Fibrinogen Philadelphia. A hereditary hypodysfibrinogenemia characterized by fibrinogen hypercatabolism.

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J Clin Invest. 1974;53(2):600-611. https://doi.org/10.1172/JCI107595.

Research Article

A new, autosomally inherited abnormal fibrinogen associated with hypofibrinogenemia has been described in several members of a family. Plasma fibrinogen measured either as thrombin-clottable protein or by immunodiffusion revealed a fibrinogen level ranging between 60 and 90 mg/100 ml. The thrombin time of plasma or purified fibrinogen was prolonged and only partially corrected by the addition of calcium. Purified fibrinogen prolonged the thrombin time of normal plasma. Fibrinopeptide release by thrombin was normal in rate and amount, but fibrin monomer aggregation was grossly disturbed, especially in a high ionic strength medium. We have designated this fibrinogen "fibrinogen Philadelphia." Acrylamide gel electrophoresis of mixtures of [121I]normal and [125I]abnormal fibrinogens revealed a slight increase in the anodal mobility of fibrinogen Philadelphia. Similarly, DEAE-cellulose chromatography showed slightly stronger binding of fibrinogen Philadelphia than normal. To elucidate the mechanism responsible for the low plasma fibrinogen concentration, simultaneous metabolic studies of autologous (patient) and homologous (normal) fibrinogen, labeled with 125I and 121I, respectively, were performed in two affected subjects. Autologous fibrinogen half-life was short and the fractional catabolic rate was markedly increased in both family members. In contrast, homologous fibrinogen half-life and fractional catabolic rate were normal. These metabolic studies demonstrate that rapid degradation of fibrinogen Philadelphia is largely responsible for the depressed levels of a plasma fibrinogen. This represents the first example of a […]

Fibrinogen Philadelphia

A HEREDITARY HYPODYSFIBRINOGENEMIA CHARACTERIZED BY FIBRINOGEN HYPERCATABOLISM

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ABSTRACT A new, autosomally inherited abnormal fibrinogen associated with hypofibrinogenemia has been described in several members of a family. Plasma fibrinogen measured either as thrombin-clottable protein or by immunodiffusion revealed a fibrinogen level ranging between 60 and 90 mg/100 ml. The thrombin time of plasma or purified fibrinogen was prolonged and only partially corrected by the addition of calcium. Purified fibrinogen prolonged the thrombin time of normal plasma. Fibrinopeptide release by thrombin was normal in rate and amount, but fibrin monomer aggregation was grossly disturbed, especially in a high ionic strength medium. We have designated this fibrinogen "fibrinogen Philadelphia." Acrylamide gel electrophoresis of mixtures of [^{131}I]normal and [^{125}I]abnormal fibrinogens revealed a slight increase in the anodal mobility of fibrinogen Philadelphia. Similarly, DEAE-cellulose chromatography showed slightly stronger binding of fibrinogen Philadelphia than normal. To elucidate the mechanism responsible for the low plasma fibrinogen concentration, simultaneous metabolic studies of autologous (patient) and homologous (normal) fibrinogen, labeled with ^{125}I and ^{131}I, respectively, were performed in two affected subjects. Autologous fibrinogen half-life was short and the fractional catabolic rate was markedly increased in both family members. In contrast, homologous fibrinogen half-life and fractional catabolic rate were normal. These metabolic studies demonstrate that rapid degradation of fibrinogen Philadelphia is largely responsible for the depressed levels of a plasma fibrinogen. This represents the first example of a mutant plasma protein in which the molecular defect is associated with an altered catabolism.

This work was presented in part at the 64th Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., April 29, 1972 and published in abstract form (*J. Clin. Invest.* **51**: 62 *a*, 1972).

Received for publication 18 June 1973 and in revised form 15 October 1973.

INTRODUCTION

Inherited functional abnormalities of fibrinogen have been described in several families (1–21). Clinical expression of the defect is variable, ranging from no clinical manifestations (1–11), to bleeding (9, 12–20) and even thrombotic episodes (14, 20, 21). Wound dehiscence also has been reported (1, 3). The most frequent abnormalities of coagulation tests are prolonged prothrombin and thrombin times and an inhibitory effect of patient plasma or fibrinogen on the thrombin time of normal plasma. The functional defect in these dysfibrinogenemias has been related either to an abnormality in the proteolytic release of fibrinopeptides from fibrinogen by thrombin or to an abnormality in the subsequent aggregation of fibrin monomers. The plasma fibrinogen concentration, when measured by methods based on thrombin-clottable protein, has generally been low (22), but assay by chemical or immunochemical methods has resulted in normal values in all cases but one (12).

We have recently had the opportunity to study a family with an abnormal fibrinogen characterized by disturbed fibrin monomer aggregation and a low circulating plasma fibrinogen concentration determined by both biological and immunochemical methods. Radioisotopic studies have demonstrated a much more rapid catabolism of this mutant fibrinogen that the normal species, apparently accounting for the depressed fibrinogen levels found in affected members. These fea-

tures differentiate this abnormal fibrinogen from previously described dysfibrinogenemias, and we propose that it be designated "fibrinogen Philadelphia."

METHODS

Blood was collected with plastic syringes into 1/9 vol 0.1 M trisodium citrate and transferred to plastic tubes. After centrifugation for 15 min at 4°C and 2,500 *g*, the platelet-poor plasma was removed and either tested immediately or quick-frozen in acetone-dry ice and stored at −85°C.

Fibrinogen purification. Normal fibrinogen was purified from ACD plasma obtained from healthy Australia antigen negative donors by the method of Kazal, Amsel, Miller, and Tocantins (23). The abnormal fibrinogen was purified by the same method from ACD plasma obtained from the propositus (J. S.) and her son (D. S.). Normal and D. S. fibrinogens were lyophilized and stored at −85°C; J. S. fibrinogen was quick-frozen and stored at the same temperature. The amount of fibrinogen present in the purified materials was measured spectrophotometrically at 280 nm, pH 7.1, by assuming an $A_{280}^{1\%}$ of 13.9, the figure obtained by Kazal et al. (23). When normal fibrinogen is prepared by this technique, the protein is consistently more than 94% clottable.

