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Evidence That Fitzgerald Factor Counteracts Inhibition by Kaolin or Ellagic Acid of the Amidolytic Properties of a Plasma Kallikrein

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Fitzgerald trait, an asymptomatic disorder, is associated with abnormalities of surface-mediated plasma reactions, including coagulation via the intrinsic pathway, augmentation of the clot-promoting properties of factor VII, kaolin-mediated fibrinolysis, kinin generation, and enhancement of vascular permeability by diluted plasma (PF/Dil). These abnormalities can be corrected by Fitzgerald factor, an agent probably identical with high molecular weight kininogen found in normal, but not Fitzgerald-trait plasma. Our preparations of Fitzgerald factor possessed a second property. Amidolysis of α-*N*-benzoyl-L-proline-L-phenylalanine-L-arginine-*p*-nitroanilide by a plasma kallikrein (activated Fletcher factor) was inhibited by kaolin or solutions of ellagic acid. Addition of preparations of Fitzgerald factor to kaolin or to solutions of ellagic acid counteracted their inhibitory properties. The action of these preparations was duplicated by solutions of cytochrome C or IgG, suggesting that these agents may inhibit the negative charges of kaolin or ellagic acid. Fitzgerald factor enhanced amidolysis of both normal and Fitzgerald-trait plasmas exposed to kaolin, effects not duplicated by cytochrome C or IgG. Whether or not the two properties of our preparations of Fitzgerald factor are related to the same agent is not yet certain. The relationship between these observations and the biologic role of Fitzgerald factor remains to be investigated.

FITZGERALD FACTOR is an agent in normal human plasma that shortens the abnormally long partial thromboplastin time of individuals with Fitzgerald trait, whose plasmas appear to be functionally deficient in this substance.[1,2] Fitzgerald factor has been identified with high molecular weight (MW) kininogen.[2-5] How it influences the partial thromboplastin time is not known, but earlier studies have suggested that it participates in clotting subsequent to activation of Hageman factor (factor XII) and Fletcher factor (a plasma prekallikrein).[5]

The synthetic amide α-*N*-benzoyl-L-proline-L-phenylalanine-L-arginine-*p*-nitroanilide (PPAN) is hydrolyzed by a plasma kallikrein.[6] The data presented demonstrate that hydrolysis is inhibited by kaolin or ellagic acid, components of reagents used to measure the partial thromboplastin time and other surface-mediated reactions. Preparations of Fitzgerald factor, a substrate of plasma kallikrein, counteract this inhibition, perhaps through an action shared by cytochrome C and IgG, positively charged proteins. Thus, this property of Fitz-

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gerald factor must be distinguished from its specific capacity to correct defective surface-mediated reactions in Fitzgerald-trait plasma.

MATERIALS AND METHODS

Unless otherwise noted, pooled normal citrated plasma, kaolin, ellagic acid, purified plasma kallikrein, and barbital-saline buffer were prepared or obtained as described earlier.[7,8] Plasma from the index individual with Fitzgerald trait was obtained through the courtesy of Dr. Robert Waldmann and Dr. Joseph P. Abraham, Henry Ford Hospital, Detroit, Mich. Fletcher-trait plasma was kindly supplied by Dr. C. Abildgaard, University of California, Davis. Two batches of plasma kallikrein were used. One released 90 μmole of methanol/ml/hr from *p*-toluene sulfonyl-L-arginine methyl ester[8] and had a specific activity of 360 μmole methanol released/mg protein/hr, while another released 7.5 μmole of methanol/ml/hr and had a specific activity of 197 μmole methanol released/mg protein/hr. The plasma kallikrein preparations contained traces of activated plasma thromboplastin antecedent (PTA, factor XI) and Hageman factor-cofactor. The plasma kallikrein was diluted suitably with phosphate-saline buffer before use.

Fitzgerald factor (high MW kininogen) was prepared at room temperature by chromatography of citrated normal human plasma, adsorbed with Cγ alumina gel, successively upon QAE Sephadex A-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), Sephadex G-200 (Pharmacia), and SP-Sephadex C-50 (Pharmacia) by a technique described elsewhere.[5,9] Two lots were used, one containing 10.8 U/ml of barbital-saline buffer (specific activity, 11.0 U/mg protein), and the other, filtered twice through Sephadex G-200, 7.6 U/ml (12.4 U/mg protein), 1 unit being the amount detected in 1 ml of pooled normal human plasma.[5] These preparations were not homogeneous when examined by analytic disc gel electrophoresis, possessing a major band of material stained by Coomassie brilliant blue that had Fitzgerald factor-like activity, and several minor bands. They were devoid of other recognized clotting factors, plasminogen, or prekallikrein.

