Familial Thrombosis Due to Antithrombin III Deficiency

By Ewa Marciniak, Claude H. Farley, and Philip A. DeSimone

A large kindred from eastern Kentucky, with extensive history of recurrent venous thrombosis and pulmonary embolism, was studied. Low antithrombin III titers, ranging from 26% to 49% of normal values, were found in plasma of nine members in three consecutive generations; another five members, four of whom were not available for study, are suspected of having the biochemical defect. There was a good correlation between clinical symptoms and antithrombin III deficiency, although three of the younger members with the defect still remained free of thrombosis. In serum of the affected subjects antithrombin III was almost completely utilized, which indicates that stoichiometric binding to coagulation enzymes dominates under biological conditions. Antithrombin and antifactor Xa activities residing in the macroglobulin region of plasma and serum remained unchanged. The responsiveness to heparin in vitro and in vivo confirmed the evidence that antithrombin III is the sole blood component through which heparin exerts its anticoagulant effect. In five affected members therapy with oral anticoagulants increased very significantly the level of antithrombin III in plasma and contributed to a remarkable increase of residual antithrombin III in serum. This objective improvement after warfarin therapy may create significant difficulties in the laboratory diagnosis of antithrombin III deficiency.

AT THE PRESENT time antithrombin III is recognized as an α2 globulin capable of inactivating both thrombin and factor Xa in blood. This antiproteinase has been also found identical with so-called heparin cofactor. Reports on hereditary deficiency of antithrombin III have been restricted to a single Norwegian family studied by Egeberg and Abildgaard. A second large kindred from the Appalachian Mountains, unusually susceptible to thromboembolic disease in consequence of a biochemical defect, is the subject of the present study. The defect, inherited as an autosomal dominant, was limited to a single protein fraction bearing the characteristics of antithrombin III. In the affected subjects we found a reduced neutralization of thrombin and factor Xa both in the absence and in the presence of heparin. During the therapy with oral anticoagulants the level of antithrombin III in members with the defect has been significantly increased. These findings substantiate the outstanding biological role of antithrombin III in the support of blood fluidity and indicate that a decrease in vitamin-K-dependent coagulation factors abates the utilization of antithrombin III in vitro and probably also in vivo.

MATERIALS AND METHODS

Plasma was obtained by collecting 9 volumes of a fasting blood sample into 1 volume of citric acid-sodium citrate (0.1 M) applying a two-syringe technique. Immediately after drawing, blood...
was centrifuged in a refrigerated centrifuge at 1200 g for 20 min. Plastic syringes, centrifuge tubes, and storage containers were used. Serum was obtained from blood clotted in a glass tube and incubated for 2 hr at 37°C prior to centrifugation. Ether extract of bovine brain, suspended in saline at a concentration of 0.03%, was used as partial thromboplastin. This was mixed with an equal volume of 4% (w/v) kaolin suspension for activated partial thromboplastin time (aPTTₐ) determination. Sodium heparin (Upjohn) was used. Purified bovine factor Xa, purified bovine thrombin, and purified bovine antithrombin III were obtained as described before.

Prothrombin time was performed by the method of Quick. Thrombin activity was standardized in Iowa units. Two-stage prothrombin assay was performed according to the method of Ware and Seegers. Factors X and Xa were determined as described previously; the activity in 1 ml of normal human plasma was accepted for 100 U. Factor V, VIII, and IX assays were carried out by one-stage technique on a substrate plasma deficient in the respective coagulation factor. Thrombin time determination was performed by mixing equal volumes of plasma and purified thrombin solution of varying activity, indicated in the text. Antitrypsin determination was according to Homer et al.

Gel Filtration

A sample of plasma or serum, exactly 5 ml in volume, was applied to the Sephadex G-150 column (Pharmacia) 45 x 2.5 cm, conditioned and eluted with 0.1 M NaCl in 0.04 M Tris-HCl buffer, pH 7.8. An up-flow adaptor was used giving the elution rate of 20-25 ml/hr. The effluent was collected in fractions, exactly 3 ml in volume.

