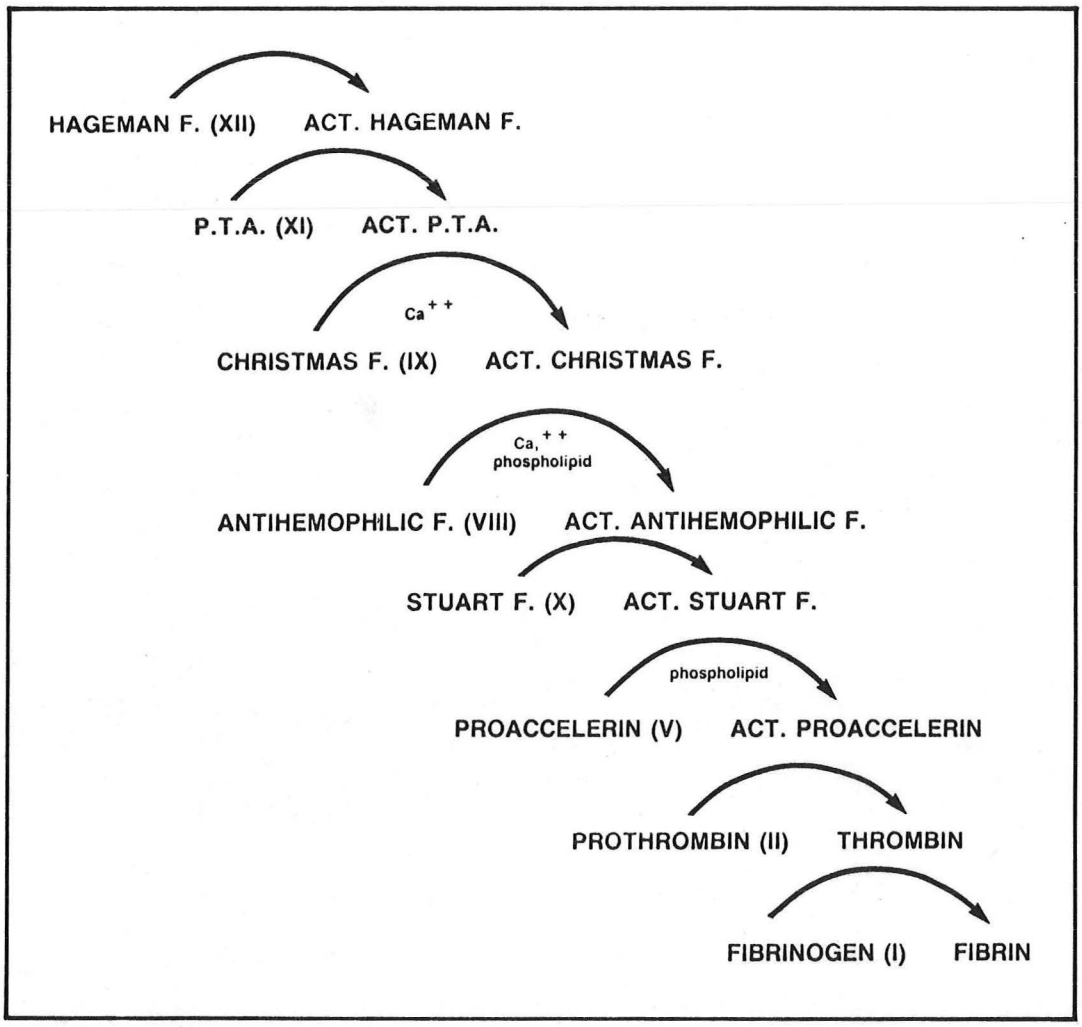
# *NATURE'S ANTICOAGULANTS: CONTROLLING THE CLOTTING CASCADE*

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The most catastrophic disasters in medicine result from the sudden thrombotic occlusion of a blood vessel. The common result is a stroke, a myocardial infarction, a dead leg or a pulmonary infarct. Some people develop thrombi spontaneously without any underlying disease of the blood vessel. Others develop thrombi in the presence of only minimal vascular disease or stasis. And still others are highly resistant to thrombotic occlusion even though they have partial vascular obstruction that persists for many years. What are the factors that dictate how readily each person's blood will clot? Why should some people's blood clot more readily than others?

Answers to these questions are beginning to emerge from new knowledge about natural anticoagulants that circulate in plasma. These circulating proteins inhibit the clotting cascade, thereby preventing the inappropriate formation of thrombi. These natural anticoagulants are absolutely required if errant blood clotting is to be avoided. When these proteins are reduced in activity, either as a result of genetic defects, or as a result of acquired deficiency states, plasma becomes hypercoagulable, and thrombi form spontaneously.

In this Grand Rounds I will discuss some of these newly recognized circulating anticoagulants and I will review the literature describing the clinical consequences of the deficiency states. The audience should realize that this is a rapidly advancing field of research, and that new information is being added every day. Hopefully, when the story is finally assembled, we will understand most of the factors responsible for inappropriate clotting of the blood.

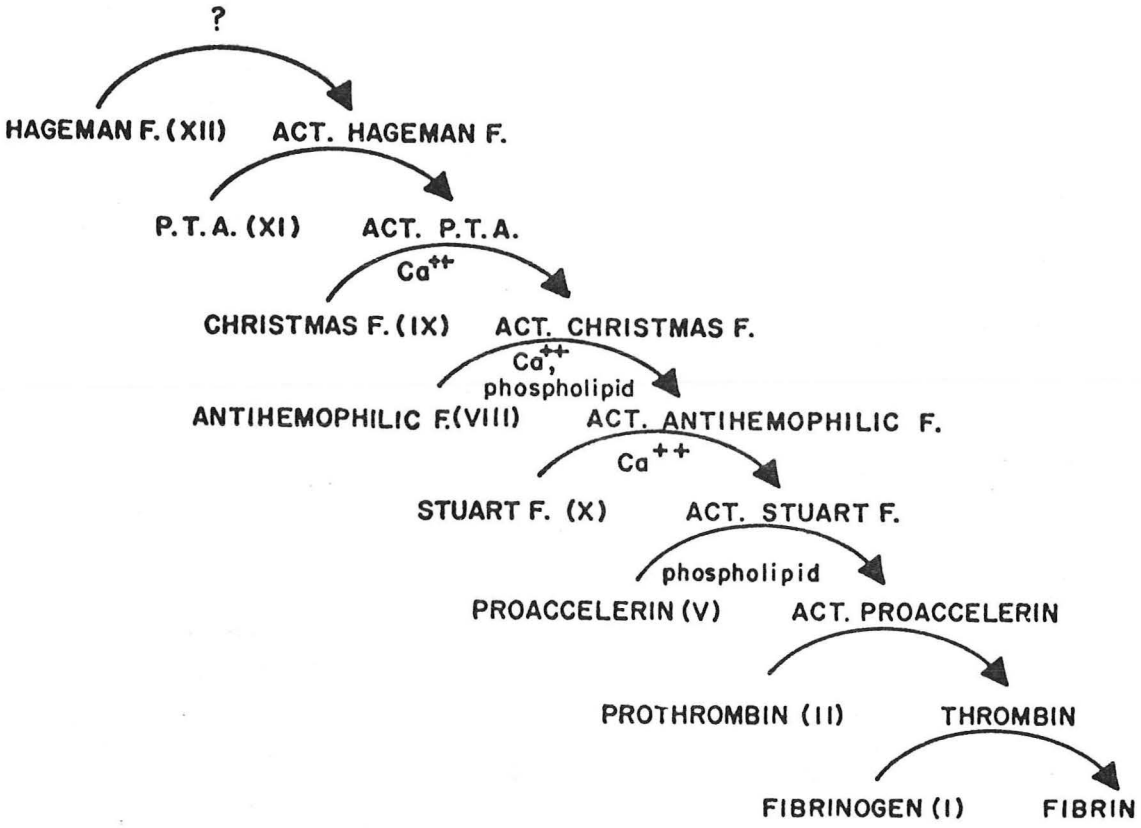
In 1964 Earl Davie and Oscar Ratnoff wrote a classic two-page paper in Science which boiled down 30 years of research on blood clotting (1). They proposed a grand scheme that linked all of the previously described partial reactions in a single chain of events. They called their scheme the "waterfall sequence for intrinsic blood clotting." Their original figure is shown in Fig 1. It is the basis of the pathway that we teach students even today.

The crux of this mechanism lies in the observation that plasma contains a large number of coagulation factors, most of which are proteolytic enzymes that circulate in an inactive form. The intrinsic clotting cascade is triggered when blood comes into contact with a foreign substance, or when endothelium is damaged and blood comes into contact with subendothelial tissues. This opens the floodgate and allows the waterfall to begin. The earliest steps were poorly understood in 1964, and are not perfectly understood even today. Eventually, however, an inactive plasma protease called factor XII is activated to become an activated protease. Activated factor XII cleaves factor XI to convert it into an active protease and so on. Eventually prothrombin is converted to thrombin which cleaves fibrinogen and actually forms the clot. This scheme is not precisely correct in all details, but it has served as the paradigm by which all subsequent blood clotting reactions have been evaluated.

Over the ensuing 20 years since the Davie and Ratnoff paper all of these clotting factors have been purified. In the last few years the amino acid sequences for most of these proteins have been obtained, either from sequencing the protein itself or from sequencing a cloned copy of the messenger RNA.

Fig. 2 summarizes the results of these efforts (2). All of the proteolytic enzymes of the clotting cascade have a common structure. They all contain a common sequence near the carboxyl-terminal end of the protein. This sequence, which is shown in black in Fig. 2, is a serine protease. It is homologous to the proteolytic enzyme trypsin, and it cleaves peptide bonds by a similar chemical

Figure 1. Waterfall sequence for intrinsic blood clotting as proposed by Davie and Ratnoff (Science 145, 1310-1312, 1964).

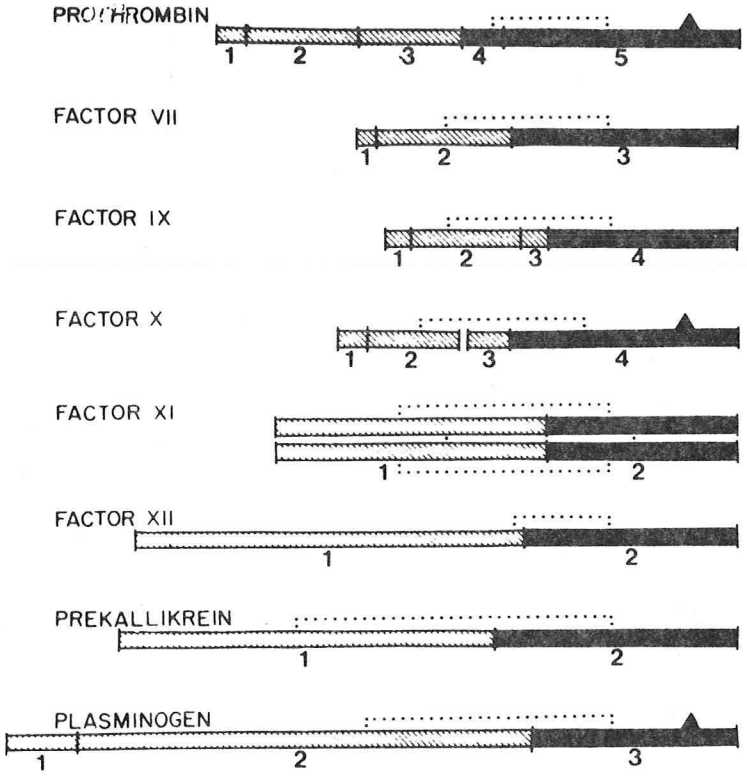



mechanism. The amino-terminal part of each of the clotting proteins differs, and this gives specificity to each protein. When these proteins are secreted into plasma their proteolytic activities are not expressed because the active site is occluded by a segment of the peptide chain. When one of the factors is activated a proteolytic clip is made between the proteolytic domain and the amino-terminal domain. In most cases the two domains remain associated with each other because they are linked by disulfide bonds, which are shown as dashed lines on the slide. However, the proteolytic clip removes a short peptide from the region between the two domains and this unblocks the proteolytic activity.

Certain of the blood clotting factors have an additional feature. They have an unusual modified amino acid near the amino terminus of the protein. The amino acid is called gamma-carboxyglutamic acid, and its structure is shown in Fig. 3.

The amino acid glutamic acid or glutamate is a normal constituent of proteins. Several of the blood clotting factors contain a modification of several glutamate residues near the amino terminus. In this modification an additional carboxyl group is added to the gamma carbon of glutamate. When this happens the glutamate develops a paired negative charges because it has two free carboxyl groups. This structure has a high affinity for binding calcium ions. The calcium ions form a bridge between the gamma carboxyglutamate and phospholipid molecules on the surfaces of platelets. Clotting factors that have gamma carboxyglutamate bind tightly to phospholipids in the presence of calcium.

Figure 2. Schematic structures for the coagulation proteinase zymogens. Each proteinase may be divided into a carboxyl-terminal region of approximately 250 residues which contains the active site (shown in solid black) and an amino-terminal region (shown by the cross-hatched zone) which varies from approximately 150 residues to 582 residues.



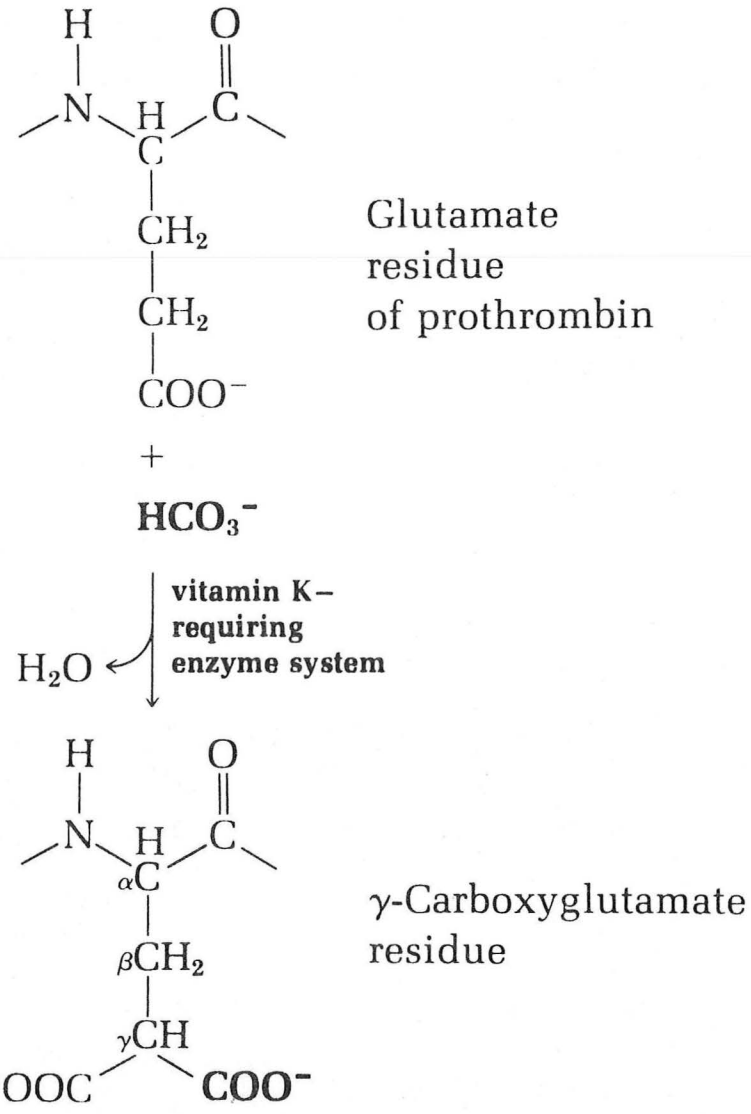

The gamma carboxylation reaction is mediated by an enzyme that uses vitamin K as a cofactor. When vitamin K is diminished, or when vitamin K antagonists such as coumarin are given to patients, this vitamin K-dependent enzyme system does not work. The clotting factors are made without the gamma carboxyglutamate residues and their activity in blood clotting is markedly diminished.