Coagulation studies. Platelets were counted by the method of Brecher and Cronkite (24). The bleeding time was determined by the method of Ivy, Nelson, and Bucher (25). Partial thromboplastin time and prothrombin time were done by standard methods (26). Plasma prothrombin was assayed by the two-stage technique of Ware and Seegers (27) slightly modified (28). Factor V was measured by the correction of the prolonged prothrombin time of aged oxalated plasma (29). Factors VII and X were each measured by the correction of the one-stage prothrombin time of congenitally deficient substrate plasmas. Factor X was also assayed with Diagen substrate plasma[1] (30). Factors XII, XI, IX, and VIII activities were measured by the kaolin-activated partial thromboplastin time (31) with artificially depleted plasma (32) for the Factor XI assay and congenitally deficient plasmas (with less than 1% activity) for the others. Factor XIII activity was estimated by incubating a 1-ml clot with 5 ml of 5 M urea and observing for dissolution at 24 h. The euglobulin lysis time was performed by the method of Blix and Aas (33). This test was repeated after the propositus' plasma was enriched with her own purified fibrinogen to bring the plasma fibrinogen concentration within the normal range. The presence in plasma of fibrin monomer complexes was tested by the serial dilution protamine sulfate test (34). Serum fibrinogen-fibrin-related antigens were measured by a latex agglutination test (35).

The thrombin clotting time was performed by adding to 0.1 ml of plasma 0.2 ml of imidazole buffered saline (0.15 M NaCl, 0.045 M imidazole, pH 7.4) and 0.1 ml of bovine thrombin (10 U/ml imidazole buffered saline).[2] In some experiments the imidazole buffered saline contained 0.01 M $CaCl_2$. Thrombin clotting times were also done on normal and propositus purified fibrinogen. Fibrinogen was dissolved in 0.055 M sodium citrate, pH 7.4, at a concentration of 1.30 mg/ml. 0.1 ml of this solution was mixed with 0.2 ml of imidazole buffered saline and 0.1 ml of bovine thrombin (10 U/ml imidazole buffered saline). Inhibition of the thrombin time of normal plasma was tested by mixing normal plasma with various amounts of normal or propositus plasma or normal or propositus purified fibrinogen. The purified fibrinogen solutions had equivalent concentrations (1.3 mg/ml) so that the total amount of fibrinogen was constant in the test system. Reptilase times were performed with venom from *Bothrops atrox*[3] diluted with distilled water. The system was identical to the one described for the thrombin time method, except that 0.1 ml (20 μg/ml) of Reptilase was substituted for thrombin.

Fibrinogen concentration was determined by thrombin clottability with the method of Ellis and Stransky (36), ammonium sulphate precipitation (37), and quantitative immunodiffusion, by using commercial antibody-containing agar plates.[4]

Fibrinopeptide release during coagulation was investigated by a method similar to that described by Gralnick, Givelber, Shainoff, and Finlayson (17). Lyophilized fibrinogen, containing sodium citrate, was dissolved in distilled water to a concentration of 3.5 mg/ml fibrinogen and 0.055 M citrate. 0.5-ml aliquots of this fibrinogen solution (1.75 mg) were added to a series of tubes containing 0.05 ml of bovine thrombin (2.5 U). At intervals 0.5 ml of 4% acetic acid was added to each tube. After the clot had dissolved, the mixture was treated with 0.05 ml 100% trichloroacetic acid (TCA).[5] 30 min later the samples were centrifuged, and the protein present in the supernate was measured by the Folin-Lowry method (38), as modified by Gralnick et al. (17). Fibrinopeptides were also measured as the amount of TCA-soluble arginine present in the supernate (17).

Fibrin monomer aggregation studies were done by several methods. One was similar to the procedure described by von Felten, Frick, and Straub (39). 100 ml of plasma, containing 0.5% Na EDTA and 25,000 U Trasylol[6] was diluted 1:5 with 0.15 M NaCl and clotted with 100 U of human thrombin.[7] The mixture was incubated for 24 h at 4°C, after which the clots were recovered by centrifugation. Fibrin monomers remaining in the supernate were precipitated with 0.3 vol 50% ethanol, kept at 4°C for 18 h, and recovered by centrifugation at 2,000 *g* for 15 min. The clot as well as the precipitated fibrin monomers were each washed five times with 0.15 M NaCl containing 0.025% Na EDTA. The fibrin clots and the precipitated monomers were dissolved in 5 M urea and dialyzed at 4°C for 72 h against several changes of pH 4.6 barbital-sodium acetate buffer, ionic strength 0.05. The fibrin monomer concentration after dialysis was adjusted to 100 mg/100 ml. 0.5 ml of this solution (0.5 mg) was transferred to a cuvette to which 0.5 ml of phosphate buffer, pH 6.8, and ionic strength, 0.05, was added. Absorbance at 350 nm was followed over 30 min in a Gilford spectrophotometer.[8] Fibrin monomer aggregation was also measured at a final ionic strength of 0.20, by adding to the monomers an equal volume of pH 6.8 phosphate buffer with an ionic strength of 0.35. Final pH of the aggregation mixtures was 6.7.

The aggregation of fibrin monomers was also examined by a method similar to that used in the study of Fibrinogen

[1] Diagnostic Reagents, Ltd., Thame, Oxon, England.

[2] Upjohn Co., Kalamazoo, Mich.

[3] Abbott Scientific Products Div., Abbott Laboratories, South Pasadena, Calif.

[4] Behringwerke AG, Marburg-Lahn, West Germany.

