

**The Significance of Plasma Protein C
in Collagen Diseases
With And Without Vasculitis**

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in *Internal Medicine*

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ

خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ

اقْرَأْ وَرَبُّكَ الْأَكْرَمُ الَّذِي عَلَّمَ بِالْقَلَمِ

عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ



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List of Abbreviations

Ag	: Antigen
APC, PCa	: Activated protein C.
APTT	: Activated partial thromboplastin time
ATIII	: Antithrombin III
AVH	: Acute viral hepatitis
Ca++, Ca 2+	: Calcium ions
C2BP	: C4b - binding protein
DIC	: Disseminated intravascular coagulation
ELISA	: Enzyme linked immunosorbent assay
F	: Factor
FDPS	: Fibrinogen, fibrin degradation products
Gla	: Glutamic acid
HC	: Heavy chain
HMWK	: High molecular weight kininogen
HRG	: Histidine rich glycogen
MCTD	: Mixed connective tissue disease
Mol. Wt., M.W.	: Molecular weight
OC	: Oral contraceptives
OV	: Oesophageal varices
PAF	: Platelet activating factor
PC	: Protein C
PCI	: Protein C inhibitor
PSBP	: Protein S. binding protein
PT	: Prothrombin time
RA	: Rheumatoid arthritis
SLE	: Systemic lupus erythematosus
TF	: Tissue factor
t-PA	: Tissue - type plasminogen activator
u-PA	: Urokinase - type plasminogen activator

INTRODUCTION &
AIM OF THE WORK

INTRODUCTION

Protein C is the zymogen of a serine protease involved in blood coagulation that has been isolated from both bovine and human plasma (*Stenflo, 1976; Kiesel, 1979*). It was discovered by Stenflo as a result of his interest in vitamin K dependent plasma proteins which could not be detected with coagulation assays. Protein C received its present name because it was purified from a protein fraction (pool C) obtained after gradient elution of a prothrombin complex concentrate on a DEAE-Sephadex column (*Stenflo, 1976*).

Activated protein C destroys the activity of activated factors V and VIIIc (*Kiesel et al., 1977; Marlar et al., 1982*) and stimulates fibrinolysis by inducing a rise in plasma plasminogen activator activity (*Zolton and Seegers, 1973; Comp and Esmon, 1981*). This rise in plasma plasminogen activator activity is due to a neutralizing effect of activated protein C on a circulating inhibitor of t-PA (*Van Hinsberg et al., 1985*).

The significance of protein C was evaluated in intravascular coagulopathy, nephrotic syndrome, heart disease, and diabetes mellitus.

The involvement of protein C in disseminated intravascular coagulation (DIC) has been demonstrated by the presence of low concentrations of protein C in patients with clinical conditions associated with DIC (*Griffin et al., 1982; Mannucci and Vigano, 1982*).

Pabinger-Fasching et al. (1985) showed that protein C levels are not reduced in patients with nephrotic syndrome, on the contrary, they observed a significant elevation of protein C antigen and protein C activity.

Patients with acute myocardial infarction often develop venous thrombosis (*Jespersen et al., 1983*). *Gram and Jespersen (1985)* found that protein C showed insignificant fluctuations in patients with and without deep venous thrombosis after acute myocardial infarction. They concluded that protein C is of little value as possible indicator for the presence of deep venous thrombosis at early stages of the disease when clinical signs are absent and when antithrombotic prophylaxis should preferably be initiated (*Gram and Jespersen, 1985*).

Muntean and Borkenstein (1985) studied protein c level in diabetic children with poor control of diabetes, they used ELISA method. They found that plasma levels of protein C antigen concentration was significantly elevated in diabetic children. It did not differ in the groups with different durations of clinical diabetes. The mechanism leading to elevation of protein C in diabetics was unclear, but might be a sign of increased turnover of protein C in these patients.

