

**SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY**

PBL SEMINAR: NORMAL HEMOSTASIS – An Overview

- ❑ Hemostasis is the cessation of bleeding from a cut or severed vessel
 - It encompasses Blood Clotting (Coagulation) and involves: Blood Vessels, Platelets Aggregation, and Plasma Proteins that can cause both formation and dissolution of Platelet Aggregates
- ❑ Normal Hemostasis is dependent upon the complex interaction of: Blood Vasculature, Platelets, Plasma Coagulation and Fibrinolytic Proteins
- ❑ Normal mechanism of Hemostasis can be separated into 4 interrelated phases for easier understanding of these complex processes
 - Generally the 4 phases can occur simultaneously and not sequentially, since they play intimate and often mutually interdependent roles in coagulation.

What are the 4 interrelated phases in normal mechanism of Hemostasis?

The 4 interrelated phase are as following:

- ❑ Constriction of injured vessel to diminish blood flow distal to the injury
- ❑ Formation of Loose and Temporary Platelet Aggregate at the site of injury, i.e.,
 - ❑ Formation of Hemostatic plug in Hemostasis or
 - ❑ Formation of Thrombus in Thrombosis
- ❑ Formation of Fibrin mesh (via the Coagulation Cascade) that binds to the platelet aggregate, forming more stable Hemostatic plug (clot) or Thrombus
- ❑ Dissolution of the Hemostatic plug or Thrombus by Plasmin.

BLOOD VESSEL WALL:

How are the blood vessels involved in Hemostasis? (See Table 1)

- ❑ Following injury to the vessel wall, the initial event is Vasoconstriction, which is a transient, locally induced phenomenon
 - Vasoconstriction retards extra-vascular blood loss,
 - Vasoconstriction slows local blood flow, enhancing the adherence of platelets to exposed sub-endothelial surfaces and the activation of Coagulation process
- ❑ Normal Endothelium prevents Hemostasis by:
 - Providing a physical barrier and
 - Secreting products, which inhibit platelets activation and aggregation
 - Endothelial cells:
 - Synthesize Prostacyclin I₂ (PGI₂), a potent inhibitor of Platelet Aggregation and an antagonist of Thromboxane A₂.
 - Prostacyclin act by stimulating the activity of Adenylyl Cyclase (in the surface of Platelets), which increases the level of Cyclic AMP within the Platelets, causing reduction in Calcium uptake into Platelets and inhibition of Platelet activation.

- Possess the enzyme ADPase that catalyzes the hydrolysis of ADP, thus, reducing the aggregating effect of ADP on Platelets.

FORMATION OF PRIMARY PLATELET PLUG:

What processes and components are involved in formation of primary platelet plug?

- Process involved in formation of Primary Platelet Plug include:
 - Platelet Adhesion,
 - Platelet Activation
 - Platelet Aggregation

- Some components involved include:
 - Platelets, Blood vessel wall and von Willebrand factor (vWf – **This is a Glycoprotein secreted by endothelial cells into the plasma, it stabilizes Factor VIII and binds to Collagen and the Sub-endothelium**).

Briefly describe the processes involved in formation of primary platelet plug

Platelet Adhesion:

- Involves the recognition and adhesion of platelets to exposed sub-endothelial collagen.
 - Fibrinogen and von Willebrand factor (vWf), mediate the adhesion (binding) of platelets to exposed sub-endothelium at the site of endothelial damage.

Platelet Activation:

- Adhesion of platelets to the sub-endothelium results in Activation of the Platelets.
- Activation caused the platelets to:
 - Change shape,
 - Activate the Collagen Receptors on their surface and
 - Undergo the release reaction.
 - Synthesize and release Thromboxane A₂ (TXA₂), ADP and Platelet Activating Factor (PAF),
 - These are potent Platelet Aggregating Agonists and Vasoconstrictors.

- Platelet Activation is enhanced by the generation of Thrombin through the Coagulation Cascade, (Thrombin being an important Platelet Agonist)

Platelet Aggregation:

- Platelet aggregation occurs after Platelet activation
- Thromboxane A₂, PAF, ADP and Serotonin are Platelet Agonists that causes further Activation and Recruitment of Additional Platelets, which then binds to the already adhered platelets.
- **Platelet aggregation is mediated primarily by Fibrinogen** (vWf has a secondary role),
 - **Fibrinogen** binds to Glycoprotein molecules located on Platelet membrane

- Platelet aggregation leads to formation of Primary Platelet Plug, which must be stabilized by the formation of Fibrin.
- Platelets contribute to Coagulation cascade by providing a Phospholipid surface and Receptors for the binding of Coagulation factors.

