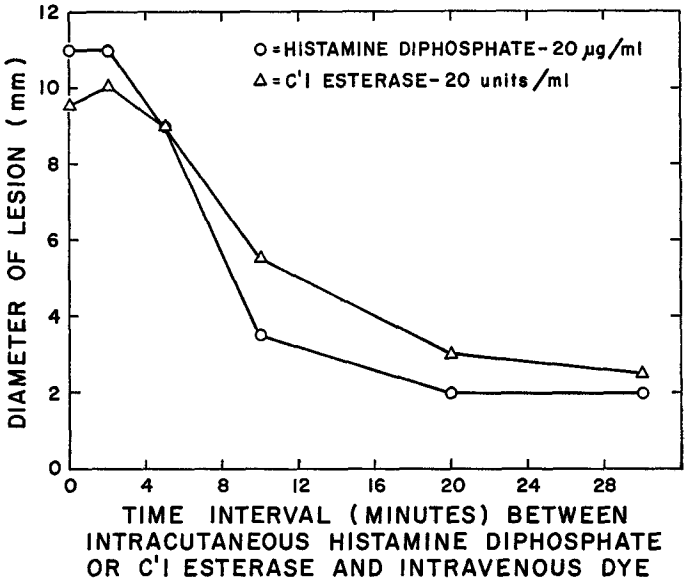
Mixture tested	Esterolytic activity	Permeability activity	
		Dilution tested	Diameter of lesion
	units/ml		*mm*
I. Subcomponents of C'1			
1 part C'1q + 1 part buffer	0	Undiluted	5.1
	0	Diluted ½	3.9
	0	Diluted ¼	4.4
1 part C'1r + 1 part buffer	0	Undiluted	6.1
1 part C'1s + 1 part buffer	0	Undiluted	5.0
1 part C'1q + 1 part C'1r	0	Undiluted	5.9
	0	Diluted ½	4.6
	0	Diluted ¼	3.6
1 part C'1q + 1 part C'1s	0	Undiluted	4.0
	0	Diluted ½	5.0
	0	Diluted ¼	3.8
1 part C'1r + 1 part C'1s	0	Undiluted	5.0
0.5 part C'1q + 0.5 part C'1r + 1 part C'1s	18.6	Undiluted	9.0
	9.3	Diluted ½	8.2
	4.7	Diluted ¼	6.5
	2.3	Diluted ⅛	5.2
II. Purified C'1 esterase	18.6		8.4
	9.3		9.1
	4.7		7.4
	2.3		4.4
III. Phosphate-buffered saline solution	0	Undiluted	3.6

7. The Duration of Action of Intracutaneously Injected C'1 Esterase and Histamine.—Pontamine sky blue was injected intravenously into guinea pigs which had been injected intracutaneously with C'1 esterase and histamine diphosphate at time intervals ranging from 0 to 30 minutes before the administration of dye. The effect of both substances was evanescent, decreasing significantly within 10 minutes after injection (Text-fig. 5).

8. Histologic Examination of Skin Sites Injected with C'1 Esterase, Histamine, or Buffer.—Hematoxylin and eosin-stained paraffin sections of skin sites



TEXT-FIG. 4. The effect of triprolidine on the permeability-increasing properties of C'1 esterase and histamine. Guinea pigs were injected intravenously with triprolidine hydrochloride, 1 mg per kg of body weight, or with saline solution. C'1 esterase and histamine diphosphate were then injected intracutaneously in the concentrations indicated on the abscissa. The size of the resultant lesions is plotted on the ordinate.



TEXT-FIG. 5. The duration of action of intracutaneously injected C'1 esterase and histamine. C'1 esterase (20 units/ml) or histamine (20 μg/ml) was injected at indicated intervals of time prior to the intravenous injection of pontamine sky blue.

excised at various times from immediately to 4 hours after the intracutaneous injection of C'1 esterase (20 units per ml), histamine diphosphate (20 μg per ml), or buffer revealed only subtle pathological alterations. Both the enzyme and histamine sites had a greater number of polymorphonuclear leukocytes aggregated in vascular spaces and free in the surrounding tissue and an increased incidence of separation of collagen fibers, as compared with buffer controls. The differences, however, were not marked.

