

# Flaujeac Trait

## DEFICIENCY OF HUMAN PLASMA KININOGEN

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**ABSTRACT** Flaujeac trait plasma resembled Hageman trait or Fletcher trait, in that the intrinsic coagulation pathway, plasma fibrinolytic pathway, kinin-forming system, permeability factor of dilution (PF/dil) phenomenon were abnormal. The defect in each assay was reconstituted by a factor separable from Hageman factor or Fletcher factor. This substance was an  $\alpha$ -globulin with an approximate mol wt of 170,000. Flaujeac plasma did not release a kinin upon incubation with kallikrein and was deficient in total kininogen antigen. Antiserum to kininogen inhibited the activity of the factor in solution. Flaujeac factor was identified as a kininogen of high molecular weight (HMW-kininogen).

The mean total kininogen antigen in four children of the proposita was 51% (range 34-62%) of normal. A functional coagulation assay of HMW-kininogen in the children was 34% (range 23-55%). The results were consistent with autosomal recessive inheritance.

The plasma pathways of intrinsic coagulation, fibrinolysis, kinin formation, and PF/dil generation are dependent upon HMW-kininogen. We believe this is the first demonstration of biological function for a kininogen apart from its role as a substrate for kallikreins.

## INTRODUCTION

Since the first description of Hageman trait as a coagulation factor deficiency (1), evidence has accumulated to show that Hageman factor participates in other reactions as well. These reactions include the plasma fibrinolytic pathway (2, 3), the kinin-forming system (4), and the generation of permeability reactions upon dilution of plasma, a phenomenon recognized by Miles and his

associates (5, 6) and attributed to a plasma globulin (PF/dil).<sup>1</sup>

In 1972, a second defect in the early-reacting components of the intrinsic coagulation pathway, known as Fletcher trait (7), was found to demonstrate abnormalities similar to those of Hageman factor deficiency (8). Fletcher trait was established as the lack of biologic activity of plasma prekallikrein (8, 9). It is inherited as an autosomal recessive defect.

Lacombe et al. (10, 11) have recently recognized the deficiency of yet another procoagulant, participating in the "contact phase" of coagulation. The deficiency was observed in an asymptomatic French Caucasian woman born of a consanguineous marriage. This report extends that work and demonstrates reduced fibrinolysis, kinin formation, and generation of PF/dil. Experiments are described which show that Flaujeac trait (identified by the surname of the proposita) represents the deficiency of human plasma kininogens and is inherited as an autosomal recessive defect. Of the two forms of plasma kininogen that were deficient in Flaujeac plasma, one, high mol wt (HMW-) kininogen, reconstituted each of the deficits of this unusual plasma.

## METHODS

**Reagents and chemicals.** Synthetic bradykinin (BRS 640, Sandoz Ltd., Basel, Switzerland), hexadimethrine bromide (Polybrene, Aldrich Chemical Co., Inc., Milwaukee, Wis.) and Ionagar No. 2 (Colab Laboratories Inc., Chicago Heights, Ill.) were purchased from their suppliers. Rabbit brain cephalin, a platelet substitute in coagulation assays, was obtained from Sigma Chemical Co. (St. Louis, Mo.).

**Human plasmas.** Blood was obtained by venipuncture from the proposita, Mme. Flaujeac, and four of her children.

<sup>1</sup> *Abbreviations used in this paper:* ACD, acid citrate dextrose; APTT, activated partial thromboplastin time; HMW-, high molecular weight; LMW-, low molecular weight; PF/dil, permeability factor of dilution; PTA, plasma thromboplastin antecedent, Factor XI.

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Nine parts blood was mixed with one part 3.8% citrate or acid-citrate dextrose (ACD). Plasma was separated by centrifugation and frozen on dry ice for shipment from Paris. Upon receipt, it was transferred to a freezer at  $-70^{\circ}\text{C}$  until use. Normal pooled plasma from 16 donors was treated identically.

Plasma from two patients deficient in Hageman factor activity was generously supplied by Dr. S. Goodnight, University of Oregon Health Sciences Center. Plasma deficient in Fletcher factor was obtained from two members of the original Fletcher family (7) through the courtesy of Dr. W. Hathaway, University of Colorado. Plasma thromboplastin antecedent (PTA, Factor XI)-deficient plasma was obtained from Dr. S. Rapaport, Veterans Administration Hospital, La Jolla, Calif. All deficient plasmas were kept at  $-70^{\circ}\text{C}$  until use.

**Biological reagents.** Human fibrinogen, 90% clottable (Grade L, AB Kabi, Stockholm, Sweden) and bovine thrombin, topical (Parke, Davis & Company, Detroit, Mich.) were purchased from their suppliers. A granular kallikrein from hog pancreas was supplied by Prof. Dr. G. Haberland (Bayer AG, Wuppertal, W. Germany).

**Antisera.** Sheep antiserum to human kininogen was a gift of Dr. J. V. Pierce, National Heart and Lung Institute, NIH. Normal sheep serum was purchased from a local supplier.

Antisera to various human plasma  $\alpha$ -globulins were obtained commercially from Behring Diagnostics (American Hoechst Corp., Somerville, N. J.). The antigens included C $\bar{\text{I}}$  inhibitor, inter- $\alpha$ -trypsin inhibitor,  $\alpha_1$ -antitrypsin, antithrombin III,  $\alpha_2$  macroglobulin,  $\alpha_1$ -B glycoprotein,  $\alpha_1$ -T glycoprotein, Zn- $\alpha_2$ -glycoprotein,  $\alpha_2$ -HS glycoprotein, Gc globulin,  $\alpha_1$ -lipoprotein,  $\alpha_2$  AP glycoprotein,  $\alpha_1$ -acid glycoprotein, C-reactive protein, and ceruloplasmin.

