Dysfibrinogenemia Associated with Liver Disease

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ABSTRACT To test the possibility that a functionally abnormal fibrinogen may exist in some patients with liver disease, we studied the plasma and purified fibrinogens of five patients whose plasma thrombin times were prolonged at least 40% over normal controls. In no patient was there evidence of disseminated intravascular coagulation and/or fibrinolysis. No abnormalities were detected by immunoelectrophoresis of plasmas or purified fibrinogens. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reduced patient fibrinogens showed normal mobility and amount of $A\alpha$, $B\beta$, and γ chains. Alkaline polyacrylamide gel electrophoresis and gradient elution, DEAE-cellulose chromatography of admixtures of radioiodinated patient ^{125}I-fibrinogen and normal ^{131}I-fibrinogen showed identical mobility in the gel and simultaneous elution from the column, respectively. Thrombin and Reptilase (Abbott Scientific Products Div., Abbott Laboratories, South Pasadena, Calif.) times of purified patient fibrinogens were prolonged, and calcium ions improved but did not completely correct these defects. Increasing amounts of thrombin progressively shortened the clotting times of patient fibrinogens but not to the level of normal. Addition of equal amounts of patient fibrinogen to normal fibrinogen resulted in a prolongation of the thrombin time of the normal protein. Thrombin-induced fibrinopeptide release was normal. Fibrin monomers prepared from patient plasmas and purified fibrinogens demonstrated impaired aggregation at low (0.12) and high (0.24) ionic strength. These studies demonstrate that some patients with liver disease and prolonged plasma thrombin times have a dysfibrinogenemia functionally characterized by an abnormality of fibrin monomer polymerization.

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INTRODUCTION

Most coagulation proteins are synthesized by the liver, and patients with liver disease often exhibit multiple coagulation defects (1, 2). Prolongation of the plasma thrombin time in the presence of normal or even elevated levels of plasma fibrinogen is often observed in these patients. In several cases, this abnormality has been explained on the basis of increased antithrombin activity in the plasma, probably due to circulating fibrinogen-fibrin degradation products (3) whose clearance by the diseased liver may be delayed (4). It has also been suggested that the fibrinogen molecule itself is abnormal in patients with liver disease. In one study of plasmas of patients with cirrhosis and hepatitis, impairment of thrombin-induced fibrin monomer aggregation was described. However, when studies were performed with the euglobulin fraction of the same plasmas, the functional abnormality was no longer demonstrated (5). Since the system utilized did not involve purified protein, the influence of other plasma factors cannot be excluded in these studies.

To examine the possibility that an abnormal fibrinogen may exist in patients with liver disease, we have studied the fibrinogen of five patients with clinically and histologically documented liver disease (three with alcoholic liver disease and two with postnecrotic cirrhosis of undetermined etiology). The plasma thrombin times in all five patients were at least 40% longer than normal in the absence of clinical and laboratory evidence of disseminated intravascular coagulation and/or fibrinolysis.

METHODS

Blood was collected with plastic syringes into 1/9 volume 3.8% sodium citrate or 2% disodium EDTA. Samples were then centrifuged at 4°C for 15 min at 2,500 *g*. The platelet-poor plasma was removed and tested immediately, or quick-frozen in acetone-dry ice, and then stored at −80°C.

Fibrinogen purification. Normal fibrinogen was purified from Anticoagulant Citrate Dextrose Solution U. S. P.

(Fenwal Inc., Ashland, Maine) plasma obtained from healthy hepatitis-associated antigen-negative donors by the method of Kazal et al. (6), modified by the addition of epsilon-aminocaproic acid to a final concentration of 0.1 M in plasma and buffers. Patient fibrinogen was purified by the same method from plasma collected in 3.8% sodium citrate. The purified fibrinogen dissolved in 0.055 M sodium citrate, pH 7.4, was quick-frozen in acetone-dry ice and stored at −80°C. The concentration of fibrinogen present in the purified material was measured spectrophotometrically at 280 nm, using an $A_{280}^{1\%}$ of 13.9, the figure obtained by Kazal et al. (6). The recovery of fibrinogen by this method varied between 55 and 65%, and the fibrinogen was 96% clottable.