A more convincing histologic demonstration of the pathophysiological abnormality induced by C'1 esterase was provided by use of saccharated iron oxide as a marker. Skin sites from guinea pigs which had received 16 mg of iron equivalent intravenously were excised 10 minutes after intracutaneous injection of 0.1 ml of C'1 esterase (40 units per ml), histamine diphosphate (40 μg per ml), or buffer. Closely adjacent paraffin sections were stained with hematoxylin and eosin or Turnbull blue–iron stain (Figs. 2 *a* and 2 *b*). The iron stain revealed the presence of iron in and immediately surrounding the walls of small vessels. This finding was striking and involved many small vessels in skin sites which had received enzyme or histamine but was detectable only in the immediate area of the needle tract in buffer controls. The caliber and type of vessel affected were consistent with postcapillary venules, reported by Majno and Palade (34) to be the target organ for the permeability-enhancing activity of histamine.

Specimens of skin from the above experiment were also fixed in Caulfield's solution, embedded in methacrylate, and examined with the electron microscope (Figs. 3 and 4). The findings confirmed and extended those obtained by optical microscopy. In skin sites which had received C'1 esterase, iron was visualized in endothelial junctions and at the basement membrane of small vessels, compatible in appearance with postcapillary venules. Large amounts of iron were also observed on the pericyte side of the basement membrane. Iron was not found in the vesicles of endothelial cells. The same pattern of distribution of iron was noted in skin sites which had received histamine. The latter observations were in accordance with those of Majno and Palade (34).

DISCUSSION

Many mechanisms have been invoked to explain the increase in vascular permeability which is an important element in the reaction of tissues to injury. These proposed mechanisms are based in common upon the assumption that a chemical agent is either generated at the site of injury or released preformed from some loose combination. For example, the local release of histamine or a histamine-like substance (35), 5-hydroxytryptamine (36), and certain polypeptides (37-39) has been thought to contribute to the increase in vascular permeability in inflammation.

Considerable information exists concerning the participation of plasma in increasing vascular permeability. Thus, homologous or certain heterologous plasmas, diluted with saline solution in glass tubes, evolve the capacity to enhance vascular permeability in guinea pig skin (24). The agent responsible for the enhancement of permeability, designated PF/Dil or globulin permeability factor by Miles, is inhibited by treating the plasma with diisopropylphosphofluoridate or by the simultaneous local injection of soy bean trypsin inhibitor, but not by the intravenous injection of antihistaminic compounds (32). PF/Dil is thought to be a hydrolytic enzyme.

A second permeability-increasing property of human plasma can be demonstrated by its intracutaneous injection undiluted into guinea pig skin (40). This "PF/Nat" is not antagonized by local soy bean trypsin inhibitor or by systemic antihistaminic agents. The nature of PF/Nat is not yet elucidated, though Elder and Wilhelm (40) suggested that this factor may be related to that property of human plasma which may produce necrosis in guinea pig skin (41).

A third possible permeability factor may evolve in plasma under experimental conditions. Plasma contains an inactive agent, kallikreinogen, which can be activated by several means to form kallikrein, a substance with enzyme-like properties (42). In turn, kallikrein releases at least two biologically active polypeptide "plasma kinins," bradykinin (kallidin I) and kallidin (kallidin II) from a precursor in plasma globulin (43, 44). Among their other properties, these polypeptides increase vascular permeability in guinea pig skin (39, 45, 46). Kallikrein, too, increases vascular permeability, presumably through the production of plasma kinins. The permeability-enhancing activity of bradykinin is not affected by soy bean trypsin inhibitor, while that of kallikrein, its liberator, is inhibited (46). In all likelihood, PF/Dil and kallikrein are not identical (47), though PF/Dil may function as an activator of kallikreinogen (48).

Another possible permeability factor is said to be released from undiluted guinea pig serum upon incubation with insoluble antigen-antibody aggregates (2). The release of the permeability-increasing property does not seem to be related to the formation of PF/Dil or plasma kinins, nor is its activity significantly inhibited by antihistaminic agents. Whether the activity studied by Davies and Lowe can be ascribed to the elaboration of anaphylatoxin is not clear. Anaphylatoxin refers to the toxic agent or agents which appear in guinea pig (3, 49) or rat (50) sera treated with such diverse agents as antigen-antibody aggregates, polysaccharides, and polyvinyl pyrrolidine. Among its other described properties, anaphylatoxin is said to increase vascular permeability and contract smooth muscle. Anaphylatoxin may exert at least part of its effect by releasing histamine-like activity in tissue (51). Antihistaminic drugs block its effect (52).