[5] *Abbreviations used in this paper:* SDS-DTT, sodium dodecylsulfate-dithiothreitol; TCA, trichloroacetic acid.

[6] Bayer, Leverkusen, West Germany.

[7] Fibrindex, Ortho Diagnostics, Raritan, N. J.

[8] Gilford Instrument Laboratories, Inc., Oberlin, Ohio.

Cleveland (3). The low fibrinogen level in D. S. plasma was increased to normal by the addition of his own purified fibrinogen. In this experiment 0.5 ml of normal or D. S. plasma was mixed with 1 ml of barbital-sodium acetate buffer, pH 2.6, bringing the mixture to a final pH of 5.2. Human thrombin (100 U) was added to the acidified plasma, and the mixture was incubated at 37°C. At timed intervals 0.2 ml of barbital-sodium acetate buffer, pH 9.6, containing 15 U heparin, was added to 0.2 ml of the incubation mixture (final pH 7.2), and the clotting time was recorded. To examine the influence of D. S. fibrin monomers on the aggregation of normal monomers, 0.25 ml of normal plasma was mixed with 0.25 ml of D. S. plasma, and the same procedure was followed.

Electrophoretic and chromatographic studies. Immunoelectrophoretic studies were performed in 1% agarose at pH 8.6 and pH 8.2 in barbital buffers with commercial rabbit antihuman fibrinogen antiserum.[4] Polyacrylamide gel electrophoresis was done by the method of Davis (40), with 7.5% gels and pH 8.5 Tris-glycine or pH 7.5 barbital-HCl buffers, and also in sodium dodecylsulfate-dithiothreitol (SDS-DTT) as described by McDonagh, Messel, McDonagh, Murano, and Blomback (41). Densitometry was performed on the stained gels with a Gilford gel-scanner.[7] In some experiments [^{131}I]fibrinogen purified from normal plasma and [^{125}I]fibrinogen purified from the propositus or her son's plasma were mixed and run in the gel. After electrophoresis the gels were stained with Coomassie blue and cut into 2-mm segments. The gel segments were placed inside glass tubes, and the radioactivity present in each gel segment was counted in a two-channel Nuclear-Chicago counter.[9]

DEAE-cellulose[10] chromatography was performed at 4°C by using a continuous concave gradient with a starting buffer of pH 8.6, 0.005 M Tris-phosphate and a final buffer of pH 4.1, 0.50 M Tris-phosphate (42). In some experiments purified normal fibrinogen plus trace amounts of propositus [^{125}I]fibrinogen were dialyzed together and chromatographed. In other experiments trace amounts of normal [^{131}I]fibrinogen were added to propositus purified fibrinogen before dialysis and chromatography. Absorbance at 280 nm, radioactivity, pH, and conductivity were measured on column effluents. The influence of iodine labeling on the chromatographic characteristics of fibrinogen was examined by chromatographing mixtures of [^{125}I]fibrinogen and nonlabeled fibrinogen on DEAE-cellulose columns and measuring absorbance and radioactivity of effluent.

Metabolic studies. Autologous purified patient fibrinogens were labeled with ^{125}I, and normal fibrinogen was labeled with ^{131}I by the iodine monochloride method of McFarlane (43) as previously described (44). Unbound iodine was removed by passage of each protein through a 2 × 90-cm column of Sephadex G-100[11] equilibrated with sterile, pyrogen-free buffer consisting of 0.25 M sodium chloride and 0.01 M trisodium citrate, pH 7.0. The labeled proteins were free of unbound iodine, and each protein contained an average of less than ½ atom of iodine per molecule. Specific activities ranged between 2 and 4 μCi/mg of protein. Clottability of the labeled fibrinogen, measured by the method of Regoeczi (45), ranged between 92 and 96%. Metabolic studies of D. S. and J. S. were done separately, 3 mo apart, and fibrinogens were labeled immediately before injection. Just before injection, the labeled proteins were passed through a G2, 0.22-μm Millipore filter.[12] 20–25 μCi of each protein were injected through separate syringes, and 5-ml blood samples were drawn 15 min and 4 h later and once daily for another 6 days. In all samples, more than 95% of the plasma radioactivity was clottable. Catabolic and synthetic rates of fibrinogen were calculated from the plasma radioactivity disappearance curves by the method of Matthews (46). Fractional catabolic rates were also calculated from the 24-h urinary excretion of radioactivity (47). 24 h after the labeled fibrinogens were injected, 5 ml of plasma from each subject was filtered through a 2.5 × 90-cm column of Sepharose 4-B,[10] equilibrated with 0.25 M NaCl-0.01 M trisodium citrate, pH 7.0. Absorbance at 280 nm and ^{131}I and ^{125}I radioactivity were measured in the effluent. Patients received 10 drops of saturated potassium iodide solution orally twice daily for 1–2 days before and once daily during the study. Hematocrit, platelet count, and fibrinogen concentration were measured and found to be stable throughout the study. Patients were afebrile and were not taking any medication during the study.

RESULTS

Clinical data. The family pedigree is shown in Fig. 1. The propositus (J. S.) is a 46-yr-old white woman with a long history of excessive bleeding after minor trauma, tonsillectomy, and tooth extractions. She bled profusely after her two deliveries, requiring blood transfusions. After the diagnosis of hypodysfibrinogenemia was established, she underwent a total hysterectomy under cover of plasma infusions without abnormal blood loss. Her surgical incision healed normally. The propositus' sister (N. L.) has never had bleeding manifestation. The propositus' son is 21 yr old and has no history of excessive bleeding. He underwent an appendectomy at age 14 without complications.

As shown in Fig. 1, three members of this family in two different generations have a low plasma fibrino-

[9] Nuclear-Chicago Corp., Des Plaines, Ill.

[10] Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.

[11] Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

[12] Millipore Corp., Bedford, Mass.

