THROMBOCYTE FUNCTION: HEMOSTASIS

Hematopoietic System and Disorders (MED202)

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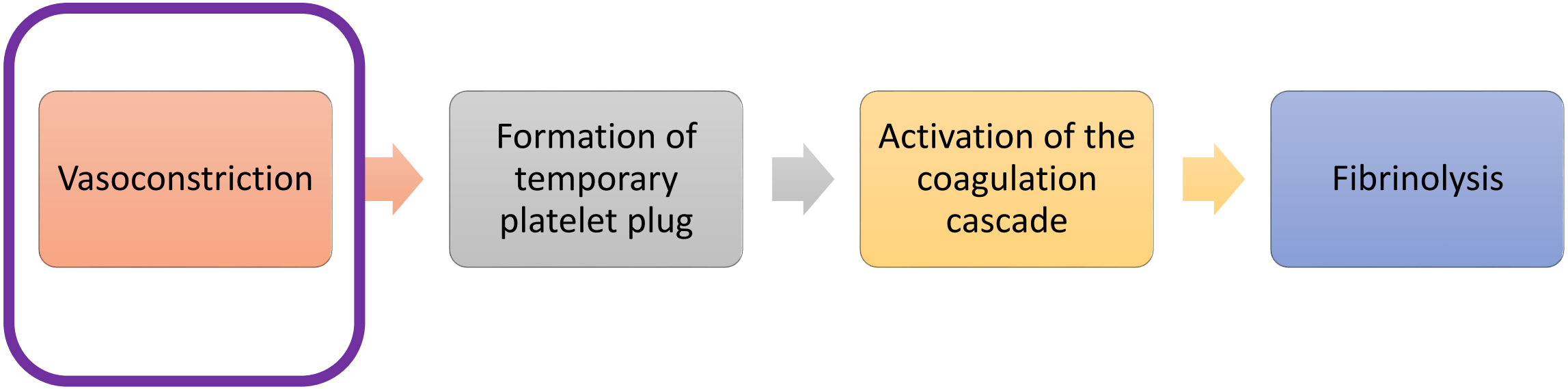
Lecture Outline

- Mechanism of hemostasis
  - Vasoconstriction
  - Formation and regulation of thrombocyte-fibrin plug
  - Mechanisms of blood coagulation (extrinsic and intrinsic pathways)
  - Mechanisms of fibrinolysis
- Anticoagulant mechanisms
- Procoagulant-anticoagulant factors

Functional characteristics of platelets

- ✓**Actin** and **myosin** molecules, and **thrombosthenin** can cause the platelets to contract
- ✓Residuals of both the ***endoplasmic reticulum*** and the **Golgi apparatus** that synthesize various enzymes and especially store large quantities of calcium ions.
- ✓Mitochondria and enzyme systems that are capable of forming **ATP** and **ADP**
- ✓Enzyme systems that synthesize **prostaglandins**, which are local hormones that cause many vascular and other local tissue reactions
- ✓**Fibrin-stabilizing factor**, in relation to blood coagulation
- ✓A **growth factor** that causes vascular endothelial cells, vascular smooth muscle cells, and fibroblasts to multiply and grow, thus helping repair of damaged vascular walls

Hemostasis: Preventing blood loss, stop bleeding

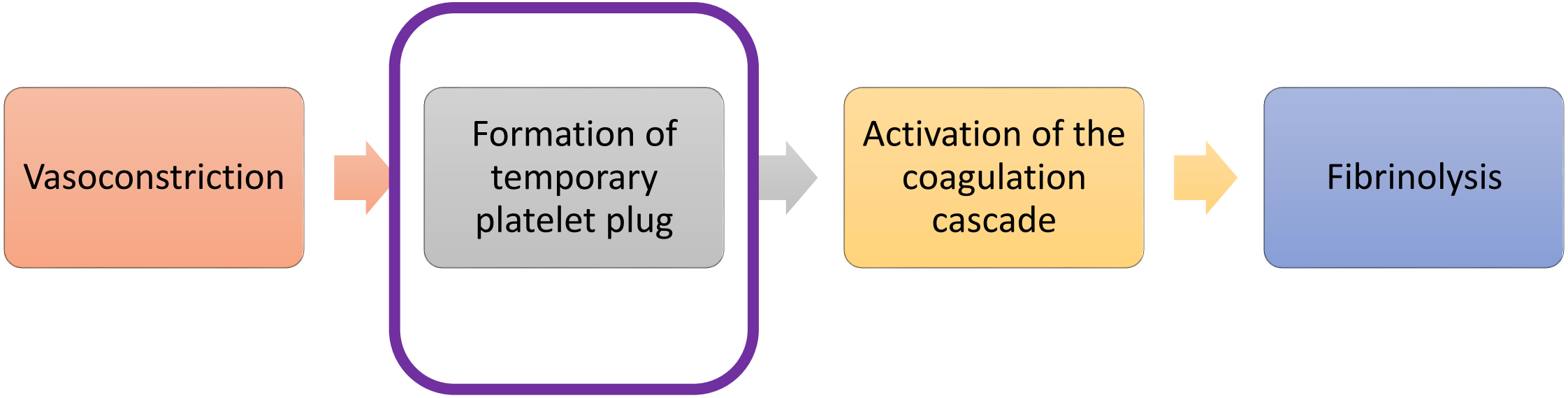


1. Vasoconstriction

- ✓Occurs immediately after a blood vessel has been damaged.
- ✓Is the fastest occurring stage
- ✓Trauma to the vessel causes smooth muscle in the vessel wall to contract.
- ✓Reduces the flow of blood from the ruptured vessel.