FORMATION OF FIBRIN MESH (COAGULATION CASCADE)

BLOOD CLOTTING PATHWAYS (COAGULATION CASCADE):

- ❑ Coagulation Cascade is an example of a medically relevant process that is controlled by a sequence of critically regulated enzymatic reactions
- ❑ Formation and Regression of Clots are tightly regulated processes involving many activating and inhibiting factors (**See Table 2** for the names of various Factors)
- ❑ Factors needed for Clot formation, after initiation, are present in blood.
- ❑ Vitamin K is required to regulate the functions of some clotting factors.
- ❑ Clot formation has been likened to a "Cascade", in which Inactive Blood Clotting Factors (Zymogens) are activated into Proteolytic enzymes that selectively attack the next Zymogen in the sequence, converting it into an Active Clotting Factor (Enzyme).
- ❑ Coagulation Cascade also involves:
 - Cofactors: Factor V (FV) and Factor VIII (FVIII),
 - Calcium,
 - Platelets (Platelets provide a source of Phospholipid and a binding surface upon which the Coagulation Cascade proceeds)

What are some of the advantages of a Cascade regulatory response in clotting pathway?

- ❑ Some advantages of a **“Cascade” regulatory response in clotting pathway** include:
 - Initiation of large response requires very low amount of Activator
 - Activation of Cascade does not occur in the absence of an activator, thus, the response is usually localized to the sight of activation
 - Each step of the Cascade Amplifies the response, thus ensuring that the response is both large and rapid

What component parts (pathways) make up the Coagulation Cascade?

- ❑ Coagulation Cascade is usually separated into 2 components parts (Pathways):
 - Extrinsic Pathway and
 - Intrinsic Pathway,
 - Both pathways are Initiated by different Activators
 - Both Pathways converge to activate a Final Common Pathway, which then leads to the final clot formation (**Fig. 1**).

Give a brief description of the Extrinsic Pathway (See Figs. 1 and 2):

- ❑ Extrinsic pathway involves the **Tissue factor-Factor VIIa complex (TF-FVIIa)**, which in the presence of Ca^{2+} ions activates Factor X (FX)
- ❑ Extrinsic pathway is short-lived and is primarily responsible for Initiation of the Coagulation Cascade
- ❑ Tissue Factor (TF) also called Thromboplastin or Factor III (FIII), normally found in circulating blood, triggers the clotting activation pathway
- ❑ TF is a membrane bound Glycoprotein
- ❑ When exposed upon vessel injury, TF tightly binds Factor VII (FVII)
- ❑ While bound to TF, FVII is activated (FVIIa) by trace amounts of circulating, Active Proteases
- ❑ Complex of TF, FVIIa and Calcium, catalyzes the activation of FX of the Common Pathway
- ❑ TF-FVIIa complex can also catalyze the activation of FIX in the Intrinsic Pathway

What is the function of EPI (also called LACI)?

- ❑ Extrinsic Pathway Inhibitor (EPI), or Lipoprotein-Associated Coagulation Inhibitor (**LACI**), is a circulating Lipoprotein that inhibits the conversion of FX to FXa by the TF-FVIIa complex.
 - This is the reason why individual affected with Hemophilia who have normal Extrinsic Pathway do not form effective Clots

Give a brief description of the Intrinsic Pathway (See Figs. 1 and 2):

- ❑ Factors involved in the Intrinsic pathway includes:
 - High-Molecular Weight Kininogen (HMW Kininogen),
 - Prekallikrein, and
 - Factors XII, XI, IX and VIII
 - FVIII is an important modifier protein
- ❑ Conversion of Prekallikrein to Kallikrein occurs when a complex of FXII, Prekallikrein, and HMW Kininogen makes contact with an abnormal surface, such as, Collagen in an open wound,
- ❑ Kallikrein then converts FXII to FXIIa
- ❑ FVIIIa together with Calcium and Platelet Phospholipid interacts with FIXa to significantly accelerate the conversion of FX to FXa
- ❑ Thrombin (FIIa) enhances the Intrinsic Pathway by feedback activation of FXI and FVIII
- ❑ Intrinsic Pathway acts to significantly Amplify the Coagulation Cascade

Give a brief description of the Common Pathway (Figs 1 and 2):

- ❑ Common Pathway involves FX-mediated conversion of Prothrombin (FII) to Thrombin (FIIa)
 - This process is facilitated by FVa, Calcium and Platelet Phospholipids
- ❑ FIIa then converts Fibrinogen (FI) to Fibrin monomers (FIa)
- ❑ Fibrin monomers then spontaneously polymerize to form soluble Fibrin Polymer
- ❑ FIIa also converts FXIII to Factor XIIIa
- ❑ Factor XIIIa together with Calcium, serves to Cross-link and stabilize soluble Fibrin polymer, forming Cross-linked (Insoluble) Fibrin mesh

What is the role of Vitamin K in the functions of some Clotting Factors?