**Gel filtration** was performed with Sephadex G-200 hydrated in distilled water at  $100^{\circ}\text{C}$ . The expanded gel was exposed to 50  $\mu\text{g}/\text{ml}$  hexadimethrine bromide, containing 0.05% sodium azide, overnight and finally equilibrated with 0.15 M NaCl containing 0.01 M Tris buffer, pH 7.5, and 0.001 M EDTA, pH 7.5, and 0.05% sodium azide. The gel was packed under 15 cm  $\text{H}_2\text{O}$  pressure in a  $2.6 \times 80$  cm column (Pharmacia Fine Chemicals AB, Uppsala, Sweden). A sample of 2.5–3 ml was applied at the surface of the gel bed and the effluent was maintained at a flow rate of 17 ml/h with a pump (Variperpex; LKB-Produkter AB, Bromma, Sweden).

**Block electrophoresis** in Pevikon (Mercer Glass Works, Inc., New York) was performed after the beads were first moistened in distilled water, exposed to 50  $\mu\text{g}/\text{ml}$  hexadimethrine bromide for 1 h, washed thoroughly with distilled water, and finally suspended in barbital buffer  $\Gamma/2 = 0.05$ , pH 8.6. The 3-ml sample of plasma was dialyzed against the electrophoresis buffer in a dialysis casing, pre-rinsed with hexadimethrine bromide. The sample was introduced into a narrow 4-mm slot at the origin and electrophoresis was performed at room temperature at 8 V/cm for 18 h. Bromphenol blue dye was added to the sample to facilitate visual localization of albumin.

**Assay methods.** The following assays have been previously described (9): kaolin-activated partial thromboplastin time (APTT), kaolin-activated kinin generation, and permeability reactions given by diluted plasma (PF/dil).

The release of kinin from plasma by a glandular kallikrein was measured by bioassay on the rat uterus. 100  $\mu\text{l}$  kallikrein, 500  $\mu\text{g}/\text{ml}$ , was incubated with 50  $\mu\text{l}$  plasma for 30 s at  $22^{\circ}\text{C}$ , and 50  $\mu\text{l}$  of the reaction mixture was transferred to a muscle bath containing a rat uterine horn suspended in oxygenated Tyrode's solution at  $37^{\circ}\text{C}$ .

The ability of normal pooled plasma to correct Flaujeac plasma or plasmas deficient in Hageman factor or Fletcher factor was tested as described (9). The quantity of Flaujeac factor in an unknown solution could be compared to normal plasma when the APTT fell within the dose-dependent part of the response. For purposes of comparison, the quantity of the factor present in 1 ml of normal pooled plasma is designated as one unit of activity.

The ability of preincubation with kaolin to shorten the APTT was performed by addition of 50  $\mu\text{l}$  of the kaolin-cephalin mixture with 50  $\mu\text{l}$  test plasma for specified intervals. The incubation mixture was then recalcified with 0.05 M  $\text{CaCl}_2$  and the clotting time was recorded.

Flaujeac factor was first quantitatively assayed by coagulation methods before use in attempting to reconstitute the kaolin-activated euglobulin lysis test, kaolin-activated generation of kinin, or the PF/dil reactions. Mixtures were then prepared by twofold dilution so as to contain various amounts of Flaujeac factor in the reaction mixture.

The kaolin-activated euglobulin lysis assay (9) was modified slightly to reduce the volume of plasma required for the assay. To 0.1 ml plasma was added 0.05 ml kaolin 8 mg/ml and 1.8 ml 0.01 M sodium acetate buffer, pH 4.8. The tubes were incubated at  $37^{\circ}\text{C}$  and gently inverted at 5-min intervals to maintain the euglobulin and kaolin in suspension. After 1 h, the samples were centrifuged at 3,000 rpm, the supernate was discarded, and the pellet of kaolin and euglobulin was suspended in 0.1 ml of 0.15 M NaCl containing 0.05 M Tris buffer, pH 7.5. 0.1 ml of a 0.4% fibrinogen solution and 0.05 ml thrombin, 50 NIH U/ml, were added and the mixture was transferred to a  $37^{\circ}\text{C}$  water bath. The time required for lysis of the clot was recorded.

**Immunochemical assays.** Double diffusion in agar was performed in precast agar plates containing azide as a preservative (style B, Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.). Antiserum to kininogen was used at a dilution of 1:3; antisera for other  $\alpha$ -globulins were used without further dilution.

Electroimmunodiffusion followed the method of Laurell (12), employing Ionagar No. 2 at a concentration of 1.5%. 0.2 ml of antiserum to kininogen 1:3 was used for each 10-ml agar plate.

Partially purified Flaujeac factor, recovered from Sephadex G-200 gel filtration of normal plasma, was incubated with antiserum to kininogen at dilutions of 1:32–1:512. Controls included Flaujeac factor incubated with normal sheep globulin 1:32–1:512 or saline buffered with 0.01 M Tris, pH 7.5, and incubated with anti-kininogen or normal sheep globulin. Incubation was carried out at  $37^{\circ}\text{C}$  for 15 min, then at  $4^{\circ}\text{C}$  for 1 h. No visible precipitate formed upon centrifugation. The presence of Flaujeac factor was then assayed quantitatively by the APTT.

Normal sheep serum and sheep antiserum to kininogen were found to contain a substance that corrected the coagulation defect found in Flaujeac plasma. The factor was removed from normal or immune sheep serum by incubation at room temperature for 1 h with an equal volume of DEAE Sephadex A-50 beads suspended in 0.15 M NaCl. The supernate obtained after centrifugation of the Sephadex beads contained 1% or less Flaujeac factor.

