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Fitzgerald Trait: *Deficiency of a Hitherto Unrecognized Agent, Fitzgerald Factor, Participating in Surface-Mediated Reactions of Clotting, Fibrinolysis, Generation of Kinins, and the Property of Diluted Plasma Enhancing Vascular Permeability (PF/Dil)*

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The prolonged partial thromboplastin time observed in the plasma of a 71-yr-old asymptomatic man was related to the deficiency of a hitherto unrecognized agent. The patient's plasma also exhibited impaired surface-mediated fibrinolysis and esterolytic activity and impaired generation of kinins and of the property enhancing vascular permeability designated PF/Dil. The patient's plasma contained normal amounts of all known clotting factors except Fletcher factor (a plasma prekallikrein) which was present at a concentration of 10-15% of pooled normal plasma. Fletcher trait plasma, however, contained normal amounts of the agent missing from the patient's plasma and corrected the defects in clotting, fibrinolysis, and vascular permeability. Fletcher trait plasma was less effective in correcting generation of kinins and esterolytic activity, presumably because of the patient's partial deficiency of prekallikrein. The site of action of the factor deficient in the patient's plasma appeared to be subsequent to the activation of Hageman factor and plasma prekallikrein. A fraction of normal plasma, devoid of other clotting factors, corrected the defect in clotting in the patient's plasma; a similar fraction of the patient's plasma did not correct this abnormality. No evidence yet exists pointing to the familial nature of the patient's defect. Tentatively, the patient's disorder may be referred to by his surname as Fitzgerald trait, and the agent apparently deficient in his plasma as Fitzgerald factor.

Fitzgerald Trait

DEFICIENCY OF A HITHERTO UNRECOGNIZED AGENT, FITZGERALD FACTOR, PARTICIPATING IN SURFACE-MEDIATED REACTIONS OF CLOTTING, FIBRINOLYSIS, GENERATION OF KININS, AND THE PROPERTY OF DILUTED PLASMA ENHANCING VASCULAR PERMEABILITY (PF/DIL)

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ABSTRACT The prolonged partial thromboplastin time observed in the plasma of a 71-yr-old asymptomatic man was related to the deficiency of a hitherto unrecognized agent. The patient's plasma also exhibited impaired surface-mediated fibrinolysis and esterolytic activity and impaired generation of kinins and of the property enhancing vascular permeability designated PF/Dil. The patient's plasma contained normal amounts of all known clotting factors except Fletcher factor (a plasma prekallikrein) which was present at a concentration of 10–15% of pooled normal plasma. Fletcher trait plasma, however, contained normal amounts of the agent missing from the patient's plasma and corrected the defects in clotting, fibrinolysis, and vascular permeability. Fletcher trait plasma was less effective in correcting generation of kinins and esterolytic activity, presumably because of the patient's partial deficiency of prekallikrein. The site of action of the factor deficient in the patient's plasma appeared to be subsequent to the activation of Hageman factor and plasma prekallikrein. A fraction of normal plasma, devoid of other clotting factors, corrected the defect in clotting in the patient's plasma; a similar fraction of the patient's plasma did not correct this abnormality. No evidence yet exists pointing to the familial nature of the patient's defect. Tentatively, the patient's disorder may be referred to by his surname as Fitzgerald trait, and the agent apparently deficient in his plasma as Fitzgerald factor.

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INTRODUCTION

Activated Hageman factor (HF,[1] Factor XII) participates in the surface-mediated reactions of clotting, esterase- and kinin-generation, fibrinolysis, and enhancement of vascular permeability. These properties of HF are mediated or intensified by Fletcher factor, a plasma prekallikrein deficient in Fletcher trait (1, 2). In early experiments, crude activated HF appeared to activate plasma thromboplastin antecedent (PTA, Factor XI) enzymatically (3). When more purified systems became available, activation of PTA by activated HF was no longer demonstrable (4). Although activated Fletcher factor (a plasma kallikrein) is needed for optimal activation of PTA (2), incubation of a mixture of HF, PTA, prekallikrein, and kaolin still did not result in activation of PTA (4). These observations suggest that the activation of PTA requires the presence of one or more additional agents.

