CONGENITAL ABNORMALITIES OF THE FIBRINOGEN MOLECULE

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A review of fibrinogen and its conversion to fibrin would be incomplete if one were not to discuss the abnormalities associated with this important coagulation protein. Like all other coagulopathies, the abnormalities of fibrinogen can be divided into two major categories: the acquired fibrinogen abnormalities and the congenital ones.

No attempt will be made at this time to review the acquired fibrinogen abnormalities of either the quantitative or qualitative nature; they will be the subject of a future review in this journal. Instead, this essay will limit itself to the congenital abnormalities of the fibrinogen molecule itself. The congenital abnormalities associated with the stabilization phase of fibrin formation, the fibrin-stabilizing factor deficiencies, or Factor XIII deficiencies, were mentioned by Finlayson in the first issue of this volume [35].

The congenital abnormalities associated with the fibrinogen molecule can be of a quantitative nature and are then called afibrinogenemias or hypofibrinogenemias, or of a qualitative nature, referred to as dysfibrinogenemias [38, 41]. Both abnormalities result in a malfunctioning fibrin formation, although the clinical features of the quantitative disturbances seem, as a general rule, to be quite different from the ones of the qualitative defects. An attempt will be made to review both congenital abnormalities, hopefully fairly comprehensively investigating the literature on this subject matter.

AFIBRINOGENEMIA OR HYPOFIBRINOGENEMIA

Congenital afibrinogenemia is a fairly rare disease, as compared to the more frequently encountered two forms of hemophilia and the von Willebrand's syndrome. Nevertheless, well over 100 cases with this disorder have been described in the literature to this date. In 1963, Bommer et al. [21] described a patient with congenital afibrinogenemia and used the opportunity to comprehensively survey the literature for cases described up to 1959 [22]. They collected 55 cases, beginning with the first patient described in 1920 by Rabe and Salomon [115]. In 1968, Yamagata and associates [145] described a case with congenital afibrino-

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genemia and reviewed, on that occasion, the Japanese literature, adding a total of 14 cases, which were described in that country. In 1971, Egbring et al. [31] added 23 patients to the list of Bommer and associates, reviewing the literature up to 1964, thus making a total of 92 cases up to that time. The author has reviewed 35 additional cases described until 1973, bringing the total number of cases to 127 [1, 2, 5, 6, 8, 10, 20, 26, 29, 44, 52, 59, 78, 83, 90, 93, 95, 105, 107, 109, 110, 112, 113, 118, 133]. In addition to human beings, afibrinogenemia has also been observed in goats [23] and dogs [72].

Heredity

Congenital afibrinogenemia is an inherited disease that affects both sexes. In contrast to both hemophilias, this disease is thus not sex-linked. In reviewing the cases reported, there is an almost equal distribution between male and female patients, as already shown earlier [21], and the disease is transmitted from either sex to either sex. Like most other congenital coagulopathies, afibrinogenemia has an autosomal inheritance pattern, although some of the cases reported have no family history. Typical patients with afibrinogenemia, i.e., no fibrinogen demonstrable in plasma, seem to be homozygous, whereas the heterozygous carriers, although in most instances clinically asymptomatic, have clearly lower than normal levels of fibrinogen in plasma [21]. It would thus seem that heterozygotes have congenital hypofibrinogenemia, the fibrinogen being determined by routine laboratory methods. Many authors described congenital afibrinogenemia as an autosomal *recessive* disorder [22, 31, 122], which is correct when one uses clinical symptoms as the basis of one's definition. Heterozygotes are, as a general rule, and as pointed out above, clinically asymptomatic. If one follows the arguments presented by Owen and associates [108], who defined dominant as "exhibiting any evidence of a genetic defect in the heterozygote," then the disease is clearly autosomal *dominant,* since heterozygotes have hypofibrinogenemia. As reported in the case by Rabe and Salomon [115], about half of the patients described with afibrinogenemia were born from consanguinous marriages [22, 31].