Antifactor Xa Assay

One volume of the test fraction and 1 volume of saline containing 2 U of purified factor Xa per ml and 0.2% of bovine albumin were mixed and incubated at 37°C for 30 min, unless stated otherwise. After the incubation the residual procoagulant activity of factor Xa was recorded as a clotting time on the substrate bovine plasma and compared with a control value, ranging from 17 to 18 sec, and obtained by substituting buffer for the test fraction. The difference between these two evaluations, expressed in seconds, represented the activity of antifactor Xa in the test fraction. Applying this method of procedure to variable low concentrations of purified antithrombin III, a rectilinear relationship between prolongations in clotting times and antithrombin III concentrations was recorded. This did not, however, apply to higher concentrations of antithrombin III, rapidly inactivating factor Xa, in which case the incubation time had to be considerably shortened.

Antithrombin Assay

Purified thrombin, 0.1 ml of a 20 U/ml solution, was mixed with 0.1 ml of test fraction and incubated for 10 min at 37°C. In fractions obtained from plasma after heparin injection this incubation was shortened to 5 min. At the end of incubation 0.2 ml of acacia-calcium reagent was added, immediately followed by addition of 0.1 ml of fibrinogen. The clotting time was recorded and compared with the control time (saline instead of the test fraction, usually 6.0-6.5 sec). Antithrombin activity in the test fraction was expressed as a difference in seconds between these two clotting times.

Heparin Concentration

This was measured as based on the ability of heparin to potentiate the activity of purified antithrombin III. Factor Xa was used as the substrate for the inhibition. One volume of the test fraction was supplemented with 0.5 volume of purified factor Xa of high activity (20 U/ml) and with 0.5 volume of purified antithrombin III (about 10% of the activity present in normal plasma). After 5 min of incubation at 37°C the residual activity of factor Xa was recorded. It was inversely proportional to the heparin concentration in the test fraction. A reference curve was obtained with known heparin concentrations by plotting heparin concentration versus the residual factor Xa activity.

CASE REPORT

The propositus (III-16) is a 24-yr-old white male who was in excellent health until June 3, 1972, when waking from an afternoon nap he suffered a sudden onset of chest pain, dyspnea, and hemop-
tysis. After admission to the University of Kentucky Medical Center pulmonary embolism was diagnosed. No signs of peripheral thrombophlebitis were noted on admission, but venograms performed on the 3rd hospital day revealed the presence of thrombi in the left iliac and the right femoral veins. Intravenous infusion of heparin was started on the 1st hospital day and continued for 18 days. On the 8th hospital day the patient developed obvious signs of thrombophlebitis in the left leg. Chest pain and hemoptysis reoccurred and lasted for a few hours. While continuing heparin, therapy with warfarin was initiated. Symptoms of thrombophlebitis resolved slowly and disappeared completely by the 17th hospital day. Twenty-four hours after discontinuing heparin, antithrombin III in patient's plasma was evaluated and found markedly decreased. He was discharged, continuing the medication with warfarin, and has been controlled periodically over 9 mo, but no thrombotic episodes reoccurred. During the entire period of hospitalization the patient demonstrated an unusual, apprehensive attitude. He stated repeatedly that symptoms similar to those observed in him were seen previously in several members of his family causing sudden death in two of his siblings.

**History of Family H**

The family has been known to inhabit an Appalachian region of Eastern Kentucky for at least a century, and most of the members still reside there. A few of them moved to Ohio, Indiana, and Michigan. A history of consanguinity was denied. The four generations that we have examined are presented in Fig. 1. Plasma level of antithrombin III for each investigated subject is shown in Table 1.

The matriarch of the family is, at the age of 83, in considerably good health with normal antithrombin III level. Her husband died at age 70, of stroke. For many years he suffered recurrent pain, swelling, and ulcerations of legs suggestive of thrombosis.