Aim of the Work

Is to, study active protein C level in patient with collagen diseases: systemic lupus erythematosus, rheumatoid arthritis and mixed connective tissue disease with and without vasculitis.

REVIEW OF LITERATURE

HAEMOSTASIS

Coagulation occurs via two alternative pathways, the intrinsic and the extrinsic pathways (Fig. 1).

Intrinsic Pathway

The initial event of the intrinsic activation pathway is the activation of factor XII. Factor XII (Hagman factor) is activated by surface contact with collagen, kallikrine or basement membrane. All are exposed due to injury of the vascular intima. Activated factor XII activates factor XI to XIa, which in turn activates factor IX to factor IXa. Together with phospholipid surface which is supplied by platelets under physiological conditions, calcium and a cofactor protien which is factor VIII, activated factor IX converts factor X to Xa. This phase of coagulation lasts 5 to 10 minutes and when it is impaired the whole clotting time, partial thromboplastin time and a number of other coagulation tests are prolonged.

Extrinsic Pathway

In the second or extrinsic pathway, injury to the endothelial cells expose tissue factor which has the effect of stimulating the activity already present in factor VII. Factor VII, tissue factor, calcium ions and phospholipid surface converts factor X to factor Xa. This sequence is very rapid (less than 20 seconds) and its defects prolong the one-stage prothrombin time.

Once factor X is activated by either the intrinsic or the extrinsic pathway (Fig. 1), factor Xa forms a complex with phospholipid, calcium ions and activated factor V.