Human albumin (Schwarz-Mann, Orangeburg, N.Y.), cytochrome C (Nutritional Biochemical Corp., Cleveland, Ohio) and IgG (Miles Research Laboratories, Kankakee, Ill.) were dissolved at a concentration of 0.1% in phosphate-saline buffer and diluted further as required.

A stock aqueous solution of 10^{-3} *M* α-*N*-benzoyl-L-proline-L-phenylalanine-L-arginine-*p*-nitroanilide hydrochloride (PPAN, Pentapharm, Basel, Switzerland) was stored at 4°C in silicone-coated glass tubes and diluted, when appropriate, tenfold in phosphate-saline buffer before use.

Kaolin was suspended at concentrations of 10 or 12.5 mg/ml in phosphate-saline buffer.

Glacial acetic acid was reagent grade (Fisher Scientific Co., Fairlawn, N.J.). *p*-Nitroaniline (Eastman Kodak Co., Rochester, N.Y.) was dissolved at a concentration of 0.001 *M* in phosphate-saline buffer and diluted serially in the same buffer; a standard solution was prepared by mixing 0.3 ml of *p*-nitroaniline solution, 2.4 ml of phosphate-saline buffer, and 0.3 ml glacial acetic acid.

Phosphate saline buffer was 0.1 *M* sodium phosphate (pH 7.5) in 0.15 *M* sodium chloride; barbital-saline buffer was 0.025 *M* sodium barbital (pH 7.5) in 0.125 *M* sodium chloride.

The effect of Fitzgerald factor upon amidolysis by plasma kallikrein was tested by incubating 0.02 ml Fitzgerald factor, 0.08 ml kaolin (12.5 mg/ml phosphate-saline buffer) or buffer, and 0.1 ml plasma kallikrein for 0-8 min at 25°C in 12 × 75-mm polystyrene tubes. Unless otherwise noted, Fitzgerald factor was always added to kaolin before addition of plasma kallikrein. Thereafter, 2.5 ml of 10^{-4} *M* PPAN, prewarmed to 37°C, was added and the mixture was incubated at 37°C for 10 min. The reaction was stopped by addition of 0.3-ml glacial acetic acid, the mixture was centrifuged at room temperature for 10 min at 2500 rpm, and the optical density of the supernatant solution at 405 nm was measured in a 10-mm cuvette against a blank in which plasma kallikrein was added after all other reagents had been mixed. The optical density was compared with that of standard solutions of *p*-nitroaniline. In other experiments, 0.1 ml of Fitzgerald factor was mixed with 2.25 ml of 10^{-5} *M* ellagic acid in phosphate-saline buffer. A volume of 0.1 ml of plasma kallikrein was then added and the mixture was incubated at 37°C for 0-8 min, after which 0.25 ml of 10^{-3} *M* aqueous PPAN was added and incubation then continued for an additional 10 min. After addition of 0.3-ml glacial acetic acid, the optical density of the solution was read without centrifugation. The effect of human albumin, cytochrome C, or IgG was tested by substituting these proteins for Fitzgerald factor in assays containing kaolin.

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The effect of Fitzgerald factor upon amidolysis in mixtures of plasma was measured in 12 × 75-mm polystyrene tubes by pipetting, in order, 0.1 ml kaolin (10 mg/ml of phosphate-saline buffer), 0.02–0.05 ml Fletcher factor or buffer, and 0.05 or 0.1 ml plasma for 0–20 min at 25°C. Thereafter, 2.5 ml of 10^{-4} *M* PPAN in phosphate-saline buffer, prewarmed to 37°C, was added to each tube and incubation was continued for 30 min. The reaction was stopped by addition of 0.3 ml glacial acetic acid, and the optical density of the supernatant solution after centrifugation was measured at 405 nm against a blank in which plasma was added after all other reagents. Normal plasma was diluted tenfold with phosphate-saline buffer before addition to kaolin and Fitzgerald factor, while Fletcher trait and Fitzgerald-trait plasmas were tested undiluted. Other experiments were performed in which albumin, IgG, or cytochrome C were substituted for Fitzgerald factor at identical gravimetric concentrations.

