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VASCULAR PERMEABILITY FACTORS (PF/Nat AND PF/Dil)—THEIR RELATIONSHIP TO HAGEMAN FACTOR AND THE KALLIKREIN-KININ SYSTEM

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Abstract—Permeability factor/native (PF/Nat) and permeability factor/dilute (PF/Dil), the factors in diluted plasma exposed to glass which increase vascular permeability of small blood vessels, were examined for their ability to hydrolyze *p*-tosylarginine methyl ester and to activate prekallikrein. The direct arginine esterase activity of diluted plasma gave a pattern of activity and inhibition similar to that previously described for PF/Nat, while the ability of diluted plasma to activate prekallikrein was similar to that for PF/Dil. Electrophoretic analyses on polyacrylamide gel of these dilutions showed that the direct arginine esterase activity was distributed through the gel with a major peak of activity in the globulin region. PF/Dil, on the other hand, like active Hageman factor (HF) preparations, had two major peaks of activity located in the globulin and albumin regions. Studies with plasma deficient in HF, PTA (Factor XI), prekallikrein (Fletcher factor) and plasmin suggested that HF, prekallikrein and possibly PTA, but not plasmin, are required for maximal generation of these activities in plasma. Plasma deficient in C'1 esterase inhibitor generates far more direct arginine esterase activity and PF/Dil than normal plasma. A number of inhibitors of HF, of plasmin and of trypsin were examined for their ability to inhibit the activation of HF during dilution, the active HF found after dilution and plasma kallikrein. The present data would suggest that active HF and/or its fragments and PF/Dil are identical enzymes. The direct arginine esterase activity generated by glass contact in these experiments may be kallikrein bound to α-2-macroglobulin.

In 1953, MacKay *et al.* [1] demonstrated that intracutaneous injections of diluted guinea pig serum increased the vascular permeability of small blood vessels in this species. Subsequent studies [2] have shown that the activity is generated only if plasma or serum is stored in glass tubes and that it can be produced in the plasma of some but not all mammalian species. Two permeability factors were found in human serum [3]. Permeability factor/native (PF/Nat) was found in the lower dilutions of plasma, while permeability factor/dilute (PF/Dil) was found in dilutions of plasma 1:100 or greater. PF/Nat was distinguished from PF/Dil in that it was not inhibited by soybean trypsin inhibitor and that the increased vascular permeability induced by it in guinea pigs lasted for 1 hr as compared to 15–20 min for PF/Dil. PF/Dil has many properties similar to plasma kallikrein. However, in 1962, Mason and Miles [4] demonstrated that PF/Dil formed kinins from native plasma, but not from plasma heated to 60°, suggesting that PF/Dil formed kinins by activation of endogenous plasma prekallikrein.

With the demonstration by a number of workers [5–9] that active Hageman factor, or fragments derived therefrom, was capable of directly activating prekallikrein, it appeared likely that PF/Dil and active Hageman factor were identical substances. However, direct experimental proof was lacking due in large part to the relative insensitivity of the clotting and biological kinin-generating assays. With the development of a sensitive radiochemical method for the determination of active Hageman factor and its fragments [10], diluted plasma could be assayed for its ability to activate prekallikrein and for its arginine esterase activity.

The data presented in this paper support the hypothesis that active Hageman factor and/or its fragments and PF/Dil are identical enzymes and suggest that the direct arginine esterase activity generated by glass contact may be plasma kallikrein bound to α-2-macroglobulin.