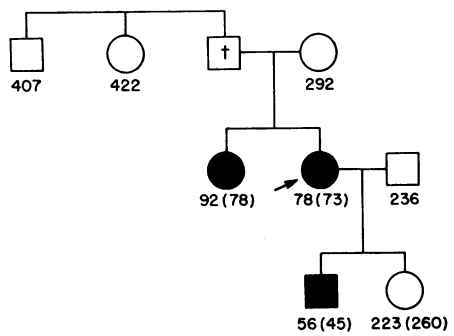

FIGURE 1 Family pedigree. The propositus is indicated by an arrow. Figures represent plasma fibrinogen concentration measured as thrombin-clottable protein or by immunodiffusion (values within parentheses).

TABLE I

Coagulation Studies on the Propositus (J. S.), her Son (D. S.), and her Sister (N. L.)

	Normal	J. S.	D. S.	N. L.
Bleeding time (minutes)	2–7	5	5½	—
Platelet count (per mm³)	150,000–350,000	230,000		
Prothrombin time (seconds)	11.5–13.5	16	16.2	16
Partial thromboplastin time (seconds)*	45–90	76	74	74
Thrombin time (seconds)	18–22	83.6	86	56
Reptilase time (seconds)	38.5	300	300	190
Fibrinogen, mg/100 ml				
Thrombin-clottable	232–444	73	45	78
Immunoreactive	‡	78	56	92
Ammonium sulphate-precipitable	160–380	78	56	—
Euglobulin lysis time (minutes)	>120	120	>120	—
Fibrinogen-fibrin-related antigen (μg/ml)	<2	<2	<2	—
Serial dilution protamine sulfate test	negative	negative	negative	—
Factors II, V, VII, VIII, IX, X, XI, XII, XIII		normal	normal	

* Nonactivated.

‡ Measured against standard known pruified fibrinogen.

gen concentration when measured either as thrombin-clottable protein or by immunodiffusion.

Coagulation studies. Prothrombin, thrombin, and Reptilase times were prolonged in J. S., D. S., and N. L., the three affected family members (Table I). Fibrinogen concentration was markedly reduced in these three subjects, with good agreement between the three methods utilized. The euglobulin lysis time was normal, and no fibrinogen-fibrin-related antigens were detected in the serum. The serial dilution protamine sulfate test was negative. The influence of calcium on the thrombin and Reptilase times of propositus (J. S.) plasma is shown in Fig. 2. The prolonged thrombin time of propositus plasma shortened considerably after the addition of calcium but still remained longer than the thrombin time of normal plasma in a calcium-free system. The Reptilase time of propositus plasma was markedly prolonged

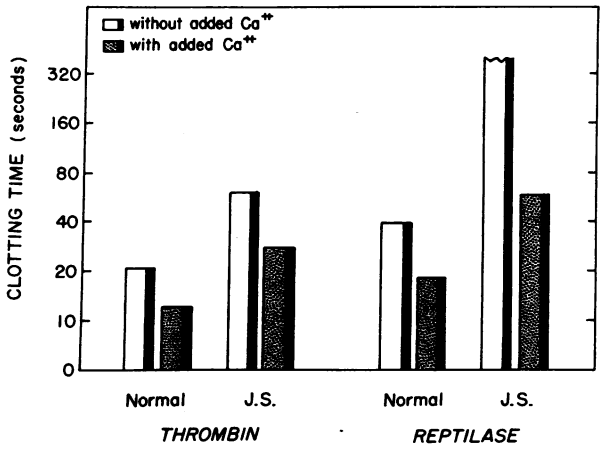

FIGURE 2 Thrombin and Reptilase times of normal and J. S. plasmas with and without the addition of calcium. The mixture consisted of 0.1 ml plasma, 0.2 ml imidazole buffered saline, and 0.1 ml bovine thrombin (10 U/ml) or Reptilase (20 μg/ml).

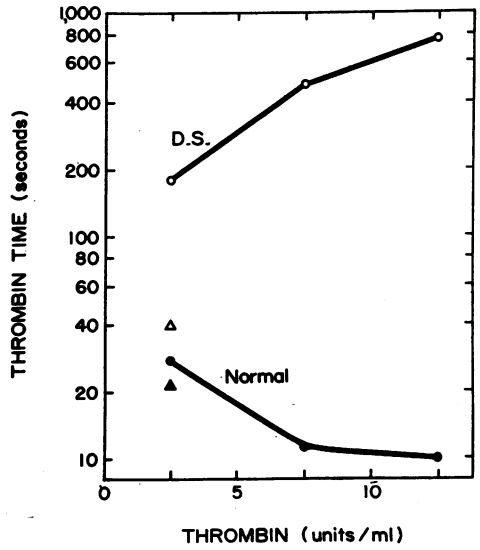

FIGURE 3 Thrombin times of purified normal and D. S. fibrinogens (fibrinogen concentrations were 130 mg/100 ml). Abscissa is given as final thrombin concentration. Solid lines represent the effect of increasing thrombin concentrations. Triangles represent the influence of normal fibrinogen (▲) or D. S. fibrinogen (△) on the thrombin time of normal plasma.

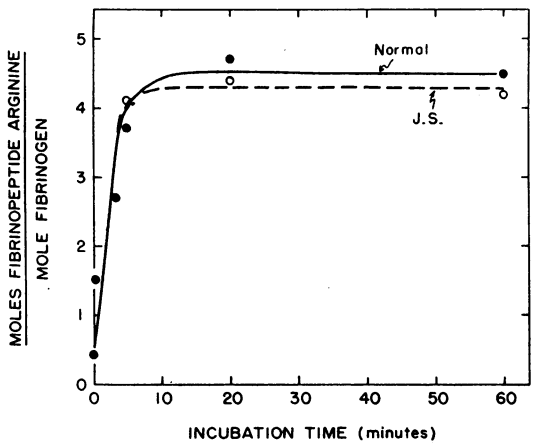

FIGURE 4 Fibrinopeptide release from normal and J. S. fibrinogen. Fibrinogen concentration was 3.5 mg/ml, thrombin concentration was 4.5 U/ml, temperature was 25°. Clots were visible in the normal at 20 s, and in J. S. at 140 s.

and was only partially corrected by the addition of calcium. The effect of increasing concentrations of thrombin on the clotting of D. S. or normal purified fibrinogen is shown in Fig. 3. While increasing concentrations of thrombin shorten the clotting time of normal fibrinogen, an opposite effect was found when D. S. fibrinogen was tested. Addition of purified D. S. fibrinogen to normal plasma caused a significant prolongation of the thrombin time, compared to mixtures of normal fibrinogen and normal plasma.

