REVIEW ARTICLES

Familial venous thrombosis

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Introduction

It is not uncommon for a patient presenting with deep venous thrombosis or pulmonary embolism to describe the occurrence of such disease in members of his or her family. The occasional familial clustering of venous thromboembolic disease has been recognized for many years; for example, Briggs in 1905 states:

'The patient, a man of 35 years, has since 1887 passed through 8 attacks of thrombophlebitis in the lower extremities... Of 16 adult individuals among this gentleman's ancestors and collateral relatives, 8 have shown a marked susceptibility to venous disease, in the manifestation of varices and haemorrhoids or in extreme liability of thrombosis in the puerperium or following acute infections'.

It is a reasonable assumption that, in some cases of familial venous thrombosis, a genetically defined abnormality will be present. Study of such families has revealed in a few cases an association between thrombosis and inherited abnormalities of the following plasma proteins: anti-thrombin III (ATIII), fibrinogen, plasminogen and protein C.

In this article, the clinical and biochemical aspects of these abnormalities are discussed. Firstly, the normal coagulation and fibrinolytic systems are briefly described to enable the reader to appreciate the likely consequences of specific abnormalities within these systems.

The coagulation and fibrinolytic systems

The mechanism whereby blood clots *in vitro* is now well understood but whether thrombosis *in vivo* occurs via this same mechanism is unknown and theories concerning thrombogenesis must necessarily be largely extrapolated from *in vitro* data. The subject has recently been reviewed (Bloom and Thomas, 1981).

Fibrin forms the structural lattice work of a venous thrombus and thus the generation of thrombin, which converts soluble fibrinogen to insoluble fibrin polymers, is likely to be an essential step in the genesis of venous thrombosis. Thrombin itself is produced by the cleavage of prothrombin, an inactive precursor, by activated factor X in the presence of a cofactor, activated factor V. Activated factor X is a common product of both the 'intrinsic' and 'extrinsic' pathways of the blood coagulation system (Fig. 1). The separation of the coagulation system into 'intrinsic' and 'extrinsic' pathways is artificial since they are in fact intertwined, but is helpful conceptually. The intrinsic pathway is initiated *in vitro* by the addition to plasma of substances such as kaolin which bear a negative surface charge; this results in the activation of factor XII which then initiates the serial activation of factors XI, IX and X in a process involving the participation of high molecular weight kininogen, prekallikrein, factor VIII (anti-haemophilic globulin) phospholipid and calcium. Factor X activation via the extrinsic pathway results from the action of a complex made up of factor VII and tissue factor, a factor released by damaged cells (Fig. 1).

Activation of coagulation factors involves the cleavage of one or more peptide bonds, resulting in structural changes to the procoagulant molecules so that in their new form they possess protease activity which is responsible for their coagulant activity. That part of the molecule responsible for the protease activity, that is, the hydrolysis of peptide bonds, is termed the active site and in the family of coagulant proteases the common feature of the active site is a central serine residue.

Platelets play an essential part in haemostasis and it is thought that they are also involved in the initiating events of venous thrombosis. Platelets provide a source of phospholipid for the intrinsic pathway and provide specific receptors for activated factors X, V and thrombin. There is also evidence

FIG. 1. Diagrammatic representation of the coagulation system based on Davie and Ratnoff (1964).

that platelets may be capable of developing the ability to directly activate both factors XI and X, thereby by-passing earlier stages in the intrinsic pathway of coagulation.

In addition to the mechanisms which bring about the production of thrombi, blood also contains mechanisms for the dissolution of thrombi. Plasminogen is the inactive precursor of plasmin, a protease of very wide specificity capable of digesting fibrin and many other proteins. Plasminogen activators, as their name suggests, are responsible for the generation of plasmin from plasminogen; a number of differing activators are thought to exist derived from endothelium, tissue, plasma (factor XII dependant) and kidney (urokinase). Mechanisms exist which allow the activation of plasminogen to take place more readily on the surface of fibrin than in plasma.

Control and balance in the coagulation and fibrinolytic systems

Haemostasis, the process of limiting blood loss from areas of vascular damage, involves the adhesion of platelets to areas of denuded endothelium and the activation of the coagulation system with the production of fibrin. Clearly, if this process were to continue unchecked then the vascular system would rapidly solidify as fibrin formation became generalized. A number of inhibitors of coagulant proteases

exist which modulate the activity of the coagulation system. Of these inhibitors, ATIII is thought to be of importance in the neutralization of coagulation factors entering the blood stream. A more recently described inhibitor of the coagulation system, protein C, is also likely to be of importance in the control of the coagulation mechanism. The process of fibrinolysis is similarly influenced by inhibitors. There are several plasma proteins able to inhibit plasmin activity *in vitro*, the most important of which *in vitro* appears to be alpha 2-antiplasmin.

There is evidence that the coagulation and fibrinolytic systems normally exist in balance. Thus, excessive activity in the coagulation system may lead to thrombosis whereas excessive activity in the fibrolytic system may lead to haemorrhage. Similarly, underactivity in the coagulation system may lead to haemorrhage, whereas underactivity in the fibrolytic system may lead to thrombosis. Thus, potential abnormalities predisposing to thrombosis may be predicted on the basis of this theory.