The clotting factors that contain gamma carboxy glutamate residues are factors VII, IX, X and prothrombin.

Fig. 4 shows the modern version of the waterfall proposed by Davie and Ratnoff (3). The intrinsic system of clotting begins when a negatively-charged surface, acting through a complex system involving kallikrein, a circulating protease, leads to a cleavage of factor XII to remove the activation peptide and convert factor XII into an active protease, designated XIIa (2). The activated form of each of the clotting factors is now designated with an "a" following the roman numeral. The activated factor XIIa then activates factor XI which activates factor IX, also known as christmas factor. Factor IX is the first of the vitamin K-dependent gamma glutamylcarboxylated clotting factors. Factor IXa binds to platelet phospholipids in the presence of calcium. The binding also requires the activated form of factor VIII. In the presence of this complex, factor IXa can activate factor X. In the presence of phospholipid and calcium, factor Xa cleaves prothrombin to thrombin. This reaction is accelerated markedly by factor Va. In contrast to the other proteins in the cascade factor VIII and factor V are not

proteases. Rather, they are factors that bind to platelet membranes and accelerate the proteolytic reactions catalyzed by factor IXa and factor Xa, respectively.

Figure 3. The attachment of an additional carboxyl group by a vitamin K-requiring enzyme system converts glutamate to γ-carboxyglutamate in prothrombin, factor VII, factor IX, factor X, protein C and protein S.

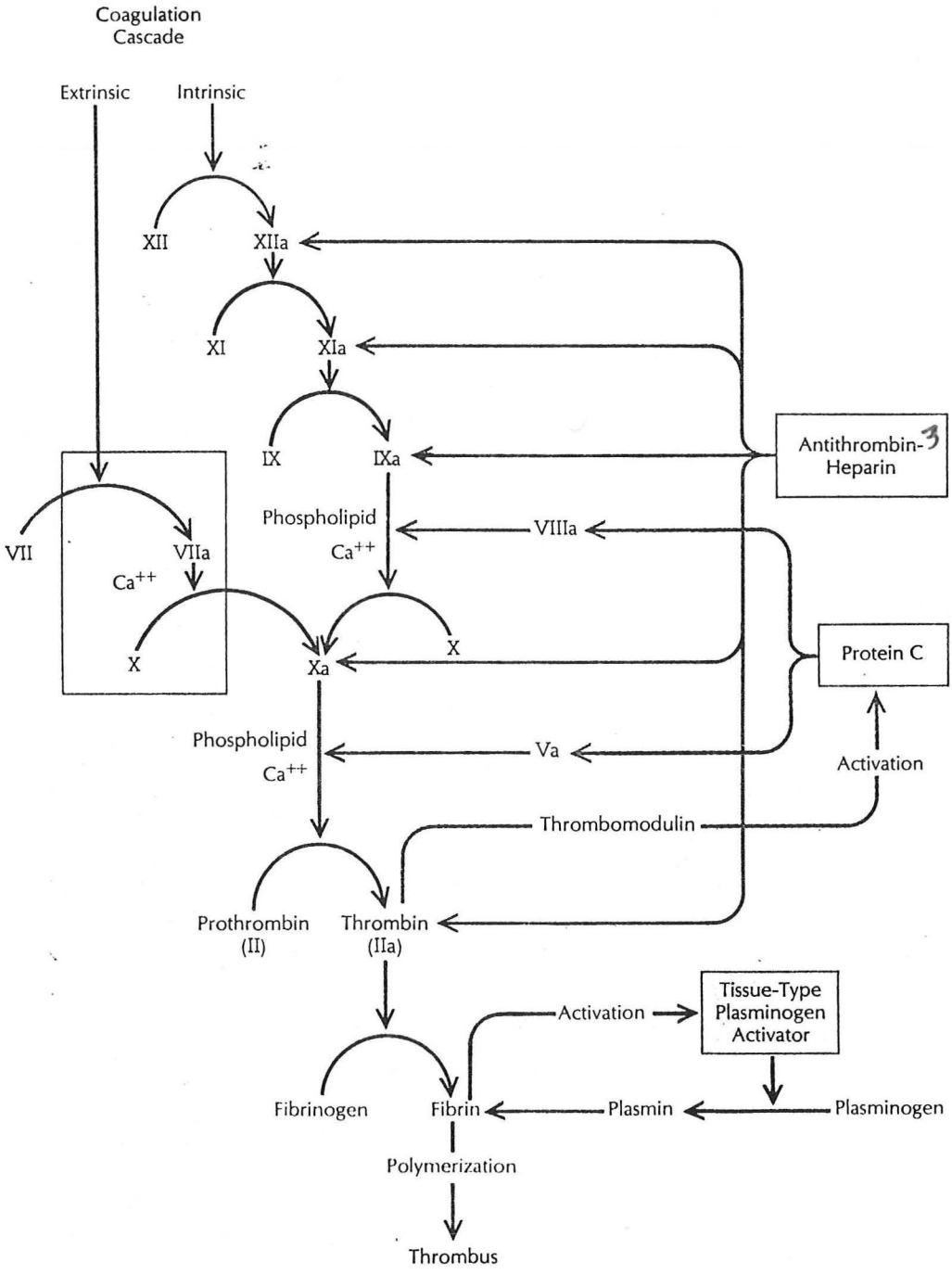



In addition to the intrinsic system for activation of prothrombin there is an extrinsic system that begins when a tissue factor called thromboplastin activates factor VII. Factor VIIa in turn catalyzes the activation of factor X.

Once thrombin is activated, it catalyzes the cleavage of fibrinogen to produce fibrin with the release of a series of fibrin peptides. The fibrin undergoes polymerization and this creates a thrombus.

The problem with a waterfall is that there is no way of stopping it. Once the floodgate is opened by the activation of factor XII or factor VII, blood clotting would continue in a chain reaction and all of the blood in the body would rapidly clot. Clearly, there must be factors that turn off the reaction and localize the thrombotic event to the site of tissue injury.

Figure 4. The coagulation cascade with its known regulatory controls. From Rosenberg and Bauer, Hospital Practice 21, 131-137, 1986.



A great deal of work over the past few years has clearly delineated three of these anticoagulant factors. The major ones are indicated in Fig. 4 (3). They include a protein called anti-thrombin III, which is activated by heparin and which in turn inactivates several elements of the clotting reaction. Another natural anticoagulant is protein C, which inactivates factor VIIIa and factor Va. Finally, there is a protein called tissue type plasminogen activator which activates the proteolytic enzyme plasminogen to form plasmin. Plasmin is not an anticoagulant per se, but rather it acts to dissolve clots after they have been formed.

All three of these proteins, anti-thrombin III, protein C, and tissue type plasminogen activator (TPA) are of intense current interest in medicine. In my Grand Rounds today, I plan to focus primarily on protein C because this is the newest of the factors to have been discovered and because it has wide implications for biology and medicine.

Protein C was actually discovered in 1960 but it went by another name. It was a protein that was extracted from blood clots and purified by a biochemist named Walter Seegers at Wayne State University. Seegers called the protein autoprothrombin-IIa (4). He showed that it could act as an anticoagulant in vitro, but his analysis of the anticoagulant mechanism was incorrect and there was not much interest in the protein throughout the 1960's and early 1970's.

In 1976, however, a major breakthrough occurred (5). A Swedish biochemist named Stenflo, who was one of the discoverers of vitamin K-dependent gammacarboxylation had developed methods to identify all of the gammacarboxylated proteins that were present in plasma. He did this by adsorbing the proteins to barium. The gammacarboxylated proteins bind tightly to barium and are thereby separated from other plasma proteins. With the barium technique Stenflo identified a new protein that contained gamma carboxyl groups and he called it protein C. He determined that protein C had an amino acid sequence that was homologous to the other serine proteases of the blood clotting cascade. However, the protein had no coagulant activity.

The next major steps were made by Earl Davie in Seattle (6), and by Charles Esmon and co-workers (7). These two scientists showed that activated protein C had anticoagulant properties. It prevented the clotting of blood that was induced by stimuli of the intrinsic or extrinsic systems.

Davie and his coworkers identified the sites at which protein C acts (6). The first site was at the level of clotting factor V. Fig. 5 shows the results of these assays. Purified factor V was incubated with increasing concentrations of activated protein C. At the indicated times the reaction was stopped and the mixture was transferred to a complete clotting mixture that lacked only factor V. Increasing amounts of activated protein C rapidly inactivated the factor V protein.

Protein C also inactivates a second factor in the blood clotting cascade, that is, factor VIII. Fig. 6 shows an example of this inactivation (8). Partially purified factor VIII from human plasma was incubated with thrombin. Thrombin cuts factor VIII proteolytically, converting it to its active form, factor VIIIa. At intervals aliquots were removed from the reaction mixture and assayed for factor VIII activity. Thrombin rapidly activated factor VIII. When protein C was added, as indicated by the arrow, the activated factor VIII was rapidly inactivated.

Figure 5. Effect of increasing concentrations of activated protein C on factor V activity. Factor V was incubated at 37°C with increasing concentrations of activated protein C. At the indicated time factor V activity was assayed. Each line represents a progressively increasing concentration of protein C. From Kisiel, et al., Biochemistry 16, 5824-5831, 1977.

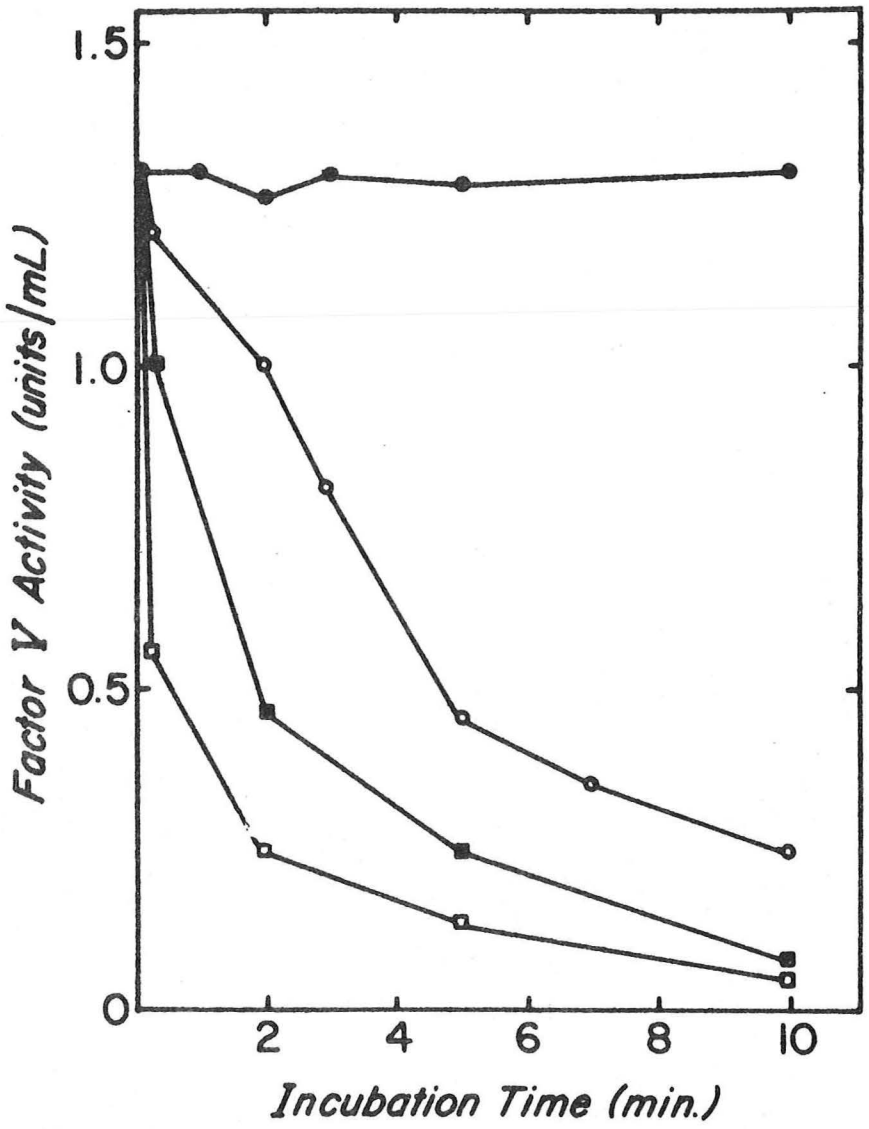
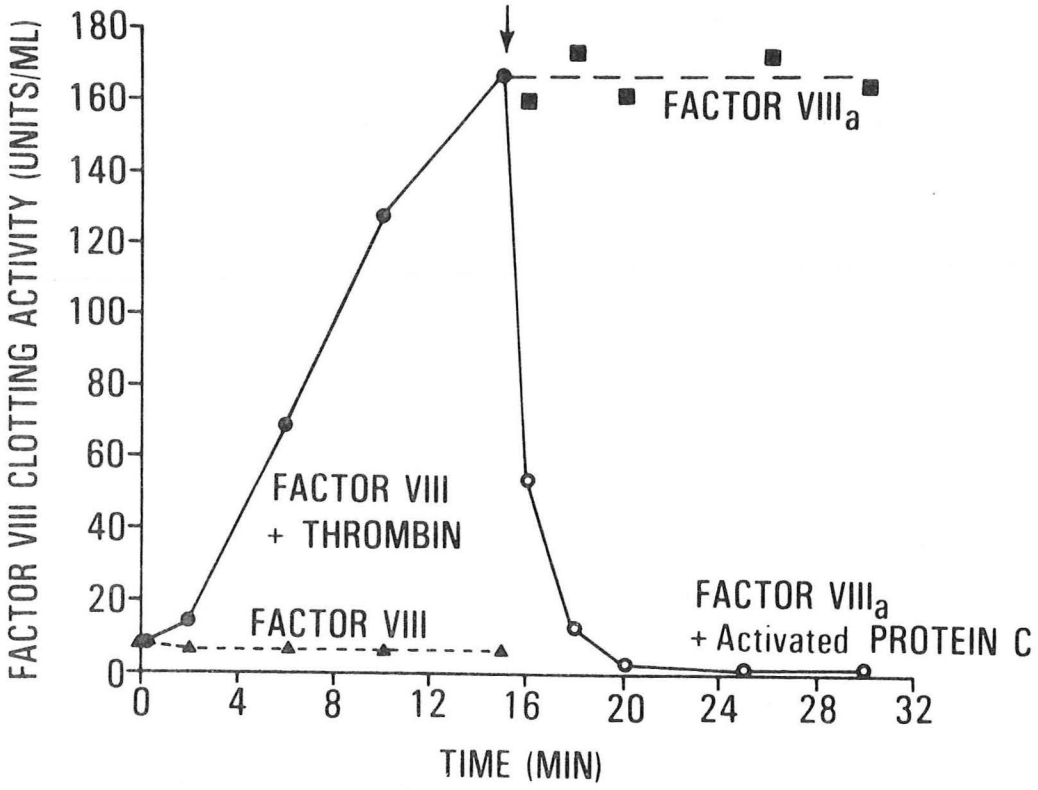



Fig. 7 shows a series of assays of the effect of activated protein C upon all of the known clotting factors in human plasma (8). You can see that protein C specifically inactivates factor Va and factor VIIIa, but it has no effect on the other clotting factors.

Other experiments showed that protein C acts by proteolytically cleaving factors Va and VIIIa (9).