- ❑ Vitamin K dependent clotting factors are: Factors II, VII, IX and X
- ❑ Other Vitamin K dependent proteins are Protein C and Protein S
- ❑ Vitamin K dependent clotting factors are synthesized in the Liver and require the Post-translational modification of Glutamate residues in their molecules
 - Posttranslational modification involves formation of Gamma-CarboxyGlutamate residues by the addition of Carboxyl groups (-COOH) to Glutamate residues in these proteins
 - Reaction is catalyzed by Specific Vitamin K dependent Carboxylase enzyme
- ❑ Vitamin K-dependent carboxylation reaction converts the Glutamate residue, a weak Chelator of Calcium into Gamma-CarboxyGlutamate residue, which is a strong Chelator of Calcium
- ❑ Normal functions of Vitamin K dependent clotting factors in the Coagulation Cascade dependent on the Calcium-binding properties of the Gamma-CarboxyGlutamate residues in their molecules

How is the Coagulation Cascade Initiated?

- Extrinsic Pathway Initiates the Coagulation Cascade when TF is exposed
 - Endothelial Cells, Sub-endothelial Tissue and Monocytes produce TF,
 - Production of TF is enhanced by Cytokines (TNF-alpha, IL-6)
- Exposed TF binds to FVII to form a complex that Activates FX
- FXa in the presence of FVa, Calcium and Platelet Phospholipid (this complex is called "**Prothrombinase Complex**") then converts Prothrombin to Thrombin (FIIa)
- Extrinsic Pathway is rapidly inhibited by the Lipoprotein-Associated Coagulation Inhibitor, called Extrinsic Pathway Inhibitor (EPI) or Tissue Factor Pathway Inhibitor (TFPI)
- However, the small amount of Thrombin generated by this pathway (before inhibition) activates FXI of the Intrinsic Pathway, which then amplifies the Coagulation Cascade

How does amplification of the Coagulation Cascade occurs?

- Coagulation Cascade is amplified by Thrombin generated via the Extrinsic Pathway
- Thrombin activates Intrinsic Pathway by activation of FXI and FVIII
- FIXa, together with FVIIIa, Calcium and Phospholipid (this complex is called "**Tenase Complex**"), significantly increases the activation of FX
- FXa in the presence of FVa, Calcium and Platelet Phospholipid ("**Prothrombinase Complex**") then converts Prothrombin to Thrombin
- Thrombin in the presence of Calcium converts Fibrinogen to soluble Fibrin Monomers,
- Fibrin Monomers then spontaneously polymerize to form soluble Fibrin Polymer
- Thrombin also converts FXIII to FXIIIa
- FXIIIa together with Calcium, serves to cross-link and stabilize soluble Fibrin Polymer, forming cross-linked (Insoluble) Fibrin Mesh

What is the Alternate pathway?

- Alternate pathway involves direct stimulation of the Intrinsic pathway
- It occurs via the direct activation of FIX by the TF-FVIIa complex.
- Alternate pathway is a minor pathway; the major stimulator of the Intrinsic Pathway is Thrombin, through activation of FXI

What are the components and functions of the Contact Pathway?

- Contact pathway is made up of:
 - Prekallikrein (Fletcher factor),
 - High-Molecular Weight Kininogen (Williams, Fitzgerald factor) and
 - FXII (Hageman factor)
- Contact system has important Anticoagulant, Pro-fibrinolytic and Pro-inflammatory roles and has minimal influence on the Coagulation cascade (by the FXIIa-mediated activation of FXI)
- Prekallikrein circulates in a 1:1 complex with High-Molecular Weight (HMW) Kininogen
- This complex assembles with FXII on the surface of cell membranes (the so-called Negative Surface for Activation), such as Collagen in an open wound.
- Activation of FXII by this complex converts Prekallikrein into Kallikrein, a potent enzyme
- Kallikrein amplifies the activation of FXII and converts HMW Kininogen into Bradykinin
 - Bradykinin is an important Vascular Mediator causing:
 - Vasodilatation,
 - Increased Vascular Permeability, and
 - Vascular Smooth Muscle Growth and Proliferation

- ❑ In an injured vessel, Bradykinin serves to stimulate vessel repair
- ❑ Bradykinin has an antithrombotic role, by inhibiting Thrombin-mediated Platelet Aggregation
- ❑ Contact system is a strong activator of Fibrinolysis
- ❑ Kallikrein and FXIIa can convert Plasminogen directly into Plasmin;
- ❑ Bradykinin is the most potent and specific stimulator of tissue Plasminogen activator release from endothelial cells

What are some of the functions of Thrombin (FIIa) in Hemostasis?