Subject II-3, the 62-yr-old uncle of the propositus, with markedly decreased antithrombin III, has a history of long-lasting swelling and pain in both legs with secondary crural ulcerations. He was hospitalized at the age of 58 with thrombophlebitis and suspicion of pulmonary embolism, and treated briefly with oral anticoagulants. Thereafter he had at least one additional incident of thrombophlebitis.

Subject II-4, the 59-yr-old father of the propositus, suffered recurrent leg thrombosis since the age of 26, and had two incidents of pulmonary embolism. When we initially evaluated his antithrombin III and found it abnormally low, he had been irregularly treated with small dose of heparin for few years.

Subject III-1 is a 34-yr-old woman, with decreased antithrombin III, who after the delivery of her third child, at the age of 25, developed severe thrombophlebitis in left leg, which recurred several times over the following few months. Treatment with oral anticoagulants lasted for 3 yr. Since then she has remained free of thrombotic episodes, although for 3 yr she took oral contraceptives.
Table 1. Antithrombin III Levels* in Plasma from Members of Family H

<table>
<thead>
<tr>
<th>Subject</th>
<th>A-III (%)</th>
<th>Subject</th>
<th>A-III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>79</td>
<td>III-9</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III-10</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III-11</td>
<td>78</td>
</tr>
<tr>
<td>II-2</td>
<td>77</td>
<td>III-12</td>
<td>31</td>
</tr>
<tr>
<td>II-3</td>
<td>31</td>
<td>III-15</td>
<td>49</td>
</tr>
<tr>
<td>II-4†</td>
<td>32</td>
<td>III-16‡</td>
<td>48</td>
</tr>
<tr>
<td>II-5</td>
<td>116</td>
<td>III-17</td>
<td>90</td>
</tr>
<tr>
<td>II-6</td>
<td>118</td>
<td>III-21</td>
<td>85</td>
</tr>
<tr>
<td>III-1</td>
<td>26</td>
<td>III-23</td>
<td>100</td>
</tr>
<tr>
<td>III-4§</td>
<td>83</td>
<td>IV-1</td>
<td>91</td>
</tr>
<tr>
<td>III-6</td>
<td>87</td>
<td>IV-2</td>
<td>82</td>
</tr>
<tr>
<td>III-7</td>
<td>89</td>
<td>IV-3</td>
<td>32</td>
</tr>
<tr>
<td>III-8</td>
<td>89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Normal range 80%-117%.
†On heparin.
‡On warfarin for 5 yr.
§Propositus, on warfarin for a few days.

Subject III-4 is a 26-yr-old man who had his first thrombosis and pulmonary embolism at the age of 15, after fracturing the left femur. Since then he has had 10 episodes of recurrent thrombophlebitis and at least three additional incidents of pulmonary embolism. Continuous therapy with warfarin was instituted at the age of 20, when multiple pulmonary embolization occurred after a plastic surgery of the leg for crural ulcers. Since the age of 21 he has remained asymptomatic but has continued the therapy with warfarin. Despite his dramatic clinical history the plasma level of antithrombin III was now found within the normal range. After observing in the other affected members of his family that a prolonged treatment with warfarin may considerably increase the biological activity of antithrombin III, we presume that this subject has the congenital biochemical defect.

Subject III-10, age 38, is the oldest brother of the propositus, with both legs amputated below the hip. He lost his left leg at the age of 23, a few months after developing pain and progressive numbness in this extremity. At that time he was told that he had Beurer's disease. The right leg was amputated at the age of 29 in consequence of severe circulatory disturbances indicative of venous obstruction with secondary pretibial ulcerations, which lasted for 4 yr. He has had delayed healing of the stump with recurrent ulcerations. We found that his antithrombin III is significantly decreased.

Subject III-12 is a 33-yr-old brother of the propositus, with decreased antithrombin III. He had a single incident of thrombophlebitis following appendectomy, at the age of 25.

Subject III-13, another brother of the propositus, is said to have had symptoms suggestive of thrombophlebitis. He died suddenly at the age of 27, when in the military service in Korea. His colleagues were alarmed when they heard him breathing with an extreme difficulty, but found him dead upon entering his room. On the autopsy a marked vascular congestion of the lungs, pulmonary edema, and right heart dilatation were noticed. This implies that pulmonary embolism was most likely the cause of a sudden death.