Study of the endothelium is hampered by the lack of accessibility relative to that of plasma, but it is likely that abnormalities of the endothelium may also predispose to the development of thrombosis.

Factors associated with familial venous thrombosis

A number of different plasma abnormalities have been associated with familial venous thrombosis.

With the exception of ATIII deficiency, arguably the best delineated example of a 'hypercoagulable' state, it is not possible to say with certainty that the abnormalities involved are responsible for the observed predisposition to thrombosis.

Increased activity of the coagulation system

An increase in the concentration of a procoagulant is unlikely *per se* to predispose to venous thrombosis since it is presumably only the activated coagulant protease which is instrumental in the thrombotic process. Nevertheless, one family has been described in which a marked increase in the plasma level of factor VIII (anti-haemophilic globulin) was associated with a high incidence of recurrent thrombosis (Penick *et al.*, 1967). In addition, a high titre of plasma factor V has been described in a further family affected with both venous and arterial occlusive disease (Gaston, 1966). It is noteworthy that both factors VIII and V act as cofactors in the coagulation system rather than as serine proteases, and are both inhibited by protein C, a deficiency of which has now been described in 2 families with a predisposition to venous thrombosis.

Protein C is a relatively recently discovered vitamin K dependent plasma protein (Stenflo, 1976). It circulates in an inactive form and may be converted to an active serine protease by thrombin (Kisiel, 1979). Activated protein C has potent anti-coagulant properties (Marlar, Kleiss and Griffin, 1981) which are believed to be related to the ability of the protease to inactivate activated factor V and VIII; in addition a stimulatory effect on fibrinolytic activity has been reported (Comp and Esmon, 1980), although the mechanism of this action is not clear. In the balance between coagulation and fibrinolysis therefore, protein C may play a part in both limiting fibrin formation by inhibition of activated V and VIII and in increasing fibrin removal by stimulation of fibrinolysis. Thus, a deficiency of protein C activity might reasonably be expected to predispose to the occurrence of venous thrombosis. A difficulty in the search for protein C deficiency in individuals with a history of venous thrombosis is that these individuals are commonly receiving therapy with oral anticoagulants which results in a decrease in the plasma concentration of all vitamin K-dependent factors including protein C. This difficulty may have been resolved by 2 methods; firstly by the establishment of normal range for plasma protein C in individuals receiving different intensities of oral anticoagulant therapy and secondly by relating the protein C concentration to that of another vitamin K dependent protein such as thrombin or factor X, and establishing normal ranges for the ratio of protein C to such proteins in patients receiving oral anticoagulant therapy (Bertina *et al.*,

1982). In the family with protein C deficiency first described (Griffin *et al.*, 1981) the 3 deficient members had all suffered with deep venous thrombosis and pulmonary emboli and one had also suffered a cerebrovascular accident and a myocardial infarction at the early ages of 43 and 45 years respectively. In the second reported family (Bertina *et al.*, 1982) all 3 siblings had suffered with superficial thrombophlebitis, 2 of these had histories of deep venous thrombosis and only one had suffered with a pulmonary embolus. Inheritance of the deficiency was consistent with an autosomal dominant mode, and a protein C antigen level of approximately half of the normal level was found in one deficient individual whilst not receiving oral anticoagulant therapy (Bertina *et al.*, 1982); thus the mode of inheritance and degree of deficiency may be similar to those seen in familial ATIII deficiency (*vide infra*).

Abnormalities of fibrinogen and the fibrinolytic system

A number of inherited abnormalities of the fibrinogen molecule have been associated with thrombosis; Beck, Charache and Jackson (1965) described a bleeding tendency in members of a family who had inherited an abnormal fibrinogen species; in addition, deep venous thrombosis and pulmonary emboli occurred in the propositus of this family who had also inherited the fibrinogen abnormality. Al-Mondhiry, Bilezikian and Nossel (1975) also described a family with an inherited fibrinogen abnormality in whom only the propositus was affected with venous thromboembolic disease. The association between an inherited fibrinogen abnormality and thrombosis is considerably more striking in the family described by Egeberg (1967); a high incidence of thrombosis in this large family is described, and abnormally short thrombin times were found in 5 members who had a history of thrombosis. An abnormal fibrinogen species was not demonstrated directly, but fibrinogen concentrate from one affected individual retained the property of clotting more quickly than normal fibrinogen concentrate on exposure to thrombin.

Although the suggestion that a decrease in activity of the fibrinolytic enzyme system may predispose to venous thrombosis has been made repeatedly, supporting evidence from family studies is lacking. Aoki *et al.* (1978) have reported the familial occurrence of a plasminogen abnormality. In affected individuals, approximately half of the plasma plasminogen was incapable of being activated to plasmin by streptokinase and 2 molecular species could be demonstrated by an iso-electric focusing technique. The 31-year-old propositus of this family had suffered severe thromboembolic events since the age of 15 years, but no history of thrombosis was found in other affected family members, including a niece in whose plasma

very little normal plasminogen was present. Wohl, Summaria and Robbins (1979) have described an abnormality of plasmin generation, believed to be congenital, in 2 unrelated individuals with a history of deep venous thrombosis. However, no family studies are reported and little clinical detail is given.