Fibrinopeptide release and fibrin monomer aggregation. Thrombin-induced fibrinopeptide release from patient compared with normal purified fibrinogen is shown in Fig. 4. Both the rate and final amount of fibrinopeptide released are entirely normal. Identical results were obtained with the Folin-Lowry method.

Fibrin monomer aggregation, on the other hand, was grossly abnormal (Fig. 5). When normal fibrin monomers are allowed to aggregate in a high (0.2) ionic strength medium, there is an initial delay in fibrin formation, followed by a rapid rise in absorbance which reaches a plateau after 20 min of incubation. D. S. fibrin monomers did not show any aggregation at this ionic strength. In a low (0.05) ionic strength buffer, both normal and D. S. fibrin monomers initiated aggregation immediately, although D. S. still showed decreased total aggregation when compared with normal.

Fibrin monomer aggregation was also studied in plasma (Fig. 6). No aggregation of D. S. fibrin monomers was found even after 40 min of incubation with thrombin, and a marked inhibitory effect was observed when D. S. and normal plasmas were admixed in this system.

Electrophoretic and chromatographic studies. Immunoelectrophoresis of plasma or fibrinogen from the

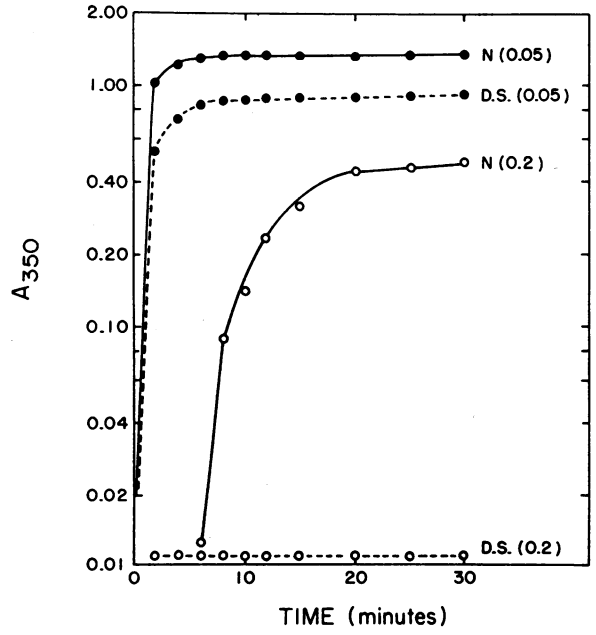

FIGURE 5 Aggregation of normal (N) and D. S. purified fibrin monomers at ionic strength of 0.2 (○) and 0.05 (●). Final pH was 6.7 and protein concentration was 0.5 mg/ml.

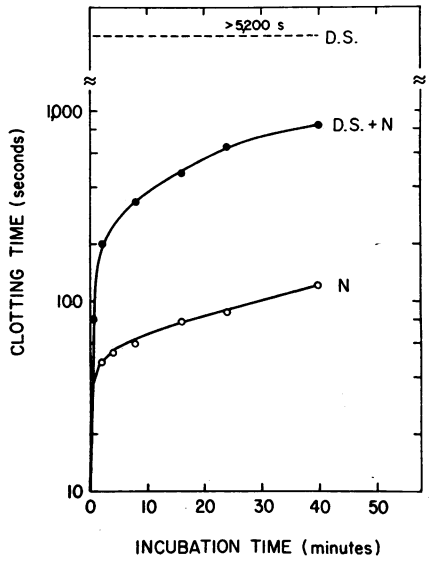

FIGURE 6 Fibrin monomer aggregation in normal plasma (○), and a mixture of equal volumes of normal and D. S. plasmas (●). Plasmas were incubated with thrombin at pH 5.2. At timed intervals (abscissa) 15 U heparin in sodium acetate-barbital buffer (pH 9.6) was added to the mixture (final pH 7.2). D. S. fibrin monomer (dashed line) did not aggregate in this system.

propositus (J. S.) or her son (D. S.) revealed an immunoprecipitin arc of normal mobility. However, the plasma precipitin arc was of distinctly decreased density compared to normal. Purified fibrinogen from J. S. and D. S. were also examined by polyacrylamide disk gel electrophoresis. No consistent differences from normal were observed. However when D. S. [^{125}I]fibrinogen was mixed with normal [^{131}I]fibrinogen and then run in the same electrophoretic system, D. S. fibrinogen consistently migrated 2–4 mm more anodally than normal (Fig. 7). In SDS-DTT polyacrylamide gels no differences were noted between the mobilities of Aα, Bβ, and γ chains of J. S. and normal fibrinogen. Densitometric tracings (Fig. 8) confirm this observation and demonstrate, in addition, the presence of intact Aα chains in J. S. fibrinogen as judged by ratios of staining intensity of Aα, Bβ, and γ bands.

