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Sandor S. Shapiro, ... , Jose Martinez, Ruth R. Holburn

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Research Article

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Congenital Dysprothrombinemia: an Inherited Structural Disorder of Human Prothrombin

SANDOR S. SHAPIRO, JOSE MARTINEZ, and RUTH R. HOLBURN

From the Cardeza Foundation for Hematologic Research, Department of Medicine, Jefferson Medical College, Philadelphia, Pennsylvania 19107

ABSTRACT A large family has been studied, 11 of whose members have half-normal plasma concentrations of biological prothrombin activity. The pattern of inheritance is autosomal. By use of a specific immunoassay, affected family members have been shown to possess normal quantities of immunoreactive prothrombin, whose immunologic properties seem identical with those of the normal zymogen. Prothrombin isolation from the plasma of one such individual gave normal yields of protein but half-normal amounts of prothrombin activity. Activation of this material in the "intrinsic" and "extrinsic" systems, in concentrated sodium citrate, or by trypsin, gives rise to half, or less, of the thrombin clotting and esterase activities expected from a comparable normal prothrombin preparation. During the clotting of blood from an affected individual, all material with the mobility of prothrombin disappears. Immunoelectrophoresis of the serum reveals a normal nonthrombin "pro piece," and an additional activation product with an electrophoretic mobility intermediate between that of prothrombin and of "pro piece." These results suggest that affected individuals are heterozygotes in whom half the prothrombin molecules synthesized are structurally abnormal, since they undergo some alterations during activation, but are incapable of releasing the active enzyme, thrombin.

INTRODUCTION

Since prothrombin is normally recognized by the biological action of its activation product, thrombin, it has hitherto not been possible, in cases of prothrombin deficiency, to distinguish between lack of synthesis of this protein and the production of an altered, biologically inactive zymogen molecule. The recent development of

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methods for the purification of human prothrombin (1-3) and the availability of specific antisera (4-7) have permitted the use of physical and immunologic techniques for the investigation of this problem. Thus, one previously reported case of congenital hypoprothrombinemia (8, 9) has been found to lack almost completely any material capable of being fractionated by a standard technique for the isolation of plasma prothrombin (10). In two other unrelated cases, the deficiency of plasma prothrombin was equally severe by biological and immunologic assays (5,¹). We have studied a large family, in which 11 members have half-normal levels of prothrombin biological activity. Affected individuals, however, possess normal circulating quantities of immunoreactive zymogen. It appears that these individuals are heterozygous for the synthesis of a defective prothrombin molecule, which is incapable of forming the enzyme thrombin. We propose that this defective molecule be termed "prothrombin Cardeza."

METHODS

Coagulation methods

For the preparation of plasma, blood was collected with plastic syringes into $\frac{1}{6}$ volume of 0.1 M sodium citrate and transferred to plastic or silicone-coated test tubes. After centrifugation for 15 min at 4°C and 2500 g, the platelet-poor plasma was removed and either tested directly or quick-frozen in acetone-dry ice and stored at -85°C.

Serum was prepared from blood collected without anticoagulant into sterile, uncoated glassware, and incubated first at 37°C for 3-6 hr, then at 4°C overnight. The clot was removed by centrifugation and the serum stored frozen.

Plasma prothrombin was assayed by the two-stage technique of Ware and Seegers (11), slightly modified (2). Results were corrected for the volume of anticoagulant used in the blood collection. This assay measures the thrombin generated from prothrombin by the action of a tissue extract and factors V, VII, and X. 1 U of thrombin is defined as that amount clotting a standard fibrinogen solution in

¹ Kattlove, H., S. S. Shapiro, and M. Spivack. Hereditary prothrombin deficiency. *N. Engl. J. Med.* In press.

15 sec, and 1 U of prothrombin is defined as that amount of zymogen giving rise to 1 U of thrombin.

Fibrinogen concentration was measured by the method of Ellis and Stransky (12). Factor V was assayed by correction of the prolonged prothrombin time of aged, oxalated human plasma (13). Factors VII and X were measured by correction, respectively, of the prothrombin time and Russell's viper venom time of congenitally deficient substrate plasmas. Assays for factors VIII, IX, and XI were done by standard one-stage methods, as previously described (14).

During prothrombin activation experiments (see below) thrombin concentrations were determined by adding 0.1 ml of a suitable dilution of test material to a tube containing 0.1 ml of fibrinogen solution and 0.3 ml of buffered acacia-calcium solution, so that, except for the absence of tissue extract and accessory factors, conditions were the same as in the clotting stage of the prothrombin two-stage assay (11, 2).