As indicated in Fig. 1, an 'intrinsic' mechanism for the activation of plasminogen involving factor XII exists (Saito, Ratnoff and Donaldson, 1974) and is deficient in those patients with inherited factor XII deficiency; it is not known if factor XII-related fibrinolysis is of importance in the development of thromboembolic disease but thrombosis has been described in patients with factor XII (Hageman factor) deficiency (Dyerberg and Stoffersen, 1980) and the initial factor XII deficient patient Mr Hageman himself died from a pulmonary embolus (Ratnoff, Busse and Sheon, 1968).

Vessel wall abnormalities

Deficient vessel wall fibrinolysis defined as a reduction in the fibrinolytic activity of the vessel wall or as an impaired capacity to release fibrinolytic activity into the circulation has been found in approximately 70% of patients with severe thromboembolic disease (Isacson and Nilsson, 1972). However, there are only 2 reports of the familial occurrence of this abnormality in association with venous thromboembolic disease (Johansson, Hedner and Nilsson, 1978b; Stead *et al.*, 1983).

In hereditary homocystinuria, a disorder often complicated by venous and arterial thrombosis, the underlying inherited abnormality is known but the mechanism responsible for the predisposition to thrombosis is not clear although it is known that homocystine may damage vascular endothelium (Harker *et al.*, 1976).

Familial antithrombin III deficiency

In contradistinction to the disorders just described, familial ATIII deficiency is the single disorder in which a causal relationship between a plasma deficiency and venous thrombosis is widely accepted. This acceptance is based upon extensive knowledge of the properties of ATIII and upon the study of many deficient kindred.

ATIII is a single chain glycoprotein with a molecular weight of approximately 60,000 (Nordenman, Nyström and Björke, 1977), migrating as an alpha-2-globulin and is probably synthesized in the liver (Hensen and Loeliger, 1963). Initially, several anti-thrombin activities were described and were distinguished by adding a numerical suffix to the term antithrombin. ATIII is the only activity to have retained the term antithrombin and indeed the suffix III is no longer absolutely necessary. ATIII functions

as a serine protease inhibitor. Complexes are formed by covalent bonding between the active site of the protease and the reactive site of ATIII; these complexes therefore are stable and devoid of either, protease or protease inhibitor activity (Owen *et al.*, 1976). In this respect, ATIII may be regarded as 'false substrate' for the protease. The rate at which ATIII forms complexes with coagulant proteases is greatly accelerated in the presence of heparin. It is this property of heparin which leads to its anticoagulant activity, since in plasma depleted of ATIII, heparin has little or no anticoagulant effect (Holmer, Soderstrom and Andersson, 1980). Inhibition of nearly all the serine proteases of the coagulation cascade, namely activated factors XII, XI, IX, X, VIII and plasmin, kallikrein and urokinase (Fig. 1) by ATIII has been demonstrated *in vitro* (Rosenberg and Damus, 1973; Highsmith and Rosenberg, 1974; Burrowes, Habal and Movat, 1975; Østerud *et al.*, 1976; Rosenberg, 1974; Stead, Kaplan and Rosenberg, 1976; Clemmensen, 1978). Although the observation of an inhibitory property *in vitro* does not in itself confirm a physiological importance for that property *in vivo*, it is a reasonable supposition that inhibition of one or more coagulant proteases by ATIII is important for the prevention of venous thrombosis in view of the clear predisposition to venous thrombosis which occurs in familial ATIII deficiency. Kinetic studies *in vitro* show that activated factors X and II are inhibited more quickly than other coagulant proteases and the central role in the coagulation cascade of these factors (Fig. 1) suggests that control of their activity will be of major physiological importance.

Measurement of ATIII

The measurement of ATIII may be divided into categories of activity, antigenic and functional. Antigenic activities are measured in assays utilising monospecific antibody and using such techniques as radial immunodiffusion, quantitative immunoelectrophoresis and radio-immunoassay systems (Chan *et al.*, 1979). Such assays of antigen generally do not distinguish between functionally active ATIII and ATIII which lacks antiprotease activity for reasons of inheritance (Sas *et al.*, 1974), proteolytic cleavage (Fish and Björke, 1979) or complex formation with protease. Clearly, measurement in antigenic assays is largely dependent upon the properties of the antibody employed and there is evidence that differing antibody preparations may measure different antigenic determinants on the ATIII molecule (McKay, 1980).

Functional assays of ATIII measure its antiprotease properties and are based upon a single principle, namely the incubation of test samples with standard quantity of protease, the measurement of

residual protease activity and the calculation of the quantity of protease inhibited (i.e., antiprotease activity) by subtraction. Many different assays have been described but thrombin (activated factor II) and activated factor X are most commonly used as the protease. When the former is used, the assay is said to measure progressive thrombin activity. Protease activity is commonly measured by clotting methods or by the use of artificial substrates (peptides which on cleavage by protease release a substance such as P-nitroaniline, the concentration of which may be easily quantitated by absorbance at a specific wavelength). The reaction of ATIII and protease may take many minutes to reach completion but the addition of heparin to the system will reduce this time to a few seconds and in addition will considerably reduce any contribution from other non-specific antiproteases; consequently many assays include heparin in the buffer system and are said to measure heparin cofactor activity.