Coagulation studies. Prothrombin time and partial thromboplastin time were done by standard methods (7). Factor V was measured by the correction of the prolonged prothrombin time of congenitally deficient plasma (8). Factor X was assayed with Diagen (Diagnostic Reagents, Ltd., Thame, Oxon, England) substrate plasma (9). Factors VIII and IX activities were measured by the kaolin-activated partial thromboplastin time (10) of congenitally deficient plasmas (with less than 1% activity), and Factor XI activity was assayed by the same method but with artificially depleted plasma (11). The presence of fibrin monomer complexes in plasma was tested by the serial dilution protamine sulfate test (12). To measure serum fibrinogen-fibrin-related antigens, 0.5 ml of citrated plasma was added to a mixture consisting of 0.02 ml of Amicar (250 mg aminocaproic acid/ml) (Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.), 0.5 ml of 0.02 M $CaCl_2$, and 0.05 ml of thrombin (100 U bovine thrombin [Parke, Davis & Company, Detroit, Mich.] per ml 50% glycerol in water). The sample was allowed to clot at room temperature for 30 min and the clot was removed by winding it onto a wooden applicator stick. The serum was then placed at 4°C for 30 min and any additional clot was removed. The resultant serum was then tested for the presence of fibrinogen-fibrin-related antigens by a latex agglutination test (13).

The thrombin clotting time was performed by adding to 0.1 ml of citrated 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). The thrombin clotting times of normal and patient purified fibrinogens were also determined. To 0.1 ml of fibrinogen at a concentration of 2.0 mg/ml in 0.02 M sodium citrate, 0.15 M NaCl, pH 7.4, was added 0.2 ml of imidazole-buffered saline and 0.1 ml of bovine thrombin (10 U/ml imidazole-buffered saline). When calcium was used, the imidazole-buffered saline contained 0.01 M $CaCl_2$. Reptilase times were performed with the lyophilized venom of *Bothrops atrox* (Abbott Scientific Products Div., Abbott Laboratories, South Pasadena, Calif.) reconstituted with distilled water. The system was identical to that described for the thrombin time except that 0.1 ml of Reptilase (20 μg/ml water) was substituted for thrombin. Thrombin times were also performed using a highly purified human thrombin obtained through the kindness of Dr. John Fenton, N. Y. State Department of Health Laboratories, Albany, N. Y.

Plasma fibrinogen concentration was measured by the thrombin clottability method of Ellis and Stransky (14), and by quantitative immunodiffusion using commercial antibody-containing agar plates (Behringwerke AG, Marburg-Lahn, West Germany).

Fibrinopeptide release of normal and patient fibrinogens was studied by measuring TCA-soluble arginine at timed intervals after the addition of thrombin (15).

Fibrin monomer aggregation studies were done by one of two methods. One was based on the procedure described by von Felten et al. (16). Plasma, or purified fibrinogen at a concentration of 2.0 mg/ml, containing disodium EDTA and 250 U/ml of Trasylol (Calbiochem, San Diego, Calif.) was diluted 1:5 with 0.15 M NaCl and treated with bovine thrombin. The mixture was incubated at 37°C for 3 h and then at 4°C for 48 h, after which the clots were recovered by centrifugation. The clots were then washed five times with 0.025% disodium EDTA in 0.15 M NaCl, and then dissolved in 5 M urea, and dialyzed against three changes of barbital-sodium acetate buffer, pH 4.6, ionic strength 0.05 at 4°C over 72 h. After dialysis, the fibrin monomer concentration was adjusted to 1.0 mg/ml. A 0.5-ml aliquot of this solution (0.5 mg) was transferred to a cuvette to which had been added 0.5 ml of 0.1 M phosphate buffer, pH 6.8, adjusted with NaCl to a final ionic strength of 0.20. Absorbance was followed at 350 nm over 30 min in a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Fibrin monomer aggregation was also measured at a final ionic strength of 0.24 by adding an aliquot of the monomer solution to an equal volume of 0.1 M phosphate buffer, pH 6.8, adjusted with NaCl to a final ionic strength of 0.43. Final pH of the aggregation mixtures was 6.7. Fibrin monomer aggregation was also measured in patients, 1, 2, and 4 by the method of Belitser et al. as modified by Gralnick et al. (15).

The formation of cross-linked fibrin was studied by clotting plasma with thrombin in the presence of calcium, incubating the clots in 9 M urea for 24 h, and then measuring the absorbance of the supernate at 280 nm (17).

Electrophoretic and chromatographic studies. Immunoelectrophoretic studies were performed in 1% agarose, at pH 8.6, in barbital buffer with commercial goat anti-human fibrinogen antiserum (Meloy Laboratories Inc., Springfield, Va.). Sodium dodecyl sulfate (SDS)[1]-polyacrylamide gel electrophoresis of normal and patient purified fibrinogens reduced with β-mercaptoethanol was performed using 7.5% gels according to the method of Weber and Osborn (18). Densitometric scans of the stained gels were made with a Gilford gel scanner. Polyacrylamide gel electrophoresis of labeled purified proteins was performed by the method of Davis (19) with 5% gels and pH 8.5 Tris-glycine. DEAE-cellulose (DE52, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) chromatography was performed at 4°C using a continuous concave gradient with Tris-phosphate buffer (20). For these experiments, admixtures of normal ^{131}I-fibrinogen and patient ^{125}I-fibrinogen, both labeled by the iodine monochloride method of McFarlane (21) modified as previously described, were run in the same gel or column (22). After electrophoresis, the gels were stained with Coomassie blue, cut into 1-mm segments, and the radioactivity in each gel segment was counted in an Isodyne model 1185 two-channel counter (Searle Analytic, Inc., Des Plaines, Ill.). Absorbance at 280 nm, radioactivity, and pH were measured on column effluents.