MATERIALS AND METHODS

In preliminary studies, citrated human plasma (ACD or 0·2 ml 20% sodium citrate/10 ml of blood) was prepared in plastic or silicone-coated equipment and activated as previously described [2] by dilution in saline in glass tubes (13 × 75 mm) to a final volume of 2·0 ml, followed by incubation for 44 hr at 4° and 1 hr at 37°. In most studies, plasma was diluted in glass tubes (11 × 100 mm) to a final volume of 1·0 ml and

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incubated for 1 hr at 37°. The direct arginine esterase activity and the prekallikrein activator activity were determined simultaneously by a radiochemical method employing 0·047 μCi, 280 mCi/mole of *p*-tosyl-L-arginine [^{3}H]methyl ester ([^{3}H]TAMe). In this technique [10], 20 μl of the dilution is added to a mixture of 10 μl of 0·5 M Tris buffer, pH 8·0, and 20 μl of either prekallikrein (0·015 esterase units) or water and incubated at room temperature for 10 min. [^{3}H]TAMe (10 μl) was added and the incubation continued for an additional 30 min at room temperature.

Gel electrophoresis using 7% polyacrylamide gel was conducted in Tris-glycine buffer, pH 8·3, according to the directions of Davis [11]. Either undiluted plasma (20 μl) or dilutions of plasma concentrated 10-fold by freeze-drying (200 μl) were applied to the gel in 10 or 20% glycerol respectively. After electrophoresis, the absorbance of the gel was measured at 280 nm in a spectrophotometer equipped with a gel scanner. The gel was cut into 1·5-mm slices and crushed in an assay tube with a microhomogenizer using 10 μl of 0·5 M Tris buffer, pH 8·0; the homogenizer was rinsed with 20–40 μl water and the gel assayed for direct arginine esterase activity in the radiochemical method. Alternatively, the gel slice was divided into two pieces and assayed in the presence or absence of prekallikrein (20 μl, 0·15 esterase units).

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Soybean trypsin inhibitor, lima bean trypsin inhibitor and ovomucoid trypsin inhibitor were purchased from Worthington Biochemical Corp.; pancreatic trypsin inhibitor, from Nutritional Biochemicals Corp.; spermine and lysozyme (egg white), from Cal-Biochem; and poly-L-lysine (mol wt 17,000 and 75,000), and epsilon-aminocaproic acid, from Schwarz/Mann. Hexadimethrine bromide was a gift from Grant H. Barlow, Abbott Laboratories, and epsilon-aminocaproic acid, hexyl ester; 4-amino-methylcyclohexane-L-carboxylic acid (*trans*-AMCHA); and *trans*-AMCHA hexyl ester were gifts from Dr. Yasushi Abiko, Daiichi Seiyaku, Tokyo, Japan.

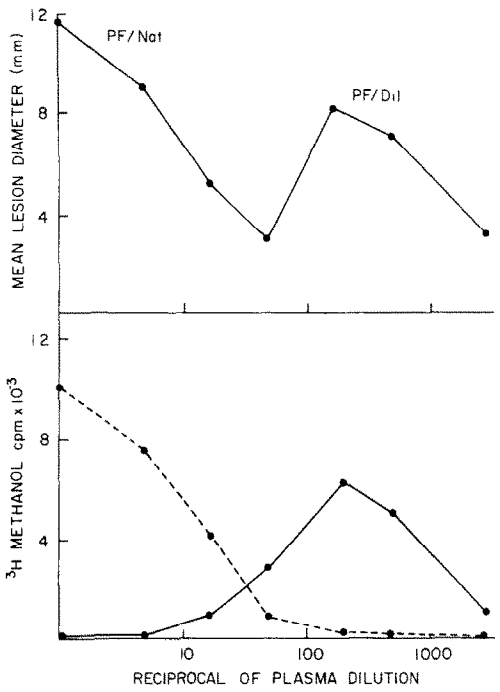

Fig. 1. Activation of PF/Nat and PF/Dil in dilutions of human plasma (final volume, 2·0 ml saline) incubated in glass tubes (13 × 75 mm) for 44 hr at 4° and then for 1 hr at 37°. Upper graph, permeability-increasing activity in guinea pig skin from Ratnoff and Miles [2] (reproduced with permission from *Br. J. exp. Path.*). Lower graph, direct arginine esterase activity (●——●) and prekallikrein activation (●——●).