Subject III-20, a sister of the propositus, died suddenly at the age of 18, with obvious signs of pulmonary embolism. Shortly before death she was released from the local hospital where she received treatment for bilateral pneumonia.

Two individuals in the third generation (III-9, age 13, and III-15, age 26) and one in the fourth (IV-3, age 9) have the biochemical defect but have not shown any thromboembolic symptoms. They are of considerably young age. Since all of the older members of the family with the defect have had symptoms, it can be predicted with a fair degree of probability that these three will also in the future develop the clinical disease.
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RESULTS

Routine Laboratory Evaluations

The propositus and subjects II-3, II-4, and III-1 were evaluated for evidence of hemostatic abnormalities. The platelet count, fibrinogen level, PTT, euglobulin lysis time, and thrombin time with thrombin solutions ranging from 2 to 20 U of activity per ml gave normal results. In addition, subjects II-3 and III-1 before the therapy with oral anticoagulants had a normal prothrombin time and two-stage prothrombin level; determinations of factors V, VIII, IX, and X were also within normal range. The thromboplastin generation test with II-3 plasma and serum, utilizing platelet substitute, was normal. Cellulose acetate electrophoresis of serum from the propositus and subject III-1 showed no abnormalities. Serum levels of antitrypsin in subjects II-3, II-4, and III-1 were increased, assuming, respectively, values of 1.72, 2.22, and 1.69 mg trypsin inactivated per ml of serum (normal range 1.19 ± 0.18).

Evaluation of Antithrombin III

Since in human blood activities directed against thrombin and against factor Xa are not limited to antithrombin III, but have been found also in connection with macroglobulins, it was desirable to fractionate each specimen before analysis. For this purpose plasma or serum was filtered on Sephadex G-150 and antiproteinases were analyzed in isolated fractions.

Figure 2 compares the result obtained by measuring antithrombin and antifactor Xa in normal plasma and serum with representative results from one of the affected subjects (III-1). Antithrombin III, which is eluted from Sephadex with the last protein peak, was of significantly lower activity in III-1 plasma than in normal plasma. In

![Fig. 2. Antithrombin (triangles) and antifactor Xa (solid circles) evaluations in plasmas and sera after gel filtration on Sephadex G-150.](image)
normal serum a partial utilization of antithrombin III during blood coagulation was apparent. A total utilization took place in case of III-I serum from which antithrombin III was virtually absent. This finding is of particular importance since it reflects an extensive functional involvement of antithrombin III during the process of blood coagulation. The capacity of antithrombin in the macromolecular fraction from III-I serum was slightly greater than in control. In the macroglobulin fraction from plasma antithrombin was not analyzed since a considerable adsorption of thrombin on fibrin that forms in these fractions interferes with the assay. Antifactor Xa in the macromolecular region revealed activity in normal plasma and serum similar to that in abnormal plasma and serum.

The procedure of gel filtration was standardized and applied to plasma samples from several normal subjects and to plasma from members of family I-I. Occasionally serum samples were also analyzed. We limited the activity studies in filtered fractions to antifactor Xa assay because it gave in our hands steady results of a more reproducible character than results obtained in the evaluation of antithrombin. The capacity of antithrombin III in a given plasma sample was expressed as the sum of prolongations in clotting time (during 30-min incubation with factor Xa) in all fractions located within the last protein peak. This value in the analysis of 14 normal plasmas ranged from 240 to 350 sec with a mean of 299.6 ± 30.4 sec. Accepting antithrombin III activity in normal plasma for 100% we thus obtained a normal range of 80%–117% and a standard deviation of 10.1%. A normal plasma in which antithrombin III concentration was evaluated as 92% was diluted with the buffer used in filtration and samples containing 80%, 50%, 40%, and 25% of plasma were subsequently analyzed. Antithrombin III titers of 73%, 43%, 35%, and 25% respectively, were obtained confirming the usefulness of the method for quantitative analysis.