RESULTS

The Effect of Fitzgerald Factor Upon Amidolysis by a Plasma Kallikrein

Hydrolysis of PPAN by plasma kallikrein was inhibited by kaolin (Table 1); kaolin was almost completely inhibitory at concentrations in the enzyme–substrate mixture as low as 0.1 mg/ml. PPAN was not hydrolyzed by preparations of Fitzgerald factor at a concentration of 0.08 U/ml in a mixture of this agent and PPAN. Addition of Fitzgerald factor at concentrations as low as 0.3 U/ml to kaolin before addition of plasma kallikrein (i.e., at concentrations of Fitzgerald factor in the enzyme–substrate mixture of 0.02 U/ml) significantly reduced inhibition of amidolysis by kaolin; lesser concentrations of Fitzgerald factor were ineffective under these conditions. This property of our Fitzgerald factor preparations appeared in the same fractions as those containing Fitzgerald factor, as measured in clotting assays, upon chromatography with both anion and cation exchangers. Fitzgerald factor did not appreciably alter amidolysis by kallikrein in the absence of kaolin.

In similar experiments, plasma kallikrein and kaolin were incubated at 25°C for intervals up to 8 min before addition of Fitzgerald factor and PPAN. In-

Table 1. The Effect of Fitzgerald Factor Upon Inhibition by Kaolin of Amidolysis by a Plasma Kallikrein

Fitzgerald Factor* (U/ml)	Kaolin	p-Nitroaniline Released (nmole/ml/10 min)
0.08	0	22.9
0.04	0	22.9
0.02	0	22.0
0	0	19.7
0.08	+	18.2
0.04	+	14.1
0.02	+	7.9
0	+	0.7

*In order, 0.02-ml Fitzgerald factor (10.8, 5.4, or 2.7 U/ml; specific activity, 11.3 U/mg/protein) or phosphate-saline buffer, 0.08 mg kaolin (12.5 mg/ml) or buffer, and 0.1 ml of kallikrein (9.0 μmoles of methanol released/ml/hr; specific activity, 360 μmole methanol released/mg protein/hr) were mixed and incubated at 25°C for 1 min. Next, 2.5 ml 10^{-4} M PPAN was added and incubation continued at 37°C in 12 × 75-mm polystyrene tubes for 10 min. Thereafter, 0.3 ml glacial acetic acid was added, and, after centrifugation, the optical density of the supernatant fluid was measured in comparison to that of *p*-nitroaniline. See Methods. The concentration of Fitzgerald factor recorded in the table is that in the enzyme–substrate mixture.

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Table 2. The Inhibitory Effect of Kaolin Upon Amidolysis by a Plasma Kallikrein

Preliminary Incubation Mixture	Preliminary Incubation Period (min)	Second Addition	p-Nitroaniline Released (nmole/ml/10 min)
Kallikrein + kaolin*	0	Fitzgerald F.	19.0
	1		14.4
	2		12.8
	4		8.9
	8		5.3
Fitzgerald F. + kaolin†	0	Kallikrein	22.6
	1		23.7
	2		26.1
	4		26.2
	8		25.9

*0.1 ml plasma kallikrein (9.0 μmole methanol released/ml/hr; specific activity 360 μmole methanol released/mg protein/hr) was incubated at 25°C for 8 min with 0.08 mg kaolin (12.5 mg/ml). At the intervals noted, 0.02 ml Fitzgerald factor (10.8 U/ml; specific activity, 11.0 U/mg protein) was added. After a total elapsed time of 8 min, 2.5 ml of 10^{-4} M PPAN was added and the mixture was incubated at 37°C for 10 min. Thereafter, 0.3 ml glacial acetic acid was added and, after centrifugation, the optical density of the supernatant fluid was measured in comparison to that of p-nitroaniline. See Methods.

†0.02 ml of Fitzgerald factor was incubated at 25°C for 8 min with 0.08 ml of kaolin. At the intervals noted, 0.1 ml of plasma kallikrein was added. After a total elapsed time of 8 min, 2.5 ml of 10^{-4} M PPAN was added, and the procedure outlined in * was carried out.