- ❑ Thrombin (Factor IIa) plays very important role in Hemostasis by functioning both as a Thrombotic and Anti-thrombotic molecule

FIIa as Thrombotic molecule:

- ❑ FIIa promotes coagulation in several ways, the most obvious is conversion of FI to FIIa
- ❑ Production of FIIa depends mainly on the conversion of large amounts of FII to FIIa, which requires the complex interaction between Intrinsic and Extrinsic pathways
- ❑ FIIa is a Platelet agonist, promoting platelet aggregation
- ❑ FIIa activates FV and FVIII, which are necessary cofactors for the "Prothrombinase" and "Tenase" complexes, respectively
- ❑ FIIa activates FXIII, which is essential for the cross-linking of Fibrin polymer to produce a stable, 3-dimensional Fibrin lattice
- ❑ FIIa inhibits Fibrinolysis by generation of a Thrombin-Activatable Fibrinolytic Inhibitor (TAFI)

FIIa as Anti-thrombotic molecule:

- ❑ FIIa conversely, acts as its own inhibitor
- ❑ FIIa binding to Thrombomodulin on endothelial cells to activate both Proteins C and S, which are Anticoagulants
- ❑ Proteins C and S inhibit activation of FV and FVIII limiting production of FIIa

DISSOLUTION OF CLOT

What is the role of Plasmin in dissolution of Clot (Fig. 3a & 3b)?

(What causes dissolution of clots?)

- ❑ Plasmin is the main enzyme responsible for degrading Fibrin and Fibrinogen (Fibrinolysis – the dissolution of clot)
 - Plasminogen is the inactive form in which Plasmin circulates in plasma
- ❑ Coagulation system is normally in a state of dynamic equilibrium in which Fibrin Clots are constantly being laid down and dissolved
- ❑ As the coagulation cascade is activated, tissue Plasminogen Activator (tPA) is released from endothelial cells
 - Release of tPA is stimulated by a variety of factors, including Hypoxia and Bradykinin
- ❑ Plasminogen binds to both Fibrinogen and Fibrin
- ❑ Plasminogen is incorporated in Clots as they are produced
- ❑ Tissue Plasminogen Activator (tPA) binds to Plasminogen within the Clot, converting it into Plasmin
- ❑ Plasmin then degrades both Fibrinogen and Fibrin (soluble and cross-linked) in the Clot, releasing Fibrin(ogen) degradation products (FDP)

- ❑ Fibrin is required for activation of Plasminogen by tPA,
 - This serves to limit the production of Plasmin to the site of Thrombus formation
 - Localization of Plasmin to site of Thrombus formation is important, because, Plasmin is fairly non-specific in its activity and will not only destroy Fibrin, but also other factors, including FV, FVIII and Fibrinogen
- ❑ Other Plasminogen activators independent of tPA include: Urokinase, FXII and Kallikrein
- ❑ FXIIa and Kallikrein can:
 - Convert Plasminogen into Plasmin directly
 - Produce Bradykinin from HMW Kininogen in the Contact portion of the Intrinsic Pathway of Coagulation;
 - ❑ Bradykinin is the most specific and potent stimulus for tPA release

SOME INHIBITORS OF HEMOSTASIS:

What is Antithrombin III and how is it functionally related to Heparin?

- ❑ Antithrombin III (ATIII) is a Natural Anticoagulant
- ❑ ATIII is an alpha₂-globulin produced in the Liver
- ❑ ATIII specifically inhibits FIIa,
- ❑ ATIII can also inhibit FIXa, FXa, FXIa and FXIIa,
- ❑ Heparin enhances the binding of ATIII to FIIa
- ❑ Heparin binds to a specific site on ATIII, inducing a conformational change and promoting the binding of ATIII to FIIa and the other clotting factors
- ❑ ATIII-FIIa complexes are then removed by the Monocyte-Macrophage system
- ❑ This provides the basis for the use of Heparin as an Anticoagulant

What is the role of Heparin cofactor II?

- ❑ Heparin cofactor II is a specific Thrombin antagonist
- ❑ Heparin is required for activation of Heparin Cofactor II

What are the functions of Protein C?

- ❑ Protein C is a Vitamin-K dependent Protease that is produced in the Liver
- ❑ Activated Protein C (an active protease) Inactivates FVa and FVIIIa

What is the function of Protein S?

- ❑ Protein S is a Vitamin-K dependent Protease that is synthesized in Endothelial cells, Megakaryocytes and Hepatocytes,
- ❑ Protein S stimulates the action of protein C

What is the role of Thrombomodulin?

- ❑ Thrombomodulin is a modifier of FIIa
 - It converts FIIa from a factor that is crucial to clot formation to one that inhibits clot formation
 - Thrombomodulin binds and changes the substrate specificity of FIIa
- ❑ FIIa bound to Thrombomodulin no longer carries out its normal functions, instead it activates circulating Protein C, which then inactivates FVa and FVIIIa

What are some of the inhibitors of Fibrinolysis?

Some inhibitors of Fibrinolysis include:

- ❑ TAFI (Thrombin-Activated Fibrinolytic Inhibitor):
 - Thrombin down regulates the Fibrinolytic system by activating TAFI

- TAFI then prevents the binding of Plasminogen to Fibrin, thus inhibiting its conversion to Plasmin
- Alpha2-Antiplasmin binds free Plasmin (that is not bound to Fibrin) and causes its removal by the Monocyte-Macrophage system, preventing widespread Fibrinolysis
- Plasminogen Activator Inhibitor (PAI-1 and PAI-2):
 - PAI-1 and PAI-2 are both Inhibitors of tissue Plasminogen Activator (tPA)
 - PAI-1 and PAI-2 are released by endothelial cells
 - They limit production of Plasmin by binding to tPA.