The studies described in the present report suggest that human plasma may contain the precursor of still another factor which may increase vascular permeability in guinea pig skin. The intracutaneous injection of purified preparations of the esterase derived from the first component of complement regularly enhanced vascular permeability. The effect of C'1 esterase was inhibited by pretreatment with a naturally occurring serum inhibitor of this enzyme or with DFP, but not by soy bean trypsin inhibitor. Moreover, treat-

ment of C'1 esterase by heat or extremes of pH inactivated its esterolytic and permeability-enhancing properties in parallel. The permeability-enhancing activity was a function of activated C'1 esterase; its inactive precursor (C'1s) had no demonstrable effect, but permeability-increasing activity was generated *pari passu* with esterolytic activity when C'1s was incubated with C'1q and C'1r, both of which are needed for its activation (26).

The mechanism through which activated C'1 esterase increased vascular permeability is not yet clear. The preparations tested were devoid of activated Hageman factor, a blood clotting factor which increases vascular permeability by activating PF/Dil (53, 54). The lack of inhibition by soy bean trypsin inhibitor distinguished C'1 esterase from PF/Dil (32) and from kallikrein (55).

The data presented are compatible with the view that activated C'1 esterase releases a histamine-like substance within guinea pig skin. Intravenously injected triprolidine, an antihistaminic compound, dramatically inhibited the effect of C'1 esterase, in contradistinction to its ineffectiveness against PF/Nat, PF/Dil, kallikrein, plasma kinins, and the factor released by antigen-antibody aggregates described by Davies and Lowe (2). The appearance of the blue spots produced by the injection of C'1 esterase in guinea pigs injected with pontamine sky blue was similar in the gross to that produced by histamine. The evanescence of the increase in vascular permeability which followed the injection of C'1 esterase resembled that which followed histamine, but the duration of increased vascular permeability is by no means specific (32). The histologic and electron microscopic nature of the changes produced were also similar to those of histamine but again were non-specific.

These observations do no more than to suggest a possible relationship between C'1 esterase and the release of histamine. Incubation of C'1 esterase with guinea pig lung (56) or rat peritoneal mast cells (57) in the presence or absence of normal serum did not release histamine under the conditions employed (58). In these experiments, the test system was highly reactive to appropriate antigen or antibody. Whether in fact histamine-like activity might be evoked by C'1 esterase in other situations remains to be determined.

The relationship between the permeability-increasing activity of C'1 esterase and several other conditions must be considered. Mention has already been made of the enhancement of vascular permeability by anaphylatoxin. Studies by Osler and his associates (59) have demonstrated that anaphylatoxins are elaborated in rat serum treated with antigen-antibody aggregates only if complement is present, as postulated many years before by Friedberger (3) and Friedemann (49). Although Osler emphasized the importance of the third component of complement in the elaboration of anaphylatoxin, his studies are compatible with an earlier role for C'1 esterase in the phenomenon.

The intraperitoneal injection of fresh human plasma provokes peritoneal exudation in rats (7). How this heterologous plasma induces increased per-

meability in the vessels lining the peritoneal cavity is not known, but Greisman's experiments suggest the participation of complement. A specific role for C'1 esterase was not delineated.

Passive cutaneous anaphylaxis,— a phenomenon in which local vascular permeability is enhanced by the intracutaneous injection of specific antibody to an animal which has been injected intravenously with antigen,— may be inhibited by antihistaminic agents (1). None the less, whether histamine has a role in passive cutaneous anaphylaxis is disputed (60, 61). Experiments have been described in which complement, particularly the third component, appears to participate in passive cutaneous anaphylaxis in the rat (5), but the role of complement has been disputed on the basis of recent evidence (6 *a*).

Finally the possible participation of C'1 esterase in the pathogenesis of attacks of hereditary angioneurotic edema is intriguing. In this disorder the patient suffers repeated attacks of severe, localized edema involving particularly the skin, gastrointestinal tract, and larynx. The serum of such individuals lacks detectable amounts of the inhibitor of C'1 esterase normally present (62, 63). Moreover, during a bout of edema, free C'1 esterase may be found in the patient's serum (64). It is tempting to relate the episodes of edema, presumably the result of locally increased vascular permeability, to the unopposed release of C'1 esterase. However, Landerman and coworkers (65) have reported a deficiency of serum inhibitor of kallikrein in patients with hereditary angioneurotic edema and Kagan and Becker (66) have shown that purified serum inhibitor of C'1 esterase will block the activities of PF/Dil and kallikrein. The precise mechanism of edema formation in this genetically determined disease is therefore not yet clear.