RESULTS

Coagulation studies. The plasma thrombin times were prolonged in three patients who had normal levels of plasma fibrinogen and in two patients with slightly decreased plasma fibrinogen (Table I). The serial dilution protamine sulfate test was negative. Fibrinogen-fibrin-related antigens were negative or

[1] *Abbreviation used in this paper*: SDS, sodium dodecyl sulfate.

TABLE I
Coagulation Studies

Test	Normal	Patient 1*	2*	3‡	4*	5‡
Thrombin time, *s*	20–23	38.0	40.0	32.9	47.7	39.0
Fibrinogen, *mg/100 ml*						
Thrombin-clottable	232–444	303	438	157	220	270
Immunoreactive	§	347	426	138	174	280
Serial dilution protamine sulfate test	Negative	Negative	Negative	Negative	Negative	Negative
Fibrinogen-fibrin-related antigen, *μg/ml*	≤8	Negative	Negative	Negative	4	Negative
Prothrombin time, *s*	12–15	18.8	18.5	14.0	16.3	16.1
Partial thromboplastin time nonactivated, *s*	60–90	84.7	75.0	97.5	80.1	84.0
Factor V, *U/ml*	0.47–1.53	0.56	0.72	0.46	0.56	0.80
Factor VIII, *U/ml*	0.48–1.52	1.64	1.64	1.64	4.99	1.72
Factor IX, *U/ml*	0.62–1.38	0.83	1.04	0.72	0.77	0.78
Factor X, *U/ml*	0.58–1.42	0.56	0.40	0.39	0.56	0.50
Factor XI, *U/ml*	0.52–1.48	0.94	1.17	0.98	0.70	0.66

* Alcoholic liver disease.
‡ Postnecrotic cirrhosis of undetermined etiology.
§ Measured against standard normal plasma.

within the range of normal for this method. Of the individual coagulation factors tested, the only abnormal findings common to all patients were a decreased level of Factor X and an increased level of Factor VIII. The thrombin and Reptilase times of purified patient fibrinogens adjusted to concentrations of 2.0 mg/ml are shown in Table II. Both thrombin and Reptilase times were prolonged. The prolongation of the thrombin time was similar in the respective patient plasma and purified fibrinogen systems. The addition of calcium improved but did not always completely correct the thrombin and Reptilase times of the patient fibrinogens. Similar prolongation of the thrombin time was noted in two patients whose fibrinogens were treated with human thrombin. The effect of increasing concentrations of thrombin on the clotting of two patient fibrinogens is shown in Fig. 1. Increasing concentrations of thrombin shorten the clotting time of the patient fibrinogens, but not to the level of the normal at the concentrations of thrombin employed. The addition of equal amounts of purified patient fibrinogen to purified normal fibrinogen caused a significant prolongation of the thrombin time of the normal fibrinogen (Fig. 1).

Thrombin-induced fibrinopeptide release of normal and patient purified fibrinogens is shown in Fig. 2. Fibrinopeptide release was normal in rate and amount for each of the patient fibrinogens tested.

Fibrin monomer aggregation was studied in each of the patients by treating both plasma and purified fibrinogen with thrombin according to a modification of the method of von Felten et al. (16) described above. Patient fibrin monomers derived from plasma and from purified fibrinogen showed impaired aggregation at both low and high ionic strength. Results for purified fibrinogen are shown in Fig. 3. The normal represents the mean of duplicate determinations of purified fibrinogen from each of two normal donors. Fibrin monomer aggregation in patients 1, 2, and 4 tested according to a modification of the method of Belitser et al. (15) was also impaired at low and high ionic strength (Fig. 4). The degree of impairment of fibrin monomer aggregation appears to correlate with the extent of prolongation of the patient plasma

TABLE II
Thrombin and Reptilase Times of Purified Normal and Patient Fibrinogens

	Thrombin time		Reptilase time	
	Without Ca^{++}	With Ca^{++}	Without Ca^{++}	With Ca^{++}
	s		*s*	
Normal*	23.2±2.0	12.3±1.0	45.3±3.2	19.5±0.8
Patient 1	41.7	16.3	95.0	25.2
2	39.8	15.2	92.5	27.8
3	41.2	12.5	75.0	21.2
4	75.1	15.7	>240.0	35.0
5	31.5	12.1	81.0	22.6

Fibrinogen concentrations were 2.0 mg/ml in 0.02 M sodium citrate, 0.15 M sodium chloride, pH 7.4. The mixture consisted of 0.1 ml of fibrinogen solution, 0.2 ml imidazole-buffered saline, and 0.1 ml bovine thrombin (10 U/ml) or Reptilase (20 μg/ml). Where indicated, $CaCl_2$ was 0.005 M final concentration.
* The normal is expressed as the mean±1 SD.