Simultaneous gradient elution chromatography on DEAE-cellulose of normal [^{131}I]fibrinogen mixed with D. S. or J. S. fibrinogen revealed some differences. Both normal and abnormal fibrinogens eluted in two major peaks, as previously described by Finlayson and Mosesson (42). However, the abnormal fibrinogen first peak eluted two tubes later than the normal fibrinogen first peak (Fig. 9 A). A similar difference may be present in the second peak. These differences cannot be ascribed to the iodination procedure itself since labeled

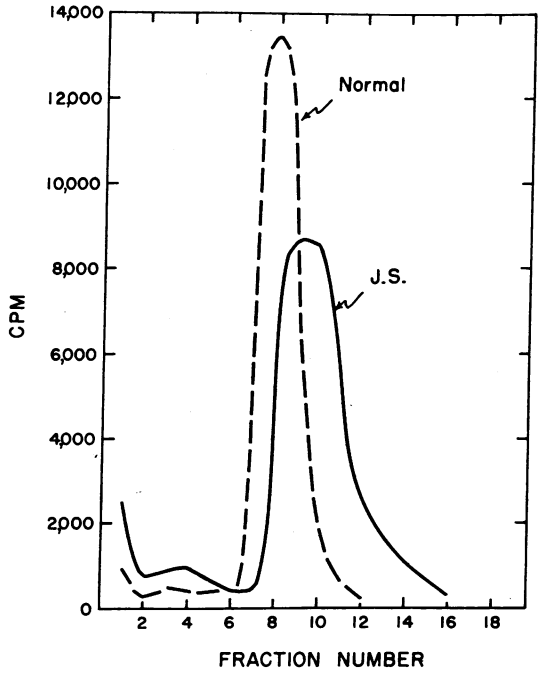

FIGURE 7 Electrophoretic mobility of [^{131}I]normal fibrinogen mixed with [^{125}I]J. S. fibrinogen in 5% polyacrylamide gel. The gel was cut into 2-mm segments and ^{131}I and ^{125}I radioactivity counted.

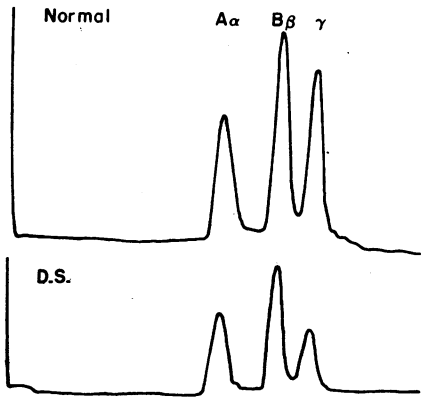

FIGURE 8 Densitometric scans of SDS polyacrylamide gels of reduced normal and D. S. fibrinogens. The anode is to the right.

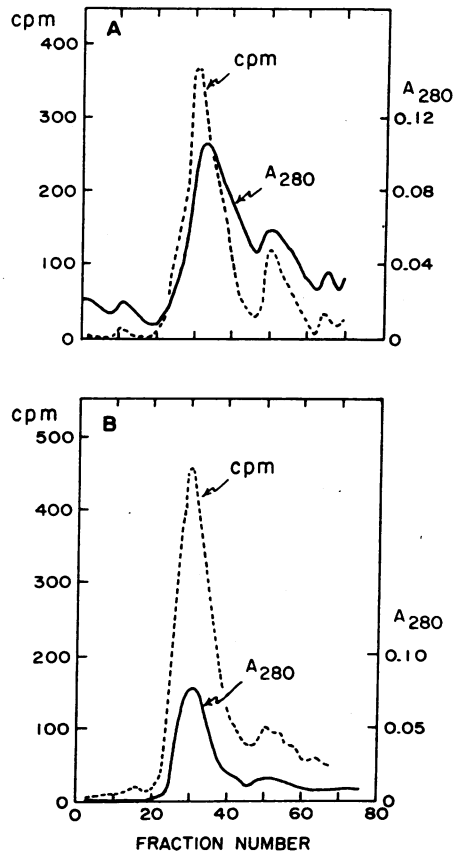

FIGURE 9 A. DEAE-cellulose chromatography of 10 mg of J. S. fibrinogen (solid line), mixed with tracer amounts of [^{131}I]normal fibrinogen (dashed line). B. Similar chromatography of 10 mg of normal fibrinogen (solid line) mixed with tracer amounts of [^{131}I]normal fibrinogen (dashed line). A concave gradient from 0.005 M Tris phosphate, pH 8.6, to 0.5 M Tris phosphate, pH 4.2, was used for both experiments.

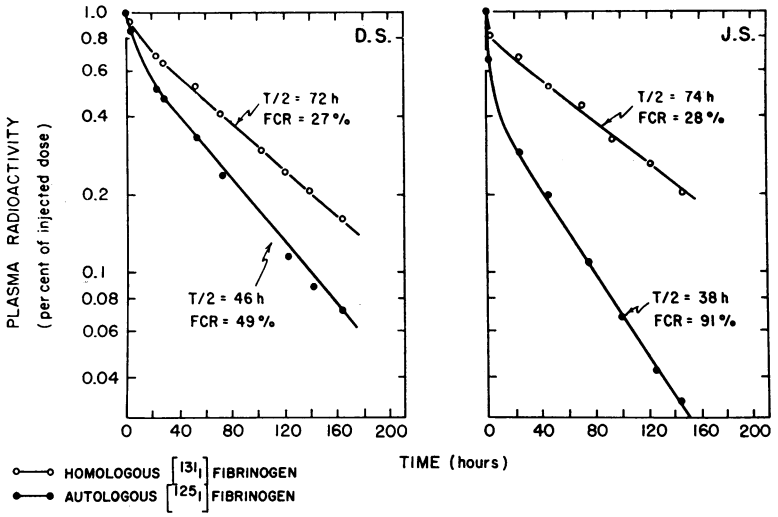

FIGURE 10 Plasma radioactivity disappearance curves of [^{125}I]autologous and [^{131}I]homologous fibrinogens in subjects D. S. and J. S. T/2, half-life; FCR, fractional catabolic rate.

and nonlabeled fibrinogens eluted at the same position in this gradient system (Fig. 9 B).