Clinical Symptomatology

Patients with congenital afibrinogenemia suffer, as a general rule, from a hemorrhagic diathesis. In contrast, patients with hypofibrinogenemia seem to not suffer from bleedings. It is interesting to note that most patients with this disorder begin to develop a bleeding diathesis in their first days of life. Melena, hematomas at local sites where forceps were placed, vomiting of blood, mucosal bleedings, and hemorrhages from the umbilicus have been reported. The latter bleeding problem prevailed in about 50% of all cases [22]. The same high incidence of umbilical hemorrhages has, interestingly, also been described for Factor XIII deficiencies [30]. Profuse bleedings following minor trauma, an easy bruising tendency and gingival hemorrhages are fairly common [22]. Internal bleedings have been observed; they are, however, fairly rare. According to Egbring and co-workers [31], joint bleedings are more common than usually anticipated, and 21% of their tabulated cases suffered from this particular problem. Menstrual bleedings were in some cases prolonged [31], and in others they were normal [84]. Several patients with afibrinogenemia died from cerebral hemorrhages [31].

As in other congenital coagulopathies, periods of increased spontaneous bleedings are followed by periods of relatively few problems, although the coagulation abnormalities do not parallel these periodic changes [31]. In most instances of afibrinogenemic hemorrhages, the bleedings are precipitated by minor trauma, although "spontaneous" bleedings (trauma not noticed by the patient) have been reported. In comparison to the hemophilias, the overall incidence of spontaneous hemorrhages seem to be less.

Whereas many years ago the prognosis of patients with congenital afibrinogenemia was guarded [137], improvement in diagnostic capabilities and improvements in therapy have altered markedly their life expectancy.

Laboratory Diagnosis

Due to the missing substrate for the fibrin clot, the blood and plasma of patients with congenital afibrinogenemia is incoagulable, and no clot will form when either thrombin or tissue thromboplastin is added. This means that whole blood clotting times, plasma recalcification times, partial thromboplastin times, prothrombin times, snake venom times, Reptilase times, and thrombin times are not measurable because no clots will form in response to the formation or addition of thrombin. The addition of normal plasma or purified fibrinogen will normalize all of the listed whole blood and plasma clotting times, indicating normal coagulation factor distribution, other than fibrinogen, and normal thrombin formation from prothrombin. Occasionally, slightly decreased levels of prothrombin [22, 90], Factor VII [25, 60] and Factor V and Factor VIII [25] have been described. In one instance [113], the hypofibrinogenemia was associated with hemophilia and thrombocytopenia. The fibrinolytic system seems to be functioning normally in these patients [4, 49, 145]. Circulating anticoagulants have not been described associated with congenital afibrinogenemia or hypofibrinogenemia.

A mild to moderate thrombocytopenia seems to be observed more frequently. Bommer et al. [22] found 13 cases in their collection of patients; Yamagata and co-workers [145] found it in seven of 14 cases, and Egbring et al. [31] observed it in three of their collection of 23 patients. Only rarely, however, did the platelet count drop below 100,000/cmm. Whereas capillary fragility, usually measured by Rumpel-Leede tests, seemed to be normal in the majority of cases (for exception see [22]), the bleeding times, either measured by the Duke or Ivy procedures, are prolonged in a large number of patients with congenital afibrinogenemia. Bommer et al. [22] found bleeding times abnormal in 38% of their reported cases, including their own patient. Yamagata et al. [145] described it in eight of their collected 14 patients, and Egbring et al. [31] reported it in ten of 18 cases where bleeding times had been measured. Since bleeding times assess the reactions leading to the formation of the first hemostatic plug or platelet plug, thus the facilitation of primary hemostasis, the findings in patients with congenital afibrinogenemia suggest a role of fibrinogen in the primary hemostatic events. This problem has been studied by several investigators, both with the *in vitro* effect of fibrinogen on platelet function [7, 94, 127] and with platelet function in plasma of afibrinogenemic patients [7, 50, 55, 63, 68, 81, 117, 142]. Washed platelets did not aggregate well in the absence of fibrinogen [7, 94, 127] when ADP was used as a stimulus; the addition of purified fibrinogen corrected, in part, the abnormal aggregation response. However, other plasma proteins, especially gamma globulin,