The specificity of protein C for factor V and factor VIII is fascinating because factor V and factor VIII are the only proteins of the clotting cascade that

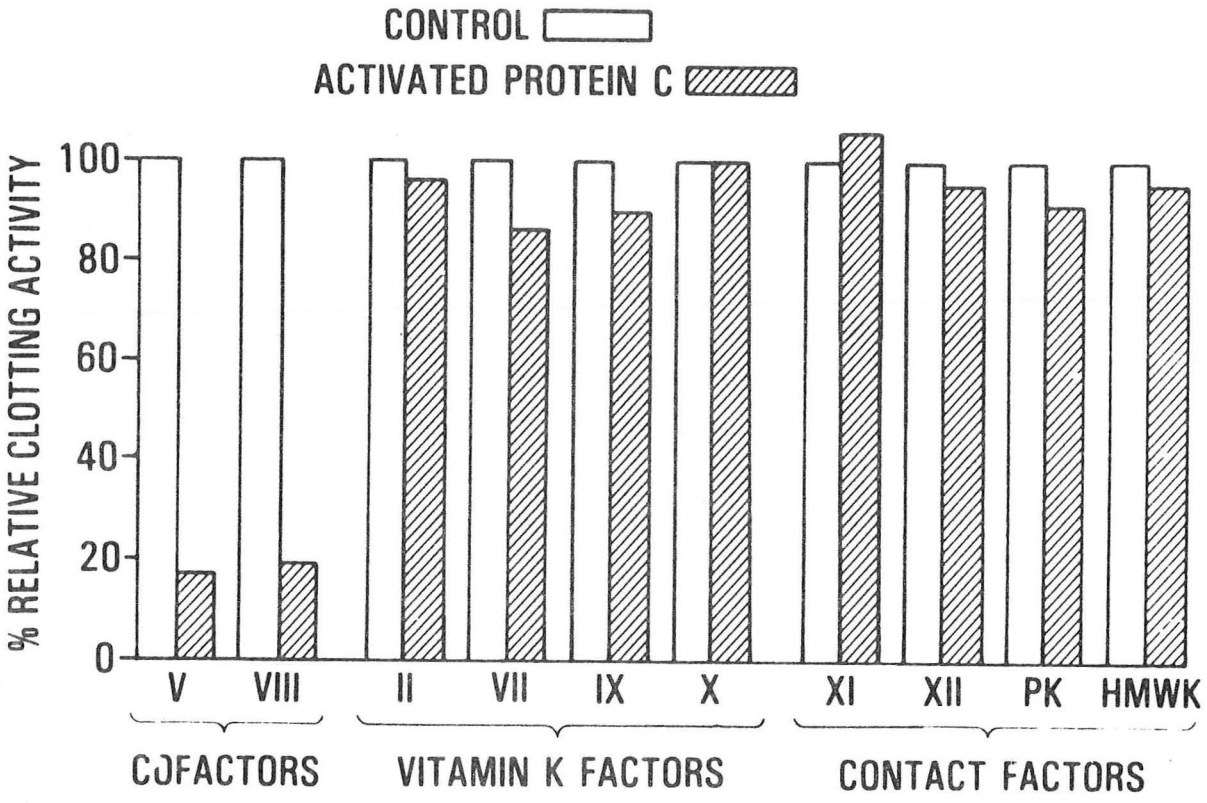
Figure 6. Activation of human factor VIII by thrombin and its inactivation by activated protein C. Partially purified factor VIII was activated with human thrombin. At 15 min (arrow) the thrombin was removed and activated protein C, phospholipid and calcium were added. Controls included unactivated factor VIII plus buffer (dashed line, solid triangles) and thrombin activated factor VIII (dashed line, solid squares). From Marlar, et al., Blood, 59, 1067-1072, 1982.




are not proteolytic enzymes. As shown in Fig. 8, factor Va and factor VIIIa both bind to platelets and act as receptors for other clotting factors. Factor Va serves as the receptor for factor Xa and prothrombin. This binding accelerates the conversion of prothrombin to thrombin by 100,000-fold. Factor VIIIa behaves in a similar way with respect to the activation of factor IX. Therefore, protein C specifically destroys the two clotting factors that act as receptors on the surface of platelets. Interestingly, neither factor VIII nor factor V is a gamma carboxy glutamate containing protein. Thus we have a paradoxical situation in which a gamma carboxy glutamate containing protein (protein C) acts as an anticoagulant, opposing the effects of clotting proteins that lack gamma carboxy glutamic acid (factors V and VIII). This paradoxical situation has important consequences for human disease, as will be discussed later.

The original studies of protein C were performed with material that was isolated from clotted blood. When protein C was isolated from unclotted plasma it had no proteolytic activity. Clearly, protein C had to be activated in order to be effective. But what was the activator? To understand this we must go back to a very old observation in clotting physiology. In the 1950's it was observed that administration of thrombin to experimental animals led to a hemorrhagic state. Why should thrombin, a blood clotting factor, cause a hemorrhagic diathesis? The first idea was that thrombin acts by converting all the fibrinogen to fibrin, thus depleting the blood of fibrinogen. However, this was not always the case. One

Figure 7. Effect of activated protein C on coagulation factors in normal human plasma. Activated protein C was incubated with plasma in the presence of calcium. After 3 min the reaction was stopped by the addition of an antibody to protein C, and the various clotting factor activities were measured. From Marlar, et al., Blood 59, 1067-1072, 1982.

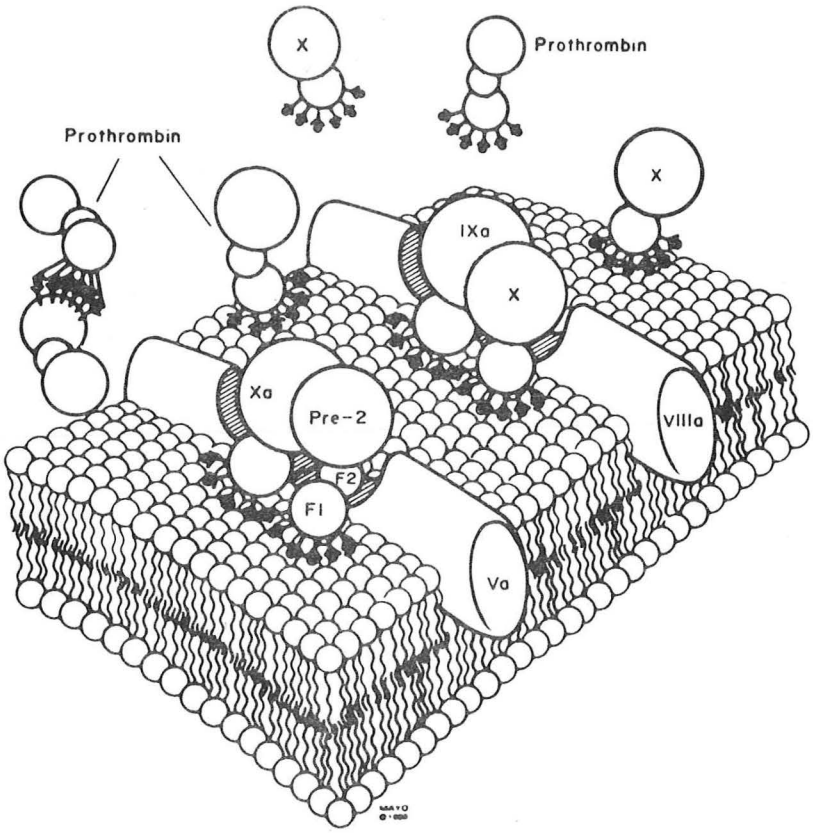



could devise conditions in which the infusion of thrombin led to an anticoagulant state before there was significant consumption of fibrinogen. So there was a suspicion that thrombin had the capacity to activate some unknown anticoagulant protein (10).

When protein C was discovered it was realized that protein C might be the anticoagulant that was activated by thrombin (6,8,9). Fig. 9 shows an example of such activation (9). Purified human protein C was treated with thrombin and digests were removed and tested for protein C activity. The activation is dramatic.

Fig. 10 shows the mechanism by which thrombin activates protein C (6). Purified protein C was incubated with thrombin for various times and aliquots were removed and subjected to electrophoresis in the presence of sodium dodecyl sulfate and a reducing agent that disrupts disulfide bonds. This electrophoresis reveals that protein C is a dimer. It contains two chains, a light chain which runs rapidly on this gel and a heavy chain which runs slowly. The two chains are linked by a disulfide bond which is disrupted by treatment with the reducing agent. When protein C is incubated with thrombin the heavy chain is converted to a form that migrates faster on this polyacrylamide gel. This change indicates that thrombin has proteolytically attacked protein C and has removed a small peptide from the heavy chain. As this small peptide is removed protein C becomes activated. Thrombin has no effect on the light chain.

Figure 8. Factor Va and factor VIIIa both bind to platelet surfaces and serve as attachment sites for other clotting factors.

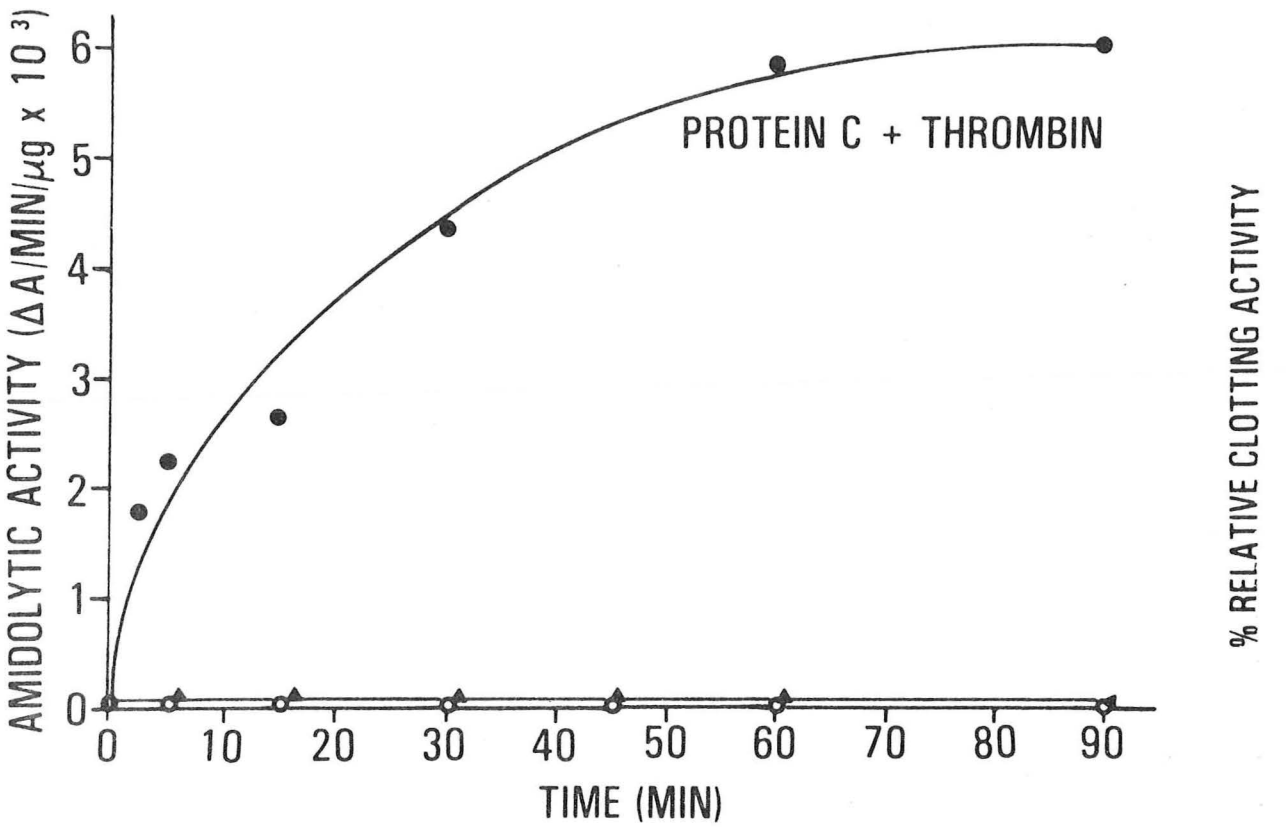



This type of proteolytic activation is strongly reminiscent of the activation of the blood clotting factors of the coagulation cascade. The unusual element about protein C is that it is a dimer. Most of the other blood clotting factors are monomers. When they are activated the protease cuts a piece out of the middle cleaving the proteins into dimers. However, protein C appears to start out life as a dimer, and thrombin only removes a little piece from one chain.

A molecular understanding of this phenomenon emerged recently when the gene for protein C was isolated by molecular cloning (11). The nucleotide sequence of the gene revealed the amino acid sequence of the protein. The results are shown in Fig. 11.

As expected from the previously known properties of the protein, the amino acid sequence of protein C is highly homologous to the other vitamin K-dependent proteins. In Fig. 11 the sequence of protein C is compared with that of factor IX. The gene sequence revealed that protein C is initially synthesized as a single chain protein. It is cleaved proteolytically during secretion to create the heavy and light chains. The light chain of protein C is strongly homologous to the amino-terminal region of factor IX. Both amino-terminal regions contain gamma carboxy glutamic acid. Both of them also contain a sequence that is very rich in cysteine residues. These cysteine residues are bonded together in intrachain disulfide bonds and this creates a highly convoluted structure. A similar cysteine-rich region is present in all of the blood clotting proteases. Interestingly, the same sequence is also present in other proteins such as the precursor for epidermal growth factor, and the receptor for low density lipoprotein. The function of this convoluted cysteine-rich structure is not

Figure 9. Activation of human protein C by thrombin. Protein C activity was measured by its proteolytic activity. From Marlar, et al., Blood 59, 1067-1072, 1982.



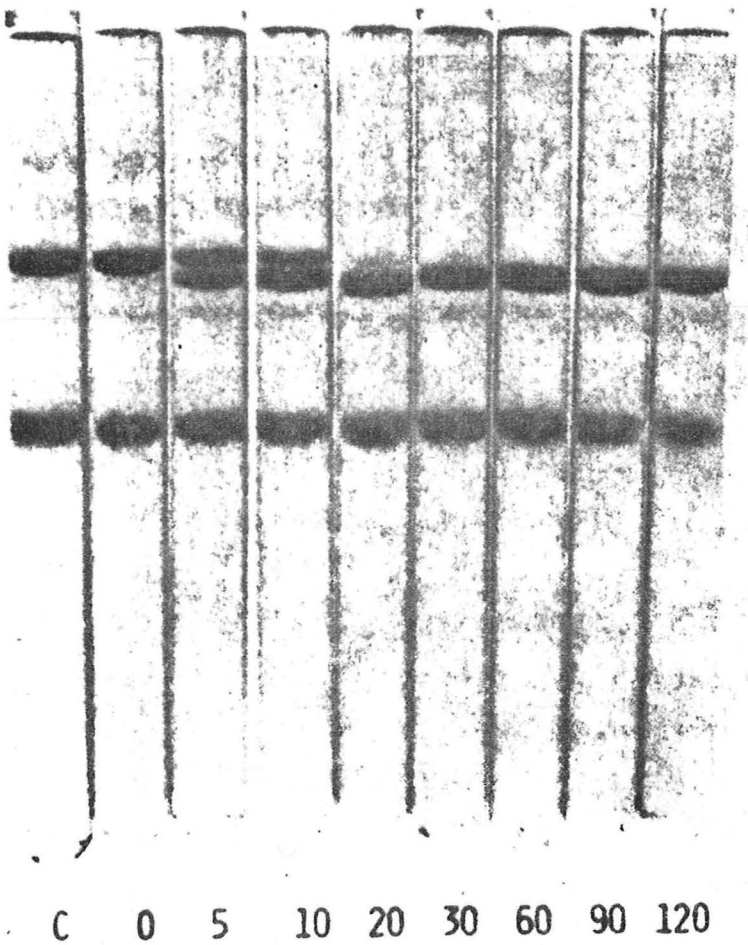

understood in any of these proteins. Amino acid 156 is the site of cleavage of the heavy and light chains during secretion. The two chains are held together by a single disulfide bond. The heavy chain contains the catalytic domain that is homologous to the serine proteases. When the protein is secreted this catalytic domain is inactive. However, thrombin activates it by clipping the heavy chain at the point indicated. This releases a short peptide that previously had blocked the active site. When this peptide is released the heavy chain becomes smaller in weight as shown previously. The release of this peptide exposes the active catalytic site and protein C becomes an active protease that is capable of destroying factors Va and VIIIa and thus acting as an anticoagulant.