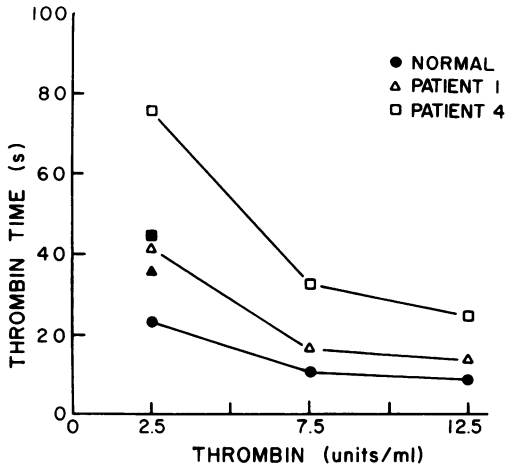

FIGURE 1 Thrombin times of purified normal and patients (1 and 4) fibrinogens with increasing amounts of thrombin. Fibrinogen concentrations were 2.0 mg/ml. Abscissa is given as final thrombin concentration. (▲) represents effect of patient 1 fibrinogen and (■) represents effect of patient 4 fibrinogen on the thrombin time of normal fibrinogen.

thrombin time except for patient 3. The lack of correlation for patient 3 is unclear at present.

Electrophoretic and chromatographic studies. Immunoelectrophoresis of each patient plasma and purified fibrinogen revealed an immunoprecipitin arc of normal mobility. SDS-polyacrylamide gel electrophoresis of each of the five reduced patient fibrinogens showed normal mobility and amount of Aα, Bβ, and γ chains with no evidence of proteolysis. Results for patients 1 and 4 are shown in Fig. 5. Alkaline polyacrylamide gel electrophoresis and DEAE-cellulose chromatography were performed with radiolabeled fibrinogen of patients 1 and 4. Patient ^{125}I-fibrinogen and normal ^{131}I-fibrinogen migrated identically when studied by alkaline polyacrylamide gel electrophoresis (Fig. 6). Gradient elution chromatography on DEAE-cellulose of a mixture of patient ^{125}I-fibrinogen and normal ^{131}I-fibrinogen revealed simultaneous elution in two major peaks (Fig. 7) as previously described for normal fibrinogen (20).

DISCUSSION

Most of the dysproteinemias described in patients with liver disease have been due to quantitative abnormalities. However, the presence of qualitative abnormalities of plasma proteins in this group of patients is becoming increasingly recognized. In several patients with hepatoma, an acquired abnormality of fibrin monomer polymerization has been reported (23, 24). High levels of an R-type B_{12}-binding protein with increased sialic acid content have also been demonstrated in patients with hepatoma (25). In addition,

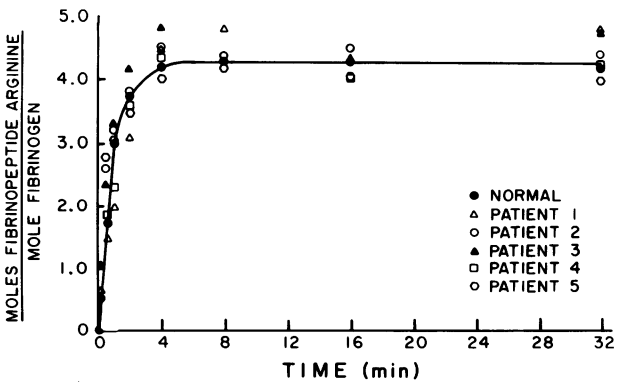

FIGURE 2 Fibrinopeptide release from purified normal and patient fibrinogens. Fibrinogen concentration was 2.7 mg/ml, thrombin concentration was 1.25 U/ml, and temperature was 25°C.