A generally accepted standard for ATIII activities is not yet available and therefore, as in the assay of many other coagulation proteins, a pool of plasma made up from the plasma of over 20 healthy individuals is used as a standard and arbitrarily assigned a value of 100%. Normal values for ATIII in our laboratory are as follows: radial immunodiffusion $102 \pm 12\%$; heparin cofactor assay $100 \pm 8\%$; progressive antithrombin activity $96 \pm 13.5\%$.

Antithrombin III deficiencies

Familial ATIII deficiency was first described by Egeberg in 1965. He noted a decrease in plasma progressive antithrombin and heparin cofactor activities to approximately half normal values in those members of a large family who had suffered venous thromboembolic events. Following the purification of ATIII and the production of antisera, it was possible to show that the level of ATIII antigen in this family was also reduced to a level approximately half normal (Abildgaard, Fagerhol and Egeberg, 1970). Thus, in this family, the prototype of classical ATIII deficiency (Table 1, type 1), a quantitative deficiency of functionally normal ATIII is seen (Fig. 2). Many families with such a deficiency have subsequently been described.

The second variant of familial ATIII deficiency (Table 1, type 2) was first reported by Sas and colleagues (1974). They describe a *qualitative* defi-

ciency of ATIII, the plasma of affected family members having a normal level of ATIII antigen but a reduced level of progressive antithrombin and heparin cofactor activities. In this family, it was possible to identify 2 molecular species of ATIII by the technique of crossed immunoelectrophoresis in the presence of heparin (Sas, Pepper and Cash, 1975) strongly suggesting that in this type of deficiency normal ATIII coexists with a molecular species of

FIG. 2. Heparin cofactor activity and antigenic levels of ATIII in members of 2 deficient families (families DM and MS, Winter *et al.*, 1982b). Reproduced with the permission of the Editor of *Annals of Human Genetics*. ○ = deficient; ● = normal.

ATIII which is functionally inactive. A number of other families with a similar deficiency have been described (Brozovic, Stirling and Hamlyn, 1978; Nagy *et al.*, 1979; Sorensen *et al.*, 1980; Barbui and Rodeghiero, 1981) and it appears that the predisposition to venous thrombosis in these families is similar to that seen in families with classical ATIII deficiency. This observation is of some theoretical importance since it strongly suggests that it is specifically the lack of antiprotease activity which is responsible for the predisposition to thrombosis.

In the third type of ATIII deficiency (Table 1, type 3), the plasma from affected family members has normal antigenic and progressive antithrombin activities, but is deficient in heparin cofactor activity (Wolf *et al.*, 1979; Penner *et al.*, 1979; Tran *et al.*, 1980). In this variant, it appears that the abnormal

TABLE 1. Three types of antithrombin III deficiency

	ATIII antigen	Progressive antithrombin activity	Heparin cofactor activity
Type 1 (Classical deficiency)	Reduced	Reduced	Reduced
Type 2	Normal	Reduced	Reduced
Type 3	Normal	Normal	Reduced

ATIII possesses antigenic and antiprotease activities, but is deficient in the property of heparin-related acceleration of antiprotease activity. The existence of a predisposition to thrombosis in such a deficiency would strongly support a physiological role for heparin or related glycosaminoglycans in haemostasis and thrombosis. However any tendency to venous thrombosis in families described to date appears to be mild.

Thus, it is clear that estimation of heparin cofactor activity will detect all 3 types of deficiency, estimation of progressive antithrombin activity will detect Types 1 and 2 whereas an assay of antigen will detect only Type 1 or classical ATIII deficiency.

Diagnosis of classical ATIII deficiency

Published estimates of ATIII antigen in the plasma of patients with classical ATIII deficiency have seldom been in excess of 60% of normal pool plasma values and our experience with 3 such families is in agreement with this (Table 2). Serum ATIII activities have been measured in a number of families but our experience does not support this practice; we have found no difficulty in distinguishing deficient from normal individuals by estimating plasma ATIII levels, whereas serum values in our families have shown an overlap between deficient and unaffected (Table 2). A number of conditions are capable of giving rise to *acquired* plasma deficiency of ATIII namely, liver disease, extensive venous thromboembolism, disseminated intravascular coagulation, nephrotic syndrome, therapy with heparin or asparaginase and extensive plasma exchange. In the absence of these conditions, plasma levels of ATIII antigen below 60% of normal are almost diagnostic of the classical deficiency, and family studies should be undertaken. Where the deficiency is found in a number of family members, there is no doubting the diagnosis. Difficulty occurs where a reduced plasma ATIII level is found in an individual without either strong family history of venous thromboembolic disease, or demonstrable deficiency in other family members. Clearly, in such an individual, a thorough search for an acquired cause for the deficiency must

be undertaken. However, there is no doubt that spontaneous mutation takes place and congenital abnormalities of chromosome one giving rise to a deficiency of plasma ATIII have been described (Winter *et al.*, 1982b).