It is really quite remarkable that nature has used a single motif for the design of a procoagulant protein such as factor IX and also for an anticoagulant such as protein C. The evolutionary mechanism by which a single ancestor gave rise to a procoagulant and an anticoagulant protein is a fascinating example of evolutionary ingenuity.

When the activation of protein C was recognized in the mid 1970's a major problem remained. Although thrombin could activate protein C in vitro the rate of the reaction was extremely slow. It required 20 to 30 min under the most optimal circumstances. Clearly, if protein C were to function as an anticoagulant in the body there must be some mechanism for enhancing the rate of this activation.

The major breakthrough in this area came from the work of Charles Esmon at the Oklahoma Medical Research Foundation in Tulsa (12). Fig. 12 illustrates Esmon's

Figure 10. Sodium dodecyl sulfate-polyacrylamide gel electrophresis of protein C following activation by thrombin. Protein C was incubated with thrombin and aliquots were removed for electrophoresis at the times (min) shown at the bottom. The heavy chain of protein C was cleaved by thrombin as indicated. From Kisiel, et al., Biochemistry 16, 5824-5831, 1977.




experiment. Esmon cleverly realized that if protein C were to be activated in the body, the activation would likely be mediated by some component on endothelial surfaces. To test this hypothesis he used what is known as a Langendorff heart preparation. An isolated rabbit heart was perfused through the coronary circulation. The apex of the heart was sliced off and the blood emerging from the capillaries was allowed to drip into a beaker. Fractions collected in the beaker were tested for their ability to prevent the coagulation of plasma in the activated partial thromboplasmin time test. When the solution perfused through the coronaries contained thrombin and protein C the eluate markedly prolonged the clotting time, extending it from 23 sec to 60 sec. On the other hand, when the perfusate contained only protein C or only thrombin there was no effect. When thrombin and protein C were mixed with the perfusion solution, (modified Hanks' solution) and incubated in vitro without perfusion through the heart there was no anticoagulant effect. When thrombin alone was put through the perfusion then incubated with protein C there was similarly no effect. When another clotting factor (factor Xa) and protein C were perfused through the myocardium there was no activation of protein C.

Figure 11. Amino acid sequence for human protein C and factor IX. From Foster, et al., Proc. Natl. Acad. Sci. USA 82, 4673-4677, 1985.

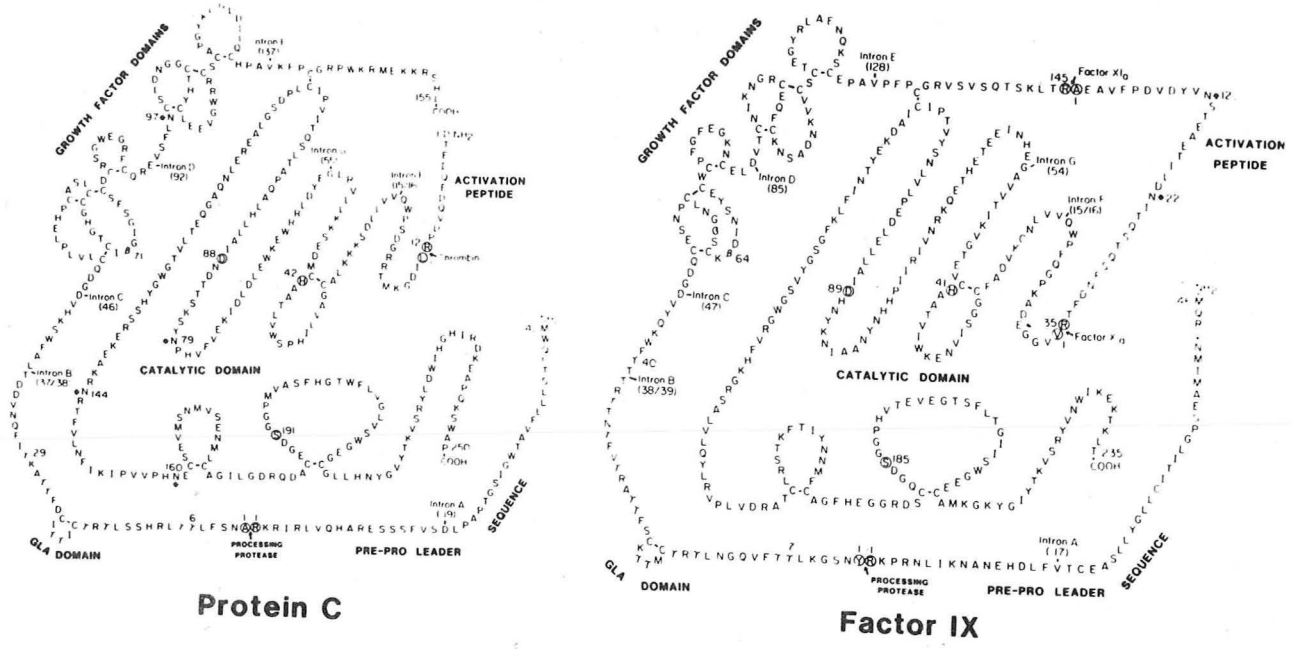



Figure 12. Isolated heart preparations were perfused with a buffered solution containing the indicated ingredients. Fractions were collected and tested for their anticoagulant activity. Asterisk indicates a solution in which thrombin and protein C were mixed but were not used to perfuse the heart. From Esmon and Owen, Proc. Natl. Acad. Sci. 78, 2249-2252, 1981.

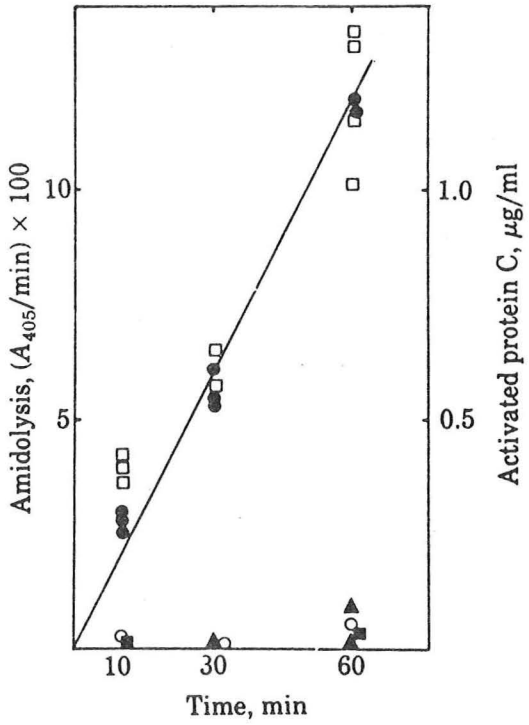
**Table 1. Requirements for generation of anticoagulant activity during perfusion of the isolated heart**

| Perfusion mixture | Clotting time, sec |
|---|---|
| Thrombin + protein C | 60 ± 2.8 |
| Protein C | 23 ± 0.8 |
| Thrombin | 22 ± 1.5 |
| Modified Hanks' solution* | 23 ± 0.6 |
| Thrombin† | 23 ± 0.4 |
| Factor Xa + protein C‡ | 23 ± 0.8 |

Considered together, these data indicated that activation of protein C required perfusion of thrombin together with protein C through an endothelial bed. The speed of this reaction was markedly increased by a perfusion through the capillaries because the capillaries have a very high ratio of surface area to blood volume. The ratio of surface area to blood volume is a thousand times higher in the microcirculation than it is in the circulation of large vessels.

To confirm that the endothelial cells were the source of the activating factor Esmon and coworkers incubated thrombin and protein C in the presence of cultured endothelial cells (12). The results are shown in Fig. 13. When protein C and thrombin were incubated together on top of a confluent monolayer of endothelial cells there was rapid activation of protein C. Two different assays were used.

Figure 13. Enhancement by cultured endothelial cells of thrombin-catalyzed activation of protein C. Monolayers of endothelial cells were incubated with a solution containing protein C and thrombin. The mixture was then removed and assayed for anticoagulant activity (open squares) and protein C-mediated proteolytic activity (closed circles). A second set of cultures received a 175-fold excess of inactivated thrombin (closed triangles). The inactivated thrombin prevented the active thrombin from binding to thrombomodulin and thus prevented the activation of protein C. From Esmon and Owen, Proc. Natl. Acad. Sci. 78, 2249-2252, 1981.




The left scale shows the proteolytic activity of protein C as measured with a specific synthetic substrate. The scale on the right shows the anticoagulant activity of protein C. Both assays showed that protein C was rapidly activated by thrombin in the presence of the cultured endothelial cells. Furthermore, the activation of protein C was prevented when an inactivated form of thrombin was included in the mixture. This was a very important result. Inactivated thrombin does not prevent active thrombin from activating protein slowly in the absence of endothelium. However, inactivated thrombin totally abolished the acceleration of protein C activation that occurred on the endothelial surface.

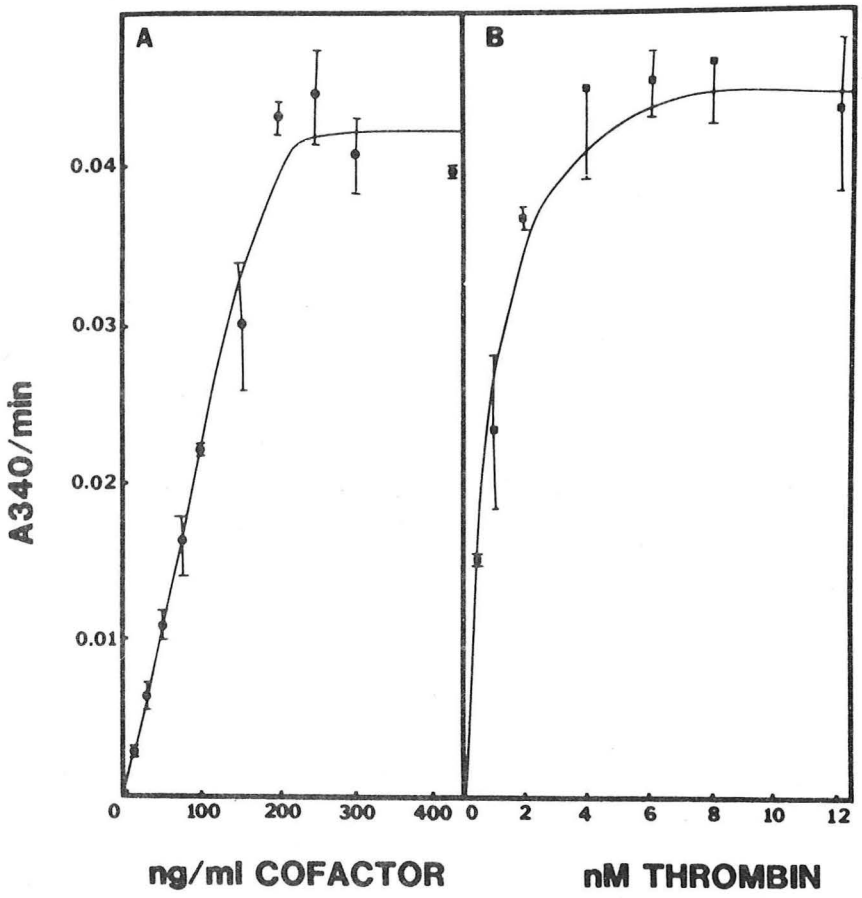
Esmon interpreted these results to indicate that the endothelial cells contain a receptor that binds thrombin and increases its activity in cleaving protein C. Inactivated thrombin competes with the active thrombin for the

receptor, thereby preventing the endothelial cell surface from activating the thrombin.

The existence of a thrombin receptor on endothelial cell surfaces was supported by time sequence experiments (12). If thrombin were perfused through the Langendorf heart first, and then protein C were perfused later, the protein C was still activated. The endothelial cells appeared capable of retaining the thrombin in an active form, apparently because the thrombin was bound to a receptor on endothelium.

Esmon followed up this physiology experiment by turning to biochemistry. He actually isolated the thrombin receptor (13). The results are shown in Fig. 14. To isolate this receptor, Esmon used rabbit lung, which is rich in endothelium. He solubilized the membrane proteins with detergents and passed the mixture over a

Figure 14. Rate of protein C activation with respect to cofactor (thrombomodulin) (left) or thrombin (right). Thrombomodulin was purified from rabbit lung. The activity of protein C was determined by assay of its proteolytic activity. From Esmon, et al., J. Biol. Chem. 257, 859-864, 1982.




Sephadex column that contained inactivated thrombin. The receptor bound to the thrombin and was retained on the column. The receptor was subsequently eluted from the column in an active form and could be used for biochemical assays.

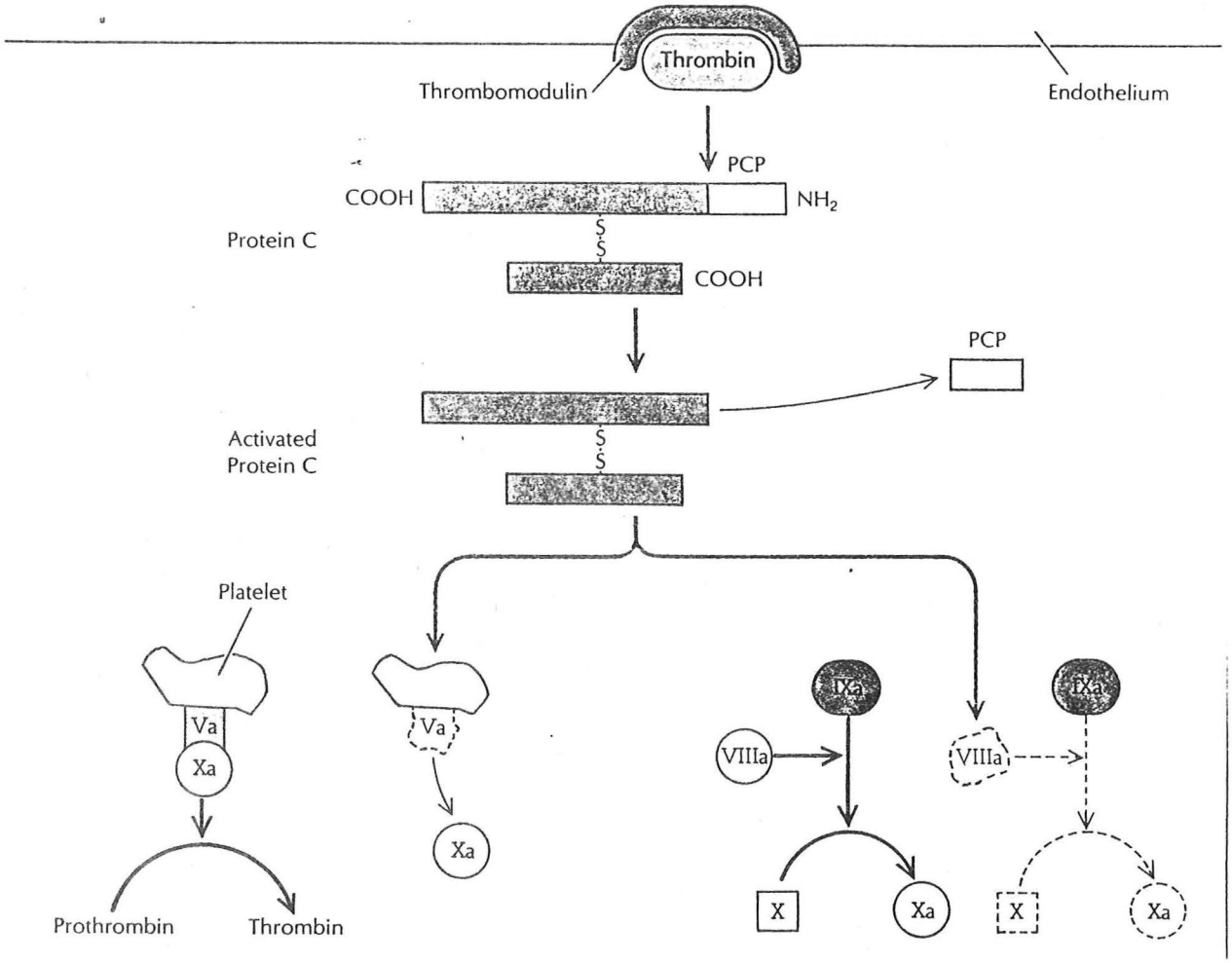
The purified receptor, which is called "cofactor" in Fig. 14, retained its ability to promote the activation of protein C by thrombin even when assayed in detergent solution. The left panel shows the rate of activation by thrombin in the presence of increasing amounts of cofactor. With no cofactor added the activation is extremely slow. With cofactor added the rate increases many many fold. The

right panel shows that even though the cofactor is present the activation is strongly dependent on thrombin. So activation of protein C requires the endothelial cofactor, as well as thrombin.