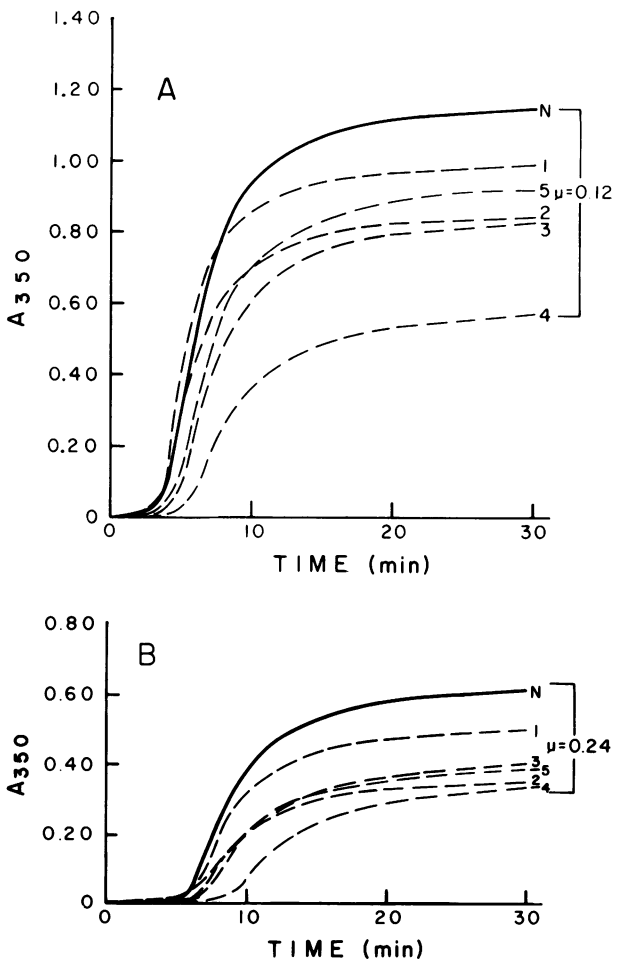

FIGURE 3 Aggregation of fibrin monomers of purified normal (N) and patient fibrinogens at ionic strength of 0.12 and 0.24. Final pH was 6.7 and protein concentration was 0.5 mg/ml (μ = ionic strength).

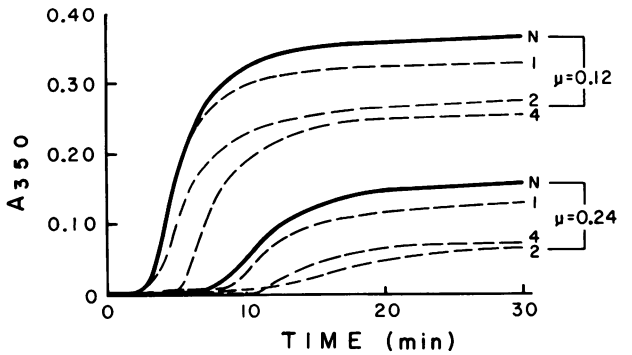

FIGURE 4 Aggregation of fibrin monomers of purified normal (N) and patients 1, 2, and 4 fibrinogens at ionic strength of 0.12 and 0.24. Final pH was 6.7 and protein concentration was 0.21 mg/ml (μ = ionic strength).

increased levels of partially desialylated thyroxine-binding globulin have been described in patients with alcoholic and biliary cirrhosis, primary and metastatic liver carcinoma, and hepatitis, presumably due to decreased removal of the altered protein by damaged hepatocyte membranes (26).

We have studied the plasma and purified fibrinogens of five patients (three with alcoholic liver disease and two with postnecrotic cirrhosis) who had prolonga-

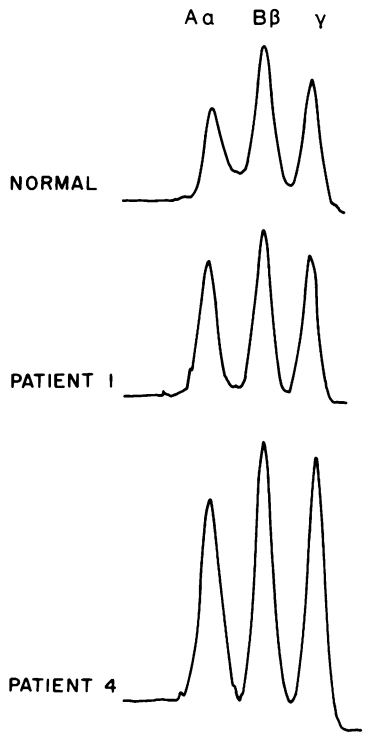

FIGURE 5 Densitometric scans of SDS-polyacrylamide gels of reduced normal and patients 1 and 4 fibrinogens. The anode is to the right.