This report describes studies in an asymptomatic 71-yr-old man in whom a defect in the intrinsic pathway of thrombin formation was detected by chance (5). His plasma was apparently deficient in a hitherto unrecog-

[1] *Abbreviations used in this paper:* AHF, antihemophilic factor; HBr, hexadimethrine bromide; HF, Hageman factor; PF/Dil, property of diluted plasma enhancing vascular permeability; PTA, plasma thromboplastin antecedent; PTT, partial thromboplastin time; TAME, *p*-toluenesulfonyl-L-arginine methyl ester.

nized factor needed for expression of the functions of activated HF (6). With his permission we have named his disorder Fitzgerald trait, and the agent functionally deficient in his plasma, Fitzgerald factor. A similar patient, described by Colman, Bagdasarian, Talamo, and Kaplan (7) while our studies were in progress, appears to have an identical defect.

METHODS

Unless otherwise noted, human citrated plasma, animal plasmas, purified clotting factors, and reagents were prepared or obtained as described earlier (8–11). Fletcher trait plasma was kindly supplied by Dr. C. Abildgaard, University of California, Davis.

The effect of Celite adsorption of normal plasma upon various clotting factor activities was studied using Celite 512 (diatomaceous earth, a gift of Johns-Manville Products Corp., Celite division, New York) as described by Nossel (12).

A crude fraction that corrected the patient's clotting defect was separated from normal or HF-deficient plasma, adsorbed for 10 min at room temperature with 1/10 vol of alumina Cγ gel (Calbiochem, San Diego, Calif.). The adsorbed plasma was mixed with 1/100 vol of 0.01 M sodium EDTA and 5 mg of hexadimethrine bromide (HBr) (Aldrich Chem. Co. Inc., Milwaukee, Wis.) per ml and was dialyzed against 0.1 M sodium chloride in 0.025 M Tris-chloride buffer (pH 8.0) containing 0.0001 M EDTA and 0.05 mg HBr per ml. This material was chromatographed on a column of QAE-Sephadex A-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), as described earlier (8), except that the starting buffer contained 0.1 M sodium chloride and elution was via a linear gradient to 0.5 M NaCl in the same buffer. The molecular weight of fractions with correcting activity was examined by filtering the concentrated, pooled active fractions from a QAE-Sephadex A-50 column through a column of Sephadex G-150 (Pharmacia Fine Chemicals Inc.) equilibrated with a barbital-saline buffer (8). In one experiment with HF-deficient plasma, fractions with corrective activity were concentrated, dialyzed against 0.05 M sodium acetate buffer (pH 5.2) containing 0.15 M sodium chloride and 0.0001 M EDTA, and chromatographed upon SP-Sephadex C-50 (Pharmacia Fine Chemicals Inc.), as described earlier (8).

Batch preparation of the corrective fraction of normal plasma was performed as follows. 2-ml plasma was adsorbed with alumina Cγ gel, mixed with EDTA and HBr, and dialyzed against 0.1 M sodium chloride in a Tris-chloride-EDTA-HBr buffer as described above. The dialyzed plasma was mixed for 10 min at room temperature with an equal volume of QAE-Sephadex A-50, equilibrated with the same buffer in a 12 × 75-mm polystyrene tube. After centrifugation, the supernatant plasma was discarded and the precipitate was washed repeatedly with 10-ml portions of buffer until the washings no longer formed a visible precipitate upon addition of an equal volume of 10% trichloroacetic acid. The washed QAE-Sephadex was then eluted twice for 10 min with 2-ml portions of 0.5 M sodium chloride in a Tris-chloride-EDTA-HBr buffer; each eluate was diluted and assayed for Fitzgerald factor activity. The patient's plasma was fractionated in the same way.