SOME ANTI-COAGULANTS:

Briefly explain the anti-coagulant function of Heparin?

- Heparin is a Glycosaminoglycan
- Heparin is usually released by mast cells when injury occurs
- Heparin is an anticoagulant that binds to and increases the inhibitory activity of Antithrombin III
- Protamine reverses the effect of heparin

Briefly explain the anti-coagulant action of Dicumarol and Warfarin?

- Dicumarol and Warfarin:
 - Analogs of Vitamin K used clinically as Anticoagulants
 - Inhibit the Vitamin K dependent Gamma-Carboxylation of Glutamate residues in the Amino-terminal regions of Vitamin K dependent clotting factors (FII, FVII, FIX, and FX, and also of Protein C and Protein S)
 - Causes the conversion of available Vitamin K to the Epoxide form that cannot participate in Carboxylation reaction
 - Act by inhibiting the Dithiol-dependent Vitamin K Reductase that catalyzes the conversion of the Epoxide form of Vitamin K to the biological active Dihydroquinone form
- In the presence of Dicoumarol and Warfarin clotting Factors II, VII, IX, and X, and also Protein C and Protein S:
 - Do not undergo Posttranslational modification,
 - Cannot bind Calcium, and
 - Cannot participate in blood coagulation
- Dicumarol and Warfarin have no effect on blood clotting In Vitro (in test tubes)

How does Aspirin (Acetylsalicylic acid) affect the action of Platelets?

- Aspirin is an effective Anti-platelet drug, because it effectively inhibits Cyclooxygenase produced in Platelets
- Aspirin acts by irreversibly Acetylates and thus Inhibits the Platelet Cyclooxygenase system involved in the formation of Thromboxane A₂,
 - Thromboxane A₂ is a potent Aggregator of Platelets
 - Thromboxane A₂ is also a Vasoconstrictor
- Aspirin also Inhibits Cyclooxygenase involve in the production of Prostacyclin (PGI₂) in Endothelial cells

- Prostacyclin I_2 prevents Platelet Aggregation
 - Prostacyclin I_2 is a Vasodilator
- Unlike Platelets, Endothelial cells are able to very quickly regenerate Cyclooxygenase and continue production of Prostacyclin
- The overall effect of Aspirin action is:
 - Inhibition of Thromboxane A_2 produced by Platelets, and
 - Consequently the Inhibition of Platelet Aggregation

TABLE 1

Molecules synthesized by endothelial cells that play a role in the regulation of thrombosis and fibrinolysis.¹

Molecule	Action
ADPase (an ectoenzyme)	Degrades ADP (an activator of platelets) to AMP + P _i .
Endothelium-derived relaxing factor (nitric oxide)	Inhibits platelet adhesion and activation by elevating levels of cyclic GMP.
Heparan sulfate (a glycosaminoglycan)	Anticoagulant; combines with antithrombin III to inhibit thrombin.
Prostacyclin (PGI ₂ , a prostaglandin)	Inhibits platelet aggregation by elevating levels of cAMP.
Thrombomodulin (a glycoprotein)	Binds protein C, which is then cleaved by thrombin to yield activated protein C; this in combination with protein S degrades factors Va and VIIIa, limiting their actions.
Tissue plasminogen activator (t-PA, a protease)	Activates plasminogen to plasmin, which digests fibrin; the action of t-PA is opposed by plasminogen activator inhibitor-1 (PAI-1).

¹Adapted from Wu KK: Endothelial cells in hemostasis, thrombosis and inflammation. Hosp Pract (Apr) 1992;27:145.

TABLE 2

Numerical system for nomenclature of blood clotting factors. The numbers indicate the order in which the factors have been discovered and bear no relationship to the order in which they act.

Factor	Common Name
I	Fibrinogen
II	Prothrombin
III	Tissue factor
IV	Ca ²⁺
V	Proaccelerin, labile factor, accelerator (Ac-) globulin
VII ¹	Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin
VIII	Antihemophilic factor A, antihemophilic globulin (AHG)
IX	Antihemophilic factor B, Christmas factor, plasma thromboplastin component (PTC)
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin stabilizing factor (FSF), fibrinoligase

¹There is no factor VI.

The functions of the proteins involved in blood coagulation.

Zymogens of serine proteases

Factor XII	Binds to exposed collagen at site of vessel wall injury; activated by high-MW kininogen and kallikrein.
Factor XI	Activated by factor XIIa.
Factor IX	Activated by factor XIa in presence of Ca ²⁺ .
Factor VII	Activated by thrombin in presence of Ca ²⁺ .
Factor X	Activated on surface of activated platelets by prothrombinase complex (Ca ²⁺ , factors VIIIa and IXa) and by factor VIIa in presence of tissue factor and Ca ²⁺ .
Factor II	Activated on surface of activated platelets by prothrombinase complex (Ca ²⁺ , factors Va and Xa). [Factors II, VII, IX, and X are Gla-containing zymogens.] (Gla = γ -carboxyglutamate.)