Table 1 shows the values obtained in plasma of family H members. Nine out of 24 analyzed subjects gave values that were significantly lower than those in the normal group, ranging from 26%–49% of normal activity. Six of the biochemically abnormal members had history of thrombosis. The seventh member with extensive history of thrombosis (III-4) revealed normal antithrombin III level, but, as mentioned before, he has been on long-term medication with warfarin. The effect of this medication on antithrombin III in other members of the family H is documented below. Also the values originally obtained in the propositus and in his father (II-4), although distinctly below normal, might have been to some extent altered by the therapy with oral anticoagulants.

**Effect of Heparin In Vitro and In Vivo**

In vitro studies were performed on fractions from plasma and serum isolated by gel filtration. Factor Xa was used as a substrate for inactivation. After addition of heparin the inactivation of factor Xa was greatly accelerated, but limited to fractions located in the antithrombin III region (Fig. 3). Plasma fractions from subject II-4 showed a diminished responsiveness to heparin. Fractions from II-4 serum which contained practically no antithrombin III were almost totally resistant to heparin. These results are in agreement with the assumption that antithrombin III is the substance through which heparin exerts its anticoagulant effect in blood.

The responsiveness in vivo was evaluated after intravenous injection of 100 U of
heparin/kg. In subjects II-3 and II-4 the hypocoagulable state, when evaluated by thrombin time assay, was of much shorter duration and lower intensity than in normal volunteers (Fig. 4). This was, however, less pronounced when we monitored the effect of heparin by PTTa assay (Fig. 5). Particularly in the case of patient III-1, the inhibition in PTTa after heparin was better expressed than in some of the controls and did not correlate with her low antithrombin III level. Unfortunately, she was not followed
up by thrombin time evaluations. However, we could demonstrate the insufficiency of antithrombin III in her plasma even during the high heparin concentration in blood. As shown in Fig. 6, the analysis of plasma obtained 15 min after heparin injection revealed a higher activity of heparin in subject III-1 than in the normal volunteer. At the same time, however, antithrombin III fraction of the affected subject remained insignificant in its antithrombin activity, which was in sharp contrast to the eminent elevation in the control. The discrepancy between this result and the marked hypo-coagulability of whole plasma in subject III-1 was apparently due to the fact that before the fractionation heparin at high concentration remained in contact with antithrombin III compensating for its low level. During the fractionation on Sephadex most of the heparin had been separated in association with other protein structures, eliciting the defect.
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Table 2. The Effect of Therapy With Warfarin on Antithrombin III Level in Deficient Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Duration of Therapy</th>
<th>Prothrombin Time (sec)</th>
<th>A-III Level in Plasma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-3</td>
<td>Before</td>
<td>12.9</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>18.3</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>4 mo</td>
<td>17.3</td>
<td>56</td>
</tr>
<tr>
<td>II-4</td>
<td>Before*</td>
<td>12.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>23.0</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>3 wk</td>
<td>23.0</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>20.0</td>
<td>79</td>
</tr>
<tr>
<td>III-1</td>
<td>Before</td>
<td>11.6</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>20.0</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>1 mo</td>
<td>20.1</td>
<td>68</td>
</tr>
<tr>
<td>III-15</td>
<td>Before</td>
<td>11.8</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>6 mo</td>
<td>18.5</td>
<td>76</td>
</tr>
<tr>
<td>III-16</td>
<td>14 days</td>
<td>22.0</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>20 days</td>
<td>18.5</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>4 mo</td>
<td>18.2</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>6½ mo</td>
<td>19.8</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>9 mo</td>
<td>22.6</td>
<td>61</td>
</tr>
</tbody>
</table>

*On heparin.

Results of Therapy With Oral Anticoagulants

The propositus and subsequently four additional members of his family have been treated with warfarin in the conventional manner. In response to this therapy a significant increase in antithrombin III activity in plasma has been observed (Table 2). Even after a few days of treatment, subject III-1 showed more than twice as much of antithrombin III as she had before. In subject III-4 almost normal values were recorded after 2 mo of the therapy. The propositus also gave objective evidence of improvement over several months of treatment. In his case, however, the assessment is not quite accurate, since the prewarfarin level of antithrombin III remains unknown.

