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Enhancement of Vascular Permeability by Mixtures of C'1 Esterase and Normal Serum.* (29226)

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Recent investigations have demonstrated that purified human C'1 esterase, an enzyme derived from the first component (C'1) of complement, has the ability to increase vascular permeability in guinea pig skin(1). By means of inhibitors, inactivation by heat and pH, and use of subcomponents of C'1, it was shown that the permeability activity was referable to C'1 esterase rather than an extraneous factor in the preparation. Histopathological studies, including electron microscopy, and the suppressive effect of an anti-histaminic drug were consistent with the hypothesis that enhanced vascular permeability might be mediated by release of a histamine-like substance.

The present study was undertaken to test the possibility that C'1 esterase functioned as a permeability factor by interacting with other humoral substances to form an agent capable of releasing a pharmacologic effector of inflammation. Experiments were performed in which concentrations of C'1 esterase, subthreshold for direct action in guinea pig skin, were added to guinea pig serum *in vitro* and the mixtures then tested for permeability activity *in vivo*. The data to be presented, in fact, provide evidence for the participation of serum constituents in the mechanism of action of C'1 esterase. Preliminary experiments further suggest the possible identity of these serum constituents with the complement system.

Materials and methods. Vascular permeability was tested in guinea pig skin according to Miles and Wilhelm(2), as further described by Ratnoff and Lepow(1). *Phosphate buffered saline* (PBS), solution A of Dulbecco(3), was used as diluent in all permeability assays. *Purified human C'1 esterase* was prepared and assayed by the methods of Haines and Lepow(4). The preparations employed

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had specific activities of 200-400 units of C'1 esterase/optical density unit at 280 mμ. *Guinea pig serum* was obtained from normal 700-900 g animals by heart puncture with siliconized needles and syringes. The blood was transferred to siliconized tubes and separated at once by centrifugation in the cold. The resulting plasma was allowed to clot overnight at 5°C in clear lusteroid tubes and the resulting serum expressed from the clot and frozen at —60°C until used(5). *Guinea pig serum reagents R1, R2, R3* and *R4,* deficient in C'1, C'2, C'3, and C'4, respectively, were prepared essentially as described by Mayer(6) with modifications summarized by Wedgwood(7). *Complement fixed serum* was prepared as described by Lepow and Pillemer(8). Hemolytic complement titrations ($C'H_{50}$) were performed according to the method described by Mayer(6), except that 2.5×10^8 sensitized erythrocytes in a reaction volume of 4.0 ml were employed and triethanolamine buffered saline containing optimal Ca^{++} and Mg^{++}(9) was substituted for barbital buffer. The source and manner of use of *soy bean trypsin inhibitor (SBTI), diisopropylphosphofluoridate (DFP), human serum inhibitor of C'1 esterase, histamine diphosphate,* and *tripolidine hydrochloride (Actidil®)* were exactly as indicated by Ratnoff and Lepow(1).

Results. As reported previously(1), intracutaneous injection of 0.1 ml of C'1 esterase containing 5 units/ml or less had little or no effect on vascular permeability, as compared with buffer (PBS) controls. Similarly, normal guinea pig serum diluted with an equal volume of PBS was without significant activity, provided that the serum was obtained in the manner described. However, mixtures of equal volumes of subthreshold concentrations of C'1 esterase and undiluted guinea pig serum resulted in generation of a permeability enhancing property not present in either reactant alone. The magnitude of this effect was dependent on the concentration of C'1 esterase employed. The data in Fig. 1 represent the average of 30 experiments in which many different pools of normal guinea pig serum and several preparations of human C'1 esterase were employed. If extended, the two curves in Fig. 1 would meet at an enzyme concentration of about 12 units/ml.

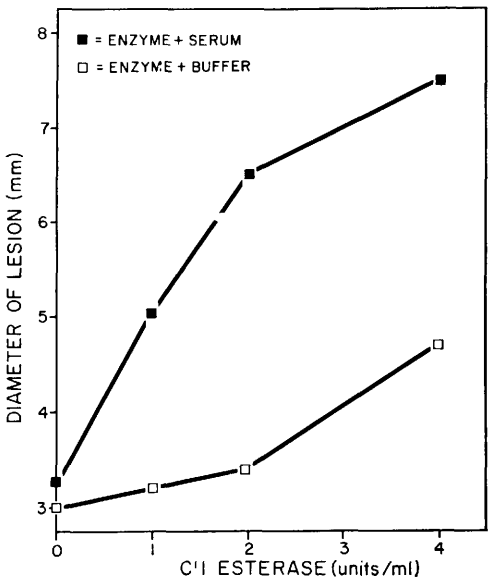

FIG. 1. Generation of a permeability factor from mixtures of equal volumes of human C'1 esterase and normal guinea pig serum; the abscissa defines final concentration of C'1 esterase in reaction mixtures.

Generation of permeability activity was also a function of serum concentration. Mixtures of equal volumes of subthreshold doses of C'1 esterase and ½ dilutions of serum were somewhat less potent than mixtures of enzyme and undiluted serum. At a dilution of ¼ (final dilution of ⅛), the serum itself began to acquire permeability activity, a property not enhanced significantly by small concentrations of C'1 esterase.

Enhancement of vascular permeability occurred when subthreshold concentrations of C'1 esterase were mixed with serum at 0°C and injected as rapidly as possible. No change in activity was found when similar mixtures were incubated at 37°C for periods up to one hour before testing.