## RESULTS

**Abnormal contact activation of Flaujeac plasma.** Mme. Flaujeac's plasma had a prolonged APTT. The prolonged APTT was corrected by mixing Flaujeac plasma with normal plasma which excludes a circulating

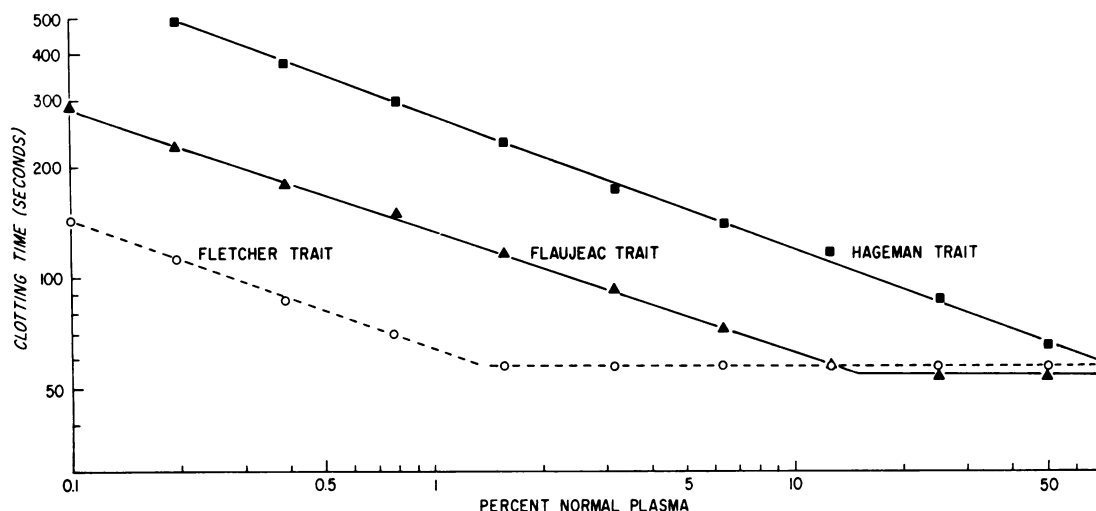


FIGURE 1 Correction of Flaujeac plasma by normal plasma. A dose-related response was given by normal plasma in concentrations less than 12.5%. The dose-dependent correction of Hageman trait and Fletcher trait plasmas is shown for comparison.

anticoagulant (Table I). The addition of 12.5% normal plasma to Flaujeac plasma gave full correction of the APTT (Fig. 1). Concentrations of normal plasma less than 12.5% gave a linear dose-response relationship. In contrast, the minimum concentrations of normal plasma that fully corrected Hageman factor-deficient plasma or Fletcher factor-deficient plasma were approximately 50 and 1.5% respectively (Fig. 1).

Mixtures of Flaujeac plasma with reference plasmas with deficient activity of Hageman factor, Fletcher factor, or PTA (Factor XI) gave mutual correction of the APTT (Table I). By a reference plasma one-stage assay method, the content of Hageman factor in the proposita's plasma was 70-90%. The content of Fletcher factor was 65-70%. Although preincubation of Fletcher factor-deficient plasma for 10 min with kaolin led to

normalization of the APTT, Flaujeac trait plasma gave little correction after prolonged incubation with kaolin (Fig. 2).

*Failure of plasmin generation in Flaujeac plasma.* The kaolin-activated euglobulin lysis test was abnormally prolonged in Flaujeac plasma (Table II) but was normalized upon mixing with Fletcher trait or Hageman trait plasmas (Table II). Therefore, the substance missing in Flaujeac plasma and required for the plasma fibrinolytic pathway differed from Hageman factor or Fletcher factor (prekallikrein).

*Absence of kaolin-induced kinin generation in Flaujeac plasma.* Neither Flaujeac trait plasma nor Hage-

TABLE I  
*Prolonged APTT of Flaujeac Trait Plasma and its Correction upon Mixing with Normal Plasma or Plasma Deficient in Hageman Factor, Fletcher Factor, or Coagulation Factor XI*

Plasma source	Kaolin-APTT
	s
Normal plasma pool	57
Hageman trait	726
Fletcher trait	198
Flaujeac trait	618
Hageman trait + Fletcher trait (5:1)	63
Hageman trait + Flaujeac trait (1:1)	66
Fletcher trait + Flaujeac trait (1:1)	66
PTA deficiency + Flaujeac trait (1:1)	71
Normal pool + Flaujeac trait (1:1)	61

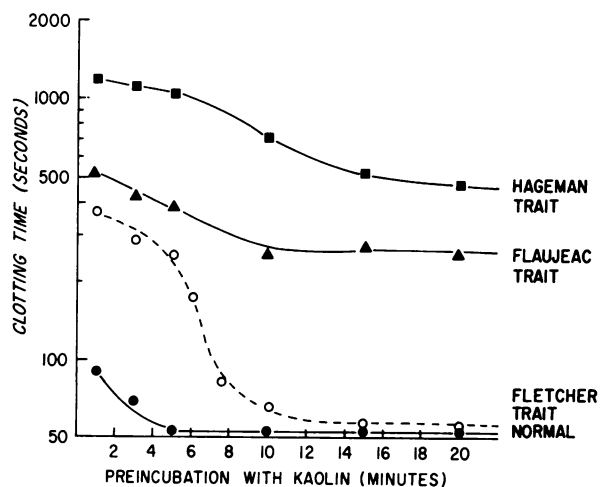


FIGURE 2 Effect of preincubation with kaolin on the APTT of Flaujeac plasma. The results obtained with normal, Hageman trait, and Fletcher trait plasmas are also given.