The observations which we have recorded represent the first known *in vivo* effect of C'1 esterase *per se*. The mechanism of enhancement of vascular permeability by C'1 esterase and its role in initiation of inflammation in man remain to be explored.

SUMMARY

Purified preparations of the esterase derived from the first component of complement (C'1 esterase) increased vascular permeability in guinea pig skin, an effect inhibited by triprolidine, an antihistaminic agent, but not by soy bean trypsin inhibitor. The permeability-increasing and esterolytic properties of C'1 esterase were inhibited in parallel by the serum inhibitor of C'1 esterase, diisopropylphosphofluoridate and extremes of temperature and pH. Moreover, the permeability-increasing and esterolytic properties evolved in parallel when C'1 esterase was generated from its subcomponents. How C'1 esterase induces changes in vascular permeability remains unexplained, although the possibility that its action is mediated through a histamine-like agent is attractive.

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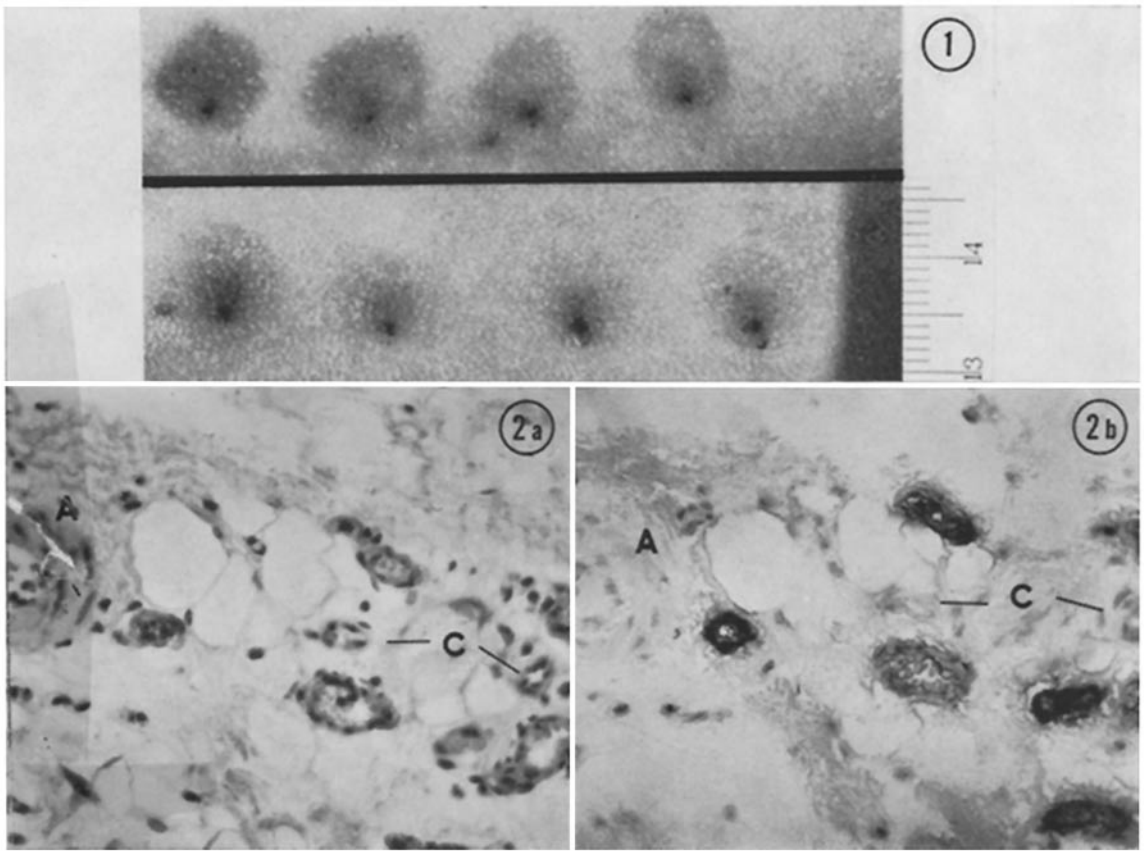
EXPLANATION OF PLATES

PLATE 77

FIG. 1. The effect of triprolidine on the permeability-increasing property of C'1 esterase. The photograph on the bottom is of an animal injected intravenously with triprolidine, 0.1 mg per kg of body weight, and that on the top of an animal injected intravenously with saline solution. The animals were injected intracutaneously with 0.1 ml of solutions of C'1 esterase containing 40, 20, 10, and 5 units per ml, reading from left to right in each case. This photograph demonstrates not only the inhibition of the reaction by triprolidine, but also the halo-like appearance of the lesion produced by the smaller amounts of C'1 esterase. Each division of the scale on the right is 1 mm.