Procoagulant assays for various clotting factors (8), immunologic assays for HF (13) and AHF (Factor VIII) (14), kaolin-activated plasma esterolytic activity for *p*-toluenesulfonyl-L-arginine methyl ester (TAME) (15), kaolin-induced fibrinolytic activity (10), antiplasmin (10), $C\bar{1}$ inactivator titer (16), and vascular permeability (17) were assayed as described earlier. Immunodiffusion was performed in a 0.9% agarose gel in a 0.05 M barbital buffer (pH 8.6) for 2 days at room temperature. A functional assay for the patient's deficient factor, based upon the kaolin-activated partial thromboplastin time (PTT) was devised. A volume of 0.1 ml each of the test sample, suitably diluted in barbital-saline buffer, a mixture of kaolin (10 mg/ml) and phospholipid (0.1% in saline, Centrolex "O," the gift of Central Soya Co., Inc., Chicago, Ill.), and the patient's plasma were incubated together for 1 min at 37°C in a 10 × 75-mm glass tube; studies performed subsequent to those in the present report suggest that a preliminary period of incubation of 8 min is superior to a 1-min period. The mixture was recalcified with 0.1 ml 0.025 M calcium chloride, and the clotting time was measured. The clotting time was converted to percentage activity by comparison with a calibration curve prepared by assaying serial dilutions of a standard pool of normal plasma (14); a linear relationship existed between the logarithm of the clotting time and the logarithm of 1/20 to 1/200 dilutions of the pooled plasma.

The effect upon the PTT of the patient's plasma by the addition of trypsin-activated PTA, kaolin-activated HF, and kallikrein (10), and the effect upon the Thrombotest time of incubating plasma with kaolin at 0°C were studied as described (18).

Generation of kinin-like activity was tested on the estrous rat uterus, adding atropine sulfate (1 μg per ml) to the deJalon's solution (19). In some experiments, kinin generation was estimated from the duration of delay between addition of the sample and the onset of contraction, an inverse function of the concentration of kinins (20). The effect of addition of ellagic acid or HF-fragments upon kinin generation was tested as described in Table II. The effect of plasma kallikrein upon kinin generation was tested in a mixture of 0.025 ml kallikrein (68 μmol MeOH released/ml per h, specific activity 755 μmol MeOH/mg protein per h), 0.05 ml test plasma, 0.05 ml 0.01 M *o*-phenanthroline (Fisher Scientific Co., Inc., Pittsburgh, Pa.), and 0.35 ml barbital-saline buffer (pH 7.4) at 37°C in 12 × 75-mm polystyrene tubes, testing samples for kinin-like activity. Kininogen in boiled plasma was measured as described by Diniz and Carvalho (21). Kinase was estimated by a modification of an earlier technique (22), incubating 5 μg of bradykinin (the gift of Sandoz Pharmaceuticals, Hanover, N. J.) in 0.05 ml barbital-saline buffer with 0.1 ml of the test plasma and 1.8 ml of the same buffer in 12 × 75-mm polystyrene tubes at 37°C. At intervals, 0.1-ml portions were added to a similar polystyrene tube containing 0.2 ml of 0.001 M *o*-phenanthroline and 1.6 ml of buffer and were tested immediately for kinin-like activity.

RESULTS

Studies on the clotting defect in Fitzgerald trait. The kaolin-activated PTT of the patient's plasma was abnormally long (> 500 s; control, 54 s), whereas the one-stage prothrombin time was normal, localizing the functional defect to the initial steps of the intrinsic pathway. Functional concentrations of HF, PTA, Christmas factor (Factor IX), and antihemophilic factor (AHF) were normal, while that of Fletcher factor was 10–15% that of pooled normal plasma (5). Immunologic assays

TABLE I

Effect of Kaolin-Activated HF, Kallikrein, and Activated PTA upon Fitzgerald Trait Plasma

	Clotting time				
	Fitzgerald trait	Hageman trait	Fletcher trait	PTA deficient	Christmas disease
	s	*s*	*s*	*s*	*s*
PTT*					
Plus kaolin-activated HF	237	59	62	>300	—
Plus kaolin-albumin	>300	>300	230	>300	—
Kaolin-activated PTT‡					
Plus kallikrein	234	213	75	151	—
Plus buffer	>300	>300	226	204	—
PTT§					
Plus activated PTA	75	77	76	75	>300
Plus buffer	>300	>300	>300	>300	>300

* 0.4 ml HF (1.25 U/ml, specific activity: 50 U/mg protein) in bovine crystalline albumin (1 mg/ml) or bovine albumin was shaken with 0.1 ml kaolin (50 mg/ml) for 10 min at 37°C in a 12×75-mm polystyrene tube. 0.1 ml kaolin-activated HF or kaolin-albumin was then added to 0.1 ml Centrolex "0" and 0.1 ml test plasma in a 10×75-mm glass tube. After 1 min incubation at 37°C, 0.1 ml 0.025 M $CaCl_2$ was added and the clotting time was determined.