TABLE II

*Abnormal Kaolin-Activated Euglobulin Lysis of Flaujeac Trait Plasma and its Correction upon Mixing with Other Plasmas Sharing a Similar Abnormality*

Plasma source	Euglobulin lysis time <i>min</i>
Buffer control	>120
Normal pool	9
Hageman trait	>120
Fletcher trait	84
Flaujeac trait	>120
Hageman trait + Fletcher trait (1:1)	13
Hageman trait + Flaujeac trait (1:1)	14
Fletcher trait + Flaujeac trait (1:1)	14
Normal pool + Flaujeac trait (1:1)	12

man nor Fletcher trait plasmas were able to generate a kinin detected by assay upon the rat uterus (Fig. 3). However, mixtures of Flaujeac plasma with either Hageman factor-deficient or Fletcher factor-deficient plasma gave mutual correction, supporting the notion that different kinin-promoting factors are lacking in each of these plasmas.

*Absent formation of PF/dil in Flaujeac plasma.* Both Hageman factor and Fletcher factor are known to be required for the formation of PF/dil in glass vessels. Upon 1:100 dilution in glass test tubes and incubation with mixing for 15 min, the Flaujeac plasma also failed to form the PF/dil. However, mixing with plasmas de-

TABLE III

*Failure of Flaujeac Plasma to Generate PF/dil*

Plasma source	Permeability diameter <i>mm</i>
Saline	3,* 3*
Normal pool	7, 8.5
Hageman trait	2,* 0
Fletcher trait	0, 0
Flaujeac trait	0, 0
Hageman trait + Fletcher trait (1:1)	9, 8
Hageman trait + Flaujeac trait (1:1)	10, 9
Fletcher trait + Flaujeac trait (1:1)	8, 9
Factor XI-deficient	10, 9

This was corrected upon mixing with plasma deficient in Hageman factor or Fletcher factor, also deficient in their ability to exhibit this permeability phenomenon.

\* Faint blueing.

ficient in Hageman factor (1:1) or Fletcher factor (1:1) fully corrected the defect (Table III).

*Characterization of the Flaujeac factor.* Further characterization of the substance(s) responsible for the abnormalities in Flaujeau plasma was performed with plasmas in which activation of components of the initial steps of the intrinsic coagulation system or kinin-forming system would be minimized. For example, gel permeation chromatography was carried out on Sephadex G-200 with Fletcher factor-deficient plasma. The fractions that eluted were tested, by the APTT, for ability

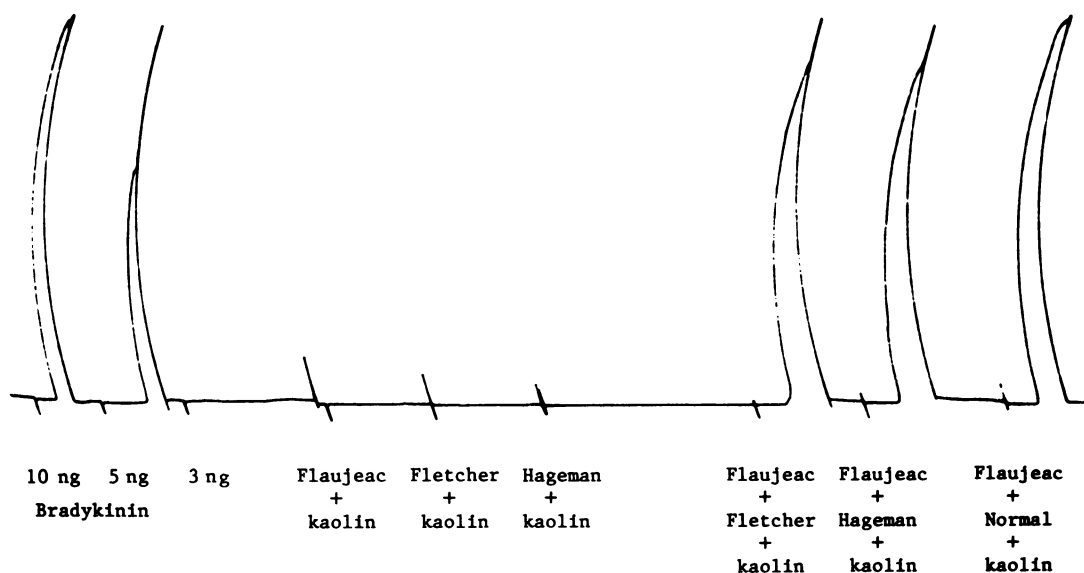


FIGURE 3 Absence of kinin generation by Flaujeac plasma upon incubation with kaolin. The defect, also shared by Hageman- and Fletcher-trait plasmas, was correctible upon mixing Flaujeac plasma with plasma deficient in Hageman factor or Fletcher factor.

to correct coagulation factor-deficient plasmas (Fig. 4). A plasma factor that corrected Flaujeac trait plasma eluted slightly ahead of the peak of gamma globulins. It corresponded to an apparent mol wt of 170,000. It was separated almost completely from Hageman factor (Fig. 4), which eluted at an apparent mol wt of 80,000. In separate experiments, for which data are not shown, Hageman factor-deficient plasma or normal human plasma was also chromatographed on Sephadex G-200.