The difference between the residual antithrombin III in serum before and during the therapy with warfarin was remarkable (Table 3). In subjects with the defect, serum-antithrombin III increased from near-zero level to a range seen in normal sera. Higher residual values, however, were observed in the serum of a patient treated with warfarin, who was without an apparent defect in antithrombin III synthesis. In this

Table 3. The Effect of Warfarin Therapy on Residual Antithrombin III in Serum

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma Prothrombin 2-stage (U)</th>
<th>A-III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In Plasma</td>
</tr>
<tr>
<td>Normal control</td>
<td>300</td>
<td>103</td>
</tr>
<tr>
<td>Control on warfarin*</td>
<td>48</td>
<td>107</td>
</tr>
<tr>
<td>II-4†</td>
<td>227</td>
<td>32</td>
</tr>
<tr>
<td>II-4 on warfarin</td>
<td>53</td>
<td>79</td>
</tr>
<tr>
<td>III-1</td>
<td>250</td>
<td>26</td>
</tr>
<tr>
<td>III-1 on warfarin</td>
<td>57</td>
<td>68</td>
</tr>
</tbody>
</table>

*Patient postaortic valve replacement, on warfarin for 5 yr.
†This patient was taking heparin irregularly at the time of analysis.
latter case we could not account for a significant increase of plasma antithrombin III above normal level. This leaves us with the impression that stern stochiometric proportions are implicated in the neutralization of coagulation enzymes in blood.

DISCUSSION

The biochemical defect in family H is apparently transmitted by an autosomal gene and has a dominant character with incomplete penetrance. Nine members are heterozygotes with antithrombin III ranging approximately from 25% to 50% of normal level, as evaluated by the anticoagulant properties. An additional five members, three of whom died before we started the investigation, are suspected of having the deficiency. Although there is a good correspondence between the presence of the biochemical defect and the occurrence of thrombosis, the clinical symptoms appear rather late in life, frequently after the age of 20. A similar hereditary abnormality was reported originally by Egeberg 8 yr ago.\(^5\) No other evidence in the literature indicates that additional families are involved. Taking into consideration the dominant character of this trait, one has to assume that the frequency of the abnormal gene must be extremely low. On the other hand, the evaluation of antithrombin III, at the present time, belongs to seldomly used laboratory procedures, and available methods are far from being satisfactory. Thus, a number of cases may escape the evidence of the investigator, despite a remarkable family history. In studies conducted on sporadic cases with thrombosis Abildgaard et al.\(^19\) observed frequently a marked decrease in plasma levels of antithrombin III, but attributed this to an enhanced consumption. Hensen and Loeliger\(^20\) recorded normal values in all their patients with thrombosis.