Prothrombin activation

Prothrombin was isolated from the fresh acid citrate dextrose (ACD)-plasma of a normal donor and family member II-7 by a previously published method (2), which involves successive adsorptions on diethylaminoethyl cellulose (DEAE-cellulose) and barium citrate, followed by precipitation with ammonium sulfate. This material ("step 3" prothrombin), which is about 95% purified, still contains significant quantities of factors VII, IX, and X, and can be activated in 25% sodium citrate (15). For some experiments prothrombin was further purified by ion-exchange chromatography on Biorex 70 (16, 2). This material ("step 5" prothrombin) is homogeneous by several physicochemical and immunologic criteria, is essentially free of all other coagulation factor activities, and activates poorly, if at all, in 25% sodium citrate. "Step 5" prothrombin has a specific activity of 1970 two-stage U/mg protein.

Intrinsic activation (17, 18). To a glass test tube containing 0.3 ml of a standard citrated normal plasma was added 0.33 mg of normal or patient II-7 "step 5" prothrombin in 0.1-0.2 ml, and the volume brought to 2.75 ml with imidazole-buffered saline, pH 7.2 (14). The mixture was warmed to 37°C and recalcified with 0.25 ml of 0.1 M CaCl₂. The initial concentrations of prothrombin activity in the normal and patient systems were 210 and 96 U/ml, respectively. Two-stage prothrombin and thrombin assays were performed at frequent intervals.

Extrinsic activation (11). "Step 3" prothrombin from a normal individual or from patient II-7 was activated with tissue extract in the standard two-stage prothrombin assay. Protein concentration of the prothrombin was the same in both activation mixtures, approximately 0.4 µg/ml.

Citrate activation (15). Normal or patient II-7 "step 3" prothrombin, at a concentration of 2.2 mg/ml, was mixed with an equal volume of 50 gm/100 ml sodium citrate solution and incubated at room temperature. Aliquots were removed for two-stage prothrombin and thrombin determinations over the subsequent 80 hr. Initial prothrombin concentrations were 2000 and 780 U/ml, respectively, for the normal and patient incubation mixtures.

The development of tosylarginylmethylester (TAME) esterase activity was also followed during citrate activation (19). Aliquots of the activation mixture were diluted in imidazole-buffered saline and the TAME esterase activity measured at 22°C in a Radiometer recording pH-stat,

according to the method of Ehrenpreis and Scheraga (20).[†] Activity is expressed as micromoles of TAME hydrolyzed per minute per milliliter of activation mixture, as determined from the initial slope of the TAME hydrolysis reaction. No TAME esterase activity was detectable at the beginning of citrate activation.

Trypsin activation (21, 22). To 1 ml of a 125 µg/ml solution of normal or patient II-7 "step 3" prothrombin were added 0.1 ml of 0.1 M CaCl₂ and 0.1 ml of a 50 µg/ml solution of trypsin in 0.001 M HCl. The mixture was incubated at 37°C and prothrombin and thrombin assays were performed over the next 60 min. Assuming a molecular weight for human prothrombin of 70,000-75,000 (10), the trypsin:prothrombin molar ratio was 1:8. Initial concentrations of prothrombin activity were 200 and 100 U/ml, respectively, in the normal and patient activation systems.

Prothrombin turnover

18 µc of normal "step 3" prothrombin-¹²⁵I was injected intravenously into patient II-7, and the disappearance of plasma radioactivity monitored. Technical details of this procedure and normal prothrombin metabolic parameters have recently been published (23).

Immunologic methods

Rabbits were immunized by the subcutaneous injection of "step 5" prothrombin in complete Freund's adjuvant once a week for 3 wk (6). Serum was harvested 5 days after the last injection and periodically thereafter. On immunoelectrophoresis (24) antisera develop a single precipitin arc against the provoking antigen and against normal citrated human plasma (Fig. 5, top). On incubation of antisera with normal citrated human plasma for 1 hr at 37°C prothrombin activity is totally neutralized, without any decrease in the levels of other coagulation factors, including the other vitamin K-dependent factors VII, IX, and X (6).

IgG fractions of rabbit antisera were prepared by repeated precipitation with ammonium sulfate at 33% saturation (25).

Immunologic analysis of prothrombin and prothrombin cleavage products was performed by immunoelectrophoresis and by the new technique of immunofixation electrophoresis (26). In the latter technique plasma or serum samples are electrophoresed in agarose gel,[‡] after which a concentrated antibody to prothrombin (the rabbit IgG fraction) is layered over the gel and allowed to remain in contact for 1-1½ hr at room temperature. At the end of this time the gel is washed, dried, and stained. Precipitin bands, resulting from the interaction of antibody with prothrombin or its cleavage products, are stained while immunologically unrelated protein is washed out of the gel. Although the quantity of antibody used in this method is greater than in immunoelectrophoresis, the direct contact between antibody and antigen results in much less diffusion and consequent better resolution of closely spaced antigenic components.