Classical ATIII deficiency is inherited in an autosomal dominant fashion, and thus the trait appears in every generation, affects the sexes equally, is passed on to an average of half of an affected individual's offspring and is never transmitted through an unaffected individual. The last mentioned criterion rule is broken by members of a recently reported South African family in which the trait appears to have been passed on by clinically and biochemically normal family members (Beukes and Heyns, 1980). The location of the ATIII gene has been identified on the long arm of chromosome one by linkage studies in ATIII deficient families; in these studies the deficient trait was linked to the Duffy blood group (known to be carried on the long arm of chromosome one) and to a variably staining area on this chromosome termed 1qh (Winter *et al.*, 1982b).

Clinical features

Individuals with ATIII deficiency demonstrate a marked predisposition to venous thrombosis, and such thrombosis and its sequelae are responsible for the clinical features of this disease. The commonest site for venous thrombosis as with unaffected individuals is the leg (Fig. 3), although thrombosis has been described in many sites including the inferior vena cava, renal, hepatic and portal veins and cerebral sinuses. Mesenteric venous thrombosis appears to be peculiarly common in this disease and is a life threatening event (Gruenberg, Smalldridge and Gruenberg, 1975; Rubinowitz and Ford, 1977). In later life, varicose veins are almost universal in affected individuals of both sexes, and varicose ulceration may occur in the third decade of life (Winter *et al.*, 1982e). The other sequelae of deep venous thrombosis namely chronic swelling of the legs, varicose eczema and pulmonary emboli are also common. A disturbing feature of the disease is sudden death, apparently due to pulmonary embolus, at least one instance of which is reported in many families,

TABLE 2. Plasma ATIII levels in three families with classical ATIII deficiency (Winter *et al.*, 1982b)

	Deficient family members				Unaffected family members			
	Number tested	Mean	Standard deviation	Range	Number tested	Mean	Standard deviation	Range
Plasma heparin cofactor activity (%)	21	47.6	4.1	42-55	39	99.6	10.3	84-128
Plasma progressive antithrombin activity (%)	12	45.4	7.9	25-54	40	95.2	10.6	75-130
Plasma antifactor Xa activity (%)	13	73.6	8.2	59-88	26	121.3	14.9	99-161
Serum heparin cofactor activity (%)	12	33.4	13.0	9-53	26	59.6	19.2	34-94
Plasma ATIII antigen (%)	21	46.3	7.2	37-56	51	96.3	9.3	79-120

occasionally in young individuals (Marciniak, Fairley and DeSimone, 1974).

PRESENTATION

FIG. 3. Presentation of thrombosis and precipitating factors in patients with documented classical ATIII deficiency. Data derived from Egeberg, 1965; Shapiro *et al.*, 1973; Van der Meer *et al.*, 1973; Marciniak *et al.*, 1974; Gruenberg *et al.*, 1975; Filip *et al.*, 1976; Carvalho *et al.*, 1976; Zucker *et al.*, 1976; Stathakis *et al.*, 1977; Hule, 1977; Odegard *et al.*, 1977; Rubinowitz *et al.*, 1977; Thuot *et al.*, 1977; Bessot *et al.*, 1978; Gyde *et al.*, 1978; Johansson *et al.*, 1978a; Juillet *et al.*, 1978; Matsuo *et al.*, 1979; Rey *et al.*, 1979; Ambruso *et al.*, 1980; Beukes *et al.*, 1980; Boyer *et al.*, 1980; Caille *et al.*, 1980; Hofman *et al.*, 1980; Leone *et al.*, 1980; Pitney *et al.*, 1980; Sas *et al.*, 1980; Schander *et al.*, 1980; Tripodi *et al.*, 1980; Scully *et al.*, 1981; Winter *et al.*, 1982b.

*The precise meaning of thrombophlebitis is not made clear in some reports. Figure reproduced with permission of the Editor of the *Quarterly Journal of Medicine*.

ATIII deficiency may be diagnosed from the analysis of cord blood at birth (Schander *et al.*, 1980) and is therefore present for many years before the onset of clinical thrombosis in most individuals (Fig. 4). Unless fatal, thrombosis settles in affected individuals and recurs intermittently, observations which suggest that a factor additional to the ATIII deficiency is necessary to cause clinical thrombosis. The majority of venous thromboembolic events are precipitated by recognized factors, the most common of which are illustrated in Fig. 3; in addition to these factors thrombosis has been associated with treat-

ment with the oestrogen-containing contraceptive pill (Filip, Eckstein and Veltkamp, 1976), diabetic keto-acidosis (Mackie *et al.*, 1978) and venography (Winter *et al.*, 1981); however a number of venous thromboembolic events have no clear precipitating factor (Fig. 3).