‡ 0.1 ml plasma kallikrein (68 μmol MeOH released/ml per h, specific activity: 755 μmol MeOH/mg protein/hour) or barbital-saline buffer was added to 0.1 ml test plasma and 0.1 ml kaolin-Centrolex "0" in a 10×75-mm glass tube. After 1 min incubation at 37°C, 0.1 ml 0.025 M $CaCl_2$ was added and the clotting time was measured.

§ 0.1 ml trypsin-activated PTA or barbital-saline buffer was added to 0.1 ml test plasma and 0.1 ml Centrolex "0" in a 10×75-mm polystyrene tube. After 1 min incubation at 37°C, 0.1 ml 0.025 M $CaCl_2$ was added and the clotting time was determined.

of HF and AHF were in agreement with these data. On immunodiffusion the concentration of Fletcher factor appeared to be diminished. Normal plasma, or plasmas deficient in HF, PTA, or Fletcher factor, at a concentration of 5%, shortened the kaolin-activated PTT from >500 to 94–106 s, thus demonstrating the defect was not due to an inhibitor; killer whale plasma, functionally deficient in HF and Fletcher factor, had approximately 65% of the corrective activity of pooled normal human plasma, while that of chickens and Muscovy ducks had less than 5%. Unlike Fletcher trait plasma (1), the PTT of the patient's plasma was not significantly shortened by prolonged incubation with kaolin. Functional activity of $C\bar{1}$ inactivator in the patient's plasma was 8.5 U/ml (normal range: 6–12 U/ml).

Addition of activated PTA shortened the PTT of the patient's plasma, PTA-deficient plasma, or HF-deficient plasma to the same degree, from >300 to 75 s, while addition of kaolin-activated HF or of a plasma kallikrein (i.e., activated Fletcher factor) was without appreciable effect (Table I), as if the patient's defect were at a step after activation of HF and Fletcher factor but before participation of activated PTA.

The agent in normal plasma correcting the patient's clotting defect was stable at −70°C for at least 2 yr, and it lost only 20% activity after heating of plasma at 56°C for 30 min. It was not significantly adsorbed from oxalated plasma by tricalcium phosphate (10 mg/ml) or from citrated plasma by alumina Cγ gel. It was not significantly removed by small amounts of Celite (1 ~ 2 mg/ml plasma), whereas most of PTA and Fletcher factor activity was adsorbed under these conditions (Fig. 1). When normal plasma was adsorbed with 5 mg Celite 512 per ml plasma, and the supernatant plasma was incubated at 37°C for 18 h, it contained approximately 50% of the factor correcting the patient's defect and 70% of HF activity, but almost no PTA and Fletcher factor activity. It was completely removed from plasma by Celite at a concentration of 40 mg/ml and could then be partially eluted by 10% sodium chloride in 0.05 M Tris-chloride buffer (pH 8.0). It was present in normal serum and in the fraction of plasma soluble at 20% and insoluble at 60% saturation with neutral ammonium sulfate. Unlike PTA, Fletcher factor and the precursor of HF-cofactor (an agent needed for surface-mediated activation of plasminogen [23]), the corrective agent was adsorbed from normal or HF-deficient plasma onto columns of QAE-Sephadex A-50, an anionic resin, from which it could be eluted at an ionic strength slightly higher than that releasing HF;

TABLE II

Effect of Addition of Ellagic Acid, HF Fragments, and Kallikrein upon Kinin Generation

Sample	Kinin activity
	nanogram bradykinin equivalent per 0.1 ml sample
Normal + ellagic acid*	11.6
HF deficient + ellagic acid	0
Fletcher trait + ellagic acid	0
Fitzgerald trait + ellagic acid	0
Fitzgerald trait + HF deficient + ellagic acid	6.0
Fitzgerald trait + Fletcher trait + ellagic acid	2.3
Fletcher trait + HF fragments‡	0
Fitzgerald trait + HF fragments	0
Fletcher trait + Fitzgerald trait + HF fragments	5.0
Fletcher trait + kallikrein§	3.6
Fitzgerald trait + kallikrein	0
Fitzgerald trait + Fletcher trait + kallikrein	2.8
Fitzgerald trait + buffer + kallikrein	0