Cofactors

Factor VIII	Activated by thrombin; factor VIIIa is a cofactor in the activation of factor X by factor IXa.
Factor V	Activated by thrombin; factor Va is a cofactor in the activation of prothrombin by factor Xa.
Tissue factor (factor III)	A protein exposed on the surface of stimulated endothelial cells that requires phospholipid to act as a cofactor for factor VII.

Fibrinogen

Factor I	Cleaved by thrombin to form fibrin clot.
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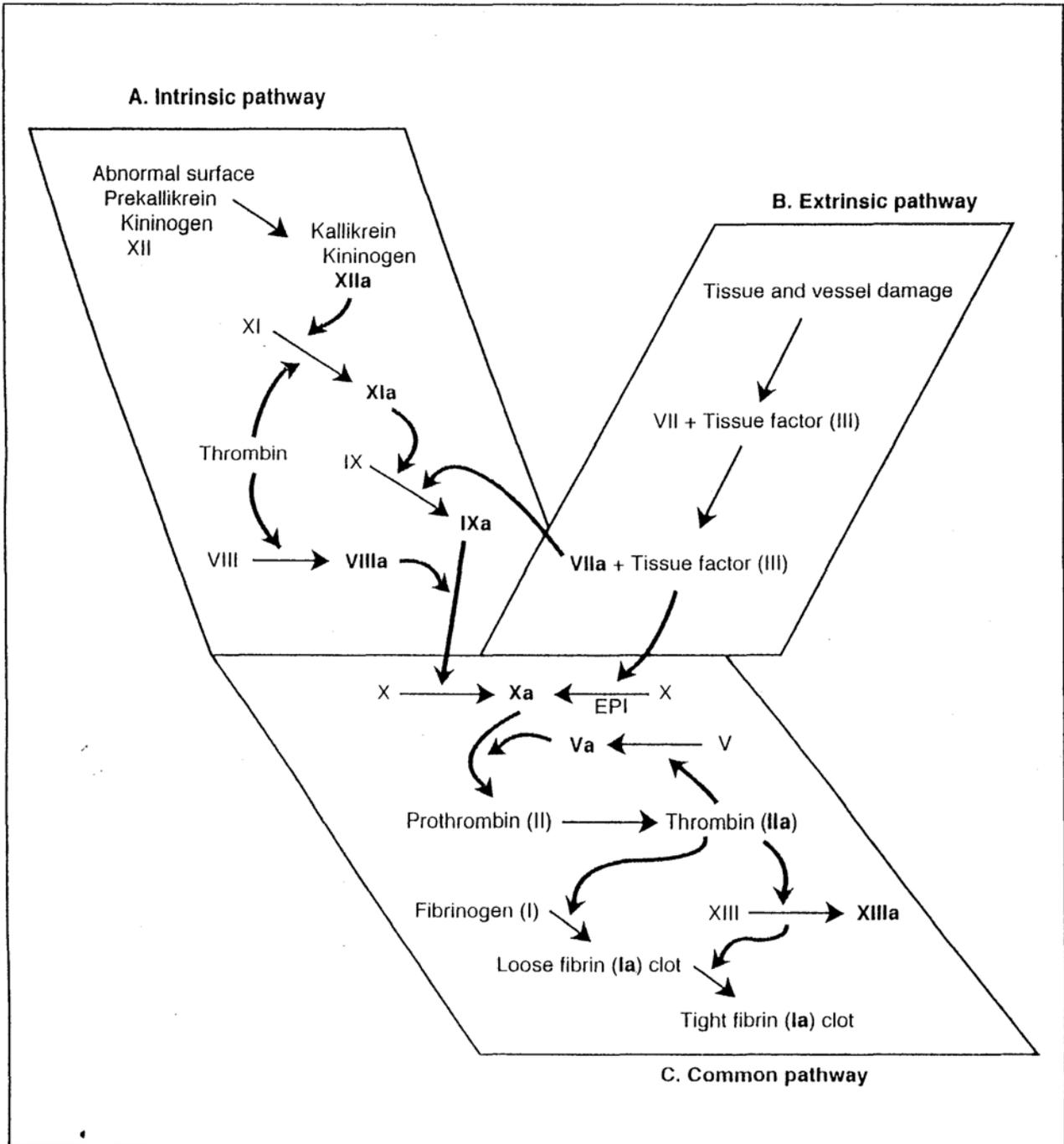
Thiol-dependent transglutaminase

Factor XIII	Activated by thrombin in presence of Ca ²⁺ ; stabilizes fibrin clot by covalent cross-linking.
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Regulatory and other proteins

Protein C	Activated to protein Ca by thrombin bound to thrombomodulin; then degrades factors VIIIa and Va.
Protein S	Acts as a cofactor of protein C; both proteins contain Gla (γ -carboxyglutamate) residues.
Thrombomodulin	Protein on the surface of endothelial cells; binds thrombin, which then activates protein C.