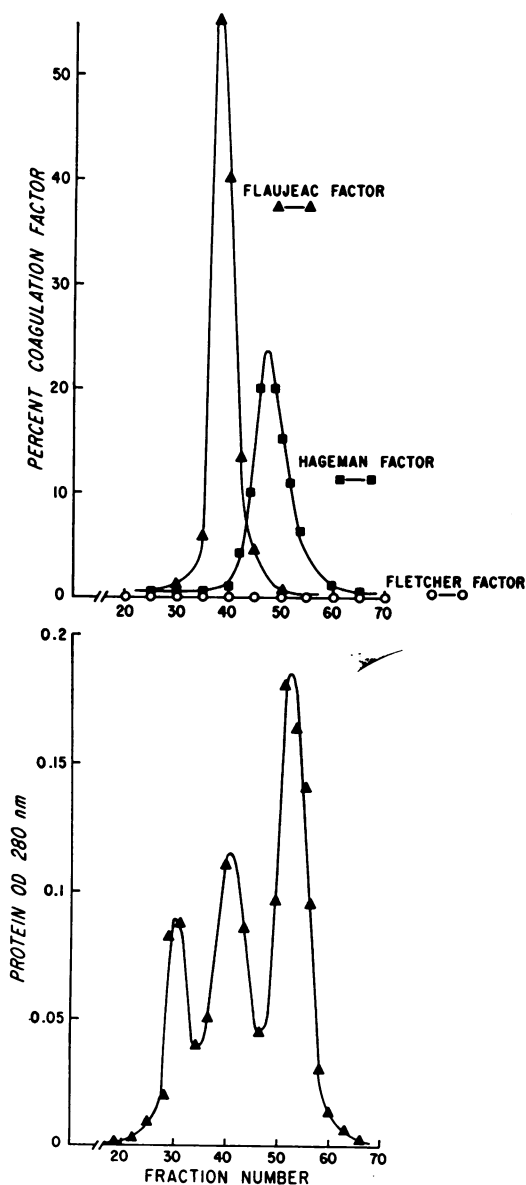


FIGURE 4 Chromatography of Fletcher trait plasma on Sephadex G-200. 50- $\mu$ l samples of fractions eluted from the column were assayed by the APTT for Flaujeac factor, Hageman factor, or Fletcher factor; the concentration relative to normal plasma is expressed on the ordinate.

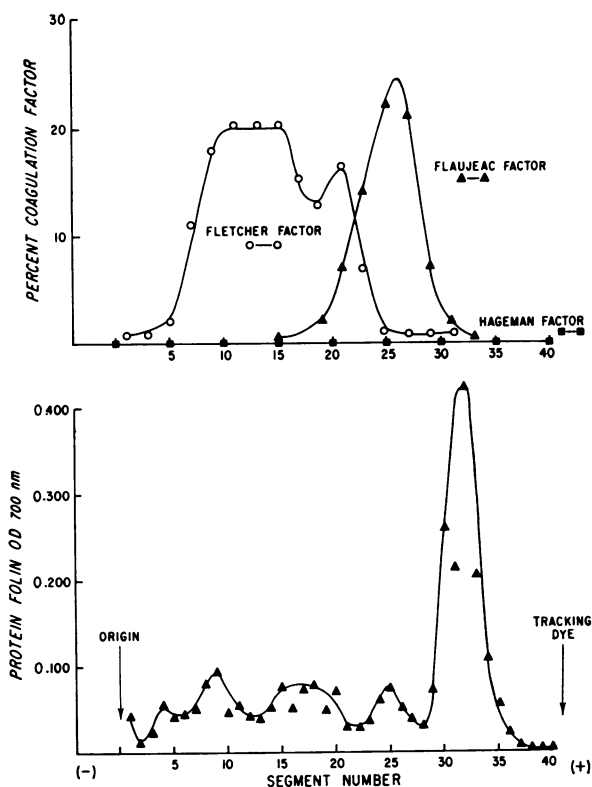


FIGURE 5 Block electrophoresis of Hageman trait plasma in Pevikon, pH 8.6. Protein was recovered from 1-cm segments and an aliquot tested for its ability to correct the APTT of Flaujeac plasma (50  $\mu$ l), Fletcher trait plasma (2.5  $\mu$ l), or Hageman trait plasma (50  $\mu$ l).

A factor that corrected the APTT of Flaujeac plasma eluted in an identical position of Fletcher trait, Hageman trait, and normal plasma.

Hageman trait plasma was subjected to zone electrophoresis in Pevikon at pH 8.6. At completion of electrophoresis, the block was cut in 1-cm segments, proteins were eluted from each segment, and fractions were assayed for the ability to correct the APTT of Flaujeac plasma. A symmetrical peak of Flaujeac factor activity was identified as an  $\alpha$ -globulin, which was distinct from fractions that contained Fletcher factor (Fig. 5).

The  $\alpha$ -globulin fractions that contained Flaujeac factor were concentrated 20-fold by ultrafiltration on a Diaflow PM 30 membrane (Amicon Corp., Lexington, Mass.) to 1.5 ml. To a 0.15-ml sample, 0.05 ml 50% sucrose was added for polyacrylamide disc gel electrophoresis. Upon completion of electrophoresis, the gel was cut in 1.3-mm segments with a gel cutter and eluted in 0.15 M sodium chloride buffered with 0.01 M Tris, pH 7.4. Under these conditions, the Flaujeac factor was recovered in the region of the  $\beta$ -globulins (Fig. 6).

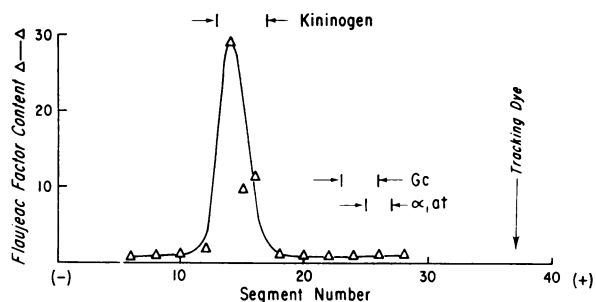


FIGURE 6 Disc gel electrophoresis of  $\alpha$ -globulins recovered from zone electrophoresis in Pevikon. Proteins eluted from segments of the gel were assayed for the Flaujeac factor by APTT. Gc globulin and  $\alpha_1$ -antitrypsin were identified by double diffusion agar. Kininogen was measured by bioassay after incubation with kallikrein.