could substitute for fibrinogen in these experiments [7]. Similar results were found in patients with congenital afibrinogenemia. Platelet aggregation of these patients in response to ADP, collagen, epinephrine and thrombin was found to be poor [7, 56, 63, 142]. It could be corrected by the addition of fibrinogen. More recent experiments by Weiss and Rogers [142] have shown, however, that the abnormal aggregation in congenital afibrinogenemia could not be demonstrated in heparinized plasma. Only citrated plasma gave this response, casting some doubt on the physiologic significance of earlier findings. Already the findings by Bang and associates [7] have raised some serious questions about the implication of previous observations. Weiss and Rogers [142] demonstrated, however, that platelet adhesion to glass surfaces was markedly impaired in patients with congenital afibrinogenemia. These observations indicate that fibrinogen may be a co-factor in the interaction of platelets with some surfaces [142], especially since the addition of fibrinogen to the patient's plasma also corrected the abnormal adhesion response. If this is assumed to be the case *in vivo* as well, it would explain why patients with congenital afibrinogenemia have prolonged bleeding times. It does not explain, however, why all patients do not have this feature, unless a correlation between plasma fibrinogen levels could be established. Afibrinogenemia, defined as a complete absence of fibrinogen in plasma, even in traces, would conceivably have abnormal bleeding times. Hypofibrinogenemia could, dependent upon the level of fibrinogen in plasma, have normal or abnormal bleeding times. Unfortunately, these correlations cannot be made at this time, because of the varying techniques used by the many authors to determine the bleeding times as well as the levels of fibrinogen in their patients. The disturbance in primary hemostasis could contribute, however, to the bleeding problem that patients with congenital afibrinogenemia display.

In order to establish the diagnosis of afibrinogenemia versus hypofibrinogenemia, it is important to consider the techniques to be used for the assay of fibrinogen. In principle, there are three groups of techniques for the determination of fibrinogen: 1) precipitation techniques, 2) immunological techniques and 3) coagulation techniques. For technical details, the reader is referred to previous reports [9, 61, 111, 135, 141]. Most sensitive for the measurement of traces of fibrinogen in plasma are the immunologic techniques, and, occasionally, traces can be found with these procedures when precipitation and clotting techniques fail to show any fibrinogen at all. It is quite common to detect traces of fibrinogen in homozygous afibrinogenemic patients, and it probably follows the observation of other congenital coagulopathies–as for example, the hemophilias–where factor levels are never "zero." These patients would be afibrinogenemic by several procedures, but hypofibrinogenemic in the strictest sense of the word by immunologic techniques. Heterozygotes, in contrast, would be hypofibrinogenemic by all procedures. The terminology can thus be confusing if one does not specify what is meant. For this reason, it is difficult to clearly establish heterozygotes and homozygotes in the cases described in the literature.

Treatment

Bleeding episodes in patients with congenital afibrinogenemia can be treated, not only with whole blood and plasma, but even better with fibrinogen concentrates. In the case of whole blood, it should not be older than five

days [71]. Fibrinogen concentrates in the form of either Cohn fraction I, cryoprecipitate or purified fibrinogen preparations lend themselves ideally to raising the plasma level of fibrinogen, without simultaneously overloading the circulatory system with fluids. Hemostasis is apparently adequate if the fibrinogen level in plasma is raised between 50 and 100 mg% [3, 92, 114, 119]. Since the half-life of fibrinogen is between three and five days [8, 43, 57, 82, 92, 136, 145], fibrinogen infusions need not be as frequent as Factor VIII infusions in hemophiliacs. It is advisable to calculate the amount of fibrinogen needed to raise the plasma level to a desired range, infuse the amount of fibrinogen and control the level by fibrinogen determinations. In this connection, it is interesting that patients may be free of bleeding episodes for a considerably longer period of time than would be expected on the basis of the half-life of the infused fibrinogen; it even exceeds the time where the last traces of fibrinogen have disappeared from plasma [31, 50].

Treatment with fibrinogen concentrates, however, is not without hazards. The formation of immunoantibodies against fibrinogen has been reported [24, 140]. A shortening of the half-life of the infused fibrinogen in these patients could signal this complication.

Another complication of blood and plasma transfusions in general, and fibrinogen concentrates in particular, is the development of homologous serum hepatitis [27, 70].