Table 3. The Inhibitory Effect of Ellagic Acid Upon Amidolysis by a Plasma Kallikrein

Preliminary Incubation Mixture	Preliminary Incubation Period (min)	Second Addition	p-Nitroaniline Released (nmole/ml/10 min)
Buffer*	0	Kallikrein	15.6
	8	Kallikrein	18.3
Buffer + ellagic acid†	0	Kallikrein	0.8
	8	Kallikrein	1.2
Kallikrein + ellagic acid‡	0	Fitzgerald F.	21.1
	8	Fitzgerald F.	5.1
Fitzgerald F. + ellagic acid§	0	Kallikrein	14.7
	8	Kallikrein	16.7

*0.235 ml phosphate–saline buffer was incubated at 37°C for 8 min in 12 × 75-mm polystyrene tubes. At 0 or 8 min, 0.1 ml of kallikrein (4.5 μmole methanol released/ml/hr; specific activity, 360 μmole methanol released/mg protein/hr) was added. After a total elapsed time of 8 min, 0.25 ml 10^{-3} M aqueous PPAN was added to each tube, the mixture was incubated for an additional 10 min, and the reaction then stopped by addition of 0.3 ml of glacial acetic acid. The optical density of the mixture was measured in comparison to p-nitroaniline. (See Methods.)

†0.1-ml buffer was incubated at 37°C for 8 min with 2.25 ml synthetic ellagic acid (10^{-5} M). At 0 or 8 min, 0.1 ml kallikrein was added. After a total elapsed time of 8 min, 0.25 ml PPAN was added, and the procedure outlined in * was then continued.

‡0.1 ml kallikrein was mixed with 2.25 ml 10^{-5} M ellagic acid and 0.1 ml Fitzgerald factor (3.0 U/ml; specific activity, 12.4 U/mg protein) was added at 0 or 8 min, and the procedure outlined in * was then continued.

§0.1 ml Fitzgerald factor was mixed with 2.25 ml 10^{-5} M ellagic acid and 0.1 ml kallikrein was added at 0 or 8 min, and the procedure outlined in * was then continued.

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cubation with kaolin progressively inhibited the amidolytic properties of plasma kallikrein (Table 2). In additional experiments, incubation of Fitzgerald factor and plasma kallikrein for 8 min before addition of kaolin did not enhance amidolysis.

The property of kaolin thought to be responsible for activation of Hageman factor is its negative surface charge. Solutions of ellagic acid are also known to activate Hageman factor.[10] Incubation of plasma kallikrein with 10^{-5} *M* ellagic acid for 8 min decreased its amidolytic activity, an effect suppressed by preliminary incubation of ellagic acid with Fitzgerald factor (Table 3). Ellagic acid itself had no effect upon the assay for *p*-nitroanaline when added after amidolysis was halted by glacial acetic acid. Thus, the effect of kaolin was not related to surface alone, but to a property shared by ellagic acid.

Were the action of our Fitzgerald factor preparations in these experiments related to its capacity to neutralize the negative surface charge of kaolin or ellagic acid, one might expect that these effects would be duplicated by equivalent concentrations of other positively charged proteins. In fact, IgG and cytochrome C were almost as effective as Fitzgerald factor, tested at the same gravimetric concentration of protein. Albumin at the same concentration was much less effective (Table 4). Neither albumin, IgG, nor cytochrome C shortened the prolonged kaolin partial thromboplastin time of Fitzgerald-trait plasmas.

Table 4. The Effect of Fitzgerald Factor, IgG, Cytochrome C, and Albumin Upon Inhibition by Kaolin of Amidolysis by a Plasma Kallikrein

Agent Tested* (μg/ml)		Kaolin	*p*-Nitroaniline Released (nmoles/ml/10 min)
Fitzgerald factor	4.6	+	11.0
	2.3	+	7.1
	1.2	+	2.5
IgG	4.6	+	5.7
	2.3	+	2.2
	1.2	+	0.6
Cytochrome C	4.6	+	7.0
	2.3	+	3.8
	1.2	+	1.4
Albumin	4.6	+	0.9
	2.3	+	2.5
	1.2	+	1.4
Buffer		+	0
Buffer		0	10.8

*0.02 ml Fitzgerald factor (0.62, 0.31, or 0.155 mg/ml; specific activity, 12.4 U/mg protein), IgG, cytochrome C, or albumin at the same gravimetric concentrations or phosphate–saline buffer was incubated at 25°C for 1 min with 0.08 mg kaolin (12.5 mg/ml) or buffer, 0.1 ml of kallikrein (7.5 μmoles of methanol released/ml/hr; specific activity, 197 μmoles methanol released/mg protein/hr). Next, 2.5 ml 10^{-4} *M* PPAN was added and incubation continued at 37°C in 12 × 75-mm polystyrene tubes for 10 min. Thereafter, 0.3 ml glacial acetic acid was added, and, after centrifugation, the optical density of the supernatant fluid was measured in comparison to *p*-nitroaniline. See Methods. The concentration of the proteins added recorded in the table is that in the enzyme–substrate mixtures.