Immunoassay of plasma prothrombin was performed by the agarose electrophoretic method of Laurell (27)[§], except that calcium was omitted from the electrophoresis buffer. In this technique plasma is migrated electrophoretically into an antibody-containing agarose gel. The height of the precipitin peaks that are formed is a measure of the plasma prothrombin content. Doubling dilutions of a standard normal plasma are always included in the same run with plasmas to

[†] We thank Dr. Louis A. Kazal for his aid in performing these measurements.

[‡] Apparatus available from Metaloglass, Inc., Boston, Mass.

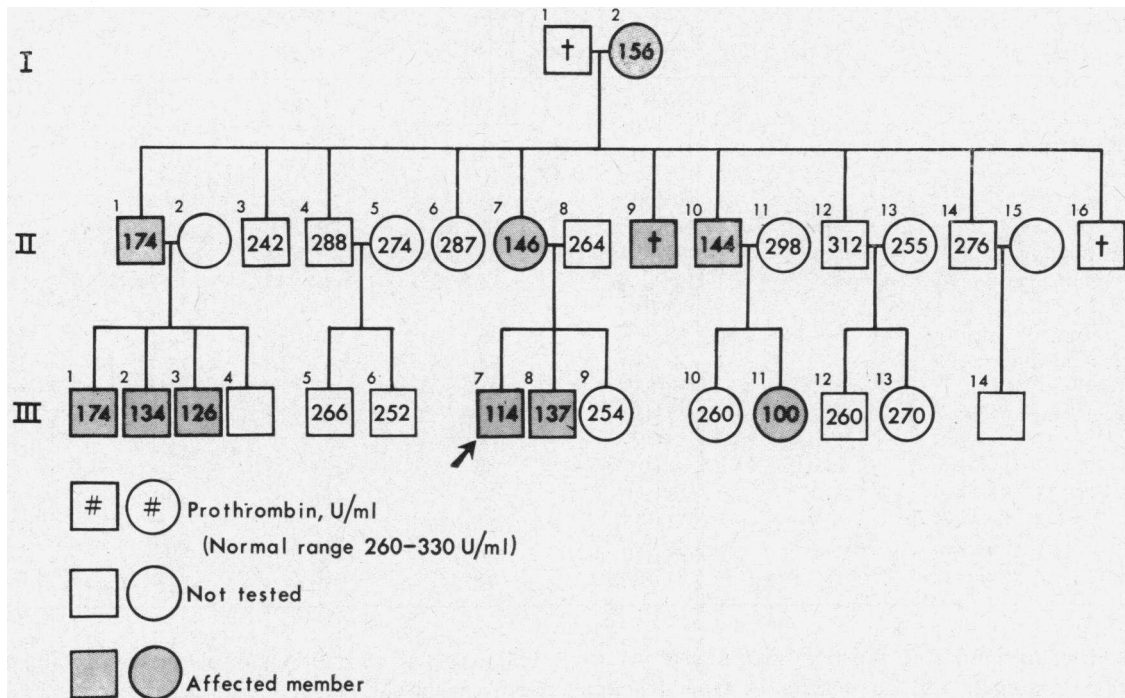


FIGURE 1 Pedigree of family with congenital dysprothrombinemia. Propositus is indicated by arrow.

be assayed, a calibration curve relating heights of peaks to concentration of plasma is constructed, and the unknowns are read from the calibration curve. In our hands the method has a reproducibility of $\pm 7\%$.

Family study

The propositus (Fig. 1, III-7) is a 13 yr old boy who was first brought to our attention at age 11, having bled excessively during a neurosurgical procedure necessitated by a subdural hemorrhage secondary to head trauma. As a result of this trauma he lost the greater part of the vision in his left eye. The only other major bleeding incident occurred at 6 yr of age, when he required two transfusions after tonsillectomy. His parents have noted occasional minor bruising since early childhood, but he has had no episodes of hemarthrosis, hematuria, gastrointestinal hemorrhage, or serious epistaxis.

Physical examination revealed a well developed, well nourished 11 yr old boy with severe visual loss in the left eye, and hyperextensible wrist and interphalangeal joints. No abnormalities were detected in the thickness or elasticity of the skin.