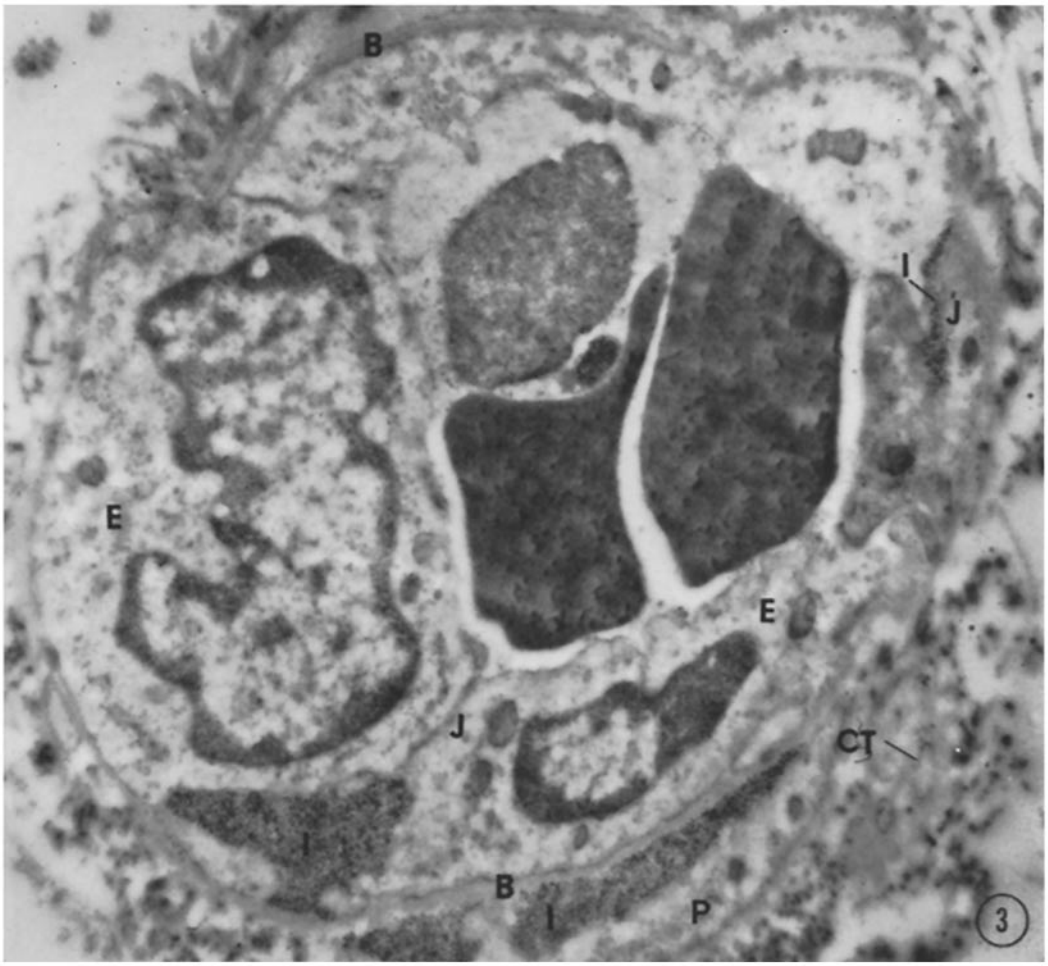
FIGS. 2 *a* and 2 *b*. Histologic appearance of guinea pig skin after the intravenous injection of saccharated iron oxide and the intracutaneous injection of C'1 esterase. × 300. Fig. 2 *a*. Hemotoxylin and eosin stain. Fig. 2 *b*. Turnbull blue–iron stain. In this photograph, iron-containing material appears black. The selective effect of C'1 esterase for vessels compatible in appearance with postcapillary venules is illustrated. Iron is not present in the artery (*A*) in the upper left hand portion of the photograph or in the two capillary-size vessels (*C*). In contrast, many of the small vessels of the size of postcapillary venules contain iron.