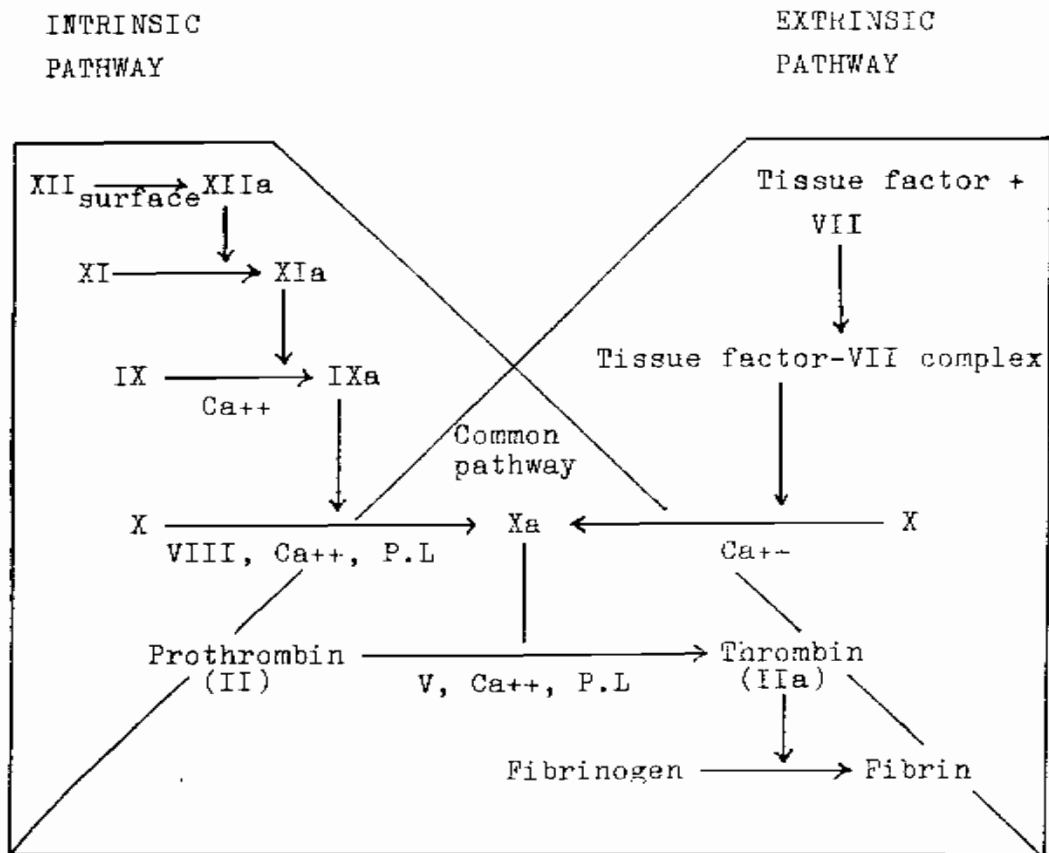


Fig (1): A diagram showing the effect of both the intrinsic and extrinsic system of coagulation on factor X activation (DiSabino et al., 1979).
P.L (Phospholipid).

This complex is called prothrombinase complex on which the enzyme thrombin is generated from its zymogen prothrombin (*Esmon et al., 1974 and Ownen et al., 1974*).

Then the thrombin which is generated cleaves two pairs of peptides from the fibrinogen molecule forming a "soluble" fibrin clot. Fibrin is stabilized through the action of thrombin-activated factor XIII which catalyses polymerisation of fibrin to produce a stable fibrin clot in the last steps in the coagulation mechanism. The action of thrombin on fibrinogen is instantaneous, but the stabilization of fibrin continues for many hours (*Lorand, 1972*).

BIOCHEMICAL STRUCTURE OF PROTEIN C AND ITS SYNTHESIS

Protein C is vitamin K-dependent glycoprotein of molecular weight 62,000. It circulates in plasma as an inactive zymogen at a concentration of 4 ug/ml (*Griffin et al., 1982*).

It consists of two polypeptide chains: a heavy chain and a light chain linked together with a disulphide bond (*Kisiel and Davie, 1981*). A single chain protein C has been isolated from the plasma (*Miletich et al., 1983*). When protein C is converted to a serine protease, activated protein C of Mol. Wt. 54,000, it is an inhibitor of blood coagulation (*Walker et al., 1979*), *Drakenberg et al. (1983)* showed that human protein C is a two chain molecule containing 11 glutamic acid and one B hydroxy aspartic acid residues in an inactive form (zymogen).

Activation by thrombin causes a single cleavage between Arginine (12) and Leucine (13) at the aminoterminal part of (HC) releasing a small peptide with Mol. Wt 1400 (*Kisiel and Davie, 1981*).

The light chain (Mol. Wt. 21,000) contain δ -carboxy glutamic acid residues (Gla) (*Stenflo and Fernlund, 1982*), that are involved in Ca^{2+} - dependent membrane association. The NH_2 -terminal part of the light chain, which contains the Gla residues is very homologous to the corresponding regions of the other vitamin K-dependent proteins (*Fernlund and Stenflo, 1982*). Vitamin K-dependent proteins are characterized as glycoproteins that are carboxylated at selected glutamic residue by a post-translational process involving at least two enzymes and vitamin K. Vitamin K functions as a cofactor

in the hepatic conversion of the N-terminus glutamic acid residues (*Suttie, 1980*).

Synthesis and Secretion of Protein C

The liver has been shown to be the primary source of plasma proteins including those of the coagulation and fibrinolytic system (*Goldsmith et al., 1980*). The soluble components of protein C pathway (protein C, protein C inhibitor and protein S) are produced by the liver parenchymal cells. This was demonstrated by using human hepatoma cell line Hep G₂ (*Fair and Marlar, 1986*).

Protein C is secreted by the liver cell as a single chain molecule of Mol. Wt. about 65,000. This single chain molecule protein C must undergo a post-secretion processing event leading to the generation of its two-chain plasma form. Both protein C and factor (VII) have high secretion rates relative to factor (X) this may reflect a normal process since both protein C and factor VII have shorter half-lives. The high synthetic rates maintain a steady state (*Fair and Marlar, 1986*).

The plasma half-life of protein C is very short (six hours) (*Epstein et al., 1984*).