Investigation of the propositus' large family revealed only one other family member who bled excessively. A maternal uncle (Fig. 1, II-9) suffered from repeated episodes of subcutaneous bleeding and died at the age of 32 of an intracerebral hemorrhage. A diagnosis of classical Ehlers-Danlos syndrome (28), had been made early in life. In addition to the connective tissue disorder, studies showed a slightly prolonged one-stage prothrombin time and a depression of two-stage plasma prothrombin activity. This patient was among those reported by Day and Zarafonitis in their study of coagulation defects in the Ehlers-Danlos syndrome

(29, case 2). Subsequent examinations failed to disclose stigmata of the Ehlers-Danlos syndrome in any other members of the family.

Detailed coagulation studies are presented in Table I. The propositus (III-7) showed a depression in two-stage prothrombin to less than half the normal level (normal range, 260-330 U/ml), as did his brother (III-8) and his mother (II-7). His father's (II-8) prothrombin level was in the normal range. The only other coagulation defect noted was the propositus' factor VIII activity of 30% of normal. No other family member tested had a subnormal level of factor VIII. Patient II-9 had normal prothrombin consumption and thromboplastin generation tests (29), but these results do not exclude the possibility of a moderate deficiency in factor VIII.

RESULTS

10 family members, in addition to the deceased uncle, II-9, had abnormally low levels of plasma prothrombin activity (Fig. 1). All members were tested at least twice, at intervals of 6 months or more.⁴ Two-stage prothrombin values of affected members ranged between 100 and 174 U/ml, with a mean of 140 U/ml, half the normal mean. Two potentially affected family members were not tested: II-16, who died in infancy of unknown causes, and III-4, who was out of the country.

⁴ Repeated testing has shown that two family members, II-3 and II-10, initially thought to be affected on the basis of single tests (*J. Clin. Invest.* 47: 89a [Abstr.]), are apparently normal.

TABLE I
Representative Coagulation Studies in Four Family Members

	Normal range	III-7	III-8	II-7	II-8
Coagulation time, <i>min</i>					
Glass	6-15	10	8.5	9.5	10.5
Silicone	20-60	23	29	39	38
Bleeding time, <i>min</i>	<7	7	2.5	2.5	2.5
Platelet count	150,000-450,000	293,000	375,000	283,000	340,000
Prothrombin time, <i>sec</i> (% of normal)	11.5-13.5 (60-100)	13.8 (55)	14.7 (45)	14.7 (45)	12.2 (80)
Partial thromboplastin time, <i>sec</i>	45-90	86	62.5	63.5	55
Prothrombin consumption, <i>sec</i>	>30	25	—	72	35
Prothrombin, <i>U/ml</i>	260-330	114	137	146	264
Fibrinogen, <i>mg/100 ml</i>	225-450	265	348	230	—
Factor V, % of normal	>60	110	77	—	—
Factor VII, % of normal	>80	94	135	—	—
Factor VIII, % of normal	48-152	30	158	158	132
Factor IX, % of normal	62-138	60	62	79	118
Factor X, % of normal	54-146	60	100	90	120
Factor XI, % of normal	52-148	68	102	112	82

No abnormalities in liver function were noted in any affected individuals, and no deficits in other vitamin K-dependent coagulation factors were observed (Table I). The propositus showed no response to parenteral vitamin K administration. Hypoprothrombinemic individuals of both sexes are represented in the three generations of this pedigree, and both sexes have transmitted the defect.

The possibility that hypoprothrombinemia might be caused by abnormally rapid prothrombin catabolism, or by the presence of a circulating prothrombin inhibitor, was excluded in two ways. (a) *In vivo* metabolism of normal "step 3" prothrombin was studied in one affected individual, II-7, and the results are depicted in Fig. 2. Biological half-life of the injected material was 3.2 days, within the normal range of 2.3-3.3 days (23). (b) *In vitro* incubation of plasma from an affected patient, III-2, with normal plasma did not result in any loss of activity. As can be seen in Table II, the two-stage prothrombin activity of the mixture was the simple sum of the individual contributions of the two plasmas.

In order to differentiate between depressed synthesis and production of a defective zymogen molecule, immunoassay of plasma prothrombin was performed by the agarose electrophoresis method of Laurell (27). A typical assay is shown in Fig. 3. The sensitivity of the method is illustrated by the standard plasma dilution curve (wells 5-9). Well 9 contains a 1:32 dilution of plasma, representing a prothrombin concentration of approximately 4 $\mu\text{g/ml}$, or a total of 20 ng in the well. With suitable adjustment of the antibody concentration in the agarose gel, prothrombin concentrations of less than 1 U/ml (0.5 $\mu\text{g/ml}$) can be detected. The first four

wells contain 1:2 dilutions of plasma from four affected family members. The precipitin peak heights are similar to that of the 1:2 dilution of standard plasma (well 5). Quantitation, on the basis of the calibration curve constructed from the standard plasma dilutions, gives values for immunoreactive prothrombin of 112, 125, 100, and 87% of normal, respectively, for the plasmas in wells 1-4. Validity of these results depends on the assumption that the electrophoretic mobilities of the immunoreactive material in patient and normal plasmas are identical.