(Ratnoff and Lepow: Complement as mediator of inflammation)

PLATE 78

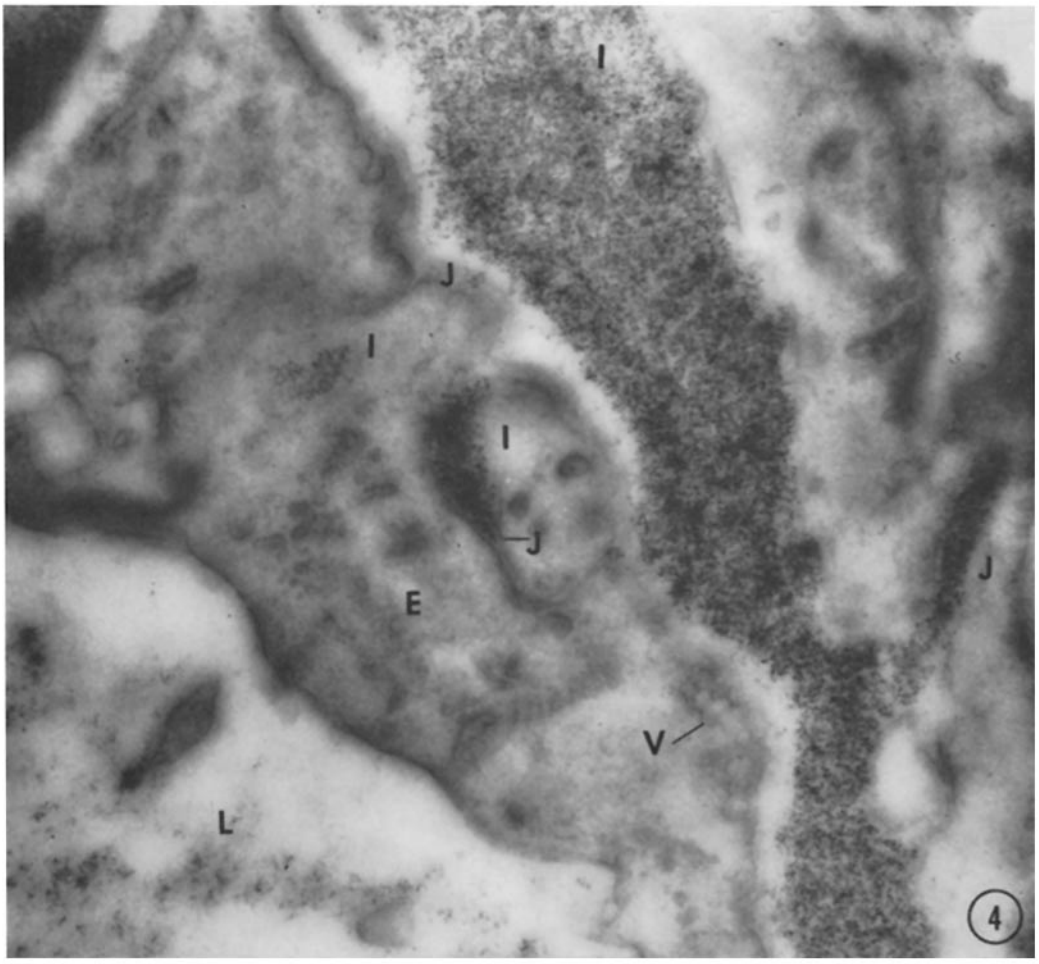
FIG. 3. Electron micrograph of a small blood vessel in guinea pig skin after the intravenous injection of saccharated iron oxide and the intracutaneous injection of C'1 esterase. Two erythrocytes are present in the lumen. The following symbols are used: *E* (endothelial cells), *J* (junction between endothelial cells), *B* (basement membrane), *P* (pericyte), *CT* (connective tissue), and *I* (saccharated iron oxide). Iron can be seen in the junction, *J*, and at both sides of the basement membrane, *B*. × 16,300.



(Ratnoff and Lepow: Complement as mediator of inflammation)

PLATE 79

FIG. 4. Electron micrograph of a portion of a small vessel similar to that shown in Fig. 3. In addition to the symbols used in Fig. 3, the following appear: *L* (lumen) and *V* (vesicle). Iron can be seen in the junction, *J*, but is absent in the cytoplasm on cytoplasmic vesicles, *V*, of endothelial cells. × 46,900.



(Ratnoff and Lepow: Complement as mediator of inflammation)