The purified cofactor has a molecular weight of 74,000 as determined by SDS polyacrylamide gels (13). Interestingly, the cofactor requires detergents to remain in solution, indicating that it is an intrinsic membrane protein. Moreover, the cofactor is extremely stable. It can be boiled in sodium dodecyl sulfate and 8 M urea as long as its disulfide bonds remain intact.

The cofactor that was isolated by Esmon was named thrombomodulin. Fig. 15 summarizes the current concepts of the mechanism by which protein C, thrombin and

Figure 15. Activation of protein C by thrombin bound to thrombomodulin. From Rosenberg and Bauer, Hospital Practice 21, 131-137, 1986.



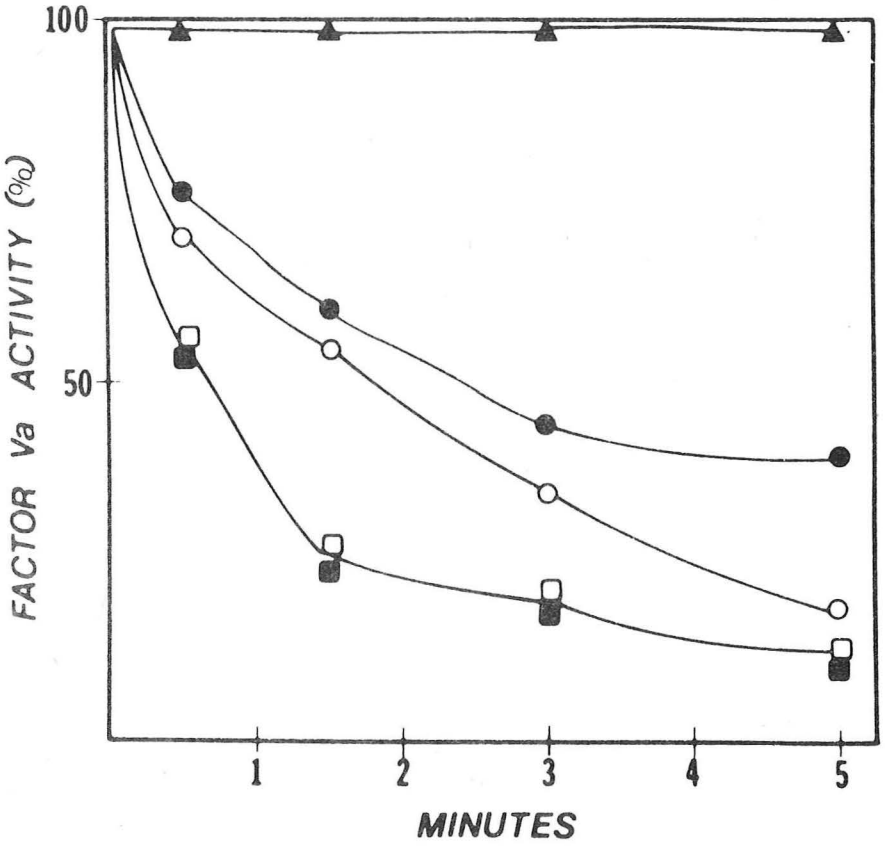

thrombomodulin interact (3). Thrombin binds to thrombomodulin on the surface of endothelium. The bound thrombin can no longer cleave fibrinogen, and hence it is inactive in promoting clotting. However, the bound thrombin is activated tremendously with respect to its ability to cleave protein C. Protein C is cleaved, releasing the short activation peptide and activating the proteolytic domain. The activated protein C now degrades factor Va and factor VIIIa, thus halting coagulation that is mediated by either the intrinsic or extrinsic coagulation systems.

The binding of thrombin to thrombomodulin creates a powerful control mechanism (9). When endothelium is damaged thrombin is activated locally and this

creates a thrombus. At the edge of the damaged endothelium excess thrombin binds to thrombomodulin that is present in the normal endothelium. The bound thrombin activates protein C and prevents propagation of the clot beyond the area of endothelial damage. This system is fiendishly cleaver, but it is also very tricky. It depends on a precise balance between the amounts of thrombomodulin, thrombin and protein C. Such a delicately balanced system might easily go wrong, and it frequently does. We will have more to say about this in a moment.

Before we can consider the clinical aspects of protein C deficiency we must first discuss an additional activator called protein S. Protein S is a circulating protein that acts synergistically with protein C to destroy factor Va and factor VIIIa. Protein S is another of the gamma carboxyglutamate containing proteins of plasma. Like the others, it was purified by Stenflo. Its activity however was not known until recently when it was observed that protein S accelerates the rate at which protein C inactivates factor Va (14,15). Fig. 16 shows the results of such

Figure 16. Effect of purified protein S on the rate of inactivation of Factor Va by activated protein C. The reaction mixture contained protein C with no protein S (closed circles) or increasing amounts of protein S. The top line (closed triangles) indicates protein S alone without protein C. From Walker, J. Biol. Chem. 255, 5521-5524, 1980.

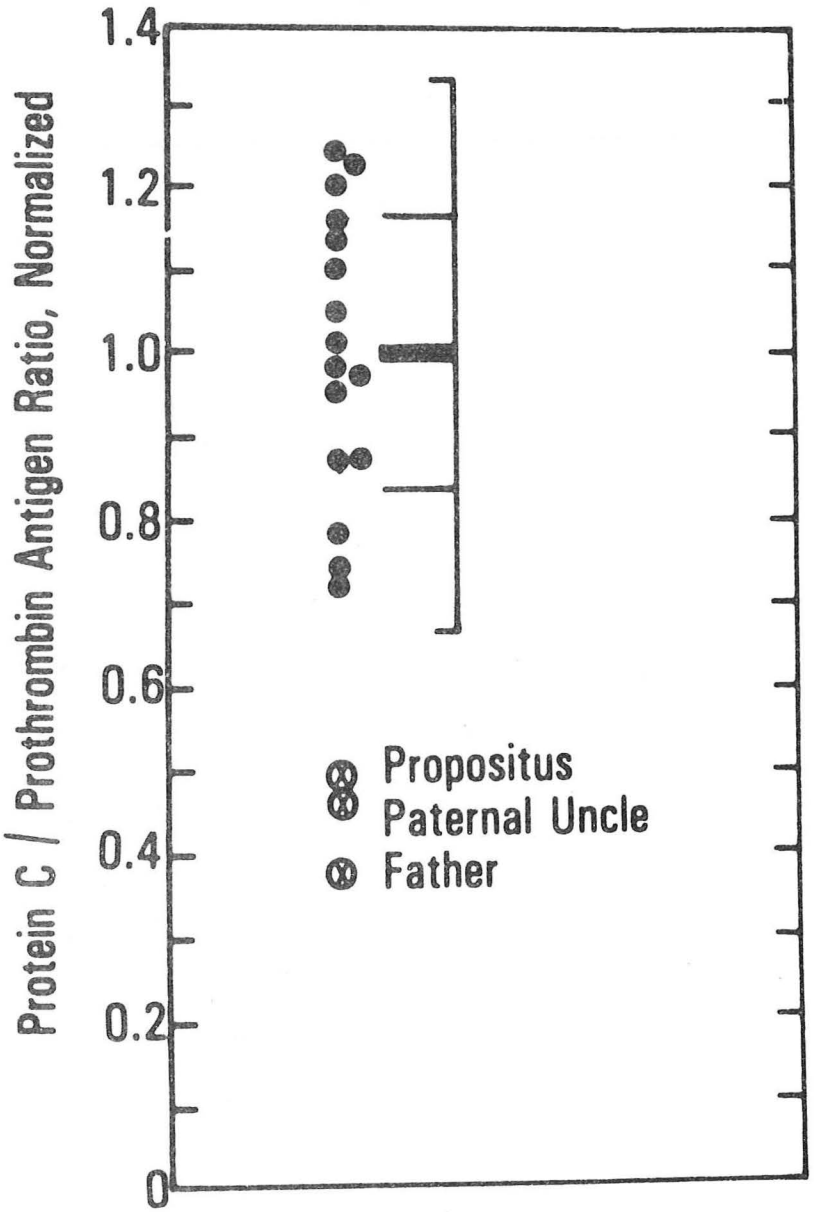



an assay (14). Increasing amounts of protein S increase the rate at which protein C inactivates factor Va. The fact that this inactivation requires both protein C and protein S creates a situation in which a genetic deficiency of either protein C or protein S might produce thrombotic disease.

Let us now turn to a discussion of the clinical relevance of all of this basic science. The protein C/protein S system was considered an elegant laboratory

curiosity until 1981 when a family was described with an inherited deficiency of protein C (16). Fig. 17 shows the amount of protein C antigen in members of that family as measured with an immunoassay. The propositus was a young caucasian man

Figure 17. Protein C antigen levels in plasmas from 3 affected members of a family with recurrent thrombotic disease. The values are expressed as a ratio of protein C antigen versus prothrombin antigen. The control subjects (closed circles) were all individuals who were taking a coumarin anticoagulant. The propositus, uncle, and father also were taking coumarin. A similar depletion was demonstrated in the propositus when he was not taking coumarin therapy. From Griffin, et al., J. Clin. Invest. 68, 1370-1373, 1981.




with recurrent thrombophlebitis. At age 17 he developed spontaneous thrombophlebitis with pulmonary emboli. At age 19 he developed recurrent thrombophlebitis after minor trauma to his leg. Subsequently he was maintained on coumadin without recurrence.

The patient's father developed spontaneous bilateral thrombophlebitis with multiple pulmonary emboli at age 24, requiring bilateral femoral vein ligation. At age 43 he had a cerebrovascular accident. At age 45 he had a myocardial infarction. After starting coumadin therapy at age 45 he became asymptomatic for the next ten years.

The paternal uncle, i.e., the father's brother, developed acute thrombophlebitis at age 20. He required multiple hospitalizations for recurrent pulmonary emboli. He also became asymptomatic after starting coumadin therapy. The paternal grandfather, i.e., the father of the father and uncle, died suddenly at age 45. He had sustained a leg injury in a fall from a horse and while he was confined to bed pulmonary infiltrates developed. His father in turn had died suddenly of a cerebrovascular accident at age 61. The maternal side of the family was negative for thrombotic disease.

As shown in Fig. 17 the propositus, his father and his paternal uncle all had protein C activities that were about 50% of normal (16). All three were taking coumadin at the time of the study. Since protein C is a vitamin K-dependent protein, coumadin lowers the activity. Therefore, the levels of protein C in the patients were compared with 16 patients from other families who were also taking coumadin. To control for variability in coumadin effect, the protein C levels were expressed in relation to the activity of prothrombin, another vitamin K-dependent protein. When related to prothrombin activity, which is a marker for the coumadin effect, the three family members had protein C levels that were about 50% of normal. A similar 50% reduction in protein C antigen was observed in the propositus at a time when he was not taking coumadin therapy, thus validating this assay. All other clotting factors were normal in these patients. In particular, antithrombin III and fibrinogen appeared to be normal and plasminogen levels were also normal.

After the original report of this family Broekmanns and coworkers described three additional families with protein C deficiency and spontaneous venous thromboembolism (17). Fig. 18 summarizes the incidence of thrombophlebitis in family members from the three families. Affected heterozygotes had superficial thrombophlebitis, deep vein thrombophlebitis, or pulmonary embolism with age of onset usually at about age 20. Fig. 19 shows the inheritance of protein C deficiency in one of the families. In this family 10 members were documented to have protein C deficiency by immunoassay. Six of the 10 had clear evidence of thromboembolic disease. Four of the 10 were asymptomatic. Clear transmission through three generations was present. In all, among the three families 18 patients were identified as having an isolated protein deficiency and 14 out of the 18 had a history of venous thromboembolism. It is striking to note that among the 18 affected members of these three families there was no occurrence of arterial thrombosis or myocardial infarction.

All of the patients described above were believed to be heterozygotes and the disease was transmitted as an autosomal domainant trait. An apparently recessive form of protein C deficiency has also been described with severe expression in the homozygous state.

The most impressive family with homozygous protein C deficiency is the one described by Seligsohn and coworkers in the N. Engl. J. Med. in 1984 (18). Fig. 20 shows the pedigree of this family. The propositus was a female infant who was born to parents who were first cousins. The infant developed hematuria several hours after birth at which time bilateral abdominal masses were palpated. A diagnosis of bilateral renal vein thrombosis was made by means of ultrasound examination. The platelet count was 130,000 per cubic millimeter the partial thromboplasmin time was normal and the prothrombin time was slightly prolonged at 14.8 sec. The fibrinogen

Figure 18. Thrombophlebitis in patients from three families with autosomal dominant forms of protein C deficiency. From Broekmans, et al., N. Engl. J. Med. 309, 340-344, 1983.