* 0.05 ml test plasma was incubated with 0.05 ml 100 μM ellagic acid, 0.05 ml 0.01 M *o*-phenanthroline, and 0.35 ml barbital-saline buffer (pH 7.4) at 37°C in a 12×75-mm polystyrene tube. After 12 min, suitable portions were tested on a uterus muscle for kinin activity in comparison with a bradykinin standard, expressing the results as nanogram bradykinin equivalent generated per 0.1 ml of the sample.

‡ 0.05 ml test plasma was incubated with 0.015 ml HF fragments (25 μg protein/ml), 0.05 ml 0.01 M *o*-phenanthroline, and 0.35 ml barbital-saline buffer at 37°C in 12 × 75-mm polystyrene tubes. After 2 min, suitable samples were tested as described above.

§ 0.05 ml test plasma was incubated with 0.025 ml plasma kallikrein (68 μmol MeOH released/ml per h), 0.05 ml 0.01 M *o*-phenanthroline, and 0.35 ml barbital-saline buffer at 37°C in 12×75-mm polystyrene tubes. After 10 min, suitable portions were tested as described above.

further purification was possible upon columns of SP-Sephadex C-50, a cationic resin. Preliminary gel filtration studies suggest a molecular weight of about 160,000. Batch fractionation of small amounts of plasma allowed ready demonstration of corrective activity in eluates of normal plasma, whereas a similar fraction of the patient's plasma lacked this property. Additional delineation of the properties of this agent has not yet been possible.

Under certain conditions, surface contact shortens the Thrombotest time of human plasmas other than those deficient in HF, Fletcher factor, or Factor VII (18, 24). When the patient's plasma was incubated with kaolin at 0°C for 4 h, the Thrombotest time did not shorten, whereas that of equal mixtures of his plasma and plasmas deficient in HF or Fletcher factor shortened significantly, from 43 to 25 and from 40 to 24 s, respectively. Thus, the patient's plasma seemed deficient in an agent other than HF, Fletcher factor, or Factor VII, required for this phenomenon.

Studies on generation of kinins and esterolytic activity in Fitzgerald trait. Kinin-like agents inducing contraction of the estrous rat uterus evolve when normal plasma is incubated with ellagic acid (10 μM), an activator of HF, a property lacking in plasmas deficient in HF or Fletcher factor. Similarly, generation of kinin-like agents was not observed in the patient's plasma, incubated with ellagic acid, but was found in equal mixtures of the patient's plasma and either HF-deficient or Fletcher trait plasma (Table II). The patient's plasma contained diminished amounts of Fletcher factor (see above); not surprisingly, kinin generation in an equal mixture of the patient's and Fletcher trait plasmas was detectable, but was less than normal.

Kinins evolve in normal or HF-deficient plasma incubated with HF fragments, but not in similarly treated Fletcher trait plasma (11). Kinin-like agents could not be detected in the patient's plasma upon incubation with HF fragments, a defect corrected by adding an equal volume of Fletcher trait plasma (Table II).

Addition of partially purified plasma kallikrein induced kinin generation in Fletcher trait plasma, but not in the patient's plasma. This was not due to an inhibitor since kinins generated in an equal mixture of the pa-

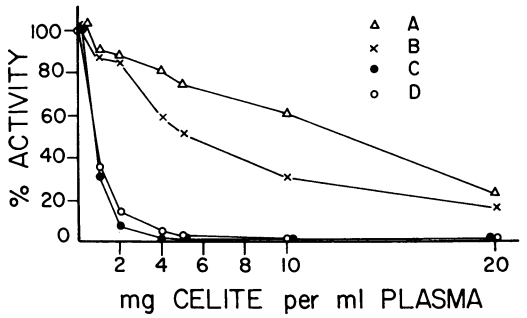

FIGURE 1 Effect of Celite adsorption upon various clotting factor activities. Normal citrated plasma was mixed with varying amounts of Celite 512 in 12 × 75-mm polystyrene tubes at 37°C for 10 min. After centrifugation, the supernatant plasma was separated and incubated at 37°C for 18 h. The following clotting factor activities were assayed in the incubated supernatant plasma which was free of activated PTA activity: (A) HF, (B) Fitzgerald factor, (C) Fletcher factor, and (D) PTA. The results were expressed as percentage of activity left in the plasma relative to that of the plasma treated similarly without Celite.