Differences in the degree of predisposition to venous thrombosis in deficient patients are difficult to estimate in view of the intermittent nature of clinical venous thrombosis, our inability to measure venous thrombosis with any accuracy and problems with assessing exposure to known and unknown precipitating factors. Nevertheless, different families and the individuals within a single family are clearly affected clinically to a lesser or greater degree. For example, Marciniak and her colleagues (1974) describe a severely affected family in which severe vascular disease of the lower extremities requiring bilateral amputation in a young man and 2 teenage deaths, apparently from pulmonary embolism, occurred. Two of the families under our care appear to be less severely affected in that no premature deaths related to thrombosis have occurred (Winter *et al.*, 1982e). A few deficient patients reach old age with apparently little disability and have received no anti-thrombotic therapy, and in a very small proportion of deficient individuals venous thrombosis does not seem to have occurred despite the exposure to potent precipitating factors (Winter *et al.*, 1982e). Such differences in the severity of predisposition to thrombosis may be the result of the random occurrence of precipitating and acquired predisposing factors, but in addition the inheritance of a particular constellation of various coagulation, fibrinolytic or endothelial factors is likely to be of importance in determining the severity of thrombotic disease.

A number of factors which might relate to the predisposition to thrombosis in ATIII deficiency have been put forward. The single best documented factor is age, as with thrombosis in unaffected individuals. It is clear that youth confers a relative immunity to the development of thrombosis (Fig. 4). Thrombosis is rare in deficient individuals before the age of 10 years but thereafter becomes increasingly common up to the age of 25 years so that by the age of 50 years less than 10% of patients have remained free of clinical venous thrombosis (Thaler and Lechner, 1981). The search for factors predisposing unaffected individuals to thrombosis has been largely unrewarding (Lowe, 1981); it is possible that in patients already predisposed to venous thrombosis by ATIII deficiency it will be more easy to identify other factors contributing to or reducing the severity of their predisposition to thrombosis. Many different coagulation tests have been performed in various families with ATIII deficiency but very few abnormalities other than the ATIII deficiency have been

FIG. 4. Histogram of age of onset of first clinical thrombotic events in patients with documented classical ATIII deficiency. Data derived from same sources as that of Fig. 3. Reproduced with permission of the Editor of the *Quarterly Journal of Medicine*.

found. Gyde and colleagues (1978) have made the suggestion that hypertriglyceridaemia may represent an additional risk factor for thrombosis in deficient individuals and there is some evidence that the activity of ATIII may be modified by lipoprotein fractions (Winter *et al.*, 1982c). However, study of the ultracentrifugally analysed lipoprotein fractions in the families under our care has not revealed any abnormality in those deficient patients who have suffered with thrombosis (Winter *et al.*, 1982e).

Of the 21 documented deficient individuals in the families under our care, all have developed clinical venous thromboembolism before the age of 40 years with the exception of one woman now aged 69 years. In this patient, one pregnancy has gone to term, a 2nd pregnancy has been terminated by an abdominal operation and an upper lobectomy has been performed for pulmonary tuberculosis. Despite these potent precipitating factors, no clinical thrombosis has occurred. The observation of a reduced level of plasma factor VIII related antigen in this woman led us to consider this and other factors in those deficient subjects who had, and those who had not suffered venous thrombosis. Comparison of these 2 groups has shown in the group without thrombosis a reduction in mean plasma factor VIII related antigen and fibrinogen and an increase in the mean plasma level of alpha 2 macroglobulin, a large molecular weight antiprotease. Plasma levels of alpha 2 macroglobulin decrease (Ganrot and Schersten, 1967) whereas those of fibrinogen and factor VIII increase (Meade and North, 1977) with increasing age; thus the observed differences may be primarily related to the lower mean age of the group without thrombosis. Nevertheless, the finding of a low factor VIII related antigen level in those individuals who have avoided venous thrombosis, suggests that such levels may be

associated with a reduced predisposition to venous thrombosis.

It is not clear if familial ATIII deficiency predisposes to arterial thrombosis in addition to that in the venous system, but, on present evidence, any such predisposition appears to be mild and a strong family history of arterial occlusion is not suggestive of a diagnosis of ATIII deficiency. Hale and colleagues (1981) have briefly described a family with mild ATIII deficiency in which the propositus, a 28-year-old man, had suffered recurrent arterial occlusive events.

A young man from one of our families suffered venous thrombosis in the legs and pulmonary emboli intermittently from the age of 22 years until his death at the age of 30 years. A post-mortem examination revealed widespread severe arteriosclerosis and extensive arterial and venous thrombosis (Winter *et al.*, 1982e). Although the diagnosis of ATIII deficiency in this man was not made, the evidence that he had inherited the deficiency is compelling. It is possible when ATIII deficiency coexists with certain risk factors for arterial disease, that potentiation occurs leading to extensive arterial disease at a young age.

Treatment

Prophylaxis

Successful prophylactic antithrombotic therapy is the primary objective in each deficient patient. In this respect, patient education and co-operation are essential so that compliance with therapy is good and so that stimuli likely to precipitate thrombosis are avoided or managed effectively. Warfarin is the drug of choice for prophylactic therapy. There are numerous anecdotal accounts of its efficacy in deficient patients and only a single report of thrombosis