Last but not least is the development of acute adverse reactions to the fibrinogen concentrates, and 14% of the patients compiled by Egbring et al. [31] who received fibrinogen showed from mild to severe reactions. Most impressive was the death of four patients due to pulmonary embolisms or myocardial infarct fairly shortly following therapy [64, 140]. In one patient, the addition of oral contraceptives was felt to be responsible for the acute thromboembolic complications [86]. In two instances [83, 140], antibodies against fibrinogen were found prior to the thromboembolic episodes. This could be causing immunoprecipitation of fibrinogen on vessel walls and, thereby, thrombosis [31]. Several years ago we described fibrinogen antibodies in patients with thrombophlebitis migrans [86], which could support an assumption that fibrinogen antibodies might be related to thromboembolic diseases. Egbring and associates [31] therefore suggested heparinization of the patients prior to the infusion of fibrinogen. In any case, the danger of immunoantibodies against fibrinogen and their possible implication in thromboembolic diseases would caution against the possible prophylactic use of fibrinogen in weekly intervals to reduce bleeding episodes in afibrinogenemic patients, as suggested by van Nuffel and Verstraete [106].

DYSFIBRINOGENEMIA

Qualitative abnormalities of the fibrinogen molecule are called dysfibrinogenemias. Characteristically, the patients with this kind of disease have normal quantities of fibrinogen circulating in their plasma, but the fibrinogen fails to form fibrin when it comes into contact with the enzyme thrombin. In order to better understand the possible kinds of abnormalities within the molecule, it is important to recall that the actual process of fibrin formation can be divided into three phases (for details see [104]). In the first phase, also called the *proteolytic phase,*

thrombin cleaves fibrinopeptide A from the A α-chain and fibrinopeptide B from the B β-chain of the individual fibrinogen molecules, which results in the formation of the fibrin monomers. Next, in the *polymerization phase,* the fibrin monomers aggregate side-to-side and end-to-end to form the actual fibrin network. In the final phase, called the *stabilization phase,* the fibrin network is stabilized by the enzyme Factor XIII, as recently reviewed by Finlayson [35]. As will be shown later, abnormalities of the fibrinogen molecule can affect each one of the phases. Since Factor XIII deficiences have already been reviewed [35], they will not be discussed here.

The first case of dysfibrinogenemia was described by Imperato and Dettori in 1958 [62]. The patient described also had hypofibrinogenemia, which is an exception rather than the rule for this disease entity. Following the report of this first patient, 26 additional families with dysfibrinogenemia have been described to date. Table 1 summarizes the reports, including the literature references. As can be seen, the abnormal fibrinogens are named according to the cities where they were found. Beck [11, 12] made this suggestion in analogy to the hemoglobinopathies, and it has been followed by all authors. It can also be noted that most cases were reported in the last three years. The reason for this is probably that only a few of the cases described have an actual bleeding diathesis. Most discoveries were made as coincidental findings. Many patients with abnormal fibrinogens may, therefore, go unnoticed, since usually only patients with a hemorrhagic tendency seek the advice

TABLE 1. Abnormal Fibrinogens

Name	*Authors*	*Year*	*References*
Parma	Imperato, Dettori	1958	62
Vancouver	Hasselback et al	1963	58, 65
Paris I	Ménaché	1963	51, 96, 97, 98
Baltimore	Beck et al	1964	11, 13, 14, 15, 16, 66, 102, 103, 126
Zürich I	von Felten et al	1965	32, 33, 34
Cleveland I	Forman et al	1967	36, 37
Detroit	Mammen et al	1968	17, 18, 19, 85, 87, 88, 89
Paris II	Samama et al	1968	99, 100, 120, 121
St. Louis	Sherman et al	1968	42, 123, 124
Zürich II	Funck et al	1970	39, 40
Louvain	Verstraete et al	1970	138, 139
Oklahoma	Hampton, Morton	1970	53, 54
Los Angeles	Zietz, Scott	1970	146
Bethesda I	Gralnick et al	1970	45, 46
Amsterdam	Janssen, Vreeken	1971	69, 70
Nancy	Streiff et al	1971	131, 132
Wiesbaden	Winckelmann et al	1971	143, 144
Troyes	Soria et al	1972	128, 129
Metz	Soria et al	1972	128, 129, 130
Giessen	Krause et al	1972	73, 74, 75, 76, 77
Bethesda II	Gralnick et al	1972	47, 48
Montreal	Lacombe et al	1972	79, 80
Vienna	Thaler	1973	134
Iowa City	Jacobsen, Hoak	1973	67
Cleveland II	Ratnoff et al	1973	28, 116
Philadelphia	Martinez et al	1974	91