**Deficiency of kininogen in Flaujeac plasma.** The content of 16  $\alpha$ -globulins in Flaujeac plasma was investigated by double diffusion in agar against specific antisera. Only the  $\alpha$ -globulin, kininogen, was grossly deficient. The content of the remaining  $\alpha$ -globulins were essentially normal ( $\alpha_1$  antitrypsin,  $\alpha_2$  macroglobulin, antithrombin III, inter- $\alpha$ -trypsin inhibitor, C1 inhibitor,  $\alpha_1$  acid glycoprotein,  $\alpha_2$  Zn-glycoprotein,  $\alpha_1$  B glycoprotein,  $\alpha_1$  T glycoprotein,  $\alpha_2$  HS glycoprotein, ceruloplasmin, Gc globulin,  $\alpha_2$  AP glycoprotein, C reactive protein, and  $\alpha_1$  lipoprotein). Comparative diffusion studies suggested that the kininogen in Flaujeac plasma was not only reduced but possibly antigenically different, since normal kininogen formed a spur with the kininogen in Flaujeac plasma (Fig. 7). However, two faint bands are seen in the Flaujeac samples that could indicate abnormal forms of kininogen in Flaujeac plasma, heterogeneity of normal kininogen in Flaujeac and other plasmas, or possibly similar but nonkininogen proteins in the Flaujeac sample.

We next tested the ability of hog pancreatic kallikrein to release a kinin from Flaujeac plasma or control plasmas. The results, given in Fig. 8, showed that, in contrast with control plasmas, no kinin was released upon incubation of Flaujeac plasma with kallikrein. However, we determined that proteins eluting from Sephadex G-200, Pevikon block electrophoresis, or disc gel electrophoresis that reconstituted Flaujeac plasma did indeed contain a kininogen, as assessed by incubation with kallikrein.

**Reconstitution of Flaujeac plasma with HMW-kininogen.** Since kininogens were deficient in Flaujeac plasma, we re-examined the two forms of kininogen that elute from Sephadex G-200 for ability to correct the APTT of Flaujeac plasma. Kininogens were recognized in two discrete zones. HMW-kininogen measured by bioassay corresponded to 170,000 mol wt and coincided exactly

with the Flaujeac factor. A low molecular weight (LMW)-kininogen detectible by bioassay or double diffusion in agar against antikinogen corresponded to 70,000 mol wt. It was unable to correct the APTT of Flaujeac plasma.

The two forms of human kininogen are also resolved by chromatography on DEAE Sephadex A-50. LMW-kininogen eluted at a relative salt concentration of 0.18 M and HMW-kininogen eluted at a relative salt concentration of 0.35 M. Again, only fractions containing HMW-kininogen were able to reconstitute the Flaujeac plasma (data not shown).

The ability of HMW-kininogen to reconstitute the PF/dil phenomenon in Flaujeac plasma is shown in Fig. 9. A dose-related correction is seen in the range of 1.5–26% of the normal content of added factor. Similarly, the ability of HMW-kininogen to correct the euglobulin lysis time after kaolin activation is depicted in Table IV.

We investigated the possibility that a fragment of HMW-kininogen, i.e., bradykinin, might shorten the APTT. 5  $\mu$ g synthetic bradykinin was added to the incubation mixture just before, or at the end of, the 3-min activation with kaolin. The mixture was then recalcified and the clotting time recorded. Bradykinin, despite its

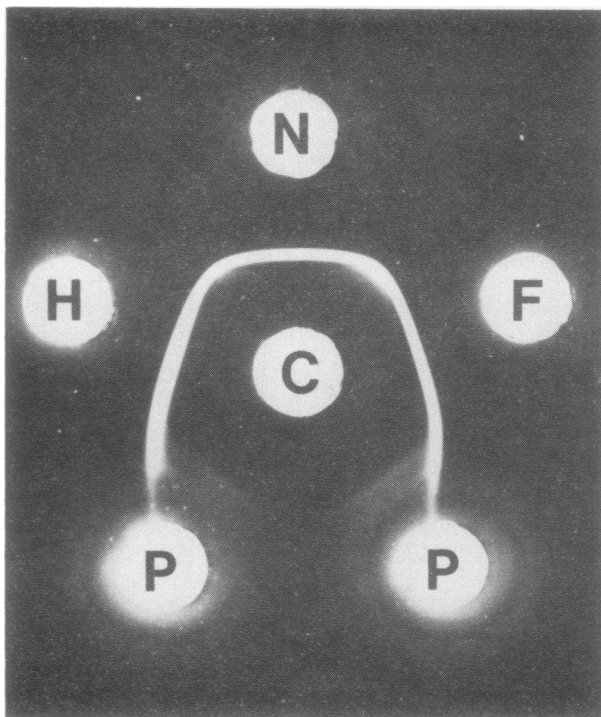


FIGURE 7 Agar diffusion of normal (N), Hageman trait (H), Fletcher trait (F), and Flaujeac trait (P) plasmas against monospecific antiserum to human kininogen (C).