occurring during therapy (Von Kaulla and Von Kaulla, 1972). We have used warfarin therapy for over 25 patient years in the deficient individuals under our care and no episode of clinical venous thrombosis has occurred during this time. Equally important, no episode of haemorrhage has occurred. Haemorrhage in a deficient individual receiving warfarin has been described only once in a patient with portal venous hypertension (Fiessinger and Aiach, 1980), and it is possible that deficient individuals may be less likely than unaffected individuals to suffer haemorrhagic complications while receiving oral anticoagulant therapy. In addition to the effects on factors II, VII, IX and X and the recently described reduction in protein C levels, there is evidence that therapy with warfarin may result in a small increase in plasma ATIII antigen (Refvem, Fagerhol and Abildgaard, 1973) and in the plasma level of ATIII measured as the ability of plasma to inhibit activated factor X (Wessler *et al.*, 1978). However, evidence suggesting that warfarin therapy does not influence plasma ATIII levels also exists (Bull *et al.*, 1980; Frost and Loveday, 1980). Small increases in plasma ATIII levels have been reported in a few individuals with classical ATIII deficiency following the institution of warfarin therapy (Marciniak *et al.*, 1974; Zucker, Gomperts and Marcus, 1976), but these increases have been small and the highest reported levels have remained without normal range; our own observations and those of other workers suggest that warfarin therapy does not give rise to any significant increase in plasma ATIII levels in patients with classical ATIII deficiency (Leone *et al.*, 1980; Longy *et al.*, 1980; Laharrague *et al.*, 1980; Tripodi *et al.*, 1980; Winter *et al.*, 1982e). Therefore, it is possible to make the diagnosis of classical ATIII deficiency in subjects receiving warfarin therapy.

The use of heparin in patients with ATIII deficiency appears doubly illogical since ATIII is the vehicle through which the anticoagulant (and presumably antithrombotic) properties of heparin are expressed and since therapy with intravenous heparin may result in a significant depression of plasma ATIII levels in normal individuals (Marciniak and Gockerman, 1977). There has been little use of heparin as a prophylactic agent in deficient patients except during pregnancy (*vide infra*).

Stanozolol is an anabolic steroid which gives rise to an increase in plasma ATIII and fibrinolytic activity in normal individuals (Davidson, Lochhead and McDonald, 1972; Walker *et al.*, 1975). Stanozolol in a patient with ATIII deficiency led to increases in plasma fibrinolytic activity and ATIII, and the cessation of previously frequent venous thromboembolic events (Fiessinger and Aiach, 1980). We have employed stanozolol as a prophylactic antithrombotic agent in 3 deficient patients and noted an increase

in plasma ATIII and fibrinolytic activities in all three individuals. Unfortunately, one patient developed spontaneous venous thrombosis within a few weeks of commencing therapy and for this reason we cannot recommend this therapy.

Thus warfarin is the only agent for which there is good evidence of efficacy in the prophylaxis of venous thrombosis in deficient individuals. Difficulties arise in the definition of those patients who will benefit from warfarin therapy. Although no age is free from risk of thrombosis, it is clear that in most patients thromboembolic events begin in the 3rd decade (Fig. 4) and thus prophylactic therapy must begin before this decade if the maximum benefit is to be achieved. Whereas thromboembolic events may be severe and frequent in one family, often families may be relatively spared and thus it appears reasonable for any anticoagulation policy to be influenced by the severity of disease in the family. We have recommended that life-long therapy with warfarin be given to the deficient patients under our care who have either suffered a thromboembolic event or who have attained the age of 20 years without a thromboembolic event, providing that recognized contraindications to therapy with oral anticoagulant drugs are absent.

Precipitating factors and therapy

Pregnancy is the major precipitating factor in females of child-bearing age. In the families under our care 15 out of 16 pregnancies in 7 deficient women were associated with thrombosis, and pregnancy was the precipitating factor of the initial thrombosis in all 6 women who were involved with the 15 pregnancies. Our experience is similar to that seen in other families, for example, thrombosis is described in association with pregnancy and the puerperium in 32 out of 47 Scandinavian pregnancies in deficient individuals (Hellgren, Tengborn and Abildgaard, 1982). It follows therefore that effective prophylactic treatment during pregnancy will be of immense value to such women.

The only substantial data concerning the treatment of pregnancy in deficient individuals is that reported by Hellgren, *et al.* (1982) who treated 9 pregnancies with twice daily subcutaneous heparin injections. The dosage of heparin (20000–45000 units/24 hr) was adjusted to provide a 5–10 second prolongation of the activated partial thromboplastin time (APTT) of a blood sample taken shortly before the next heparin injection was due. Heparin therapy was reduced or stopped and plasma ATIII levels were brought up to normal levels by infusion of ATIII concentrate for the periods of delivery or abortion. Two episodes of thromboembolism were seen in association with the 9 pregnancies which represents a considerable

reduction in incidence in comparison with historical controls. It seems reasonable to recommend this regime for pregnancy in deficient individuals emphasizing strict control of the APTT since the 2 thrombotic events noted above occurred at a time when insufficient prolongation of the APTT was present.

It is advisable for consultation between the deficient female and the physician to take place before pregnancy occurs because venous thrombosis may itself be the first indication of pregnancy in a deficient individual (Zucker, Gomperts and Marcus, 1976) and warfarin therapy during embryogenesis may be teratogenic (Hall, Pauli and Wilson, 1980). Before conception it may be of value to accustom the patient to the twice daily routine of subcutaneous heparin injections and the physician to the control of the prolongation of the APPT produced by such injections, in order to avoid problems with anticoagulation during the early months of pregnancy. It should be remembered that the heparin requirements to maintain the prolongation of the APTT will rise as pregnancy progresses.