Metabolic studies. The results of autologous and homologous fibrinogen metabolic studies performed in the propositus (J. S.) and her son (D. S.) are depicted in Fig. 10 and Table II. The normal fibrinogen metabolic data given in Table II are the result of homologous fibrinogen turnover studies performed in six healthy young men. The values obtained in this group are in close agreement with data reported by Takeda (48) and the mean values calculated from the data of several investigators (49). In both family members normal fibrinogen showed a normal half-life and fractional catabolic rate. In contrast the subjects' own fibrinogen had a shortened half-life–1.91 days in D. S. and 1.58 days in J. S. Fractional catabolic rates calculated either from the plasma radioactivity disappearance curves or from urinary radioactivity excretion were markedly increased in both family members—49% of the intravascular pool per day in D. S. and 91% in J. S. In all cases the fraction of plasma radioactivity excreted in the urine was lower in the first 24 h of the study than in any subsequent 24-h period, suggesting the absence of significant denaturation in the injected fibrinogen preparations.

When plasma samples were gel-filtered on Sepharose

TABLE II

J. S. and D. S. Fibrinogen Metabolic Studies

	Fibrinogen half-life	Intravascular distribution	Fractional catabolic rate: Data derived from: Plasma	Fractional catabolic rate: Data derived from: Urine	Fibrinogen synthesis rate
	days	*% of total pool*	*% of plasma pool/day*		*mg/kg per day*
J. S.					
Autologous fibrinogen	1.58	55.0	91.4	83.6	30.7
Homologous fibrinogen	3.08	81.0	27.9	25.7	—
D. S.					
Autologous fibrinogen	1.91	77.5	49.0	52.6	14.9
Homologous fibrinogen	3.00	88.3	27.4	26.1	—
Normal mean*	3.74	74.6	26.3	21.2‡	28.9
(Range)	3.00–4.08	60.9–89.4	21.3–32.0	20.0–22.0	17.0–46.8

* Six subjects.

‡ Three subjects.

4-B columns the ^{125}I and ^{131}I radioactivity eluted simultaneously in a single peak in the position of normal fibrinogen.

DISCUSSION

Since the initial description of an abnormal fibrinogen by Imperato and Dettori in 1958 (12), many other dysfibrinogenemias have been described. Although functional and physicochemical studies have established the distinctness of many of the abnormal fibrinogens, a specific amino acid substitution has been found only in fibrinogen Detroit (50). The distinctive features of these dysfibrinogenemias have been recently reviewed (51, 52). The functional clotting defect of an abnormal fibrinogen may be due to (*a*) an altered reactivity to the proteolytic action of thrombin, (*b*) a disturbance in the aggregation of fibrin monomers, or (*c*) a diminished ability to form covalently linked fibrin polymers under the influence of Factor XIII. In some cases a combination of the first two mechanisms has been reported (51, 52). Fibrinogen Philadelphia exhibits a low reactivity to thrombin and Reptilase that is partially corrected by the addition of calcium. The shortening of thrombin and Reptilase times under the influence of calcium probably represents an acceleration of fibrin monomer aggregation rather than of the proteolytic action of thrombin, as has previously been demonstrated for normal fibrinogen (53, 54) and fibrinogen Zurich (39). An unusual aspect of the behavior of fibrinogen Philadelphia, however, is the progressive lengthening of thrombin times with increasing concentrations of thrombin. There is no explanation at present for this behavior. Fibrinogen Philadelphia releases a normal total amount of fibrinopeptide at a normal rate when exposed to thrombin. The whole blood clot is not soluble in urea, indicating grossly normal covalent cross-linking by Factor XIII. The abnormality responsible for the coagulation defect is a delay in fibrin monomer aggregation. This abnormality is still more evident when the monomers are allowed to aggregate in a medium of high ionic strength, as previously described with another genetically abnormal fibrinogen (39). The inhibiting effect of high ionic strength is not unique to hereditary dysfibrinogenemias, however, since fetal fibrinogen (55) and an abnormal fibrinogen described in a patient with primary hepatoma (56) behave in a similar fashion. The lack of aggregation of fibrin monomers in a high ionic strength medium is suggestive of a defect in the end-to-end aggregation of fibrin monomers (57–59).

Electrophoretic and chromatographic studies of normal and abnormal fibrinogens have been very useful in the differentiation of the dysfibrinogenemias. Although fibrinogen Philadelphia has a normal migration on immunoelectrophoresis, a slight increase in the anodal mobility was noted in polyacrylamide gel electrophoresis. No abnormalities in electrophoretic mobility of $A\alpha$, $B\beta$, or γ chains were observed in SDS-DTT polyacrylamide gels. Several of the abnormal fibrinogens tested in this system have shown normal migration, except for fibrinogen Zurich, which exhibited marked heterogeneity (5) and fibrinogen Metz, which showed a slight increase in the anodal migration of the $A\alpha$-chain (9). The delayed elution of fibrinogen Philadelphia on DEAE-cellulose is similar to that of fibrinogens Nancy (10), Paris II (4), and Baltimore (60). In the latter two fibrinogens Mosesson observed heterogeneity of the major peak on DEAE-cellulose, whereas the first peak of fibrinogen Philadelphia is symmetrical. The slight abnormalities of fibrinogen Philadelphia in polyacrylamide gels and DEAE-cellulose are probably significant since they can be demonstrated in experiments involving mixtures of normal and abnormal fibrinogens. Fibrinogen Philadelphia's increased anodal migration and binding to DEAE-cellulose are suggestive of an increase of negative charge on the molecule.

In some dysfibrinogenemic families the presence of both normal and abnormal fibrinogens was demonstrated by immunoelectrophoresis, acrylamide gel electrophoresis, DEAE-cellulose chromatography, or coagulation studies (5). Fibrinogen Philadelphia showed no heterogeneity in any of these systems so that the presence of normal fibrinogen could not be demonstrated.