Fig. 1



Blood clotting pathways. Blood clot formation arises from a series of zymogen activation cascades. Two cascade pathways, the *intrinsic* and *extrinsic*, each activate a *common* cascade pathway, which leads to clot formation. The active forms in the cascades are *bold*.

Abbreviations: High MW kininogen: high molecular weight kininogen. **F**: factor, **TF**: tissue factor, Ca^{2+} : calcium, PF3: platelet phospholipid.

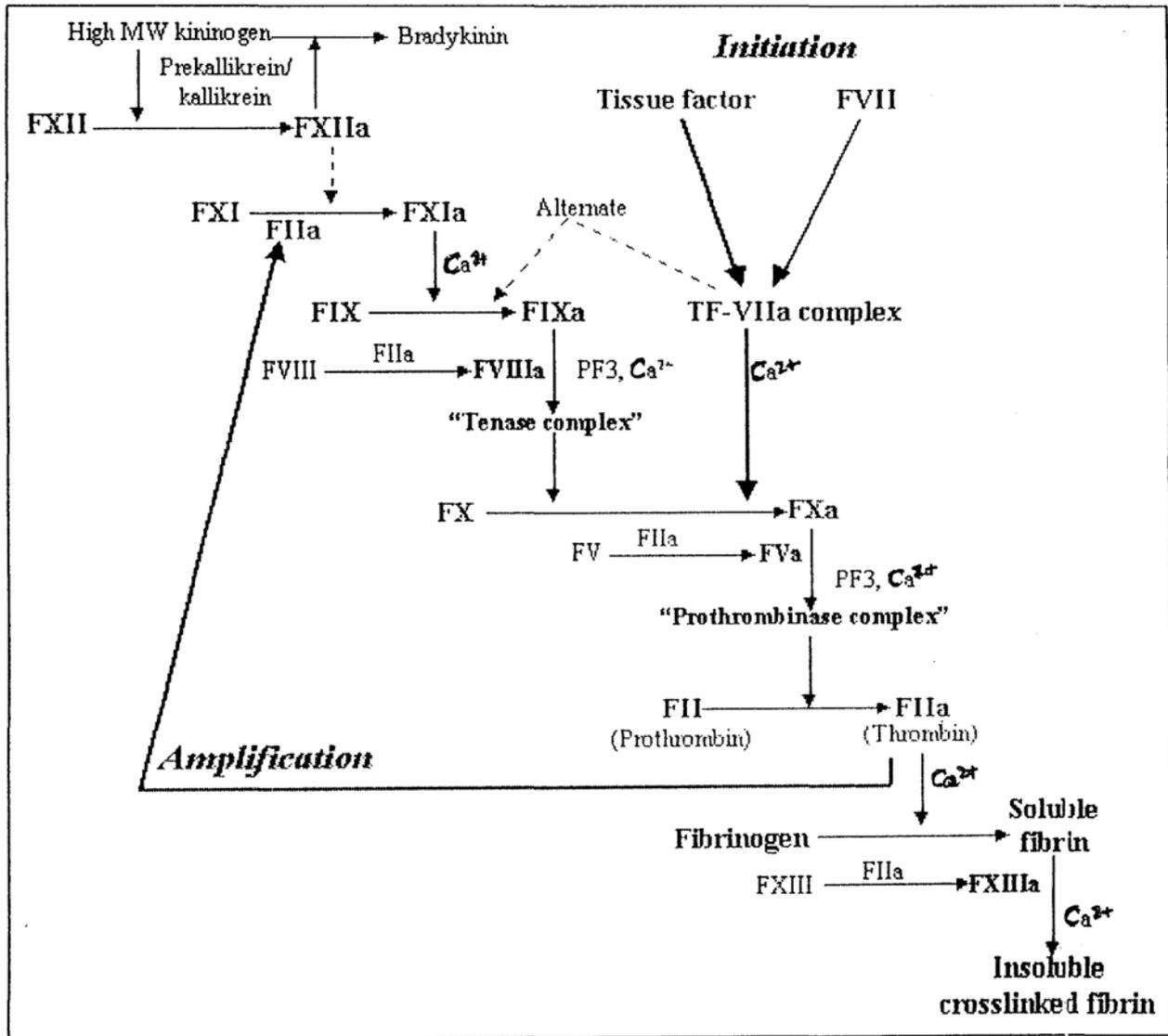
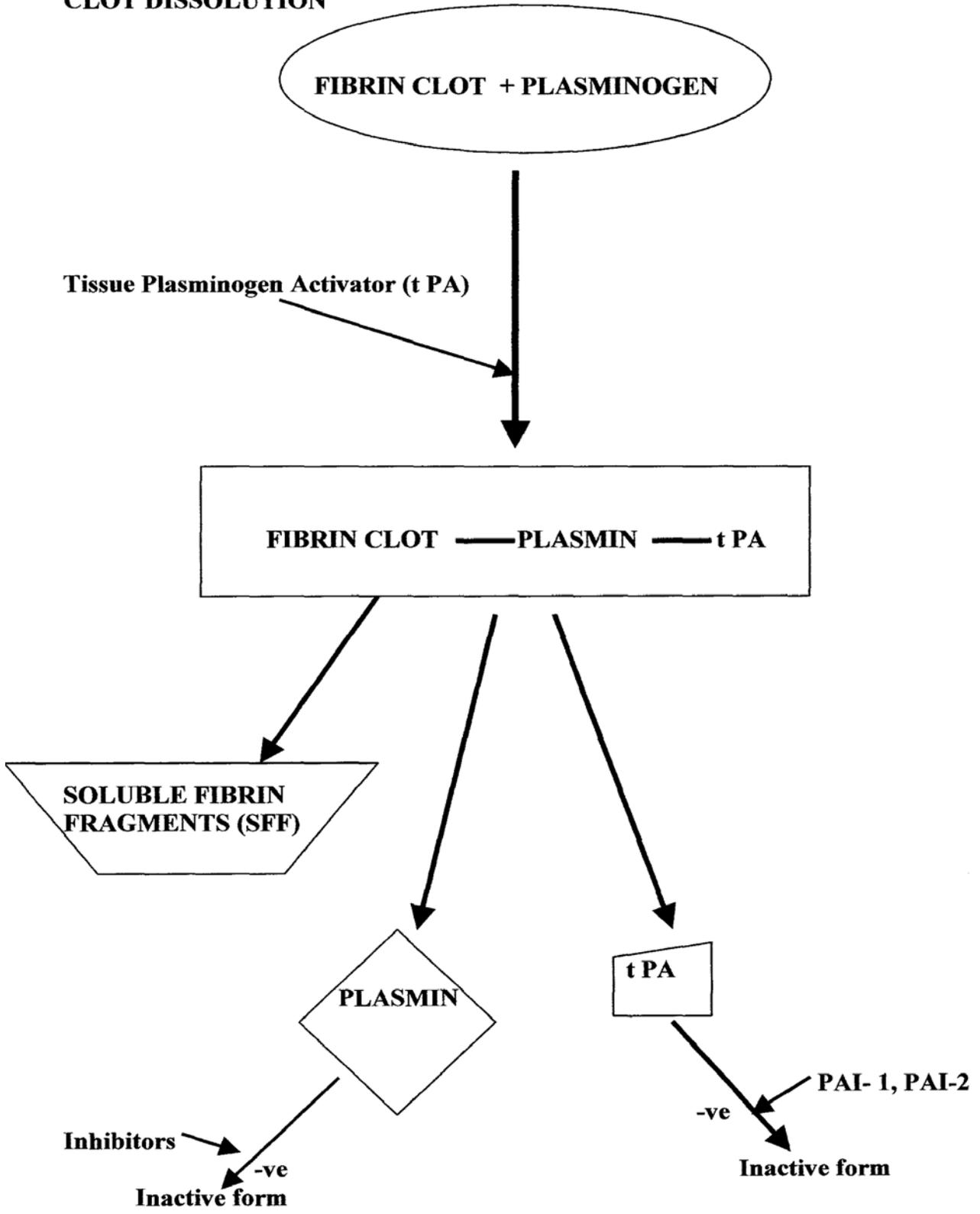