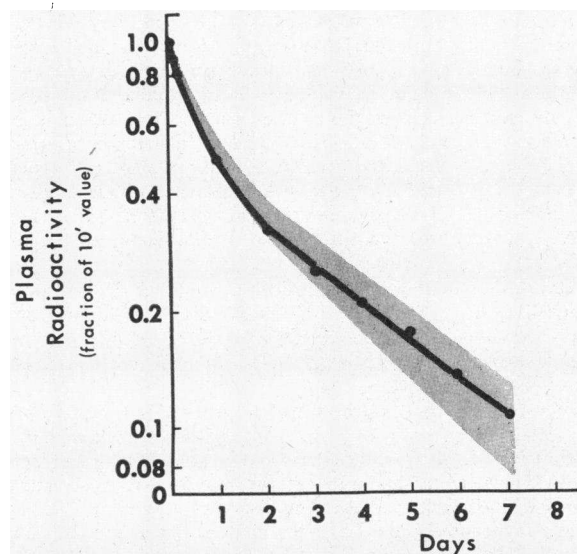


FIGURE 2 Plasma radioactivity disappearance curve in affected family member II-7, after intravenous administration of 18 μc normal prothrombin-¹²⁵I. The shaded area represents the normal range.

TABLE II
Prothrombin Concentration in Mixture of
Normal and Affected Plasma

Plasma	Prothrombin concentration <i>U/ml</i>
Normal	294
III-2	134
$\frac{1}{2}$ Normal + $\frac{1}{2}$ III-2	216

Normal and III-2 citrated plasma were incubated at 37°C separately or combined. After 1 hr two-stage prothrombin assays were performed.

Results of immunoelectrophoresis (Fig. 5, top) demonstrate that this requirement has been met. Patient plasmas contain only one immunoreactive component, whose mobility is identical with that of normal prothrombin.

The presence in patient plasma of a full complement of prothrombin molecules, in spite of half-normal levels of biological activity, was further substantiated by the results of purification experiments (Table III). "Step 3" prothrombin was prepared from approximately 550 ml of normal or patient II-7 plasma. Identical amounts of protein were recovered from both plasmas, but the isolate from patient II-7 yielded only half the biological activity of the normal isolate. This experiment was performed several times. In each case, the specific activity (two-stage units per milligram protein) was half, or less, that of the normal preparation.

Attempts to differentiate patient from normal prothrombin by immunoelectrophoresis, immunodiffusion, acrylamide gel electrophoresis, Sephadex gel filtration, and Biorex 70 chromatography, have been unsuccessful.

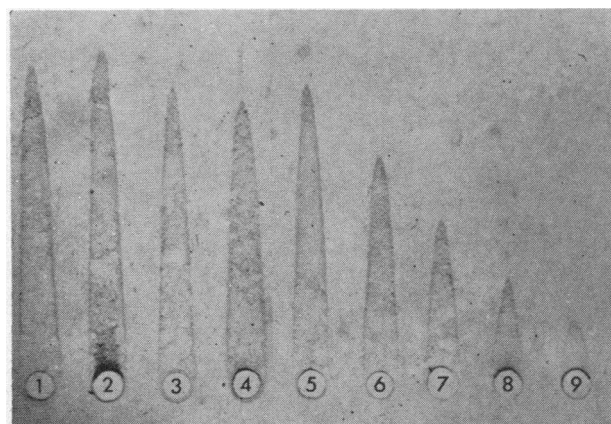


FIGURE 3 Immunoassay of plasma prothrombin by the quantitative method of Laurell (27). Sample volumes were 5 μ l. Wells 1-4: four affected family members (III-1, III-3, II-7, III-7); plasmas diluted 1:2 with electrophoresis buffer. Wells 5-9: twofold serial dilutions (1:2 to 1:32) of a standard normal plasma.