RESULTS

Generation of arginine esterase activity. When dilutions of normal human plasma in a final volume of 2·0 ml saline were stored in glass tubes (13 × 75 mm) for 44 hr at 4° and then 1 hr at 37° [2], these dilutions both hydrolyze *p*-tosyl-L-arginine methyl ester (TAMe) and activate prekallikrein (Fig. 1, lower graph). The direct esterase activity of the dilutions, as measured in the absence of prekallikrein, gave a pattern of activity similar to PF/Nat in that the greatest activity was found in undiluted plasma. On the other hand, plasma diluted 1:50 and greater was capable of activating prekallikrein, and the activities of these dilutions of plasma gave a pattern similar to that found for PF/Dil (Fig. 1, compare upper and lower graphs).

When dilutions of this same plasma in a final volume of 1·0 ml saline were stored in glass tubes (11 × 100 mm) for only 1 hr at 37°, only 50 per cent of the direct esterase activity was generated, but the amount of prekallikrein activator (PF/Dil) activity was increased (compare Fig. 1 and Fig. 2). The maximum amount of activator activity was found in the 1:100 dilution, and in subsequent experiments these latter

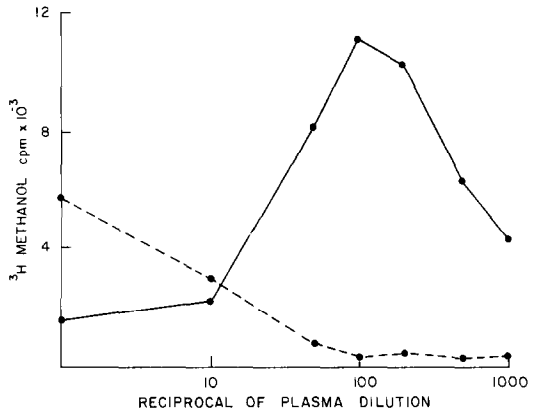

Fig. 2. Activation of direct arginine esterase activity (●——●) and PF/Dil (prekallikrein activation) (●——●) in dilutions of human plasma (final volume, 1·0 ml saline) incubated in glass tubes (11 × 100 mm) for only 1 hr at 37°.

conditions of activation were utilized unless otherwise specified.

Properties of direct arginine esterase activity. As suggested by the initial experiment, the direct arginine esterase activity in human plasma could be increased by lowering the temperature of storage in the glass tube. As shown in Table 1, plasma (1·0 ml) stored in glass tubes (11 × 100 mm) for 44 hr at 27° generated more activity than plasma stored for 1 hr at 37°, and even greater amounts of arginine esterase activity were formed in plasma stored for 44 hr at 4°. Furthermore, additional incubation for 1 hr at 37° of this latter plasma did not increase the activity.

Table 1. Generation of direct arginine esterase activity in human plasma stored at various temperatures

Treatment	Arginine esterase ^{3}H-methanol* (count/min)
Normal plasma, stored 1 hr 37°	3,937; 3,736
Normal plasma, stored 44 hr 27°	6,036; 6,932
Normal plasma, stored 44 hr 4°	14,329; 10,300
Normal plasma, stored 44 hr 4° + 1 hr 37°	9,972; 9,282

* Numbers separated by semicolons represent plasma from different individuals.

Nine normal plasmas generated from 3083 to 5642 count/min ^{3}H-methanol when incubated for 1 hr at 37° in glass tubes (3977 ± 385, mean ± S.E.) (Table 2). Plasma incubated in the absence of glass gave only 809–1545 count/min values which were similar to those obtained prior to incubation. Like PF/Nat, the direct arginine esterase activity generated by human plasma was not inhibited by soybean trypsin inhibitor (SBTI), as shown by activation of the same plasma in the presence (experimental) and absence (control) of 50 μg SBTI (Table 2).

Plasma deficient in Hageman factor did not generate direct arginine esterase activity. Furthermore, plasma from one individual deficient in prekallikrein (Fletcher factor) failed to generate activity. Plasma from two individuals with PTA (Factor XI) deficiency gave a variable response generating higher than normal values in one plasma and lower than normal in the other. Plasma depleted of plasminogen by filtration through a lysine–agarose column generated the same amount of activity both before and after treatment.