tient's and Fletcher trait plasmas upon addition of kallikrein, but not in an equal mixture of the patient's plasma and barbital-saline buffer (Table II). The patient's plasma contained adequate amounts of kininogen, demonstrated by incubation of boiled plasma with trypsin; the concentration of kininogen was estimated to be about half that of pooled normal plasma. The rate of inactivation of synthetic bradykinin was approximately the same in the patient's and in normal plasma, demonstrating the absence of excessive kininase activity.

When normal plasma is incubated with kaolin, esterolytic activity generates rapidly (25); this property, de-

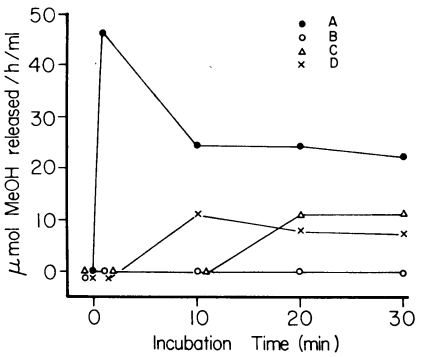

FIGURE 2 Kaolin activation of plasma TAME esterase activity in (A) normal plasma, (B) Fitzgerald trait plasma, (C) Fletcher trait plasma, and (D) 1:1 mixture of Fitzgerald trait and Fletcher trait plasma. 0.1 ml test plasma was incubated with 0.1 ml kaolin suspension (10 mg/ml 0.1 M sodium phosphate buffer, pH 7.5 in 0.15 M NaCl) in 12 × 75-mm polystyrene tubes at 25°C. At intervals, 2 ml of ice-cold 0.05 M TAME was added to the tubes and esterase activity was measured as described in the Methods section.

TABLE III
Kaolin-Induced Generation of Fibrinolytic Activity of Fitzgerald Trait

Test plasma	Lysis time
	min
Fitzgerald trait	>120
Hageman trait	>120
Fletcher trait	>120
50% Fitzgerald + 50% Hageman trait	8
50% Fitzgerald + 50% Fletcher trait	8
Normal	7

0.5 ml test plasma, 0.25 ml kaolin (8 mg/ml), and 9.25 ml 0.01 M sodium acetate buffer (pH 4.8) were incubated together in 17 × 100-mm polystyrene tubes at 37°C for 60 min. After centrifugation, the supernatant solution was discarded and the euglobulin precipitate, containing kaolin, was resuspended in 0.5 ml barbital-saline buffer. 0.2-ml portions of the euglobulin-kaolin suspension were mixed with 0.1 ml bovine fibrinogen (3 mg/ml) and 0.1 ml bovine thrombin (Parke Davis & Co., Detroit, Mich.) in 10 × 75-mm polystyrene tubes. The clot lysis time was measured at 37°C.

fective in HF-deficient and Fletcher factor-deficient plasmas (11, 15), has been ascribed to generation of a plasma kallikrein (15). Evolution of esterolytic activity, tested upon TAME, was deficient in the patient's plasma and was only partially corrected by addition of Fletcher trait plasma, presumably because the patient's plasma contained diminished amounts of prekallikrein and Fletcher trait plasma contained none (Fig. 2).

Studies on generation of fibrinolytic activity in Fitzgerald trait. Fibrinolytic activity generates in normal diluted and acidified plasma incubated with kaolin (26) an effect mediated by an agent called HF-cofactor (23) or plasminogen activator (27). Generation of fibrinolytic activity in the patient's plasma was deficient, an abnormality also observed in HF-deficient and Fletcher factor-deficient plasmas (Table III). Functional and immunodiffusion assays showed that defective generation of fibrinolytic activity in the patient's plasma was not due to deficiency of plasminogen or to increased amounts of plasmin inhibitors or fibrinogen. Defective generation of fibrinolytic activity was corrected by addition of equal volumes of HF-deficient or Fletcher factor-deficient plasma, which reduced the lysis time of fibrin clots from >120 min for each plasma alone to 8 min. Unlike Fletcher trait plasma (10), the clot lysis time of the patient's plasma was not significantly reduced by prolonged surface contact before acidification and dilution.