An unusual feature of fibrinogen Philadelphia, which serves to distinguish it from all of the previously reported dysfibrinogenemias except fibrinogen Parma (12), is the grossly depressed circulating fibrinogen concentration. Fibrinogen Parma can, nevertheless, be differentiated from fibrinogen Philadelphia since the former had no inhibitory effect on the thrombin time of normal plasma. In addition the hereditary nature of fibrinogen Parma could not be demonstrated since fibrinogen levels in the immediate family were normal. Although fibrinogen Vancouver (13) was originally classified as a hypofibrinogenemia, immunochemical studies later showed normal concentrations of plasma fibrinogen (61).

The major factors believed responsible for maintaining the steady state plasma fibrinogen concentration are (*a*) the hepatic synthesis rate, (*b*) the catabolic rate, and (*c*) the relative distribution between intravascular and extravascular compartments. Autologous fibrinogen metabolic studies performed in J. S. and D. S. indicate that rapid catabolism is in great part responsible for their depressed fibrinogen concentration. Fibrinogen hypercatabolism is known to occur in clini-

cal conditions associated with excessive activation of the coagulation (62–64) and/or the fibrinolytic systems (65, 66). The normal euglobulin lysis times, absence of fibrinogen-fibrin degradation products, the negative protamine sulfate dilution test, and particularly the homologous fibrinogen turnover studies in these subjects make such possibilities unlikely. Moreover, the disparity between the abnormal autologous and normal homologous fibrinogen catabolic rates clearly demonstrates that the rapid degradation of fibrinogen Philadelphia is due to an intrinsic molecular defect. The decreased fibrinogen levels and intravascular pools in J. S. and D. S. probably play no part in the observed findings, since it has been shown that fibrinogen catabolism is independent of both fibrinogen level and the size of intra- and extravascular fibrinogen pools (67–69).

The possibility still exists that the abnormal fibrinogen in this family shows increased sensitivity to plasmin action and that their purified fibrinogen therefore represents early products of plasmin digestion. Such a mechanism might account for faster than normal catabolism. However, the presence of intact Aα chains in fibrinogen Philadelphia seems to rule out this possibility. Furthermore, fragment X and fractions 1–8 and 1–9, the earliest plasmin degradation products of fibrinogen, have very different properties than fibrinogen Philadelphia on acrylamide gel electrophoresis and DEAE-cellulose chromatography (70).

The mechanisms involved in plasma protein degradation are poorly understood. The catabolism of most glycoproteins seems governed in part, at least, by their carbohydrate content, since removal of terminal sialic acid residues generally results in a shortened half-life (71). We are presently studying the possibility that fibrinogen Philadelphia differs from normal in its carbohydrate structure. Actual catabolism of plasma proteins seems to occur in, or in close relation to, the intravascular space. In the particular cases of fibrinogen, limited proteolytic degradation within the intravascular compartment, perhaps partly via the action of thrombin and the fibrinolytic system (72–74), may play a significant role. However, it seems likely that fibrinogen and its degradation products undergo further major intracellular proteolysis during their transit from the intravascular to the extravascular spaces (69).

Genetic hypoproteinemias may be associated with normal or rapid catabolism of the protein in question. Thus, patients with myotonic dystrophy and hypogammaglobulinemia catabolize homologous IgG rapidly (75, 76). However, their own IgG has a normal survival in normal recipients, indicating an abnormality of the catabolic apparatus rather than the IgG itself. Hypercatabolism of the C3 component of complement has been reported in two different genetic disorders (77, 78) leading to low values of C3. In both cases homologous C3 is metabolized rapidly by affected individuals, suggesting that the catabolic defect is extrinsic to the C3 molecule. Yet another mechanism for hypercatabolism is illustrated by the finding of low levels of C1q in patients with hypogammaglobulinemia (79). The hypercatabolism and increased extravascular distribution observed seem related to the unavailability for interaction with this complement component of normal quantities of specific IgG subtypes. Fibrinogen Bethesda II seems another example of hypercatabolism associated with a defect extrinsic to the protein in question, since both homologous and autologous fibrinogen are metabolized rapidly (6). The familial hypofibrinogenemias have been poorly characterized from a genetic and biochemical point of view (51, 80), and are difficult to include in this discussion. A few studies have been done only with homologous fibrinogen, and have shown normal half-lives (81–84). Thus, the possibility that some of these hypofibrinogenemias may be due to hypercatabolism of the patient's own fibrinogen has not been excluded. The reason for the lack of compensatory synthesis in these patients and in patients with fibrinogen Philadelphia is not clear. Indeed the fibrinogen synthesis in D. S. is slightly below normal. Several studies have shown that in a variety of conditions increases in hepatic fibrinogen synthesis can compensate for hypercatabolism of the same degree seen in subjects J. S. and D. S. (62, 64), maintaining plasma fibrinogen at relatively normal levels. It is possible, therefore, that the molecular defect in fibrinogen Philadelphia could give rise to a low synthesis rate. Alternatively, rapid intrahepatic catabolism of newly synthesized protein, such as occurs in some types of α_1-antitrypsin deficiency (85), may occur.

Our data indicate that fibrinogen Philadelphia is altered both in its biologic activity and in its metabolic properties. Whether the defective polymerization and the rapid catabolism are due to the the same mutation is not known at present. In any case, fibrinogen Philadelphia appears to be the first example of a hereditary plasma protein defect associated with abnormal catabolism. In view of our incomplete understanding of the catabolism of plasma proteins, this family affords a unique opportunity to investigate some of the factors governing this process.

ACKNOWLEDGMENTS

We wish to thank Mrs. Elizabeth Aukstikalnes for her expert technical assistance.

These studies were supported by research grants HE-14566 and HE-09163 and institutional grant HL-06374 from the National Institutes of Health.

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