The presence of C'1 esterase inhibitor markedly influenced the amount of direct arginine esterase activity which could be generated. As shown in Table 2, plasma from six individuals with hereditary angioneurotic edema (HANE), individuals who are deficient in this inhibitor, has much higher arginine esterase activity than found in normal plasma (13,013 ± 654, mean ± S.E.). However, in some of these plasmas, the direct arginine esterase was already preformed. For example, 14,425 cpm ^{3}H-methanol was found in one plasma

Table 2. Generation of direct arginine esterase activity in human plasma

Treatment	Arginine esterase ^{3}H-methanol* Control (count/min)	Experimental (count/min)
Normal plasma (9)†	3,083–5,642	
Normal plasma + SBTI (50 μg/ml)	3,210; 4,816	3,290; 5,486
HANE plasma + SBTI (50 μg/ml)	14,425; 15,290	15,968; 12,713
Plasma deficient		
in Hageman factor (1)	846	
in prekallikrein (Fletcher) (1)	1,316	
in PTA (Factor XI) (2)	6,147; 2,137	
in C'1 esterase inhibitor (HANE) (6)	11,206–16,831	
Normal plasma, plasminogen depleted (1)	3,333	3,075
Normal plasma, heated 1 hr 55° (3)	4,167–4,761	500–1,395

* Numbers separated by semicolons represent plasma from different individuals; those by dashes are range of activities found.

† Number of different plasmas tested.

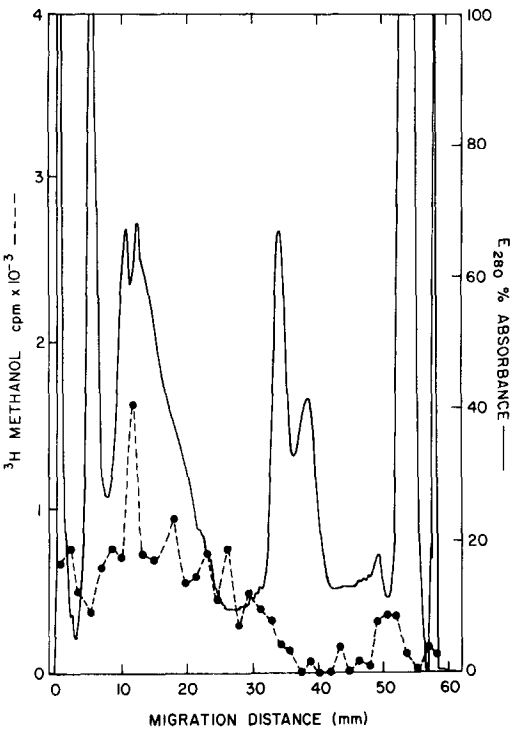

Fig. 3. Polyacrylamide gel electrophoresis of direct arginine esterase activity found in undiluted plasma activated by storage in a glass tube for 44 hr at 4°.

prior to storage in a glass tube—a count which was unaltered on storage for 1 hr at 37°. On the other hand, plasma from another individual with this disease contained 3510 cpm ^{3}H-methanol prior to glass contact and generated 15,290 cpm during incubation. Plasma heated to 55° for 1 hr, a procedure known to inactivate C'1 esterase inhibitor [13], failed to generate activity.

Polyacrylamide gel electrophoresis analyses of plasma activated by storage in a glass tube for 44 hr at 4° showed that the esterase activity was distributed from the origin to the albumin region with a major peak in the globulin region (Fig. 3) which had moved 9–11 mm. A similar pattern of activity was found with activity generated in plasma deficient in C'1 esterase inhibitor. Recoveries of activity from these gels varied from 70 to 100 per cent.