Table 1. Clinical Manifestations of Thrombotic Disease in Families 1, 2, and 3.*

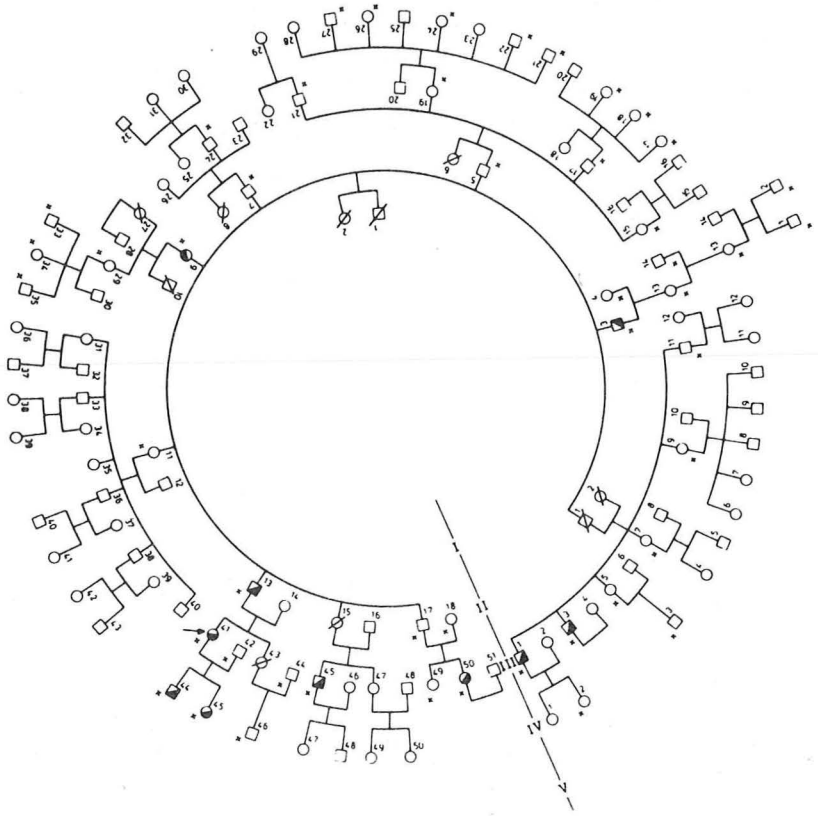
| Pedigree No. | STP | DVT | PE | Age at Onset (yr) | Precipitating Condition |
|---|---|---|---|---|---|
| Family 1 | | | | | |
| II-3 | ++ | + | + | 40 | Spontaneous |
| II-9 | ++ | − | + | 33 | Spontaneous |
| II-13 | + | ++ | + | 33 | Surgery |
| III-3 | ++ | − | − | 54 | Spontaneous |
| III-13 | − | + | + | 46 | Surgery |
| III-41 † | ++ | + | + | 23 | Pregnancy |
| III-50 | + | − | − | 19 | Intravenous injection |
| IV-13 | − | + | + | 23 | Pregnancy |
| Family 2 | | | | | |
| IV-3 | + | − | − | 28 | Spontaneous |
| IV-7 | ++ | − | − | 19 | Spontaneous |
| IV-10 | ++ | + | − | 19 | Spontaneous |
| IV-13 † | ++ | + | − | 24 | Spontaneous |
| Family 3 | | | | | |
| I-2 | + | + | − | 82 | Spontaneous |
| III-2 † | − | + | + | 20 | Spontaneous |
| III-3 | ++ | ++ | − | 16 | Trauma |
| III-5 | ++ | − | − | 18 | Spontaneous |

level was normal. Protein C antigen was nearly undetectable. The infant lived for only 34 days and died as a result of renal failure and bilateral chylothorax. Postmortum examination revealed extensive thrombosis of the inferior vena cava, both renal veins and both ileac veins. Multiple pulmonary emboli and hemorrhagic pulmonary infarcts were also observed. Partly organized thrombi were noted in the epicardial fat tissue and the sublobular veins of the liver. Platelet-fibrin thrombi were found in small blood vessels of the adrenal cortexes. No thrombi were noted in the major systemic arteries. The patient had had a sister (IV-4) who was born 4 years earlier. At 14 days of age the infant was hospitalized with convulsions. She died at 54 days of age. Postmortum examination revealed bilateral renal vein thrombosis and small infarcts of the frontal lobe of the brain.

Two other siblings of the propositus had died in the neonatal period. One died at the age of 10 days. The autopsy report indicated intracerebral hemmorhage and massive bilateral pararenal hemmorhage. Thromboses were not mentioned. Another female infant died on the 42nd postnatal day. The autopsy was uninformative.

The levels of protein C antigen were measured in many of the family members and the results are shown on this pedigree (Fig. 20). The half-filled symbols indicate individuals who have a partial deficiency of protein C averaging about 50% of normal. Both parents had a partial deficiency of protein C. The parents were 33 and 37 years old. They had no symptoms or signs of thrombosis or embolization. The maternal grandfather (II-3) died of a myocardial infarction at age 54. He had

Figure 19. Pedigree of a family with the autosomal dominant form of protein C deficiency. Half-filled symbols denote protein C deficiency. The X denotes those individuals who were investigated. From Broekmans, et al., N. Engl. J. Med. 309, 340-344, 1983.



hypertension and obesity. A second cousin of the parents (IV-20) had died of massive pulmonary embolism at age 20. Thus, of all of the putative heterozygotes in this family only one had myocardial infarction, and one had early onset pulmonary emboli.

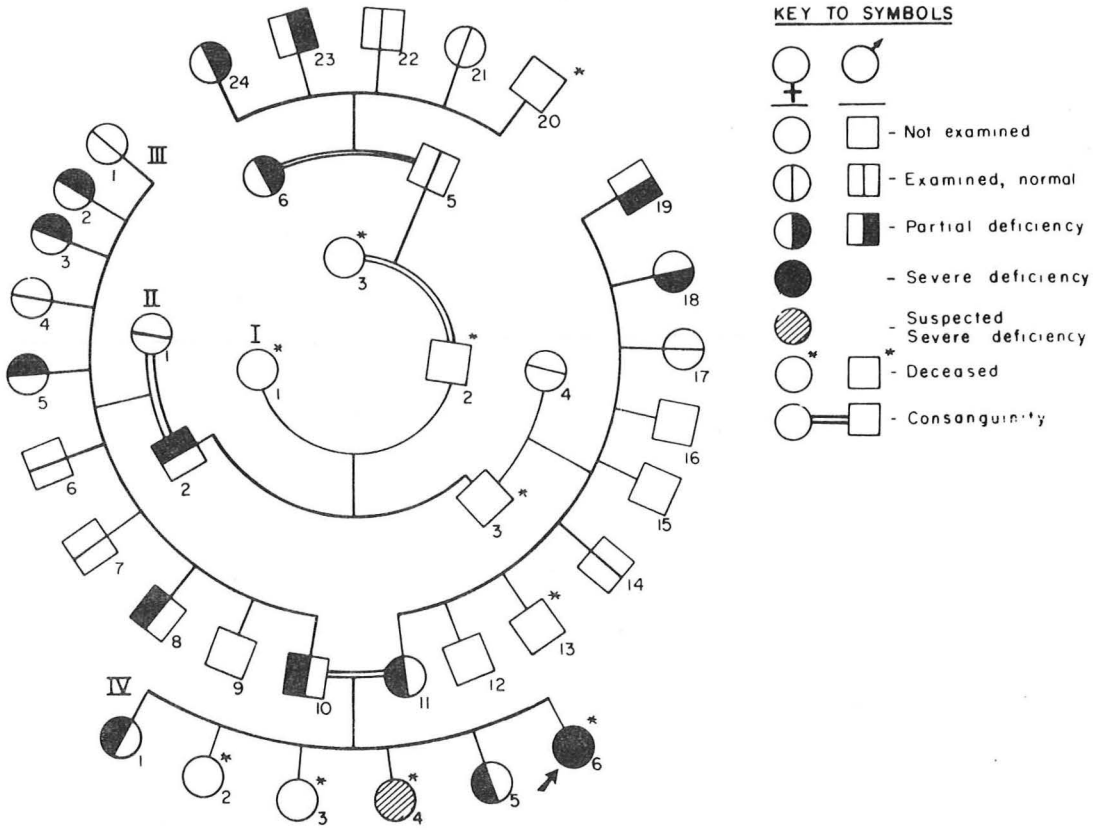
Fig. 21 shows the method used to measure protein C antigen levels in these patients (18). The technique is called Laurell rocket electrophoresis. The patient's sample is placed in a well and subjected to electrophoresis through a gel containing antibody. The height of the precipitin band corresponds to the amount of protein C antigen. The propositus has a marked deficiency, and the two parents have a moderate deficiency as compared with the pooled normal plasma shown in various dilutions on the right.

Fig. 22 summarizes the results of the protein C antigen measurements on the family. Half of the relatives were partially deficient and half were normal.

This pedigree is consistent with a co-dominant inheritance pattern. People with a 50% deficiency of protein C seem relatively asymptomatic, but the homozgotes, who have a complete deficiency of protein C, die in infancy with massive venous thromboses and some hemorrhagic symptoms.

It is not clear why the heterozygous relatives in the recessive family of Fig. 22 had so little evidence of thrombosis whereas the heterozygotes from the dominant pedigrees were symptomatic. It may well be that the mutation that produces

Figure 20. Pedigree of a family with a recessive form of protein C deficiency. Subjects whose protein C level was more than 2 sd below the normal mean were defined as partially deficient. From Seligsohn, et al., N. Engl. J. Med. 310, 559-562, 1984.




symptomatic protein C deficiency in heterozygotes is a different mutation than the one that is present in the recessive family. Now that the protein C gene has been cloned it should be possible to understand the mutations in these diseases so as to get a better idea of whether the apparent recessive and dominant forms of this disease are caused by different mutations.

In addition to a deficiency of protein C, there are also genetic defects in protein S, the protein that synergizes with protein C to inactivate factors V and VIII. Fig. 23 shows the pedigree of the first family to be described (19). The proband is a young white male. At age 14 he developed deep vein thrombosis in his left leg. At age 15 he had multiple pulmonary emboli which recurred at age 16. The patient's brother developed thrombophlebitis of the left leg following a motorcycle injury at age 15. He developed thrombosis of the right leg at age 26. In both patients all clotting tests were normal. The level of protein C antigen and the functional activity of protein C were also normal. However, both patients had a severe deficiency of protein S as determined by immunoelectrophoresis. The amount of protein S antigen was less than 5% of normal. Two siblings of these patients had normal levels of protein S antigen. However, both parents had diminished levels, consistent with heterozygosity.

These findings suggest that complete protein S deficiency is much less devastating than complete protein C deficiency. It presents in the late teens with thrombophlebitis. Subsequent to this initial report, Comp and Esmon did a

Figure 21. Immunoassay of protein C in a patient with the homozygous form of protein C deficiency and his parents. The patient is indicated by "P". The father and mother ("F" and "M") had half-normal activities of protein C. The other samples represent progressive dilutions of normal pooled plasma. The sample labeled "100" represents undiluted normal plasma and is comparable to the sample used for the patient and his parents. From Seligsohn, et al., N. Engl. J. Med. 310, 559-562, 1984.

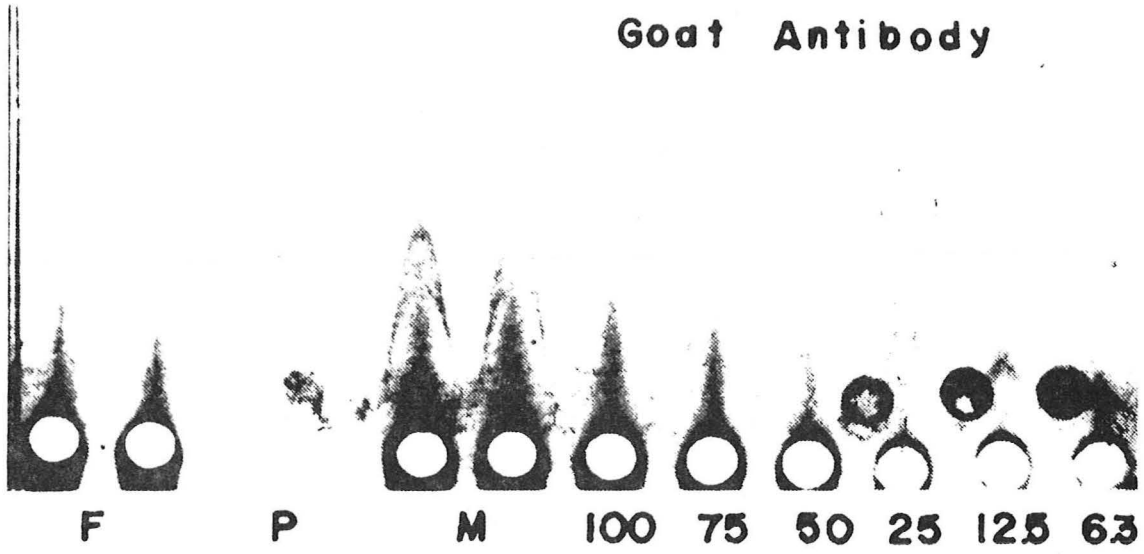



Figure 2. Electroimmunoassay of Protein C Measured with a Goat Antibody.

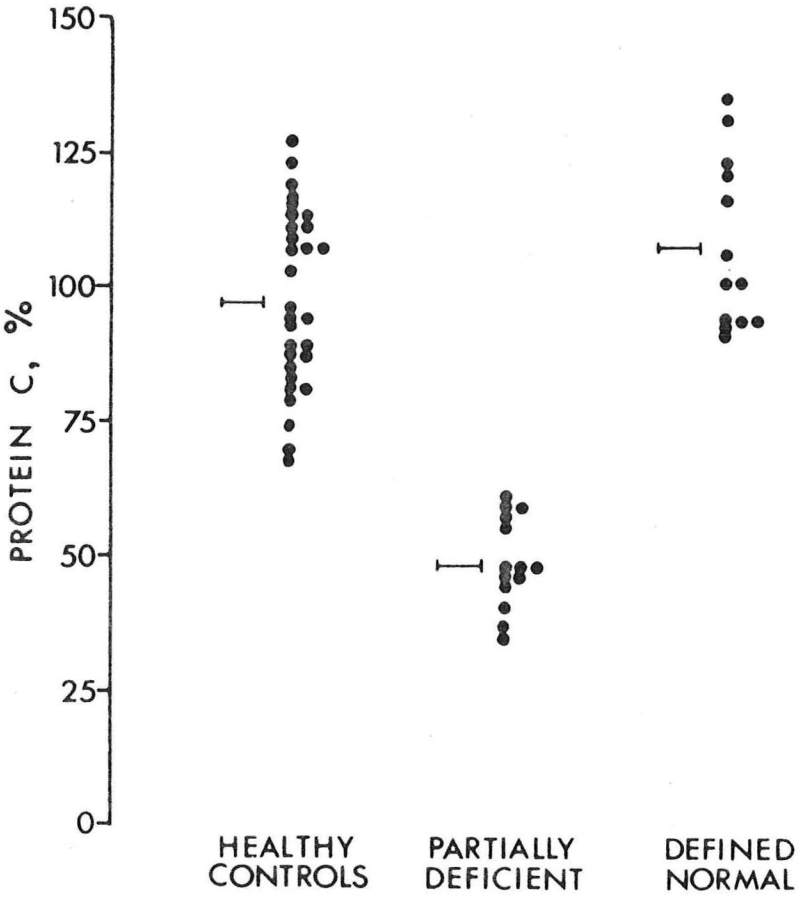
systematic survey of 120 patients who had recurrent venous thrombosis and pulmonary emboli (20). The results are shown in Fig. 24. Of 120 patients whose plasma samples were assayed, six had a substantial decrease in protein S ranging from 15 to 37%. All of the patients had normal levels of protein C, plasminogen and antithrombin III. Four of the six patients had family histories of recurrent venous thrombosis. Plasma samples were obtained from members of two of the families, and the results are shown in Fig. 25. The father of patient 6 had markedly reduced levels of protein S and had experienced at least 6 episodes of documented deep vein thrombosis. The brother of patient 6 also had recurrent episodes of venous thrombosis. In the family of patient 4 the mother was asymptomatic even though she has a markedly reduced level of protein S. However, the patient's maternal grandmother died of recurrent pulmonary emboli.

These data indicate that a familial deficiency of protein S may be relatively common in patients with early onset deep vein thrombosis. Moreover, they indicate that even a relatively mild protein S deficiency with activities in the range of 30% of normal can predispose to venous thrombosis and pulmonary embolism.

In addition to inherited deficiencies of the natural anticoagulants there are also acquired deficiencies, and these may be of even more significance for human disease. So far, all of the information on acquired deficiencies relates to protein C.