of a physician. In addition, more and more clinical laboratories adopt coagulation-screening tests to assess patients' hemostatic mechanisms prior to surgical procedures, or even as routine tests on admission to hospitals. It may be on these occasions that patients with abnormal fibrinogens are discovered.

Heredity

Like congenital afibrinogenemia, congenital dysfibrinogenemia is an inherited disease that is also transmitted from either sex to either sex. As can be seen from Table 2, the 26 cases reported with dysfibrinogenemia entail a total of 103 patients.

TABLE 2. Tabulation of the 26 Families with Dysfibrinogenemia According to Individual Cases versus Families and According to Sex

	Number of Families	*Number of Patients*	*Female*	*Male*
Individual Cases	3	3	1	2
Families	23	100	50	50
Total	26	103	51	52

Only three were isolated cases without a family history. The distribution of sex is virtually identical, 52 male patients and 51 female patients. As could be demonstrated for Fibrinogen Detroit [88], homozygotes have completely abnormal fibrinogen molecules circulating, while heterozygotes have about half normal and half abnormal molecules. The disease is therefore transmitted on an autosomal dominant basis. In contrast to congenital afibrinogenemia, consanguinous marriages are extremely rare in the history of patients with congenital dysfibrinogenemia.

Clinical Symptomatology

In contrast to patients with congenital afibrinogenemia, who, as a general rule, suffer from an extensive hemorrhagic diathesis, patients with dysfibrinogenemia only seldom display a bleeding problem. Of the 26 families with this disorder, only 13 had mild bleeding tendencies (Fibrinogen Parma, Vancouver, Baltimore, Detroit, Leuven, St. Louis, Bethesda I, Giessen, Wiesbaden, Metz, Vienna, Cleveland II and Philadelphia). In most instances, the hemorrhages were of a mild nature, with easy bruising tendencies and prolonged bleedings following minor trauma as the most common signs. The high incidence of umbilical bleedings, as reported for congenital afibrinogenemia, are virtually absent for patients with dysfibrinogenemia. The patient with Fibrinogen Detroit suffered from excessive menstrual periods. In the case of Fibrinogen St. Louis, it is possible that the bleedings are caused by Hemophilia A, which associates the abnormal fibrinogen molecule. In most instances, the coagulation abnormality was discovered coincidentally. This could, as was pointed out earlier, contribute to the fact that only so few families with dysfibrinogenemia have been described so far, in comparison to those with afibrinogenemia. It is conceivable that homozygous patients could have a bleeding problem, whereas heterozygotes who have one-half normal and one-half abnormal molecules, might not have a bleeding problem at all. The heterozygous family

members of Fibrinogen Detroit, for example, had only occasional bruises. It is difficult to tell in many reported instances of dysfibrinogenemia who might be heterozygous and who might be homozygous. Interestingly, the patients with Fibrinogen Baltimore and Paris II suffered from thromboses. Patients with Fibrinogen Cleveland I and Paris I encountered dehiscence of postoperative wounds, which was the cause of death in the patient with Fibrinogen Paris I.

Laboratory Diagnosis

The laboratory findings are greatly dependent upon whether a patient is homozygous or heterozygous. Thrombin times and Reptilase times, however, are always prolonged, regardless of a heterozygous or homozygous state. Only one patient (Fibrinogen Oklahoma) had normal thrombin times. It will be shown later that this patient has the defect in the stabilization phase. All other patients having defects in either the proteolytic phase or the polymerization phase of fibrin formation have, apparently, prolonged thrombin times. Reptilase times were measured in 19 instances and found prolonged in all of them. Fibrinogen Oklahoma has not reported Reptilase times yet, but on the basis of the defect, they are expected to be normal.