TABLE III
Prothrombin Purification

Donor	Prothrombin yield		Prothrombin specific activity
	mg	<i>U</i>	<i>U/mg</i>
Normal	33	64,358	1950
II-7	31	31,072	1002

Since it has been reported that an inactive prothrombin precursor, with anomalous calcium-binding properties, may be present in the plasma of patients treated with coumarins (30), immunoelectrophoresis of patient and normal "step 3" prothrombin was also performed in calcium-containing buffers. Again, no reproducible differences were seen between these preparations.

To investigate the possibility that the thrombin moiety exists intact within an abnormal zymogen molecule, but is not capable of being released by tissue activation, several different modes of prothrombin activation were studied (Fig. 4). Equal quantities, on a milligram basis, of patient or normal prothrombin were placed in each activation mixture, and the thrombin generated was measured. During extrinsic (tissue extract), intrinsic (plasma), citrate, and trypsin activation, patient prothrombin generated, respectively, 38, 53, 35, and 40% of the thrombin activity of the normal system. As might be expected, however, the thrombin yield expressed as a percentage of the initial two-stage prothrombin units was quite similar in normal and patient systems.

Since it has been shown that the ability of thrombin to clot fibrinogen may be abolished without interfering with

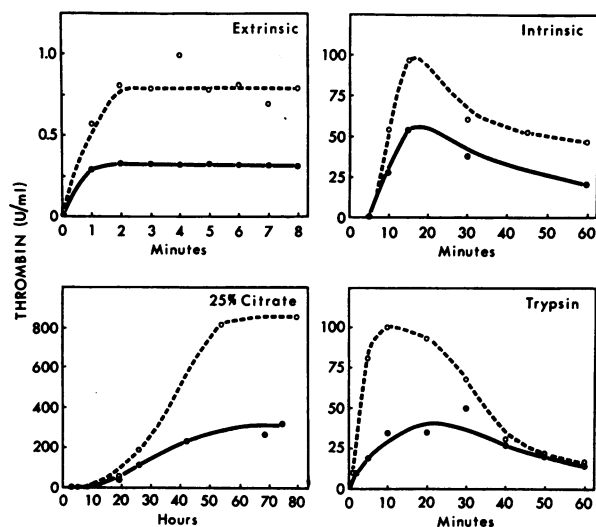


FIGURE 4 Activation of prothrombin isolated from affected family member II-7 (solid lines) and from a normal individual (broken lines).

TABLE IV

Generation of Thrombin Clotting and TAME Esterase Activity during Prothrombin Activation in 25% Sodium Citrate

Prothrombin source	Acti- vation time	Throm- bin clot- ting ac- tivity	Ester- ase ac- tivity	Ratio esterase: clotting activities
	<i>hr</i>	<i>U/ml</i>	<i>μmoles TAME hydro- lyzed/ min per ml</i>	<i>μmoles TAME hydrolyzed/min per U thrombin</i>
Normal	39	606	24.6	0.041
	46	1132	35.0	0.031
	63	1860	84.0	0.045
	70	1550	62.5	0.040
				Mean: 0.039
II-7	39	81	—	—
	46	166	5.0	0.030
	63	220	12.5	0.057
	70	240	11.3	0.047
				Mean: 0.045

its ability to hydrolyze small synthetic ester substrates (19), the generation of TAME esterase activity was followed during citrate activation. As can be seen from Table IV, no disproportionate elaboration of esterase

activity occurred, the ratio of esterase to clotting activities being the same for patient and normal activation mixtures.

The foregoing studies demonstrate that, regardless of the means of activation and of the substrate used for detecting thrombin, only half as much enzymatic activity is released from patient prothrombin as from an equal weight of normal prothrombin. These results are consistent with the existence in patient plasma of two populations of prothrombin molecules, one with full biological activity and one incapable of being activated. Although the pattern of heredity suggests this explanation, the results are equally consistent with the presence of a single population of molecules with half-normal reactivity. These alternatives were explored by examining patient serum for prothrombin activation products by immunoelectrophoretic techniques.

During normal blood coagulation prothrombin is cleaved into two major components (18). The thrombin is rapidly neutralized by plasma antithrombins and is not detectable in serum. The nonthrombin moiety, the so-called "pro piece," is easily detectable by immunologic means (Fig. 5). On immunoelectrophoresis of patient serum against antiprothrombin antiserum, it can be seen that the prothrombin precipitin arc is absent. Instead,