One striking example of the clinical consequence of acquired protein C deficiency occurs in the syndrome of coumarin-induced cutaneous necrosis. It has

Figure 22. Protein C levels measured by electroimmunoassay with a goat antibody in adult controls, partially deficient family members, and family members defined as normal. These are the results for the family shown in Fig. 20. From Seligsohn, et al., N. Engl. J. Med. 310, 559-562, 1984.




been known for many years that a small proportion of patients who begin coumarin therapy develop necrotic skin lesions within a few days after starting therapy. The lesion starts off as a ecchymotic patch, usually on an extremity or over a fatty tissue. In females it frequently involves the breast. The patch gradually enlarges and becomes necrotic. Eventually the skin sloughs off. Biopsy shows micro-thrombi in small vessels of the involved skin. Tests of clotting activity usually show only the effects of coumadin therapy.

In 1982, a 36 year old woman was described who developed skin necrosis on two different occasions when she was started on coumadin therapy (21). Realizing that protein C is a vitamin K-dependent protein, Broekmans and coworkers measured the protein C level in the patient after she had discontinued her coumadin therapy (22). They also measured the level in another patient who had experienced this syndrome. In both cases the levels of protein C were markedly reduced, being 18% of normal in one patient and 62% of normal in another. All of the other clotting factors were normal.

An even more striking case was reported McGehee, et al. (23). They observed a 33 year old man who had recurrent deep vein thrombosis of the legs. Heparin and warfarin therapy had been given previously without complications. Forty-eight

Figure 23. Family with protein S deficiency leading to recurrent thrombosis. Proband is indicated by arrow. Functional protein S activity levels are shown for each family member. From Comp, et al., J. Clin. Invest. 74, 2082-2088, 1984.

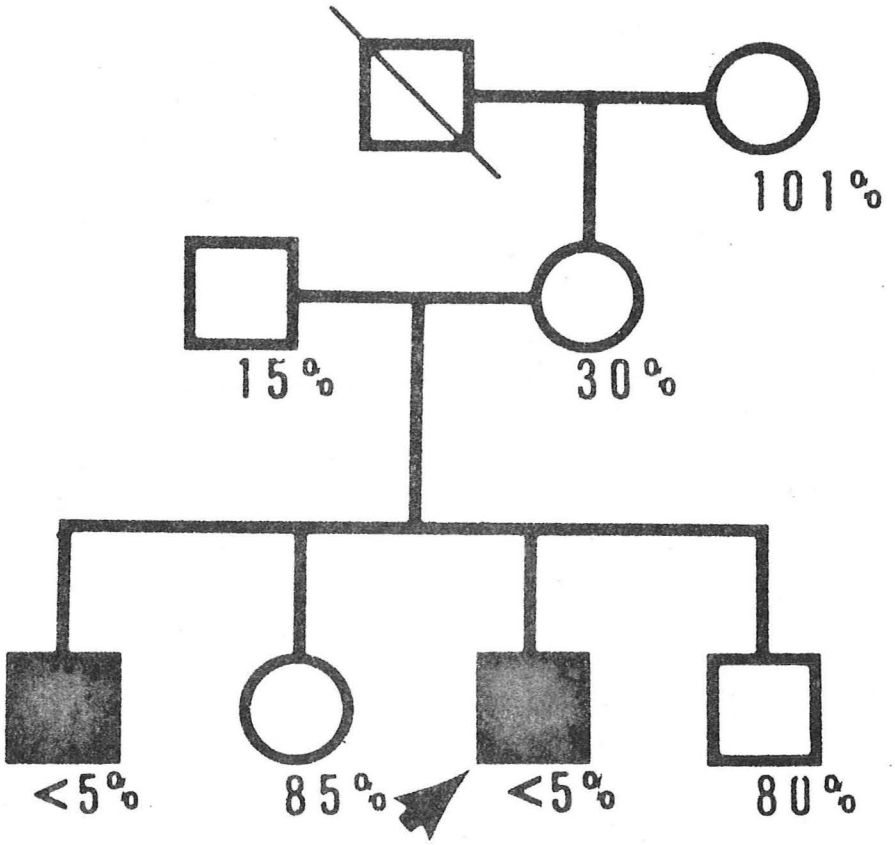
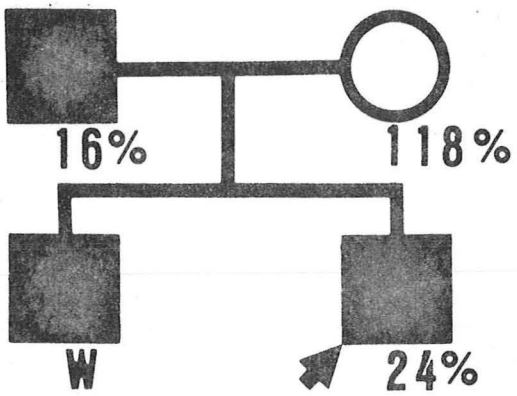



Figure 24. These six patients were part of a group of 120 patients with early-onset venous thrombosis. Six of the 120 had a deficiency of protein S. From Comp and Esmon, N. Engl. J. Med. 311, 1525-1528, 1984.

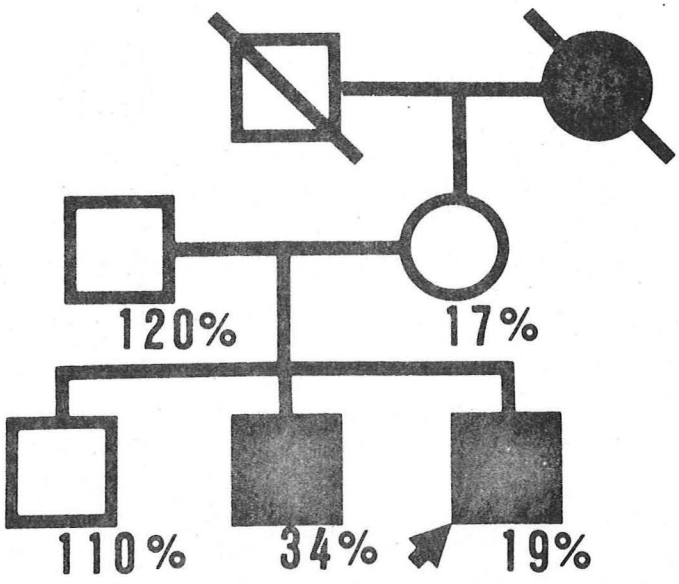
Table 1. Characteristics of Six Patients with Protein S Deficiency.

| Patient | Age/Sex | Age at Onset | Type of Thrombosis | Protein C Functional Activity (Normal, 60–138%) | Protein S Functional Activity (Normal, 63–160%) | Protein S Immunologic Levels (Normal, 61–151%) |
|---|---|---|---|---|---|---|
| | | | | | *per cent* | |
| 1 | 39/M | 24 | Recurrent deep venous thrombosis in legs | 108 | 23 | 108 |
| 2 | 31/F | 22 | Recurrent pulmonary emboli | 95 | 34 | 118 |
| 3 | 52/M | 20 | Deep venous thrombosis and pulmonary emboli | 68 | 15 | 21 |
| 4 | 19/M | 15 | Recurrent deep venous thrombosis, both legs | 107 | 19 | 31 |
| 5 | 33/M | 27 | Multiple episodes of deep venous thrombosis | 110 | 37 | 61 |
| 6 | 30/M | 19 | Recurrent deep venous thrombosis | 83 | 24 | 36 |

Figure 25. Pedigrees of two families of patients with isolated deficiency of protein S. The percentages indicate the levels of protein S as measured functionally. Solid symbols indicate recurrent venous thrombosis or pulmonary emboli. Protein S levels could not be measured in patient W because he was on long-term Warfarin therapy. From Comp and Esmon, N. Engl. J. Med. 311, 1525-1528, 1984.




FAMILY OF PATIENT 6




FAMILY OF PATIENT 4

hours after the first of two 20 mg doses of coumarin he developed necrosis of the penis, which was a frightening event. Fortunately, after treatment with heparin he recovered.

After recovery, while the patient was being treated with heparin but not coumadin, he was shown to have a diminished protein C level (34% of normal). Four

Figure 26. Protein C antigen in 2 patients with coumarin skin necrosis. From Broekmans, et al., Thromb. Haemostas. 49, 251, 1983.

*Table 1*

| | Patient 1 (♀, A. P.) | Patient 2 (♀, M. M.) | Normal |
|---|---|---|---|
| Protein C antigen (U/ml) | 0.18 | 0.62 | 0.65–1.45 |
| Factor II antigen (U/ml) | 1.00 | 1.25 | 0.65–1.45 |
| Factor X antigen (U/ml) | 1.20 | 0.92 | 0.65–1.45 |
| Factor IX antigen (U/ml) | 1.75 | 1.60 | 0.65–1.45 |
| Factor VII activity (U/ml) | 1.32 | 1.32 | 0.65–1.45 |
| Thrombotest (sec) | 36 | 35.5 | ≦39 |

Figure 27. From McGehee, et al., Annals Internal Med. 101, 59-60, 1984.

**Results of Factor X and Protein C Antigen Assays in a Patient with Coumarin Penile Necrosis, in the Patient's Family, and in Controls**

| | Factor X* | Protein C* | Ratio |
|---|---|---|---|
| | ← % → | | |
| Controls ($n$ = 31) | 98 (23)† | 94(23) | 1.04(0.33) |
| Patient | | | |
| Early‡ | 71 | 27 | 2.63 |
| After recovery | 88 | 34 | 2.59 |
| Patient's family | | | |
| Mother | 107 | 54 | 1.98 |
| Half brother | 101 | 45 | 2.24 |
| Half brother | 124 | 48 | 2.58 |
| Half sister | 106 | 41 | 2.58 |

* 100% = mean of a reference plasma pool of 15 normal persons.
† Mean values, with 2 SD in parentheses.
‡ Early sample obtained while patient was still under treatment with heparin but after vitamin K.

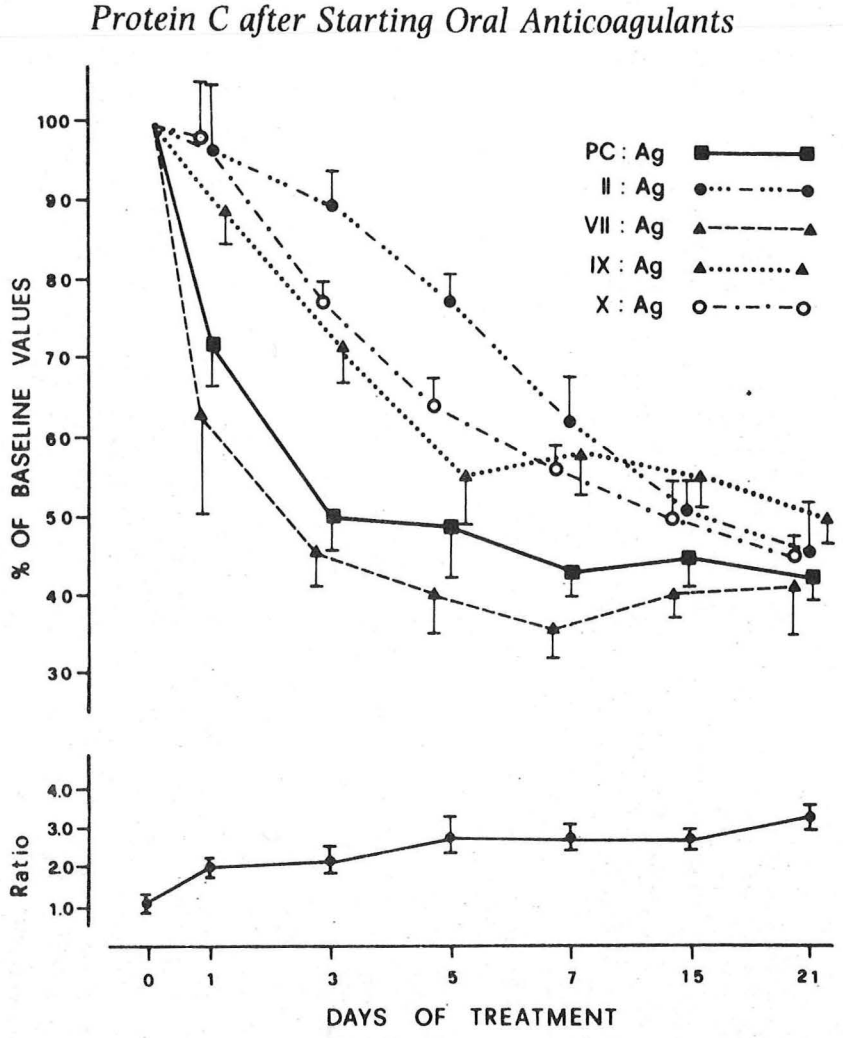
of his first degree relatives also had protein C deficiency of the same magnitude. None of these other relatives had any symptoms of venous thrombosis.

On the basis of these three cases it seems likely that patients who are heterozygotes for protein C deficiency may be predisposed to the development of

skin necrosis when coumadin therapy is initiated. However, more cases will have to be observed before this association can be considered proven.

Why should coumadin therapy cause enhanced thrombosis in patients with protein C deficiency? The likely answer comes from a consideration of the rate at which the various vitamin K-dependent clotting factors decline in plasma following coumadin therapy. An important study in this regard is shown in Fig. 28 (24). Vigano and coworkers measured the levels of all vitamin K-dependent proteins by

Figure 28. Changes in vitamin K-dependent proteins in 10 patients at the start of oral anticoagulant therapy. The bottom panel shows the relative prolongation of the clotting time as measured by the ratio of the patient clotting time versus control clotting time using the thrombotest technique. From Vigano, et al., Br. J. Hematol. 57, 213-220, 1984.




means of immunoassays after starting coumadin therapy in 10 patients. It has long been known that factor VII declines most rapidly after coumadin therapy. This is because factor VII has the most rapid turnover time of all the vitamin K-dependent proteins. Vigano and co-workers found that protein C declines just as rapidly as factor VII. Most importantly, protein C declines at a time when the major coumadin-sensitive factors, that is, prothrombin (factor II) and factor X have not yet declined.

If an individual is a heterozygote for protein C deficiency and starts with a depressed protein C level initially, then the rapid fall induced by coumadin would likely produce a transient hypercoagulable state.

Even in normal subjects, clinical anticoagulation has long been known to be delayed after starting coumadin therapy. A profound anticoagulant effect is not observed until a week after therapy when prothrombin and factor X have declined. Part of this resistance to coumadin may be attributable to the decrease in protein C. These findings provide a strong rationale for the clinical practice of continuing heparin therapy for one week after starting coumadin therapy in all patients with thrombosis. One needs the heparin to tide the patient over the transient state of imbalance when protein C has been diminished but the procoagulant factors are still present.

In addition to its reduction by coumadin therapy, protein C levels can also be reduced in disease states. The most important of these is the syndrome of disseminated intravascular coagulation. Fig. 29 shows the levels of various clotting factors measured serially in a 7 year old girl with meningococcemia and disseminated intravascular coagulation (25). Even on presentation the activity of protein C was only 50% of normal. It reached a low point of 25% of normal in 48 hr. Thereafter the activity rose as the patient recovered. The decrease in protein C was similar to the decrease observed in factor V and factor VIII in the patient's blood.

Marlar and co-workers studied the level of protein C activity and protein C antigen in plasma from 83 normal controls and 56 patients with disseminated intravascular coagulation with a variety of causative factors (Fig. 30) (25). Protein C deficiency was demonstrated in nearly all of the patients at sometime during their course.