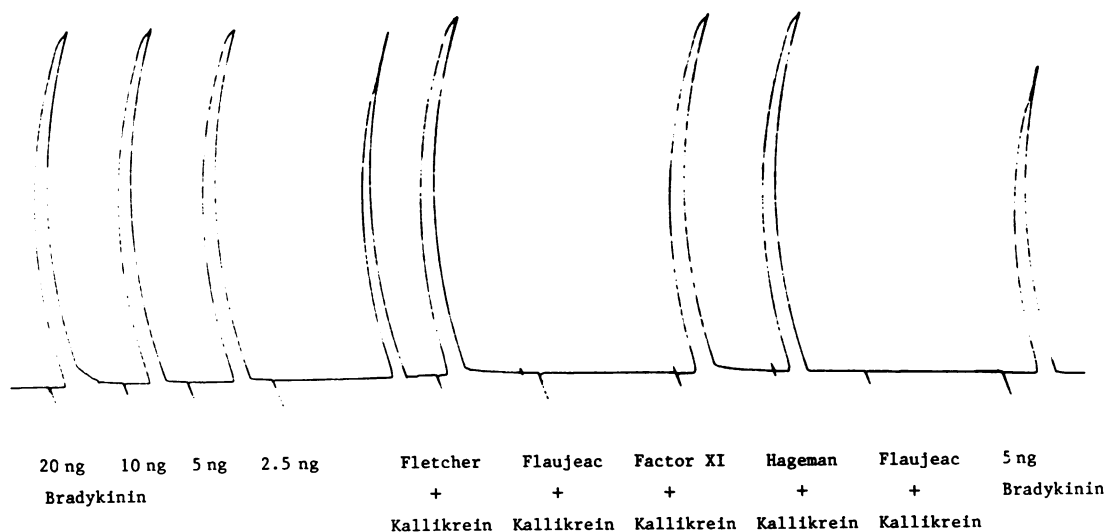


FIGURE 8 Failure of glandular kallikrein to release a kinin from Flaujeac plasma, in contrast with plasmas deficient in Fletcher factor, Factor XI, or Hageman factor.

presence in a 10-fold excess of the maximum quantity generated by normal plasma, did not influence the APTT.

*Inhibition of Flaujeac factor with antiserum to kininogen.* We studied the ability of sheep antiserum to kininogen to inhibit the clotting time of Flaujeac factor in solution. Normal sheep serum and kininogen antiserum contained Flaujeac factor, and this was removed (see Methods). Flaujeac factor, partially purified by passage over Sephadex G-200, was inhibited in the solution by the addition of antiserum to kininogen, whereas nonimmune sheep globulin was not inhibitory (Fig. 10).

*Kininogens in children of the proposita.* Total plasma kininogen (HMW-kininogen and LMW-kininogen) was measured by electroimmunodiffusion in the proposita and four of her children. The results were compared

with HMW-kininogen assayed by APTT, as shown in Table V.

## DISCUSSION

Lacombe and her co-workers described a patient with a new deficiency in the contact phase of the intrinsic coagulation pathway. They systematically excluded all known coagulation factors as responsible for the prolonged APTT (10, 11). Additionally, they recognized an abnormality of the plasma fibrinolytic pathway. The data reported here confirm these earlier observations and extend them to reveal defects in kinin generation and the permeability phenomenon of Miles and co-workers (5, 6). In this way, Flaujeac trait, named after the asymptomatic French Caucasian proposita, resembled Hageman trait and Fletcher trait. However, it is distinguished from them by the quantity of normal plasma required for full correction of the APTT, (about 12%) and the failure to normalize the APTT upon prolonged incubation with kaolin (13).

The findings reported herein lead to the conclusion

TABLE IV  
Correction of the Euglobulin Lysis Test by Addition of Flaujeac Factor

Flaujeac factor added	Euglobulin lysis time
%	min
None	154
13	9
3.2	12
0.8	81

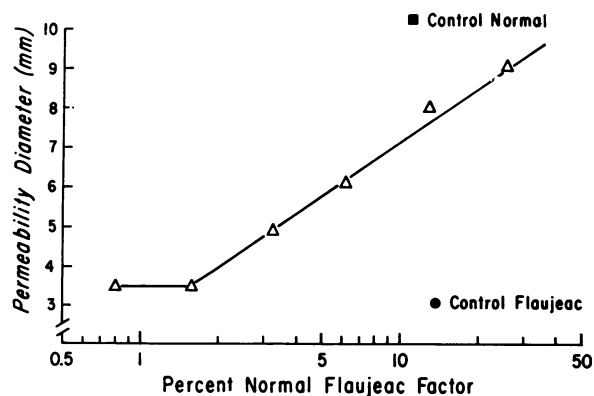


FIGURE 9 Ability of Flaujeac factor to reconstitute the PF/dil phenomenon.

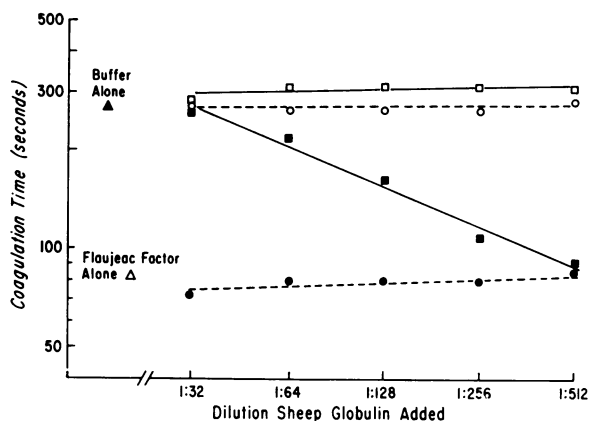


FIGURE 10 Inhibition of Flaujeac factor by sheep globulin containing antibody to kininogen. Inhibition was dose-dependent at dilutions of 1:32 through 1:512. Controls included sheep antiserum alone, normal sheep globulin alone, or after incubation with Flaujeac factor. □, buffer plus sheep antikinogen; ○, buffer plus normal sheep globulin; ■, Flaujeac factor plus sheep antikinogen; ●, Flaujeac factor plus normal sheep globulin.