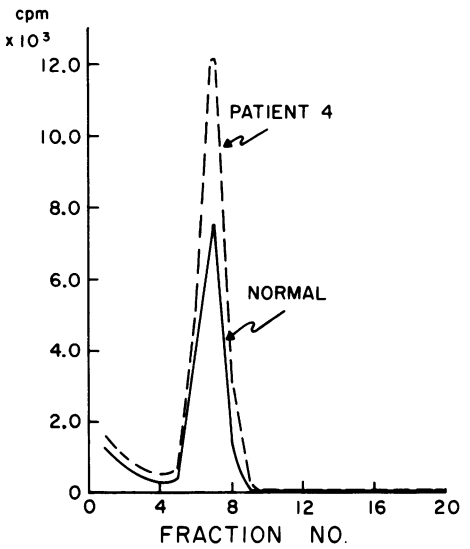

FIGURE 6 Electrophoretic mobility of ^{131}I-labeled normal fibrinogen mixed with ^{125}I-labeled patient 4 fibrinogen in 5% polyacrylamide gel. The gel was cut into 1-mm segments and ^{125}I and ^{131}I radioactivity was counted.

tion of their plasma thrombin times in the absence of clinical and laboratory evidence of disseminated intravascular coagulation and/or fibrinolysis. In all five patients we have demonstrated a functional abnormality of the fibrinogen molecule.

Functional abnormalities of fibrinogen, or dysfibrinogenemias, are initially differentiated on the basis of the abnormal clottability of fibrinogen by thrombin. The

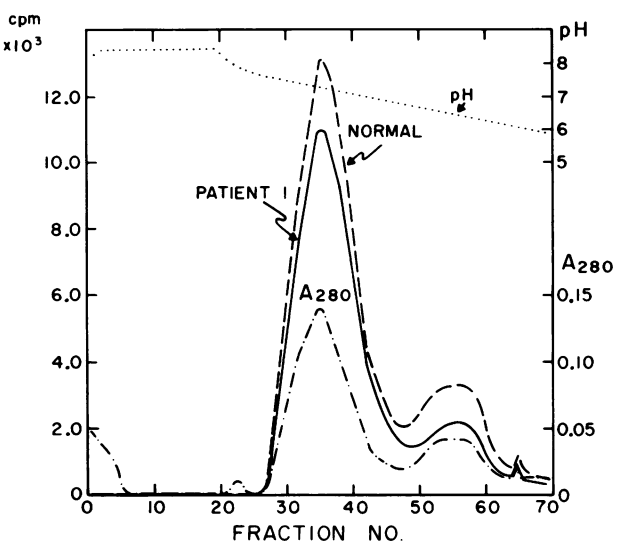

FIGURE 7 DEAE-cellulose chromatography of 15 mg of normal fibrinogen (dash-dot line) mixed with tracer amounts of ^{131}I-labeled normal fibrinogen (dashed line) and ^{125}I-labeled patient 1 fibrinogen (solid line). A concave gradient from 0.005 M Tris-phosphate, pH 8.6, to 0.5 M Tris-phosphate, pH 4.2, was used.

conversion of fibrinogen to fibrin by thrombin in the presence of Factor XIII and calcium involves three steps: (*a*) thrombin-induced fibrinopeptide release resulting in the production of fibrin monomer, (*b*) polymerization of fibrin monomers, and (*c*) formation of cross-linked fibrin. The thrombin time is affected by alterations in the first two steps of the conversion of fibrinogen to fibrin. In all five patients, the prolongation of their plasma thrombin times was comparable to the prolongation of the thrombin times of their purified fibrinogens. Reptilase times of purified patient fibrinogens were likewise prolonged. Calcium accelerated clotting in both systems but did not always completely correct the defect. Thrombin-induced fibrinopeptide release of patient fibrinogens was equal in rate and amount to that of normal fibrinogen. Therefore, the prolongation of the thrombin times could be explained by defective aggregation of patient fibrin monomers. Our studies demonstrate impaired aggregation of patient fibrin monomers prepared from plasma and from purified fibrinogen at both low and high ionic strength, and localize the functional defect specifically to the phase of monomer polymerization. The degree of impairment of fibrin monomer polymerization appears to correlate with the extent of prolongation of the thrombin times. The improvement in the thrombin times with calcium can be explained by the known enchancement of fibrin monomer aggregation by this ion (27). The third step in the conversion of fibrinogen to covalently cross-linked fibrin was grossly normal as indicated by the insolubility in 9 M urea of patient fibrinogen clotted with thrombin in the presence of Factor XIII and calcium.