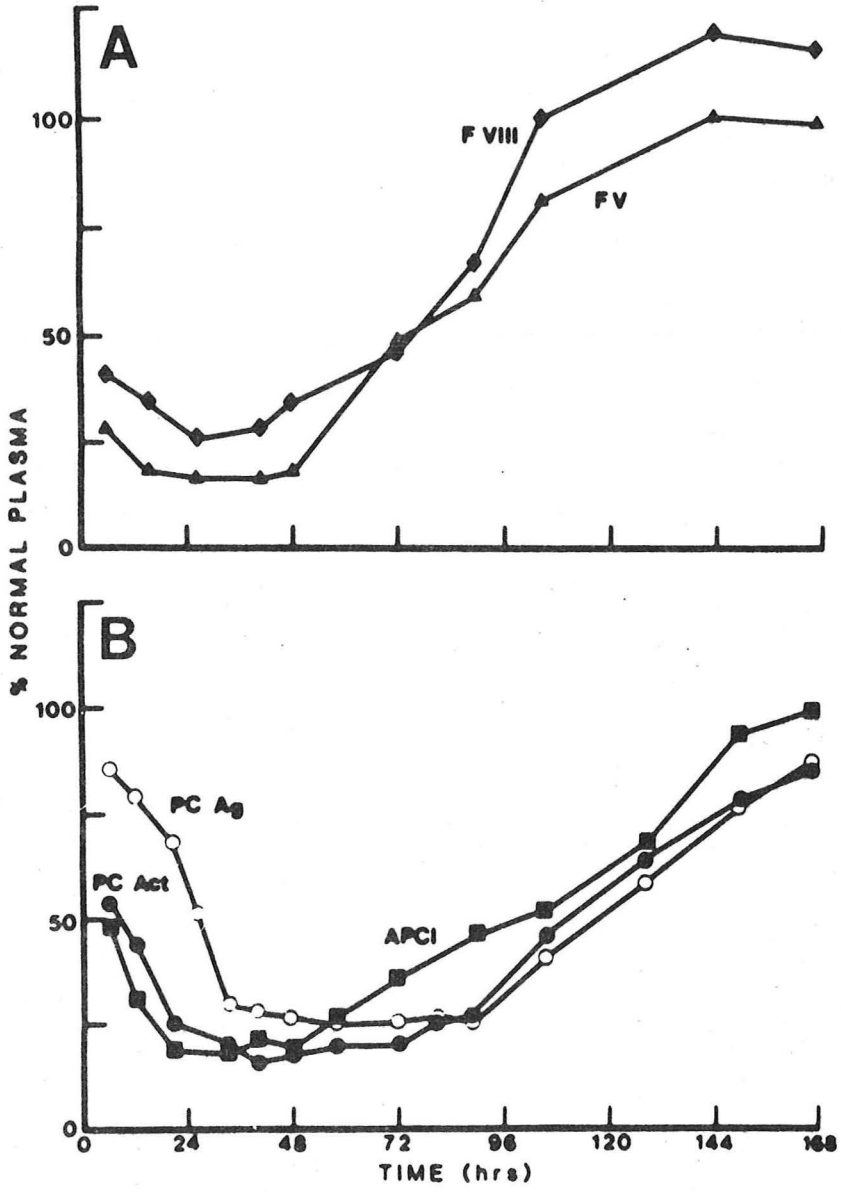
The decrease in protein C during DIC is probably due to accelerated degradation. In DIC the activation of thrombin leads to activation of protein C. Preliminary data indicates that activated protein C is rapidly cleared from the circulation thus causing a deficiency state. The deficiency of protein C may play an important role in the propagation of the DIC process. When protein C is depleted one of the major factors that fights DIC is removed from the game and this may allow DIC to continue.

These findings have exciting therapeutic applications. As mentioned previously, the gene for protein C has been isolated. It may now be possible to prepare large amounts of human protein C by genetic engineering methods in animal cells, although the attachment of gamma-carboxy glutamic acid residues will be difficult to achieve. Nevertheless, if an active form of protein C can be generated by genetic engineering companies it might have an important use in terminating DIC.

Protein C is also decreased in other diseases. It is diminished in liver disease, particularly in patients with established cirrhosis. Fig. 31 is taken from a 1982 paper in Lancet (26). The data showed that levels of protein C antigen were diminished in patients with cirrhosis, roughly in proportion to the extent of liver disease. This decrease is usually not clinically significant because the patients also have decreased levels of other clotting factors with a net result of a hypocoagulable state.

However, in another condition the depletion of protein C may be clinically signficant. Fig. 32, also taken from Mannucci (26), shows the level of protein C in patients who underwent surgery. The levels of protein C tended to decline after major operations, minor operations, and operations on cancer patients, reaching low

Figure 29. Serial time course of protein C-related proteins in the plasma of a patient with DIC resulting from meningococcemia. PC ag stands for protein C antigen levels. PC act stands for protein C activity. APCI stands for protein C inhibitor activity. From Marlar, et al., Blood 66, 59-63, 1985.




values on day 2 to 5 postoperatively. The fall in protein C levels is small, but it is nevertheless possible that this small decrease in protein C might predispose to thromboses following surgery. Again, if an active form of genetically engineered protein C were available it might be useful in the postoperative state.

Lest you think that protein C activity is altered in all patients with any disease let me show you the results of a recent study published in J. Lab. Clin. Med. in 1985 in which protein C levels were compared in patients with nephrotic syndrome and controls (27). As shown in Fig. 33, the level of protein C in patients with massive proteinuria was actually higher than in controls. Thus, we cannot implicate protein C in the hypercoagulable state that often accompanies

Figure 30. Protein C activity and antigen in patients with intravascular coagulation. From Marlar, et al., Blood 66, 59-63, 1985.

PROTEIN C ACTIVITY AND ANTIGEN IN PATIENTS WITH INTRAVASCULAR COAGULATION

| Disease | No. of Patients | Protein C Activity* | Protein C Antigen* |
|---|---|---|---|
| Normal controls | 83 | 70-130 | 70-130 |
| Infection | 27 | <6-60(100%) | 28-112(78%) |
| Malignancy | 18 | ≤6-120(94%) | 5-170(72%) |
| Leukemia | 6 | 15-75(83%) | 28-97(50%) |
| Obstetric complications | 5 | <6-55(100%) | 16-80(80%) |

*Values are expressed in units per deciliter. Parentheses show percentages of abnormal levels.

From Marlar, et al., Blood, 1985.

Figure 31. CPH = chronic persistent hepatitis; CAH = chronic active hepatitis; CIRRH = cirrhosis. Shaded area = normal range of protein C in healthy subjects. From Mannucci and Vigano, Lancet 241, 463-467, 1982.

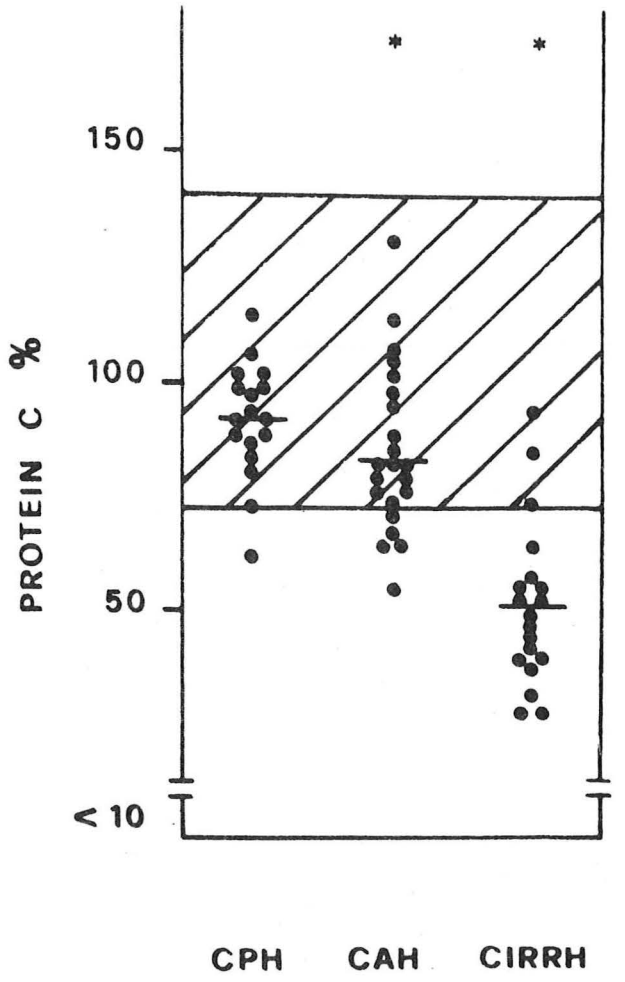



Fig. 1—Protein C in 58 patients with chronic liver diseases.

Figure 32. Changes in protein C in patients before and after major surgery, minor surgery and cancer surgery. One asterisk = $p < .05$; Two asterisks = $p < .01$. From Mannucci and Vigano, Lancet 241, 463-467, 1982.

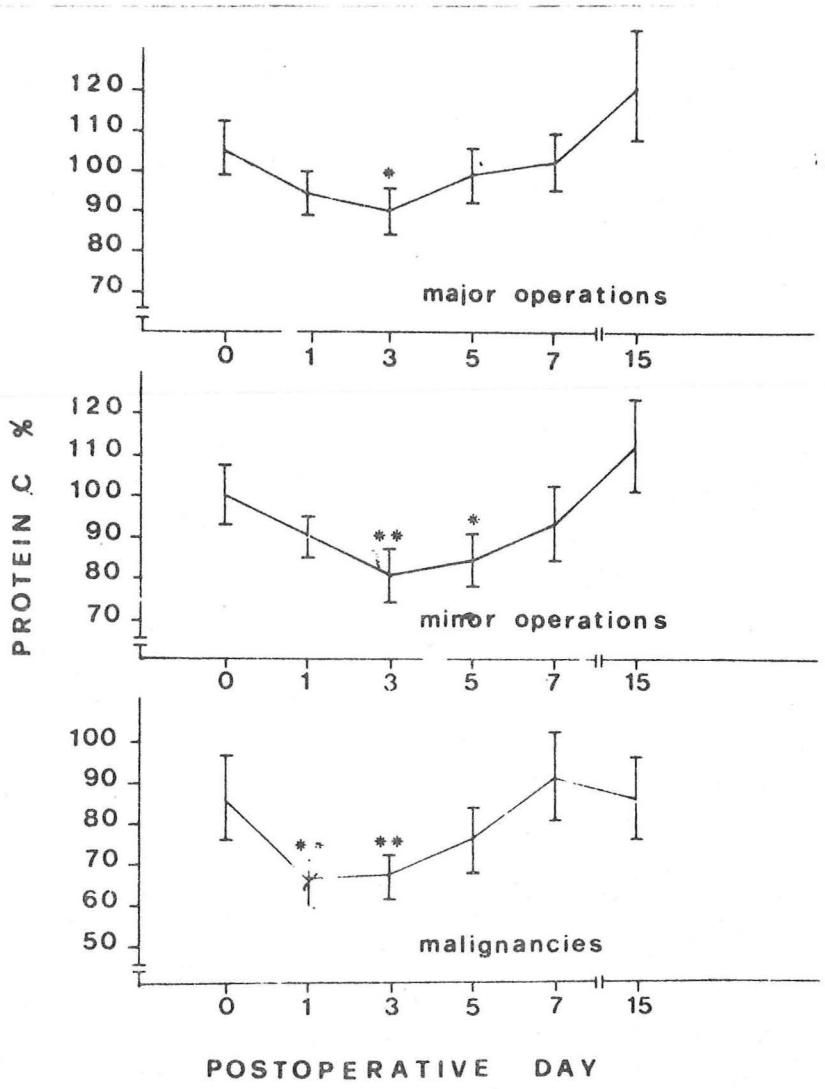
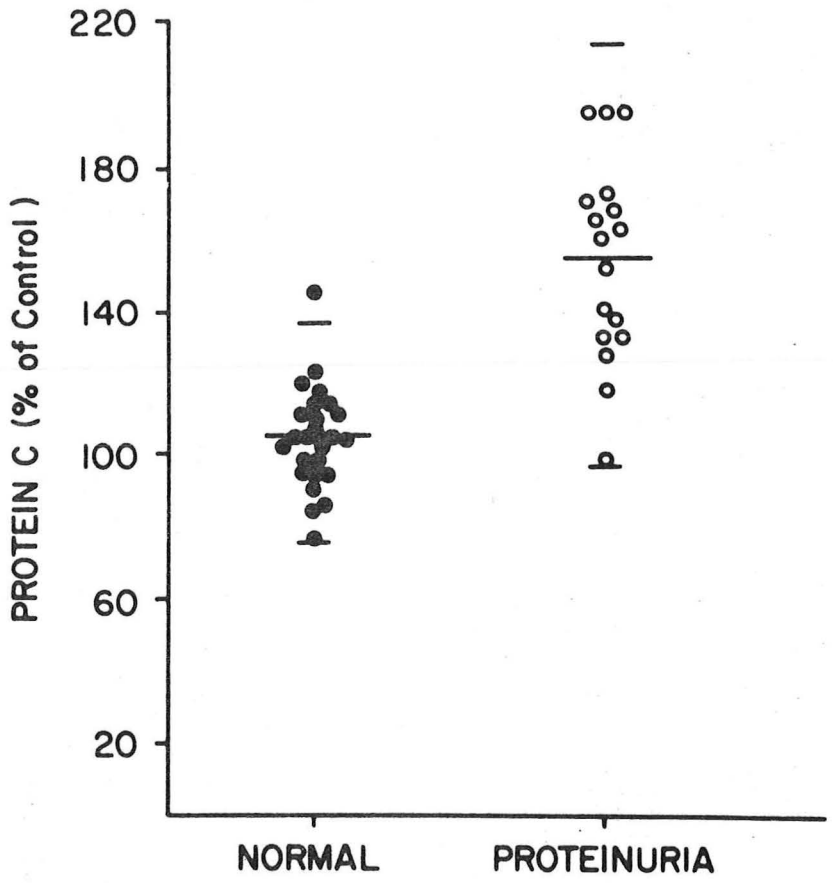


Figure 33. Plasma protein C concentrations as determined by immunoassay in 26 healthy individuals and 17 patients with proteinuria. From Cosio, et al., J. Lab. Clin. Med. 106, 218-222, 1985.




nephrotic syndrome. However, protein C has been reported to be diminished in patients with other forms of renal disease (28). Protein C is not diminished in obesity or after oral contraceptives (29) - two other conditions frequently associated with thrombosis.

Summary. The discoveries of protein C and protein S have greatly expanded our insight into the mechanism by which blood coagulation is normally controlled. Genetic and acquired deficiencies of these proteins can clearly produce hypercoagulable states. So far, only the most severe of these states have been elucidated. However, it is quite likely that further study will show milder deficiencies that predispose to thrombosis in certain patients. All patients with unexplained venous thrombosis at an early age should have measurements of protein C and protein S levels - particularly if there is a family history of thrombosis.

One of the major unresolved questions is whether defects in any of these clotting factors predispose to arterial thrombosis in contrast to venous thrombosis. Do genetic or acquired differences in these factors play any role in thrombosis of coronary or cerebral arteries? As yet, there is no evidence to implicate these factors in arterial thrombosis. In the families of patients with documented deficiencies of protein C and protein S there is abundant venous thrombosis and pulmonary embolization. However, there is no dramatic increase in heart attacks or strokes.

These findings suggest that protein C and protein S normally play a predominant role in the microcirculation and in the venous beds and are not as important in arterial systems. However, one must realize that studies of these factors are in their infancy. No one has studied a population of myocardial infarction patients and stroke patients with reference to the activities of their natural anticoagulant factors. Now that the gene for protein C has been cloned it should be possible to follow this gene through families that have a high incidence of myocardial infarction to determine whether the transmission of the gene for protein C is linked to the occurrence of myocardial infarction in certain predisposed families.

From a therapeutic point of view, one would hope that it would be possible to produce an active form of protein C and perhaps protein S in genetic expression systems. It is only through that route that we will know whether the administration of protein C and protein S has beneficial effect in various thrombotic states. This is an extremely exciting prospect.

In this talk I have not mentioned the other major anticoagulant of plasma, antithrombin III, nor tissue plasminogen activator, the other area of current excitement in thrombosis research. I refer the reader to a recent review of this field (30).

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