that Flaujeac trait plasma is deficient in plasma kininogens and that HMW-kininogen is the plasma factor that reconstitutes its biologic functions. This is supported by (a) failure of kaolin-activated kinin generation, (b) failure of kallikrein-activated kinin release, (c) reconstitution of Flaujeac plasma by fractionated plasma proteins containing HMW-kininogen, (d) reduced total kininogen antigen, 9% of normal, in the form of cross-reacting material, (e) depletion of the Flaujeac factor activity with antiserum to kininogen, and (f) same physical properties of the Flaujeac factor and HMW-kininogen. However, a possibility still exists that an additional protein is involved in these reactions that is difficult to separate from HMW-kininogen and that may

TABLE V  
Total Plasma Kininogen Antigen and Flaujeac Factor Activity in the Proposita and Four Children

	Total plasma kininogen*	Flaujeac factor activity‡
	%	%
Normal	100*	65–200‡
Mme. Flaujeac	9§	0
C. F. (female)	34	23
G. F. (male)	45	27
Ge. F. (female)	61	30
J. F. (female)	62	55

\* Measured by electroimmunodiffusion, normal pool ( $n = 16$ ).

‡ Measured by coagulation assay, normal individuals ( $n = 10$ ).

§ Present as partially cross-reacting antigen.

be neutralized by the anti-kininogen antibody used in these studies.

Despite the extensive study of kininogens as substrates of kallikreins, they have not been considered to have biological function aside from the release of the oligopeptide, bradykinin. The studies described here provide the first evidence for a second biologic function by one of the kininogens, i.e., HMW-kininogen.

Jacobsen (14) and Jacobsen and Kriz (15) first recognized the existence of kininogens of high mol wt in several species, including man. The HMW-kininogens were quite labile and were said to show a preferential susceptibility to plasma kallikrein, in contrast to LMW-kininogen. Despite scrupulous care in their efforts to purify HMW-kininogen, Jacobsen and Kriz found this component of plasma to be labile and recoverable only in low yield (15).

Yano et al. (16, 17) and Komiya et al. (18–20), working in the same laboratory, have purified HMW-kininogens from bovine plasma. A HMW-kininogen of 78,000 mol wt and a LMW-kininogen of 49,000 mol wt have been studied. Upon incubation with bovine plasma kallikrein, bovine HMW-kininogen yields two major fragments of 51,000 mol wt and 18,000 mol wt and three minor fragments containing 9 (bradykinin), 41, and 67 amino acids, respectively (21). The carbohydrate content of bovine HMW-kininogen and LMW-kininogen differ (i.e., 12.6% vs. 19.8%) (18) as do their tryptic peptide maps (20). Bovine HMW-kininogen contains tryptic peptides not shared with LMW-kininogen. Immunochemically, however, the two kininogens were identical (20).

The presence of reduced amount of kininogen antigen in Flaujeac plasma that cross-reacted with kininogen suggested that either hyperutilization of kininogen or synthesis of a defective gene product might be responsible for this material. Synthesis of an abnormal protein is favored by several observations. The half-life of Flaujeac factor in the patient, as measured by functional assay, was found to be 6.5 days (11). Consanguinity of the proposita's parents favored an inherited defect. Four of five children of the proposita, three women and one man, were studied. By electroimmunodiffusion the children had, on the average, 51% (range 34–62%) kininogen antigen when compared to a pool of 16 normal plasmas. By coagulation assay for HMW-kininogen, the children had a mean value of 34% (range 23–55%) of normal. Since both female and male offspring of the proposita are heterozygotes, the data supports an autosomal recessive inheritance pattern. We are now attempting to isolate the cross-reacting kininogen antigen in the proposita and her children to substantiate these findings further.

The relationship of the gene product HMW-kininogen



to LMW-kininogen still requires clarification. Our preliminary studies have shown that the bulk of the kininogen antigen in normal plasma is present as LMW-kininogen. Since the average content of the total kininogen antigen (HMW- and LMW-kininogen) in the Flaujeac children is halfnormal, it is tempting to speculate that the gene that determines synthesis of LMW-kininogen is somehow linked to HMW-kininogen. Alternatively, LMW-kininogen may be a fragment or breakdown product derived from HMW-kininogen.

The molecular basis for the function of HMW-kininogen in the diverse pathways of coagulation, fibrinolysis, and PF/dil formation is, as yet, obscure. Its role in the kinin-forming system self-evident, since HMW-kininogen is the preferred substrate of plasma kallikrein. In an earlier communication, it was argued that another factor in addition to Hageman factor and prekallikrein might be necessary for PF/dil formation (9). The present findings substantiate that view. They do not explain, however, the crucial aspects of the role of HMW-kininogen in the PF/dil phenomenon.

Lately, Schiffman and Lee were unable to activate their preparation of highly purified PTA with Hageman factor. They proposed that another plasma factor was required (22). It will be important to restudy the activation of PTA (8, 23) in light of our present data.

Much more work is needed to clarify the role of HMW-kininogen in the complex plasma pathways dependent upon its function.

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## ADDENDUM

After this work was completed and submitted in abstract form (24), two additional instances of a similar coagulation defect were reported by Waldmann and Abraham (25) and by Colman and his co-workers (26). We have exchanged plasma samples with these workers and have learned that mutual correction of the activated PTT test did not occur when Flaujeac trait plasma was mixed with plasma from patients identified as Fitzgerald (25) and Williams (26), suggesting the identity of the same functional deficiency in these three individuals.

Through the courtesy of Drs. H. Kato, Y. N. Han, and S. Iwanaga, we have been able to test highly purified bovine kininogens in Flaujeac plasma (data to be published). Bovine HMW-kininogen corrected the activated PTT of Flaujeac plasma in a dose dependent manner, while bovine LMW-kininogen did not give correction.

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