Several of the genetic dysfibrinogenemias have been distinguished by differences on immunoelectrophoresis and DEAE-cellulose chromatography (28). The functionally abnormal fibrinogen reported here shows normal mobility on immunoelectrophoresis of plasma and purified protein, identical mobility with normal fibrinogen on polyacrylamide gel electrophoresis, and simultaneous elution with normal fibrinogen on DEAE-cellulose chromatography. These results suggest that the patient fibrinogen has an electrical charge similar to that of normal fibrinogen. In addition, SDS-polyacrylamide gel electrophoresis of patient reduced fibrinogens showed intact Aα, Bβ, and γ chains with no evidence of proteolysis (29). Although cleavage products of fibrinogen may be present in patients with liver disease due to disseminated intravascular coagulation and/or fibrinolysis (3, 30, 31), the normal elution pattern on DEAE-cellulose chromatography and the presence of intact Aα, Bβ, and γ chains on SDS-polyacrylamide gel electrophoresis indicate that the abnormality we are describing is not secondary to proteolysis by plasmin but is intrinsic to the molecule.

The demonstration of a functional abnormality of the circulating fibrinogen molecule does not necessarily mean that the molecule secreted by the diseased liver is abnormal. It is conceivable that a normal fibrinogen is secreted by the abnormal liver and undergoes rapid alteration in the circulation due to an abnormal plasma environment.

Accumulation of altered proteins in the plasma of patients with liver disease may occur due to their impaired removal by the diseased liver. Most asialoglycoproteins are rapidly removed from the circulation by the liver as the result of binding of their terminal galactosyl residues to the hepatocyte membrane (32). Impairment of this clearance mechanism might be responsible for the finding of elevated levels of altered thyroxine-binding globulin in patients with liver disease (26). The liver also appears to play a role in the removal of activated coagulation factors (33, 34). Abnormal removal of altered coagulation proteins by the diseased liver might affect the hemostatic balance in these patients.

The occurrence of a dysfibrinogenemia in these five patients with two types of liver disease suggests that the abnormality may be a consequence of liver damage rather than a manifestation of any single type of liver disease. As can be seen from Table I, these patients exhibited a variety of coagulation abnormalities. However, since coagulation factors are usually assayed on the basis of their biological activity, what is generally interpreted as reduced levels of these factors in liver disease may reflect instead impaired biological activity of altered molecular species present in reduced, normal, or increased amounts. The protein abnormalities detected thus far in liver disease may be indicative of generalized qualitative protein abnormalities in these patients. Further investigations into the nature of these alterations may ultimately provide the basis for a better understanding of the pathogenetic mechanism responsible for the dysproteinemias found in patients with liver disease.

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REFERENCES

1. Roberts, H. R., and A. I. Cederbaum. 1972. The liver and blood coagulation: physiology and pathology. *Gastroenterology.* **63:** 297–320.