Properties of PF/Dil (prekallikrein activator activity). Additional properties associated with the generation of prekallikrein activator (active Hageman factor) are shown in Fig. 4. Like PF/Nat and PF/Dil, plasma deficient in Hageman factor did not generate activity. In addition, plasma deficient in prekallikrein appeared to generate normal amounts of activity through the 1:50 dilution, but at the higher dilutions lower than normal values were obtained. Plasma from two individuals with PTA deficiency, as with the generation of PF/Nat, gave a variable response at the lower dilutions. For example, the 1:10 dilution generated higher than normal values in one plasma and lower than normal values in the other. However, at the 1:100 and 1:200 dilutions both generated activity which was similar to or just slightly below that found in plasma from normal individuals with low activity. Plasma deficient in plasmin generated the same activity as the plasma from which it was derived.

As with the direct arginine esterase activity, the presence of C'1 esterase inhibitor markedly influenced the amount of prekallikrein activator generated. When the amount of activity found in plasma from four patients deficient in this inhibitor (Fig. 4) is compared to that from eight normal individuals, much higher than normal values are obtained particularly in the 1:10 and 1:50 dilutions. Destruction of the inhibitor in normal plasma by heating the plasma to 55° for 1 hr in a silicone-coated tube did not destroy the ability of the heated plasma to generate PF/Dil as found for direct arginine esterase activity. Rather when this plasma was diluted and activated by storage for 1 hr at 37° in a glass tube, much higher than normal values were found in the 1:10 and 1:50 dilutions as shown by comparison of unheated and heated plasmas (Table 3).

Polyacrylamide gel electrophoretic analyses of those dilutions of plasma which contain prekallikrein activator, in general, gave three peaks of activity—an in-

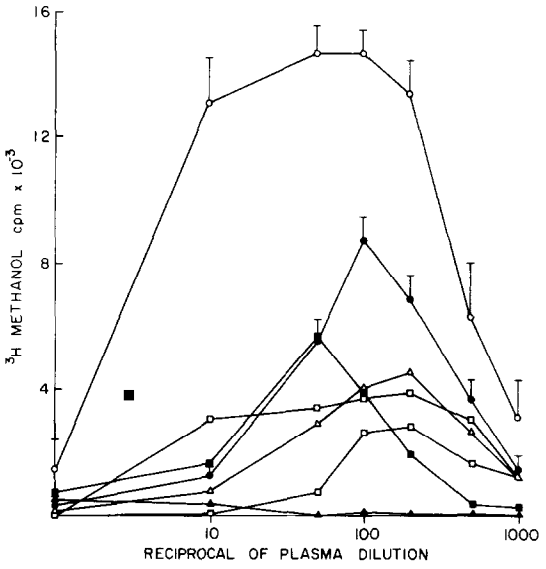

Fig. 4. Generation of PF/Dil (prekallikrein activator activity) in normal plasma (●——●) and in plasma deficient in C'1 esterase inhibitor (○——○); in prekallikrein (Fletcher factor) (■——■); in PTA (Factor XI) (□——□); in plasminogen (△——△); and in Hageman factor (Factor XII) (▲——▲). For normal and C'1 esterase inhibitor-deficient plasmas, each point represents the mean of values obtained in eight and four plasmas respectively. Vertical bars indicate standard error of the mean. Two PTA-deficient plasmas are plotted individually, and the remaining deficient plasmas represent one plasma each.

Table 3. Generation of PF/Dil(prekallikrein activator activity) in human plasma heated at 55°

Plasma dilution	Generation of arginine esterase (^{3}H-methanol)* Unheated (count/min)	Heated (count/min)
1:10	1,053; 539	12,236; 5,632
1:50	1,674; 5,059	11,897; 11,566
1:100	5,344; 8,843	9,689; 9,389
1:200	6,340; 7,868	6,738; 6,868

* Numbers separated by semicolons represent plasma from different individuals.

itial peak which was near or at the origin, a second peak which had moved 8–12 mm and a third peak which usually coincided with the albumin region (Fig. 5). In addition, in some chromatograms, small amounts of activity could be recovered throughout the gel. The amount of activity recovered in the initial peak varied throughout the gel. The amount of activity recovered in the initial peak varied from 3 to 25 per cent and could be due to activation of residual inactive Hageman factor on the gel surface. When the amount of activity recovered in the globulin region (second peak) was compared to that recovered in the albumin region (third peak), the per cent recovered varied with the dilution of plasma (Table 4). At a 1:50 dilution of plasma, 77–92 per cent of the activity was recovered in the globulin region. At the greater dilutions, most of the activity was found in the albumin region.