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Table 5. The Effect of Fitzgerald Factor Upon the Evolution of Amidolytic Activity in Kaolin-treated Normal Plasma

Preliminary Incubation Mixture*	Preliminary Incubation Period (min)	p-Nitroaniline Released (nmoles/ml/30 min)
Plasma + buffer + kaolin	0	3.5
	3	17.9
	10	13.3
	20	7.1
Plasma + Fitzgerald F. + kaolin	0	8.2
	3	17.7
	10	20.5
	20	14.1

*A mixture of 0.1 ml kaolin (10 mg/ml), 0.05 ml Fitzgerald factor (7.6 U/ml; specific activity, 12.4 U/mg protein) or phosphate–saline buffer, and 0.05 ml pooled normal plasma, diluted tenfold with phosphate–saline buffer, was incubated for 0–20 min at 25°C in 12 × 75-mm polystyrene tubes. At the intervals indicated, 2.50 ml 10^{-4} M PPAN was added, and incubation was continued at 37°C for 30 min. After addition of 0.3 ml glacial acetic acid, the tubes were centrifuged, and the optical density of the supernatant fluid was compared with that of *p*-nitroaniline.

The Effect of Fitzgerald Factor Upon Amidolysis by Kaolin-treated Plasma

When normal plasma diluted tenfold was incubated with kaolin, amidolytic activity for PPAN evolved rapidly, and then decreased in intensity (Table 5); similar results had been reported, using the synthetic ester *p*-toluenesulfonyl-L-arginine methyl ester as the substrate.[11,12] The addition of Fitzgerald factor to the mixture of plasma and kaolin increased the intensity of amidolysis at each time tested over a period of 20 min. No amidolysis was observed in the absence of kaolin. When IgG, at the same protein concentration as that of the preparation of Fitzgerald factor, was substituted for this substance, no enhancement of amidolysis was observed.

In similar experiments, amidolytic activity evolved only slowly in undiluted Fitzgerald-trait plasma incubated at 25°C with kaolin (Table 6). Addition of small amounts of Fitzgerald factor to the kaolin before addition of Fitzgerald-trait plasma induced rapid generation of amidolytic activity; maximal activation was noted at a concentration of 0.07 U of Fitzgerald factor/ml in the preliminary mixture of plasma, kaolin, and Fitzgerald factor, when tested after 1 min at 25°C. Consonant with the low titer of Fletcher factor in the Fitzgerald-trait plasma, 15% that of the normal plasma pool in clotting assays,[2] the titer of amidolytic activity evolved was much less than that in normal plasma. No activity evolved when kaolin was omitted from the mixture, nor was amidolysis enhanced when IgG, albumin, or cytochrome C were substituted for Fitzgerald factor.

No amidolytic activity evolved under these conditions in Fletcher trait plasma incubated with Fitzgerald factor at a concentration of 0.54 U/ml in the initial mixture.

DISCUSSION

Four asymptomatic individuals have now been described in whom the partial thromboplastin time (PTT) has been abnormally long despite the presence of

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Table 6. The Effect of Fitzgerald Factor Upon the Evolution of Amidolytic Activity in Kaolin-treated Fitzgerald-Trait Plasma

Preliminary Incubation Mixture*	Preliminary Incubation Period (min)	p-Nitroaniline Released (nmoles/ml/30 min)
Plasma + buffer + kaolin	0	1.3
	1	1.9
	3	1.9
	10	1.5
	20	6.2
	30	8.4
	60	8.1
Plasma + Fitzgerald F. + kaolin	0	14.3
	1	18.3
	3	11.8
	10	5.6
	20	5.7
	30	5.4
	60	5.4