Antithrombin III has the distinction of being a protein with well-defined properties, known to interact stoichiometrically with thrombin\(^21\) and with factor Xa,\(^19\) on account of which it can be visualized as an insurance agent against acute thrombotic episodes. Nevertheless, the question which remains to be answered is, to what extent normal persons benefit from these properties of antithrombin III. The existence of individuals with incomplete antithrombin III synthesis due to a selective genetic defect, many of whom develop deep vein thrombosis and pulmonary embolism without any apparent cause, not only buttresses the biological significance of antithrombin III but indicates that the tendency for intravascular coagulation has been generally underestimated. The ratio of antithrombin III capacity to the potential capacity of thrombin undoubtedly plays a very important role in the prevention of thrombosis. The liver appears to be the only organ in which antithrombin III is produced. In addition to the inborn defect, low levels are commonly observed in liver cirrhosis.\(^19\)\(^20\) The explanation for the observation that thrombosis does not appear in the cirrhotic patient to the same extent as in congenital deficiency of antithrombin III lies in the differential requirements of the two abnormalities for antithrombin III. In liver cirrhosis the synthesis of prothrombin and factor X is impaired, which lowers the requirement for the inactivator. For members of family H with normal levels of vitamin-K-dependent coagulants, higher levels of antithrombin III are required in order to support adequately the fluidity of their blood. On this assumption one would expect a significant improvement of the biologic equilibrium in patients with hereditary deficiency of antithrombin III by lowering their level of circulating procoagulants. The relatively short time during which a systematical therapy with warfarin has been administered in members of family H does not allow us yet to assess properly their clinical responsive-
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ness. Nevertheless, as a result of the therapy, not only the residual antithrombin III in serum appeared in quantities indicating a generous surplus over the capacity required for thrombin inactivation, but a significant increase in plasma levels has been recorded. The latter effect might be attributed to either the possibility that a catabolic pathway of antithrombin III is also induced by its stochiometric binding to activated coagulation enzymes, or that coumadin stimulates the synthesis. Of interest is the fact that, although an increase in normal plasma levels of antithrombin III in patients on oral anticoagulants has been noticed before,20,4 it was usually of a very moderate degree. Similar therapy in some of the subjects with the defect whom we observed raised the level of antithrombin III more than twice above the original value. At the present time we are unable to offer an explanation for this discrepancy. The fact that must be taken under consideration, however, is that a long-lasting therapy with oral anticoagulants gives an objective improvement in patients with the hereditary antithrombin III deficiency, which worsens the conditions for a laboratory diagnosis of this abnormality.

The responsiveness to heparin in vivo in members of family H reflects a direct stimulation of antithrombin III, which as a sole blood component in concert with heparin provides the anticoagulant effect. Only in the event of a complete absence of antithrombin III will this effect be abolished. Such a condition, however, in a human being is unknown and probably incompatible with life. In heterozygotes, like the members of family H, it is a question of supporting an adequate amount of heparin in order to compensate for low antithrombin III level. This amount may not differ significantly from a conventionally applied dose and is probably highly individual, since the distribution of heparin in blood depends on other plasma components than antithrombin III.15 When an abnormal type of gamma globulin appears in the circulation, the therapy with heparin may be completely futile despite a normal antithrombin III level.22 Partial neutralization of heparin by blood cell constituents23,24 found in platelets, erythrocytes, and neutrophils also may contribute to a variable responsiveness. Furthermore, the rate of heparin decay or elimination from blood, the mechanism of which remains unknown, will influence the duration and extent of the anticoagulant effect. Thus, variations in the responsiveness to heparin among persons with an inborn error of antithrombin III metabolism, as unrelated to the profoundness of the defect, are not surprising.

In the view of a high frequency of thrombosis in the family H, it seems unquestionable that antithrombin III represents the principal biochemical safeguard against fibrin deposition inside the vessels. As documented previously,10 under biological conditions thrombin constitutes the main target for this antiproteinase, factor Xa being protected by a complex formation with phospholipid and factor V. Of some concern, however, is the specificity of other natural proteinase inhibitors. Despite elevated antitrypsin activity in antithrombin III-deficient sera, we could not account for any thrombin inhibition that might have corresponded to the alpha-1 antitrypsin fraction. This contradicts some of previously published suppositions25 in regard to the role of antitrypsin in binding and disposition of thrombin. In the case of this antiprotease, however, the time of interaction26 and species specificity of the reacting components27 may contribute to outstanding discrepancies in experimental results. It is also difficult to determine critically the biological implications of macroglobulin fraction for thrombosis and hemostasis. Although a bimolecular binding of thrombin16 and factor Xa18 by components of this fraction has been documented, the inhibitory capacity of
macroglobulins for coagulation enzymes seems to remain unchanged after blood clotting, even in subjects with antithrombin III deficiency. This may imply that the biological role of macroglobulins is rather negligible. On the other hand, experimental procedures may alter significantly the physiological environment in which macroglobulins function as inactivators of coagulation enzymes.\textsuperscript{3,18}

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Familial Thrombosis Due to Antithrombin III Deficiency

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