2. Walls, W. D., and M. S. Losowsky. 1971. The hemostatic defect of liver disease. *Gastroenterology.* **60:** 108–119.
3. Merskey, C., G. J. Kleiner, and A. J. Johnson. 1966. Quantitative estimation of split products of fibrinogen in human serum, relation to diagnosis and treatment. *Blood.* **28:** 1–18.
4. Bloom, A. L. 1975. Intravascular coagulation and the liver. *Br. J. Haematol.* **30:** 1–7.
5. Soria, J., C. Soria, M. Samama, J. Coupier, M. L. Girard, J. Bousser, and G. Bilski-Pasquier. 1970. Dysfibrinogénémies acquises dans les atteintes hépatiques sévères. *Coagulation.* **3:** 37–44.
6. Kazal, L. A., S. Amsel, O. P. Miller, and L. M. Tocantins. 1963. The preparation and some properties of fibrinogen precipitated from human plasma by glycine. *Proc. Soc. Exp. Biol. Med.* **113:** 989–994.
7. Tocantins, L. M., and L. A. Kazal, editors. 1964. Blood Coagulation, Hemorrhage and Thrombosis. Grune & Stratton, Inc., New York. 2nd edition. 148–150, 103–107.
8. Wolf, P. 1953. A modification for routine laboratory use of Stefanini's method of estimating Factor V activity in human oxalated plasma. *J. Clin. Pathol. (Lond.).* **6:** 34–38.
9. Denson, K. W. 1961. The specific assay of Prower-Stuart factor and Factor VII. *Acta Haematol. (Basel).* **25:** 105–120.
10. Hardisty, R. M., and J. C. Macpherson. 1962. A one-stage Factor VIII (Antihaemophilic Globulin) assay and its use on venous and capillary plasma. *Thromb. Diath. Haemorrh.* **7:** 215–229.
11. Horowitz, H. I., W. P. Wilcox, and M. M. Fujimoto. 1963. Assay of plasma thromboplastin antecedent (PTA) with artificially depleted normal plasma. *Blood.* **22:** 35–43.
12. Niewiarowski, S., and V. Gurewich. 1971. Laboratory identification of intravascular coagulation. The serial dilution protamine sulfate test for the detection of fibrin monomer and fibrin degradation products. *J. Lab. Clin. Med.* **77:** 665–676.
13. Melliger, E. J. 1970. Detection of fibrinogen degradation products by use of antibody coated latex particles. The possibilities and limits of the method. *Thromb. Diath. Haemorrh.* **23:** 211–227.
14. Ellis, B. C., and A. Stransky. 1961. A quick and accurate method for the determination of fibrinogen in plasma. *J. Lab. Clin. Med.* **58:** 477–488.
15. Gralnick, H. R., H. M. Givelber, J. R. Shainoff, and J. S. Finlayson. 1971. Fibrinogen Bethesda: a congenital dysfibrinogenemia with delayed fibrinopeptide release. *J. Clin. Invest.* **50:** 1819–1830.
16. von Felten, A. P., P. G. Frick, and P. W. Straub. 1969. Studies on fibrin monomer aggregation in congenital dysfibrinogenaemia (fibrinogen 'Zürich'): separation of a pathological from a normal fibrin fraction. *Br. J. Haematol.* **16:** 353–361.
17. Martinez, J., J. Palascak, and C. Peters. 1977. Functional and metabolic properties of human asialofibrinogen. *J. Lab. Clin. Med.* **89:** 367–377.
18. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
19. Davis, B. J. 1964. Disc electrophoresis-II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121:** 404–427.
20. Finlayson, J. S., and M. W. Mosesson. 1963. Heterogeneity of human fibrinogen. *Biochemistry.* **2:** 42–46.
21. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature (Lond.).* **182:** 53.
22. Martinez, J., R. R. Holburn, S. S. Shapiro, and A. J. Erslev. 1974. Fibrinogen Philadelphia. A hereditary hypodysfibrinogenemia characterized by fibrinogen hypercatabolism. *J. Clin. Invest.* **53:** 600–611.
23. von Felten, A., P. W. Straub, and P. G. Frick. 1969. Dysfibrinogenemia in a patient with primary hepatoma. First observation of an acquired abnormality of fibrin monomer aggregation. *N. Engl. J. Med.* **280:** 405–409.
24. Verhaeghe, R., B. Van Damme, A. Molla, and J. Vermylen. 1972. Dysfibrinogenaemia associated with primary hepatoma. *Scand. J. Haematol.* **9:** 451–458.
25. Burger, R. L., S. Waxman, H. S. Gilbert, C. S. Mehlman, and R. H. Allen. 1975. Isolation and characterization of a novel vitamin B_{12}-binding protein associated with hepatocellular carcinoma. *J. Clin. Invest.* **56:** 1262–1270.
26. Marshall, J. S., A. M. Green, J. Pensky, S. Williams, A. Zinn, and D. M. Carlson. 1974. Measurement of circulating desialylated glycoproteins and correlation with hepatocellular damage. *J. Clin. Invest.* **54:** 555–562.
27. Boyer, M. H., J. R. Shainoff, and O. D. Ratnoff. 1972. Acceleration of fibrin polymerization by calcium ions. *Blood.* **39:** 382–387.
28. Ratnoff, O. D., and W. B. Forman. 1976. Criteria for the differentiation of dysfibrinogenemic states. *Semin. Hematol.* **13:** 141–157.
29. Mosesson, M. W., J. S. Finlayson, R. A. Umfleet, and D. Galanakis. 1972. Human fibrinogen heterogeneities. I. Structural and related studies of plasma fibrinogens which are high solubility catabolic intermediates. *J. Biol. Chem.* **247:** 5210–5219.
30. Tytgat, G. N., D. Collen, and M. Verstraete. 1971. Metabolism of fibrinogen in cirrhosis of the liver. *J. Clin. Invest.* **50:** 1690–1701.
31. Fletcher, A. P., O. Biederman, D. Moore, N. Alkjaersig, and S. Sherry. 1964. Abnormal plasminogen-plasmin system activity (fibrinolysis) in patients with hepatic cirrhosis: its cause and consequences. *J. Clin. Invest.* **43:** 681–695.
32. Hudgin, R. L., W. E. Pricer, G. Ashwell, R. J. Stockert, and A. G. Morell. 1974. The isolation and properties of a rabbit liver binding protein specific for asialoglycoproteins. *J. Biol. Chem.* **249:** 5536–5543.
33. Deykin, D. 1966. The role of the liver in serum-induced hypercoagulability. *J. Clin. Invest.* **45:** 256–263.
34. Deykin, D., F. Cochios, G. DeCamp, and A. Lopez. 1968. Hepatic removal of activated Factor X by the perfused rabbit liver. *Am. J. Physiol.* **214:** 414–419.