Ogston, Ogston, Ratnoff, and Forbes (23) demonstrated that HF-induced fibrinolysis required the presence not only of HF-cofactor but also of an ill-defined additional agent. This substance, previously found in

TABLE IV

Effect of Ellagic Acid on the Development of Permeability-Enhancing Activity in Fitzgerald Trait Plasma

Plasma (diluted 1/100)	Diluent	Permeability-enhancing activity 0 min	12 min
		mm	*mm*
Normal	Buffer	2.7	2.8
Normal	Ellagic acid	2.8	6.3
Hageman trait	Ellagic acid	2.4	2.4
Fletcher trait	Ellagic acid	3.3	1.6
Fitzgerald trait	Ellagic acid	2.8	1.7
50% Fitzgerald trait + 50% Hageman trait	Ellagic acid	3.1	5.4
50% Fitzgerald trait + 50% Fletcher trait	Ellagic acid	2.5	5.1
Buffer	Ellagic acid	—	3.2

0.05 ml plasma was diluted with 4.95 ml 0.0001 M ellagic acid or with barbital-saline buffer in 13 × 100-mm silicone-coated glass tubes. Each tube was incubated at 37°C and, at the time indicated, 0.1-ml samples were injected intracutaneously into each of four guinea pigs. The permeability-enhancing activity was measured as described in the Methods sections as the mean diameter of blued spots.

Fletcher trait plasma (10), was also present in the patient's plasma.

Studies on the generation of PF/Dil in Fitzgerald trait. Normal plasma, diluted and incubated with ellagic acid, enhances vascular permeability in guinea pig skin (28). This property, designated PF/Dil, does not appear in similarly treated HF-deficient (28) or Fletcher factor-deficient (2, 10) plasmas, nor did it in the patient's plasma, whereas PF/Dil developed in an equal mixture of the patient's plasma and either of the other two (Table IV). The addition of ellagic acid-activated HF or HF fragments generated PF/Dil in Hageman trait plasma, but not in the patient's plasma (Table V).

Family studies in Fitzgerald trait. We do not know the range of concentration in normal plasma of the agent apparently deficient in the patient's plasma, but that of the patient's sole surviving sister was 40% that of a pool of normal plasma, and that of his only son was 70%. The titer was 120% of the pooled plasma in an individual with Fletcher trait, and ranged from 52 to 105% in four subjects with Hageman trait and from 80 to 130% in four subjects with PTA deficiency.

DISCUSSION

When human plasma is exposed to glass or similar negatively charged agents, a series of reactions ensues consequent upon the activation of HF. These reactions include (*a*) enhancement of clotting via activation of PTA and augmentation of the clot-promoting properties of factor VII, (*b*) elaboration of kinins via activation of Fletcher factor (a plasma prekallikrein), (*c*) induction of fibrinolysis via activation of HF cofactor, (*d*) enhancement of vascular permeability upon dilution of plasma, and (*e*) chemotaxis. Evidence has accumulated that Fletcher factor, activated by HF, either mediates or intensifies these various reactions (1, 2).

In the experiments reported, the prolonged activated PTT in the plasma of an asymptomatic man, Allen Fitzgerald, was related to the deficiency of an agent not identified with known clotting factors. The defect, localized to a step after activation of HF and Fletcher factor and before participation of activated PTA, was corrected by human plasma deficient in HF, PTA, or Fletcher factor, or by cetacean plasma, deficient in HF

TABLE V

Effect of Ellagic Acid-Activated HF or HF Fragments on the Development of Permeability-Enhancing Activity

Plasma (diluted 1/100)	Diluent	Permeability-enhancing activity 0 min	12 min
		mm	*mm*
Normal	Ellagic acid-HF	5.6	6.3
Hageman trait	Ellagic acid-HF	5.8	7.6
Fitzgerald trait	Ellagic acid-HF	2.9	4.6
Buffer	Ellagic acid-HF	—	4.0
Hageman trait	HF fragments	2.3	5.3
Fletcher trait	HF fragments	1.3	2.0
Fitzgerald trait	HF fragments	1.1	2.0
Buffer	HF fragments	2.6	—