Effect of inhibitors on generation of PF/Dil (Hageman factor). A number of inhibitors of Hageman factor, of plasmin and of trypsin were examined for their ability to inhibit these enzyme reactions. The per cent inhibition furnished by these inhibitors of the activation of Hageman factor during dilution, of the active Hageman factor found after dilution, and of plasma kallikrein for two different plasmas is shown in Table 5.

Table 4. Recovery of prekallikrein activator activity from polyacrylamide gels after electrophoresis

Plasma dilution	% Prekallikrein activator activity Globulin	Albumin
1:50	92; 78	8; 22
1:100	35	65
1:200	18; 29	82; 71
1:500	14	86

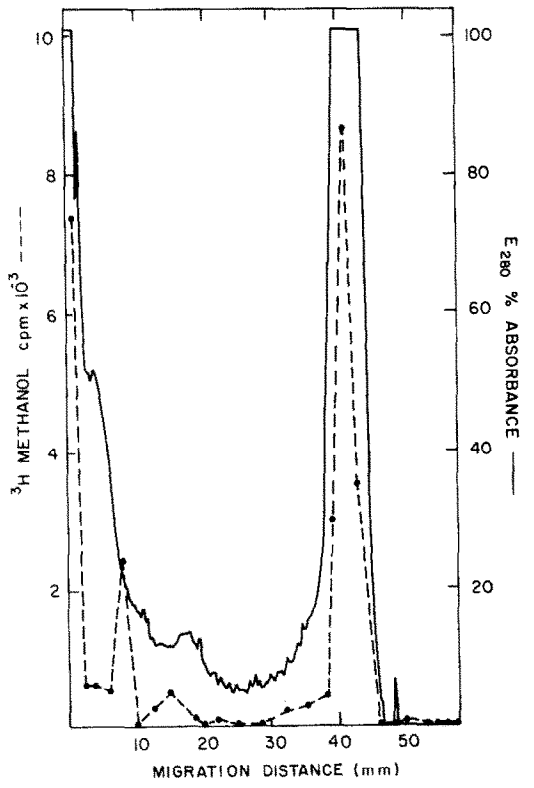

Fig. 5. Polyacrylamide gel electrophoresis of prekallikrein activator activity found in 1:200 dilution of plasma activated by storage in a glass tube for 1 hr at 37°.

With some inhibitors, more than two plasmas were tested, and for these the range of inhibitory activity is shown. Spermine and cytochrome c are considered to be specific inhibitors of the clotting activity of Hageman factor [14] in that they do not affect other components of the clotting sequence. Both of these inhibitors prevented the generation of active Hageman factor without affecting the activity of the enzyme once it had been activated or of plasma kallikrein. Similarly, hexadimethrine bromide affected only the activation of Hageman factor. Two other inhibitors of Hageman factor, polylysine (mol wt 15,000 or 170,000) and methylene blue, inhibited both the activation and the active enzyme. In addition, methylene blue partially (30–34 per cent) inhibited the activity of plasma kallikrein. These latter inhibitors, therefore, cannot be classed as inhibitors of the activation step, since their ability to inhibit this step could be due solely to inhibition of active Hageman factor.

Inhibitors of plasmin, which in most cases inhibit both the activation of plasminogen and plasmin itself, were not as effective in inhibiting the activation of Hageman factor. The hexyl ester of *trans*-AMCHA in some normal plasma caused almost complete inhibition of the activation phase, but in other plasma gave only partial inhibition. The reason for this variability is not known. Similarly, with epsilon-aminocaproic acid, a small inhibition was usually found. The other two plasmin inhibitors, the hexyl ester of epsilon-aminocaproic acid and *trans*-AMCHA, had little if any inhibitory activity.