In many instances, the thrombin times seemed to be infinite, although, eventually, a clot did form. Plasma of Fibrinogen Detroit will eventually clot when thrombin is added; however, it takes up to 12 hours before a solid clot can be observed. Several investigators purified the abnormal fibrinogen from the patients' plasma and then added thrombin. As in plasma, the purified fibrinogen did not readily convert to fibrin (Fibrinogen Baltimore, Paris I, Zürich I, Cleveland I, Detroit, Zürich II, Leuven, Bethesda I, Bethesda II, Cleveland II and Philadelphia) indicating that the defect lies with the fibrinogen molecule, rather than with an unknown substance in plasma that inhibits the conversion of fibrinogen to fibrin. The thrombin times of some abnormal fibrinogens could be shortened by adding increased quantities of thrombin (Fibrinogen Paris I, Cleveland I, Bethesda I, Bethesda II, Iowa City, Paris II and Giessen); in others this had no effect (Fibrinogen Detroit and Metz). In some abnormal fibrinogens, the addition of calcium chloride shortened the thrombin times of plasma (Fibrinogen Paris I, Cleveland I, Bethesda I, Bethesda II, Los Angeles, Leuven, St. Louis, Troyes, Iowa City, Nancy, Philadelphia and Cleveland II); in others it had no effect (Fibrinogen Detroit and Metz).

Mixing patients' plasma with normal plasma and then performing thrombin times has had varying results. In Fibrinogen Cleveland I, Bethesda I, Bethesda II, Paris I, Wiesbaden, Zürich I, Paris I, Zürich II, Detroit and Cleveland II, the thrombin times remained prolonged, indicating an inhibitory effect of the abnormal fibrinogen on the conversion of normal fibrinogen to fibrin. This phenomenon could explain why heterozygotes could have prolonged whole blood and plasma clotting times, although this did not occur in Fibrinogen Detroit. In other abnormal fibrinogens, such as Baltimore, Leuven, Parma, Metz, Vancouver, Troyes and Iowa City, no inhibitory effect could be demonstrated.

Generally speaking, the other screening tests, such as whole blood clotting times, plasma recalcification times, partial thromboplastin times, and prothrombin

times were either normal or abnormal. In the case of Fibrinogen Detroit, homozygous family members had almost infinite whole blood and plasma clotting times, obviously due to the presence of only abnormal fibrinogen molecules, whereas the heterozygous patients had normal whole blood and plasma clotting times. Normal clotting times, except for thrombin and Reptilase times, thus do not exclude the presence of an abnormal fibrinogen.

In contrast to the congenital afibrinogenemias, where there was a high incidence of thrombocytopenia, prolonged bleeding times, abnormal platelet adhesion and possibly even abnormal platelet aggregation, patients with congenital dysfibrinogenemia have so far not been described with these defects. All tests, such as bleeding times, tourniquet tests, platelet counts, thromboplastin generation tests and the quantities of all known clotting factors have been described as normal. Also, the fibrinolytic activity was reported to be normal. The only deviation from this general rule is Fibrinogen St. Louis [42, 123, 124], where the abnormal fibrinogen is found in conjunction with Hemophilia A.

The final differential diagnosis between congenital afibrinogenemia and congenital dysfibrinogenemia can be made on the basis of quantitative fibrinogen determinations. In this connection it is important to recognize that the results may vary from one procedure to the next. Table 3 lists the amounts of fibrinogen, determined by three different procedures, in the family with Fibrinogen Detroit. As can be seen, homozygous patients (P.H. and M.H.) have no fibrinogen at all demonstrable by clotting procedures. In contrast, the levels of fibrinogen are normal with precipitation techniques and immunologic procedures. Doing only one procedure could have given rise to misinterpretations. The data clearly show that the patients have normal amounts of fibrinogen protein in plasma but that this protein is inactive. The heterozygous family members (E.H., S.H. and A.H.) had about half normal amounts of fibrinogen in plasma with clotting techniques, but normal levels with precipitation and immunologic procedures. If one investigates the other families with dysfibrinogenemia as to this phenomenon, one finds that, of 26 families, 25 had decreased fibrinogen levels when clotting procedures were used (Table 4). The exception is Fibrinogen Oklahoma. Precipitation techniques were used in 20 of the families, and only three had lower than normal levels (Fibrinogen Parma, Vancouver and Philadelphia); all other families had normal fibrinogen levels by this technique. Similarly, immunologic fibrinogen determinations were performed on the plasma of 16 families; 14 were normal or even above normal, and