Conception, of course, cannot be guaranteed during a particular menstrual cycle, whereas contraception can almost be guaranteed using an oestrogen-containing oral contraceptive. Oestrogen in this form or as postmenopausal replacement therapy should be avoided in deficient patients since oestrogens can cause a depression of plasma ATIII in normal individuals (Fagerhol *et al.*, 1970) and are associated with venous thrombosis in both unaffected and deficient individuals. One family has been described in which venous thrombosis occurred in deficient individuals only when exposed to either oestrogen treatment or to high endogenous oestrogen levels during pregnancy and parturition (Brandt and Stenbjerg, 1979). Preparations containing progestogen alone are less effective contraceptives than those containing oestrogen but do not appear to reduce plasma ATIII levels (Bergsjö, Fagerhol and Abildgaard, 1972).

Surgery is a potent thrombogenic stimulus in deficient subjects and antithrombotic prophylaxis is essential. Ideally, plasma ATIII levels should be increased to normal values by infusion of human ATIII concentrate and subcutaneous heparin therapy used to cover the pre-, per- and postoperative periods. However, human ATIII concentrates have become available only recently and are likely to be in short supply. If concentrates are not available then the infusion of normal plasma will lead to a small increase in plasma ATIII (Zucker *et al.*, 1976) and it is recommended that subcutaneous heparin be used to cover the pre-, per- and postoperative periods with the introduction of warfarin as soon after surgery as reasonable. During periods where other factors likely

to precipitate thrombosis, such as immobilization, infection and trauma, are present, it appears reasonable to recommend therapy with subcutaneous heparin in the short term and warfarin in the longer term. However, it must be stressed that the recommendations regarding prophylaxis in relation to surgery made in this paragraph are based on assumptions and not upon clinical evidence.

Acute thrombosis

The management of acute venous thrombosis in ATIII deficient patients is subject to the same considerations as in unaffected subjects. Therapy may be directed towards a number of goals, namely thrombus dissolution, prevention of thrombus extension, prevention of embolization and prevention of *de novo* thrombosis at another location. A small number of French patients have received therapy with fibrinolytic agents, either urokinase or streptokinase (Bessot *et al.*, 1978; Rey *et al.*, 1979; Caille *et al.*, 1980), which was successful in that the clinical consequences of thrombotic disease improved. Fibrinolytic treatment with these agents is used less often in the U.K. than on the Continent, but the use of such agents for severe thromboembolic events in deficient patients should be considered since the thrombolytic and anticoagulant effects of these drugs do not, as with heparin, depend upon the plasma content of ATIII.

Notwithstanding the drawback of such therapy, heparin has been used for the immediate treatment of acute thrombosis in deficient patients, but a number of patients receiving such therapy in isolation have developed further thrombosis or pulmonary embolism (Marciniack *et al.*, 1974; Gruenberg *et al.*, 1975; Filip *et al.*, 1976; Rey *et al.*, 1979). In the absence of ATIII concentrates, it would be reasonable to use fibrinolytic agents in the event of life-threatening disease. Alternatively, warfarin therapy might be commenced immediately with the intravenous administration of heparin, in dosages sufficient to produce a 1.5–2.5 times prolongation of the activated partial thromboplastin time. Marked heparin resistance may occur with thrombosis of ATIII deficient patients and may be partially treated with infusions of plasma (Penner, 1980). The use of ATIII concentrates may prove to be the treatment of choice in acute venous thrombosis since their infusion will allow full and immediate anticoagulation with heparin. There is insufficient evidence to indicate the optimal therapeutic level to which plasma ATIII should be elevated, but this level is likely to be equal to or in excess of normal plasma levels. Although there is no clear evidence of their efficacy, we have used such infusions in the therapy of acute venous thrombosis in 2 deficient patients and believe that

this therapy contributed to the prevention of extension of thrombosis and clinical pulmonary embolism (Winter *et al.*, 1982d).

Any value of antiplatelet agents in the therapy or prophylaxis of venous thrombosis in deficient patient is, as yet, undefined.

Conclusions

Familial venous thrombosis is met commonly in general medical practice but in only a small proportion of these families can a clearly defined abnormality be found. ATIII deficiency is the commonest known cause of familial thrombosis, but it is likely that as antibody to protein C becomes more widely available, further families with a deficiency of this protein will be found, and the clinical evidence may indicate that protein C is another causal factor in familial thrombosis. Even if protein C deficiency proves to be as common as ATIII deficiency, many thrombophilic families will remain undefined biochemically and should provide a fertile area for the identification of factors of importance in the pathogenesis of venous thrombosis.

It is hoped that advances in therapy and prophylaxis of venous thrombosis in normal individuals will be applicable to individuals with familial venous thrombosis, in whom a causal factor can be identified. However, it may be advisable for patients with a defined abnormality of the coagulation or fibrinolytic systems to receive treatment from physicians with specialized knowledge of these systems, since in certain circumstances, certain avenues of therapy may be inadvisable.

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