Trypsin inhibitors such as pancreatic trypsin inhibitor and soybean trypsin inhibitor were so effective in

Table 5. Inhibition of activation of Hageman factor, of active Hageman factor and of plasma kallikrein

Inhibitors	(μg/ml)	Activation of Hageman factor*	% Inhibition of Active Hageman factor†	Plasma kallikrein†
Inhibitors of Hageman factor				
Spermine	500	87; 100	10; 19	<1; <1
Cytochrome c	500	77; 100	<1; <1	<1; <1
Hexadimethrine bromide	100	89; 98	14; 10	<1; <1
Polylysine (mol wt 15,000)	500	84; 100	88; 100	<1; <1
(mol wt 170,000)	500	90; 100	89; 90	<1; <1
Methylene blue	500	100; 100	75; 77	30; 34
Inhibitors of plasmin				
trans-AMCHA, hexyl ester	500	37; 73	15; 20	15; 19
ε-Aminocaproic acid	1000	20; 34	4; 29	7; 17
ε-Aminocaproic acid, hexyl ester	500	<1; 21	6; 15	<1; 9
trans-AMCHA	1000	<1; 15	<1; 3	<1; <1
Inhibitors of trypsin				
Pancreatic	5	39; 60	59; 72	86; 90
Soybean	5	97; 100	87; 89	68; 76
Lima bean	5	3; 2	18; 11	<1; <1

* Inhibitors were added to 1·0 ml saline prior to the addition of plasma (5 μl) and subsequent activation.

† Inhibitors were incubated for 10 min at room temperature with either the activated plasma or with plasma kallikrein (4·6 mTU) prior to the addition of the substrate.

their ability to inhibit plasma kallikrein that their inhibition of the activation step could be due solely to inhibition of plasma kallikrein. Lima bean trypsin inhibitor weakly inhibited active Hageman factor.

The mechanism of action of those inhibitors which inhibit only the activation of Hageman factor was also examined. In these experiments, the inhibitors were added to the glass tube; the tube was washed three times with saline; the diluted plasma was added and activated by incubation for 1 hr at 37°. Under these conditions, hexadimethrine bromide treatment caused almost complete inhibition of the activity, probably by coating the negatively charged glass surface. A similar mechanism at least in part explains the inhibition of the activation by cytochrome c as this inhibitor caused a 46 and 68 per cent inhibition of the activation of two different plasmas. Spermine, on the other hand, did not inhibit under these conditions.

DISCUSSION

The data presented in this paper provide evidence to suggest that active Hageman factor and/or its fragments and PF/Dil are identical enzymes. Plasma diluted 1:50 or greater is capable of activating prekallikrein, and the activities of these dilutions gave a pattern similar to that previously found [2, 3] for PF/Dil. Like PF/Dil [15], the activity cannot be generated unless the plasma is exposed to glass surfaces and is not formed in Hageman-deficient plasma. Also as was observed with PF/Dil [3, 16], the activity was suppressed with soybean and pancreatic trypsin inhibitors. However, whether these inhibitors are blocking the action of PF/Dil or the plasma kallikrein which is generated cannot be stated with certainty until a method for the direct measurement of active Hageman factor and its fragments is available. Similarly PF/Dil has been thought to be an arginine esterase [17, 18], but in the initial experiments the presence of the arginine ester may have prevented the inhibition of plasma kallikrein by diisopropyl fluorophosphate [19] and thus premitted the activation of Hageman factor [20, 21]. In the latter experiments, where direct arginine esterase activity was measured, the preparation of PF/Dil had been only partially purified, and may well have been a mixture of active Hageman factor and plasmin.