*A mixture of 0.1 ml kaolin (12.5 mg/ml), 0.02 ml Fitzgerald factor (10.8 U/ml; specific activity, 11.0 U/mg protein) or phosphate–saline buffer, and 0.1 ml Fitzgerald trait plasma was incubated 0–60 min at 25°C in 12 × 75-mm polystyrene tubes. At the intervals indicated, 2.50 ml 10^{-4} M PPAN was added, and incubation was continued at 37°C for 30 min. After addition of 0.3 ml glacial acetic acid, the tubes were centrifuged, and the optical density of the supernatant fluid was compared with that of *p*-nitroaniline.

all known clotting factors including Fletcher factor. These patients, variously described as having Fitzgerald trait,[2,3,5] Williams trait,[13] or Flaujeac trait[4] after the individuals studied, appear to lack an agent in plasma originally designated Fitzgerald factor,[1] but probably identical with high-molecular-weight kininogen.[2-5] Besides the abnormality measured in the PTT assay, the other surface-mediated reactions are all impaired in Fitzgerald trait.[5] The defect in Fitzgerald-trait plasma is corrected by addition of preparations of Fitzgerald factor.[5] Fitzgerald factor may be required for the activation of PTA in the presence of Hageman factor, plasma prekallikrein (Fletcher factor), and kaolin.[14,15]

The mode of action of the agents participating in surface-mediated reactions in plasma—Hageman factor, PTA, Fletcher factor, and Fitzgerald factor—may be complex. Interactions may occur among agents adsorbed to negatively charged surfaces, or between adsorbed agents and substances in the fluid phase. Furthermore, distinction must be made between the specific characteristics of the various reactants, and nonspecific activities shared by other proteins with similar properties, for example, surface charge. The experiments reported herein illustrate the importance of distinguishing between such specific and nonspecific effects. Fitzgerald factor corrects the functional defect of Fitzgerald-trait plasmas in surface-mediated systems. Besides this property, our preparations of Fitzgerald factor appear to have effects related to their electrical charge. Fitzgerald factor behaves chromatographically like a positively charged agent. Preparations of Fitzgerald factor counteract the inhibitory properties of negatively charged kaolin, widely used in the study of surface-mediated reactions. The amidolytic properties of a plasma kallikrein for PPAN are inhibited by

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kaolin, an effect prevented by prior addition of Fitzgerald factor. A similar result is obtained when ellagic acid, a soluble activator of Hageman factor, is substituted for kaolin, suggesting that the inhibitory properties of these agents are not directly related to solid surfaces, but to their negative charge. Solutions of cytochrome C and IgG, at gravimetric concentrations similar to that of Fitzgerald factor, also counteract the inhibitory properties of kaolin; albumin is much less effective. Presumably, then, our Fitzgerald factor preparations had a nonspecific effect upon these assays in contrast to their specific capacity to correct disordered surface-mediated reactions.

An alternate possibility, that Fitzgerald factor formed a complex with plasma kallikrein, and thus protected it from inhibition, could not be supported. High-molecular-weight kininogen (i.e., Fitzgerald factor) is a substrate of plasma kallikrein, but preliminary incubation of Fitzgerald factor with plasma kallikrein did not increase its capacity to counteract inhibition by kaolin.

Not unexpectedly, our preparations of Fitzgerald factor enhanced evolution of amidolytic activity in Fitzgerald-trait plasma incubated with kaolin. Our preparations, however, also enhanced amidolytic activity in normal plasma, containing Fitzgerald factor. In these experiments, Fitzgerald factor was added to kaolin before addition of plasma. Neither albumin, cytochrome C, nor IgG enhanced amidolysis in Fitzgerald trait plasma. The preparations of Fitzgerald factor, then, had two properties, an action upon the effect of kaolin or solutions of ellagic acid upon kallikrein and a specific corrective effect for the defect in Fitzgerald trait that was not shared by the other proteins tested.

Under the conditions used, no amidolytic activity evolved in Fletcher-trait plasma, deficient in the precursor of a plasma prekallikrein. The importance of these observations in assays for plasma kallikrein that are based upon evolution of esterolytic activity in kaolin-treated plasma remains to be explored.

Demonstration of the activity of our Fitzgerald factor preparations has been based upon highly artifactual systems. Whatever the physiologic role for this substance, individuals functionally deficient in Fitzgerald factor are asymptomatic. Further exploration of the role of Fitzgerald factor is needed to determine its importance in surface-mediated reactions. The recent studies warn against the assumption that, in artifactual systems containing negatively charged activators of surface-mediated reactions, the action of Fitzgerald factor is necessarily related to those specific properties defective in Fitzgerald-trait plasmas.

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