Fig: 2.

COAGULATION CASCADE

CLOT DISSOLUTION



Schematic diagram of reactions involved in Clot Dissolution

Fig. 3a

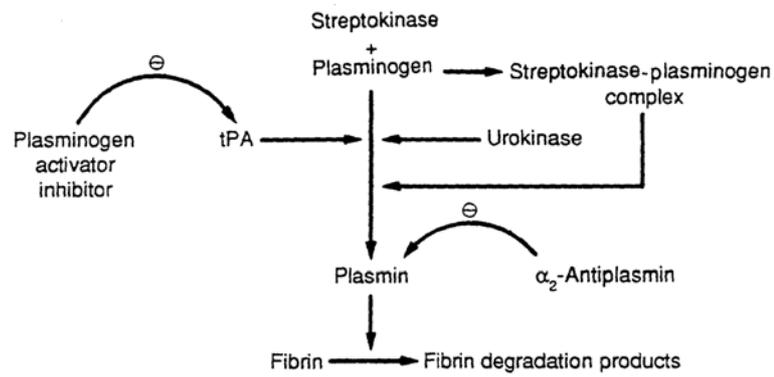


Fig. 3b

Scheme of sites of action of streptokinase, tissue plasminogen activator (t-PA), urokinase, plasminogen activator inhibitor, and α_2 -antiplasmin (the last two proteins exert inhibitory actions). Streptokinase forms a complex with plasminogen, which exhibits proteolytic activity; this cleaves some plasminogen to plasmin, initiating fibrinolysis.