0.02 ml plasma was diluted with 2 ml ellagic acid-HF (0.2 μg HF/ml 0.0001 M ellagic acid) or HF fragments (0.05 μg HF fragments/ml barbital-saline buffer) in 13 × 100-mm silicone-coated glass tubes. Each tube was incubated at 37°C, and, at the times indicated, 0.1-ml portions were injected intracutaneously into each of four guinea pigs. The permeability-enhancing activity was measured as described in the Methods section.

and Fletcher factor, but not by certain fowl plasmas. The shortening of the Thrombotest time in cold-stored plasma, the generation of kinins, esterolytic activity resembling a plasma kallikrein, kaolin-induced fibrinolytic activity, and the plasma property designated PF/Dil were all impaired.

These observations are of particular interest because previous studies have demonstrated that the generation of activated PTA (4) and of fibrinolytic activity (23) and the augmentation of factor VII activity, as measured by shortening of the Thrombotest time in cold-stored plasma (24), all require not only the presence of activated HF and Fletcher factor, but other, not yet unidentified agents. It is not yet clear whether the agent deficient in Fitzgerald trait plasma is identical with one or another of these agents, but this plasma appeared to contain adequate amounts of one additional agent required for fibrinolysis described by Ogston et al. (23).

The several defects in the patient's plasma were corrected by addition of HF-deficient or Fletcher factor-deficient plasma. Addition of Fletcher trait plasma was less effective in kinin-generating or esterolytic systems than Hageman trait plasma because the patient's plasma contained only 10–15% Fletcher factor.

Failure of purified plasma kallikrein to correct the clotting defect and to induce kinin-like properties in the patient's plasma fortified the view that the deficient agent is not Fletcher factor. Separation from the precursor of HF-cofactor is less secure because the identity of this agent is disputed, but this latter substance, like plasma prekallikrein and PTA, does not adsorb to anionic exchange resins. In contrast, a crude fraction that corrected the clotting defect could be prepared from normal plasma by adsorption and elution from anionic exchange resins. A similar fraction of the patient's plasma was deficient in this property. HF, too, is adsorbed to anionic exchange resins (29), but the corrective fraction required higher salt concentrations for elution than HF, it could readily be prepared from HF-deficient plasma, and it probably has a higher molecular weight.

The mode of action of the agent deficient in the patient's plasma is not yet clear. Experiments demonstrating that activated HF and kallikrein did not shorten the clotting time of the patient's plasma nor induce kinin generation suggest that it may participate in reactions subsequent to the activation of prekallikrein by activated HF or HF fragments. No evidence is yet available to suggest that the newly recognized agent (or agents) is activated from a precursor state during the clotting process. Nor do we know if it acts by inducing a further change in kallikrein, by altering the various proenzymes participating in surface-mediated reactions, or by serving as a necessary cofactor in the several reactions studied, including activation of PTA, augmentation of the action of factor VII, surface-induced fibrinolysis, generation of plasma esterolytic activity, kinin generation, and elaboration of the property described as PF/Dil.

These experiments, admittedly incomplete, support the view that the agent deficient in the patient's plasma is not identified with other recognized agents. Tentatively then, it can be called Fitzgerald factor until its nature is defined. In addition to HF and Fletcher factor, Fitzgerald factor appears to participate in surface-mediated biological reactions. Whether Fitzgerald factor has been described by earlier investigators is not known.

Note added in proof. Since this paper was accepted for publication, two additional cases with a clotting defect identical to that of Fitzgerald trait have been detected. Donaldson, Glueck, and Miller (30) will report that the abnormality appears to be inherited as an autosomal recessive trait. The concentration of kininogen in their patient's plasma was less than 1% of normal. Wuepper, Miller, and Lacombe (31) have described a patient, said to have Flaujeac factor deficiency, whose abnormality was corrected by fractions of plasma containing high molecular weight (HMW) kininogen. Our own patient is deficient in HMW, but not low molecular weight kininogen, and we have been unable to separate fractions with Fitzgerald factor activity from those containing HMW kininogen despite further purification.

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