Like Hageman factor [14], the activity generated in dilute plasma could be suppressed by inhibitors of the activation of Hageman factor. Thus spermine, cytochrome c, hexadimethrine bromide, polylysine and methylene blue all prevented the formation of activity in these dilutions. Polylysine and methylene blue also inhibited the active Hageman factor after it had been formed, but the other inhibitors inhibited only the activation step. Only methylene blue partially (30–34 per cent) inhibited plasma kallikrein. Hexadimethrine bromide probably prevents this activation by coating the negatively charged glass surface, since prior treatment of the glass tube with this inhibitor prevented the formation of prekallikrein activator. A similar mechanism was found to be at least partially responsible for the inhibitory activity of cytochrome c. Spermine, on the other hand, did not inhibit under these conditions and may have a direct effect on one of the enzymes involved in the activation of Hageman factor [20], although it did not inhibit either active Hageman fac-

tor or kallikrein. Inhibitors of plasmin and/or the activation of plasminogen [22] were much less effective than the inhibitors of Hageman factor and gave a variable response with plasma from different individuals. The most potent of these inhibitors was the hexyl ester of *trans*-AMCHA. Lima bean trypsin inhibitor weakly inhibited the active Hageman factor and was without effect on the activation of Hageman factor or plasma kallikrein.

Also, like active Hageman factor and its fragments [6, 7, 9], the activity found in dilutions of plasma migrated in polyacrylamide gel electrophoresis predominantly in the globulin and albumin regions. The greater the dilution of plasma, the greater was the amount recovered in the albumin region, indicating the formation of increasing amounts of the 30,000–40,000 molecular weight activator fragment. Hageman factor [7] and active Hageman factor [23] migrate in electrophoresis as beta-globulins as has been reported for partially purified preparations of PF/Dil [16, 18]. There appears to be no evidence available in the literature which is incompatible with the theory that in human plasma active Hageman factor and/or its fragments and PF/Dil are identical enzymes.

The formation of both the direct arginine esterase activity and the prekallikrein activator was found to be dependent upon not only Hageman factor but prekallikrein as well, a finding which was not unexpected in view of the results reported by Wuepper [21]. Plasma deficient in plasmin generated normal amounts of both activities, while plasma deficient in C'1 esterase inhibitor formed much higher amounts of the activities, particularly in the lower dilutions of plasma. In fact, at the 1:10 dilution, the difference between the amounts generated in normal plasma when compared to those from patients with hereditary angioneurotic edema (deficient in C'1 esterase inhibitor) was so great that either of these assays could provide a relatively simple method for the diagnosis of this disease. Two plasmas from patients with a deficiency of PTA (Factor XI) gave a variable response. It would appear likely that both the direct esterase activity and the prekallikrein activator are generated by activation of the same biochemical pathway.

However, differences between the two activities were found. The greatest amounts of arginine esterase activity are formed if plasma is stored for 44 hr at 4°, and heating the plasma to 55° for 1 hr destroyed its ability to generate the direct arginine esterase activity but increased the generation of prekallikrein activator in the lower dilutions of plasma. Also in polyacrylamide gel electrophoresis, the direct arginine esterase activity migrated predominantly in the globulin region, and no activity was found in the albumin region.

It is suggested that the direct arginine esterase activity generated by glass contact is kallikrein bound to α-2-macroglobulin. Plasma kallikrein, like other proteolytic enzymes such as thrombin, plasmin, trypsin and collagenase, binds to human α-2-macroglobulin [24, 25] although not to bovine α-2-macroglobulin [26]. The bound human kallikrein-α-2-macroglobulin complex retains 25 per cent or more of its original esterase activity which is now protected from inhibition by SBTI. These properties are similar to those found for the direct arginine esterase activity and are reminiscent of those previously described for PF/Nat [2, 3]. However, whether the direct arginine esterase activity in normal plasma and PF/Nat are identical substances remains to be established. Both the kallikrein– and the plasmin–α-2-macroglobulin complexes [27] have lost most, although not all, of their biological activity. Whether this residual activity is sufficient to induce vascular permeability activity equivalent to that of PF/Nat, particularly in normal plasma, is still to be determined.

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