TABLE 3. Levels of Fibrinogen in Family Members of Fibrinogen Detroit Using Three Different Techniques

Patient's Name	*Clotting Techniques (mg%)*	*Precipitation Techniques (mg%)*	*Immunological Techniques (mg%)*
P. H.	0	300	960
M. H.	0	296	600
E. H.	124	312	720
S. H.	96	247	880
A. H.	87	235	670
Normal	200-300	250-350	600-900

TABLE 4. Levels of Fibrinogen Determined by Three Techniques in Families with Dysfibrinogenemia

	Number of Families	*Normal*	*Decreased*
Clotting Techniques	26	1	25
Precipitation Techniques	20	17	3
Immunological Techniques	16	14	2

only two had lower than normal levels with this technique. It is thus important to recognize the principle of the techniques used for the determination of fibrinogen in plasma, especially abnormal fibrinogen.

Physical-Chemical Characteristics

The described abnormal fibrinogens were found to have various different characteristics. Whereas all fibrinogens studied migrated like normal fibrinogen in the electrophoretic field using paper or cellulose acetate as carriers, certain differences were noted when immuno-electrophoretic techniques were employed. They all gave a precipitating band with antibodies to normal human fibrinogen, but there were variations in electrophoretic mobility and in the number of bands formed. In the case of Fibrinogen Zürich I, Cleveland I, Detroit and Los Angeles, two precipitating bands were observed, possibly suggesting the presence of two species of fibrinogen molecules. Similar results were obtained with different techniques for Fibrinogen Baltimore, Paris I and Bethesda I.

Fibrinogen Paris I, Detroit, Paris II, St. Louis, Troyes, Wiesbaden, Zürich I, Zürich II, Montreal and Cleveland II migrated during immuno-electrophoresis like normal fibrinogen. An increased anodal migration was observed with Fibrinogen Baltimore, Bethesda II and Philadelphia, whereas an increased cathodal migration was described for Fibrinogen Amsterdam and Metz. Some fibrinogens were separated into their three chains and the electrophoretic mobility of the individual chains investigated. Only Fibrinogen Metz and Montreal had an α-chain which migrated more anodally than alpha chains of normal fibrinogen. The chains of Fibrinogen St. Louis, Zürich I, Troyes, Bethesda II and Philadelphia migrated normally.

Attempts to purify the abnormal fibrinogens from plasma have been successful where tried, and, with the exception of Fibrinogen Paris I, no differences from normal fibrinogens were noted in the precipitation characteristics. When submitted to chromatography, Fibrinogen Baltimore, Nancy, Paris I, Paris II and Philadelphia showed some differences from normal fibrinogen; all others eluted identically to normal fibrinogen.

Molecular weight determinations of the intact purified abnormal fibrinogens and their chains have so far not produced any differences from the figures given for normal fibrinogen and its chains.

Terminal amino acid analysis in Fibrinogen Baltimore, Paris I, St. Louis and Detroit gave normal values, and the determination of the amino acid composition of Fibrinogen Detroit yielded basically normal data, except for slightly decreased lysin and valine values. However, an incomplete digestion may be responsible for this finding.

Some interesting differences were noted in the carbohydrate composition of some abnormal fibrinogens. As can be seen from Table 5, Fibrinogen Zürich I and Detroit had a definitely lower-than-normal total carbohydrate content. A slightly

TABLE 5. Carbohydrate Composition of Some Abnormal Fibrinogens

Fibrinogen	*Hexose*	*Hexosamine*	*Sialic Acid*	*Total Carbohydrate*
Baltimore	?	?	Increased	?
Zürich I	Decreased	Normal	Normal	Decreased
Detroit	Decreased	Decreased	Decreased	Decreased
Paris II	Decreased	Normal	Increased	(Decreased)
St. Louis	Normal	Normal	Normal	Normal
Bethesda I	?	?	Normal	?
Nancy	Normal	Decreased	Increased	(Decreased)
Bethesda II	Normal	?	Normal	?

decreased content was found in Fibrinogen Paris II and Nancy. The sialic acid content of Fibrinogen Detroit was lower than normal, but it was higher than normal in Fibrinogen Baltimore, Paris II and Nancy. Mester and Szabados [101] calculated the sialic acid-hexosamine ratios and found them higher than normal for Fibrinogen Baltimore, Detroit and Paris II.