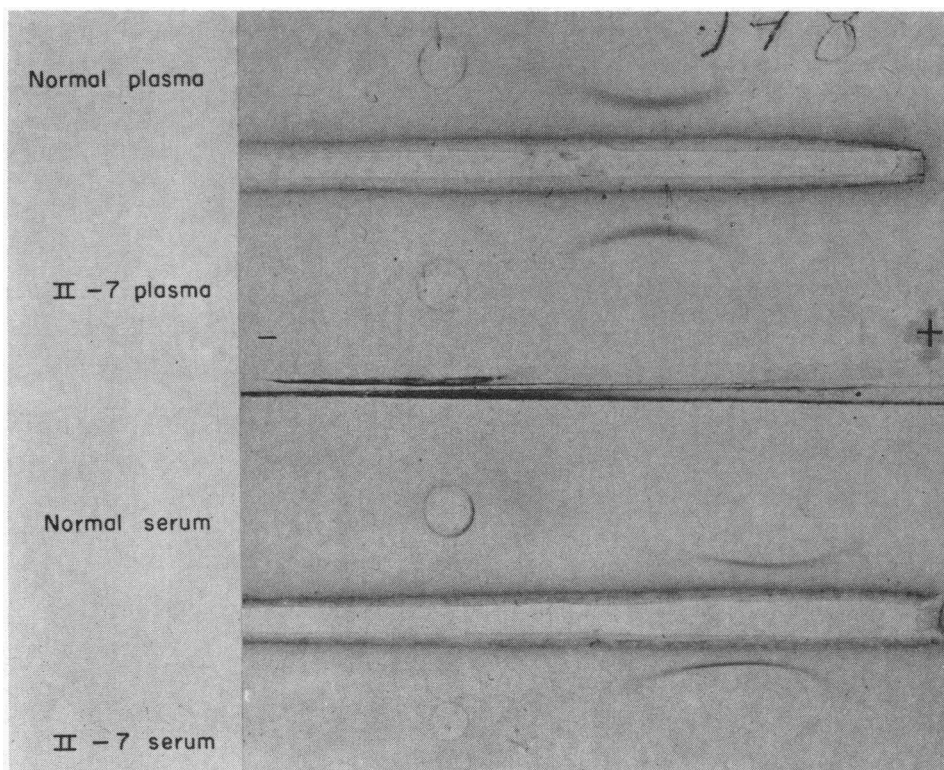


FIGURE 5 Immunoelectrophoresis of plasma and serum from a normal individual and family member II-7. The troughs contain antiserum to prothrombin. Note the abnormal cathodal extension of the precipitin arc in the serum of II-7.

a more anodally migrating arc is present whose shape and extent suggests the presence of the normal "pro piece" and a more cathodal, cross-reacting component.

Further refinement of these results is possible by the technique of agarose immunofixation electrophoresis (26), as illustrated in Fig. 6. The higher resolution of this method shows that the normal serum "pro piece" is not a single component, but rather a closely spaced double band. Patient II-7 serum is devoid of prothrombin, and contains a normal "pro piece" doublet. In addition, there is an abnormal activation product present, with a mobility intermediate between that of prothrombin and of "pro piece." Similar results were obtained in studies of sera from other affected family members.

The presence of a normal and an abnormal activation product indicates that affected family members are heterozygotes, their plasma containing normal prothrombin molecules as well as zymogen molecules capable of undergoing some steps in prothrombin activation, but incapable of forming the enzyme thrombin.

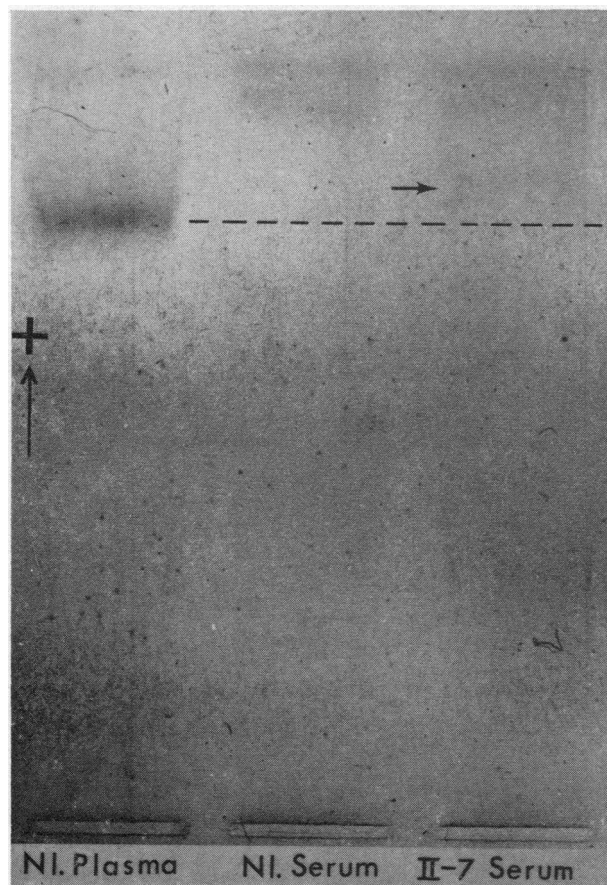


FIGURE 6 Agarose immunofixation electrophoresis (26). The arrow indicates the extra cleavage product present in the serum of II-7, NI = normal.