Generation of permeability activity from guinea pig serum by C'1 esterase was inhibited by prior inactivation of the enzyme with 10^{-2} or $10^{-3}M$ DFP or with stoichiometric concentrations of purified human serum inhibitor of C'1 esterase. SBTI, which does not affect the enzymatic activity of C'1 esterase, did not block generation of permea-

TABLE I. Correlation Between Permeability Activity and Complement Utilization in Mixtures of C'1 Esterase and Serum.*

C'1 esterase (units/ml)	Permeability activity diameter of lesion (mm)	Residual complement $C'H_{50}$ (units/ml)
4	8.9	6
2	8.4	9
1	7.7	11
.5	5.0	25
.25	4.0	61
.125	3.9	109
.063	3.0	162
—	2.8	208

* Mixtures consisted of equal volumes of C'1 esterase or buffer and normal guinea pig serum; concentrations expressed in terms of final reaction mixtures. Permeability activity was greater than that shown in Fig. 1 at corresponding concentrations of C'1 esterase. However, tabular data are averages of only 2 experiments.

bility activity. These observations were in agreement with previous findings relating enhancement of vascular permeability to the enzymatic function of C'1 esterase(1). Similarly, the permeability activity of mixtures of C'1 esterase and guinea pig serum was inhibited, in parallel with suppression of the effect of histamine, by prior intravenous treatment of test animals with 0.1 mg/kg of triprolidine, a known anti-histaminic drug.

C'1 esterase interacts with complement components in solution, resulting in inactivation of the hemolytic functions of C'2 and C'4(10). The effect of the enzyme on total hemolytic complement ($C'H_{50}$) is a reflection of this interaction. Table I demonstrates that concentrations of C'1 esterase capable of generating permeability activity produced very large reductions in the $C'H_{50}$ titer of guinea pig serum, suggesting the possibility that utilization of complement components might be related to permeability enhancement. Experiments in which the serum was first heated for 30 minutes at various temperatures before mixture with enzyme were consistent with this hypothesis. Pre-treatment of serum at 37°C or 45°C had only minimal effects on $C'H_{50}$ titer and permeability, as compared with serum held at 0°C, while temperatures of 52°C and 56°C resulted in almost complete and complete loss, respectively, of both activities (Table II). In contrast, the permeability factor produced by interaction of C'1 esterase and serum was partially stable to heating at 56°C for 30 minutes (Table III).

Discussion. The demonstration that subthreshold concentrations of C'1 esterase were capable of generating permeability increasing activity from normal guinea pig serum provides an *in vitro* experimental system for defining the serum constituents which are required and the nature of the permeability factor which is produced. Two correlative approaches described in this report suggested the possible identity of the serum constituents with components of complement. Attempts were therefore made, in experiments not presented, to test this hypothesis further by substituting for normal guinea pig serum the various complement-deficient reagents, R1, R2, R3, R4 and complement fixed serum. However, extensive trials, compromised by development of spontaneous permeability activity during preparation and by theoretical considerations discussed elsewhere(6), were inconclusive. They nevertheless point the way toward more direct and better controlled experiments with purified components of complement, an approach under current investigation.

Summary. Concentrations of human C'1 esterase which are below threshold for direct enhancement of vascular permeability in guinea pig skin have been shown capable of generating a partially heat stable (56°C, 30 minutes) permeability factor from normal

TABLE II. Effect of Heating Serum at Various Temperatures on Complement Titers and on Subsequent Ability of C'1 Esterase to Generate Permeability Activity.

C'1 esterase* (units/ml)	Permeability activity (diameter of lesion in mm) after prior treatment of serum for 30 min at 0°C	37°C	45°C	52°C	56°C
4	7.6	7.9	7.4	4.3	3.0
2	7.0	5.8	5.9	3.9	2.5
1	4.5	4.0	3.9	2.6	2.5
0	3.6	3.2	3.4	3.3	2.6
$C'H_{50}$† (units/ml)	366	306	324	14	<10

* Final concentration in reaction mixtures.
† Complement titer of undiluted serum after heating at indicated temperatures.

TABLE III. Comparison of Effect on Permeability Activity of Heating Serum Before and After Addition of C'1 Esterase.

C'1 esterase* (units/ml)	Permeability activity (diameter of lesion in mm) in mixtures containing: Unheated serum	Heated serum†	Heated (enzyme + serum)‡	No serum
4	7.3	2.7	7.3	4.4
2	6.3	3.3	4.5	3.1
1	5.5	2.2	3.0	1.9
0	3.4	2.8	3.0	2.7

* Final concentration in reaction mixtures.
† Serum heated at 56°C for 30 min before addition of C'1 esterase.
‡ Mixture of C'1 esterase and normal serum heated at 56°C for 30 min.

guinea pig serum *in vitro*. The action of this factor was blocked by treatment of test animals with an anti-histaminic drug, tripolidine. Generation of the factor from serum was prevented by inactivation of the enzymatic activity of C'1 esterase with DFP or human serum inhibitor of C'1 esterase. SBTI, without effect on C'1 esterase, did not inhibit generation. Concentrations of C'1 esterase which elaborated permeability activity in guinea pig serum also produced marked depression of the hemolytic complement titer of the serum. A relationship was also observed between the residual complement titers of heated sera and their capacity to produce a permeability factor upon mixture with C'1 esterase. The data are consistent with, but do not prove, the hypothesis that C'1 esterase mediates increased vascular permeability by interaction with the complement system.

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Effects of Bromobenzene on Mouse Tissue Sulfhydryl and Insulin-I^{131} Metabolism.* (29227)

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Enzyme studies(1,2) indicate that reduced glutathione (GSH) acts as hydrogen donor in the first step in insulin degradation, its reductive cleavage into the glycyl and phenylalanine chains. This raises the question whether the rate of insulin degradation varies in any degree as result of physiological or even unphysiological variations in intracellu-

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