Some authors investigated the fibrin clots formed from abnormal fibrinogen by means of electron microscopy and found the structures to be different from normal in Fibrinogen Baltimore, Paris I, Los Angeles, Giessen and Cleveland II. Normal clot appearance was observed for Fibrinogen Amsterdam and Paris II.

Location of the Defect

In reviewing, in the 26 families, the location of the functional defect, one finds that abnormalities have been identified with the proteolytic phase, the polymerization phase and the stabilization phase of fibrin formation (Table 6). It must be recognized that a defect in the proteolytic phase, either in the form of a delayed release of fibrinopeptide A or B, or both, generally would result in a disturbed polymerization as well, since one phase depends upon the other. So far, a disturbed release of the fibrinopeptide A from the Aα-chain has been described for Fibrinogen Baltimore, Metz, Giessen and Cleveland II. A disturbance in the release of both fibrinopeptide A and B has been found in Fibrinogen Bethesda I. A disturbed release of fibrinopeptide B alone from the B β-chain has been determined for Fibrinogen Detroit only [18]. It is interesting that the peptide B is not released in the native fibrinogen molecule, whereas it is released from the separated N-terminal disulfide knot. This suggests that positions 14 and 15 of the B β-chain are possibly not accessible to thrombin in the intact molecule, suggesting an altered molecular structure.

TABLE 6. Functional Location of the Defect in the Abnormal Fibrinogens

	Proteolytic Phase Peptide		*Polymerization Phase*	*Stabilization Phase*
	A	*B*		
Parma			Unknown	
Vancouver			Unknown	
Paris I			+	
Baltimore	+			
Zürich I			+	
Cleveland I			+	
Detroit		+		
Paris II			+	
St. Louis			+	
Zürich II			+	
Louvain			+	
Oklahoma				+
Los Angeles			+	
Bethesda I	+	+		
Amsterdam			+	
Nancy			+	
Wiesbaden			+	
Troyes			+	
Metz	+			
Giessen	+			
Bethesda II			+	
Montreal			+	
Vienna			+	
Iowa City			+	
Cleveland II	+			
Philadelphia			+	

Fibrinogen Oklahoma apparently has its defect in the stabilization phase and, therefore, the different laboratory findings, as pointed out before.

All other abnormal fibrinogens fail to polymerize after the release of the fibrinopeptides A and B. As can be seen from Table 6, this is the vast majority of cases described. The disturbed polymerization of the fibrin monomers of Fibrinogen Amsterdam can only be demonstrated in the presence of α-2 globulins, possibly indicating that this abnormal fibrinogen may be different from the others in this group.

Only two fibrinogens, Parma and Vancouver, have not been investigated as to the functional location of their defect.

The exact molecular defect causing the abnormal function is presently described only for Fibrinogen Detroit. The molecular defect apparently lies in the A α-chain. Whereas fibrinopeptide A (amino acids 1 to 16, see Fig. 4 of [104]) is properly released by the enzyme thrombin, the following tripeptide Gly-Pro-Arg (17 to 19) is not released by thrombin. Detailed analysis revealed that arginine in position 19 was replaced by a serin residue [19]. This single amino acid substitution in the A α-chain must now have a secondary effect on the molecular configuration of the molecule, in that positions 14 and 15 of the B β-chain are inaccessible to the enzyme thrombin, resulting in the failure to release fibrino-

peptide B. Furthermore, it must make certain binding sites for the carbohydrates unavailable, since Fibrinogen Detroit has markedly decreased sugars (Table 5). As a result of the single amino acid replacement, the fibrinogen is functionally abnormal, leading to the clinical bleeding problem which our patient displays. This single amino acid replacement is of course reminiscent of some homoglobinopathies.

It is hoped that with the increasing knowledge of the structure of normal fibrinogen, the exact molecular defect of the other abnormal fibrinogens will also be elucidated.

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