DISCUSSION

Interest in the genetic polymorphism of plasma proteins, and in abnormalities characterized by a deficiency in the normal biologic activity of a plasma protein, has increased greatly with recent improvements in electrophoretic and immunologic analytic techniques (31-33). It has become possible to differentiate, in many cases, between deficiency states arising from decreased protein synthesis and those caused by the production of a recognizable, though biologically inactive, protein variant. Among the proteins of the blood coagulation mechanism, three have been studied in this manner. Thus, seven families have been reported in whom genetic abnormalities of the fibrinogen molecule have been found (34-40), sometimes associated with a bleeding tendency (34, 35, 37, 40), whereas an eighth family with an altered fibrinogen has been described, whose members show an increased tendency to thrombosis (41). In the only cases in which appropriate studies have been performed (40, 42), the defect appears to be due to a single amino acid substitution. Patients with hemophilias A and B also have been investigated for the presence of circulating material cross-reactive with factors VIII or IX, using naturally occurring antibodies to these blood coagulation factors (43-49). A small percentage of patients, less than 10%, have been shown to possess normal amounts of immunoreactive material. It is quite likely that reinvestigation of patients with congenital "deficiencies" of factor VIII or factor IX, by use of a wider spectrum of more potent antibodies, will uncover a still larger number of cases of hemophilia due to synthesis of a defective protein.

Only eight well documented cases of congenital hypoprothrombinemia have been reported (9, 50, 55¹). Three of these have been studied and found as deficient in prothrombin-related protein as in biologic activity (5, 10¹). The present family represents the first in which a structural disorder of prothrombin has been found. The hereditary pattern is autosomal, and biochemical studies confirm the fact that affected individuals are heterozygotes. Heterozygosity for "prothrombin Cardeza" is associated with 50% levels of prothrombin activity and slightly prolonged prothrombin times. A bleeding tendency is not present, save for two family members in both of whom the Ehlers-Danlos syndrome was diagnosed and one of whom, the propositus, also has depressed levels of factor VIII. The presence of the Ehlers-Danlos syndrome in this family is of interest, in view of the reports of this syndrome associated with a variety of coagulation deficits (28). It is also of interest that at least two families have been reported with apparent X-linked transmission of this syndrome (56), a pattern consistent with the findings in this family. There is no evidence of a relationship between

the connective tissue disease and "prothrombin Cardeza," nor could any linkage be established between the prothrombin defect and a number of red cell and plasma protein genetic markers.⁵

The nature of the structural defect in "prothrombin Cardeza" was studied by biologic and immunologic methods. Deficient thrombin elaboration when patient plasma is activated in a normal plasma system (intrinsic activation, Fig. 4) clearly implicates an abnormality of prothrombin, rather than an abnormality of the patient's prothrombin converting mechanism. Immunoelectrophoresis demonstrates that all the patient's prothrombin disappears during blood coagulation, although only half gives rise to active thrombin and a normal serum "pro piece." The remainder is present in serum as an abnormal electrophoretic component. Preliminary studies with ¹²⁵I-labeled patient prothrombin suggest that the abnormal serum component has a molecular size only slightly smaller than prothrombin,⁶ and similar to that of the major intermediate occurring during normal intrinsic prothrombin activation (18).

The finding that the normal serum "pro piece" is a doublet is presently unexplained and requires further investigation. Since both the normal and abnormal serum components are adsorbable by prothrombin adsorbents (6), these cleavage products can be purified readily. Studies are presently underway to compare the serum activation product of "prothrombin Cardeza" with the normal "pro piece."

Note Added in Proof. Recently a second abnormal prothrombin has been discovered (Josso, F., J. M. de Sanchez, J. M. Lavergne, D. Ménaché, and J. P. Soulier. Congenital abnormality of the prothrombin molecule in four siblings: prothrombin Barcelona. *Blood*, to be published). This material is inert to tissue extracts, but can be fully activated by other means. It has not yet been possible to exchange plasma samples, but the available data suggest that prothrombin Barcelona and prothrombin Cardeza represent distinct entities.

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⁵ Schneiderman, L. J., and S. S. Shapiro. Unpublished data.

⁶ Shapiro, S. S., and J. Cooper. Unpublished observations.

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