Vasoconstriction

- ✓Perfomed by
  - Platelet-derived vasoconstrictor agents (eg. Thromboxane A_2) attached to the damaged area
  - Mechanical stimulation of the perivascular nerves
- ✓The more severely a vessel is traumatized, the greater the degree of vascular spasm.
- ✓Can last for many minutes or even hours


Vasoconstriction
Formation of temporary platelet plug
Activation of the coagulation cascade
Fibrinolysis

2. Formation of temporary platelet plug

- In physiological conditions platelet's surface is a coat of **glycoproteins** that repulses adherence to normal endothelium.
- However, the same coat causes adherence to injured areas of the vessel wall
- When platelets come in contact with a damaged vascular surface, they rapidly change their own characteristics drastically.
  - Especially with collagen fibers in the vascular wall

Formation of temporary platelet plug

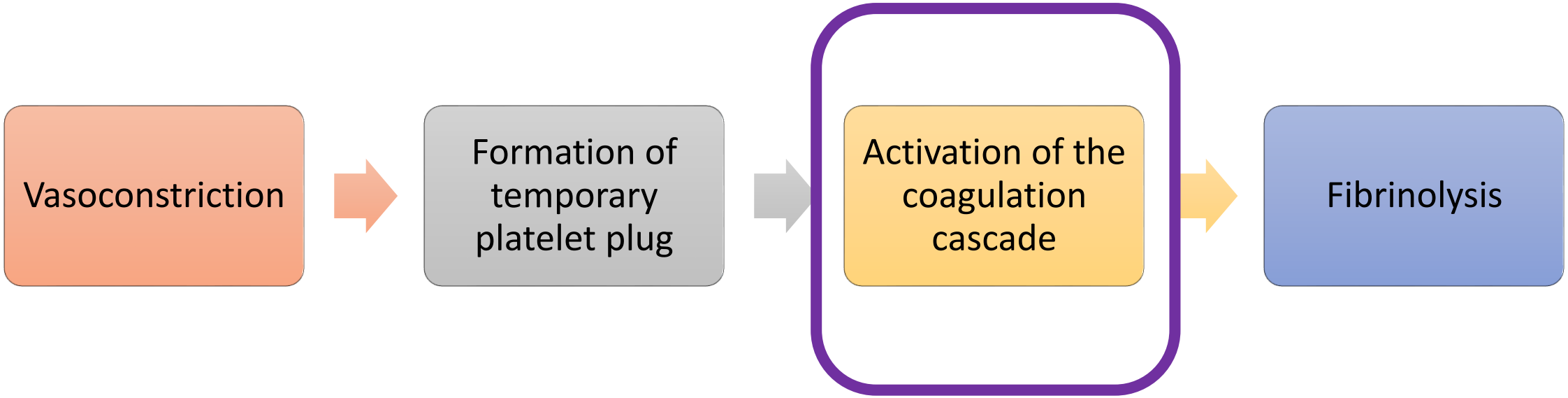
- Platelets begin to swell
- Assume irregular forms with numerous irradiating pseudopods protruding from their surfaces
- Contractile proteins contract forcefully and cause the release of granules that contain multiple active factors
  - TXA_2, ADP
- Become sticky
- Adhere to collagen in the tissues and to a protein called *von Willebrand factor (vWF)* that leaks into the traumatized tissue from the plasma

Formation of temporary platelet plug

- The ADP and TXA_2 in turn
  act on nearby platelets to activate them as well
- The stickiness of these additional platelets causes them to adhere to the original activated platelets
- **Platelet plug**

Formation of temporary platelet plug

- The plug is loose at first, but it is usually successful in blocking blood loss if the vascular opening is small.
- During the subsequent process of blood coagulation, ***fibrin threads*** form
- Fibrin threads attach tightly to the platelets, thus constructing an unyielding plug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3. Activation of Blood Coagulation Cascade

- The clot begins to develop
  - in 15 to 20 s if the trauma to the vascular wall has been severe
  - in 1 to 2 min if the trauma has been minor
  - Within 3 to 6 minutes after rupture of a vessel, the entire opening or broken end of the vessel is filled with
  - 20 minutes - an hour, the clot retracts, which closes the vessel still further

- Activator substances from
  - injured vascular wall
  - Platelets
  - blood proteins adhering to the traumatized vascular wall

initiate the clotting process

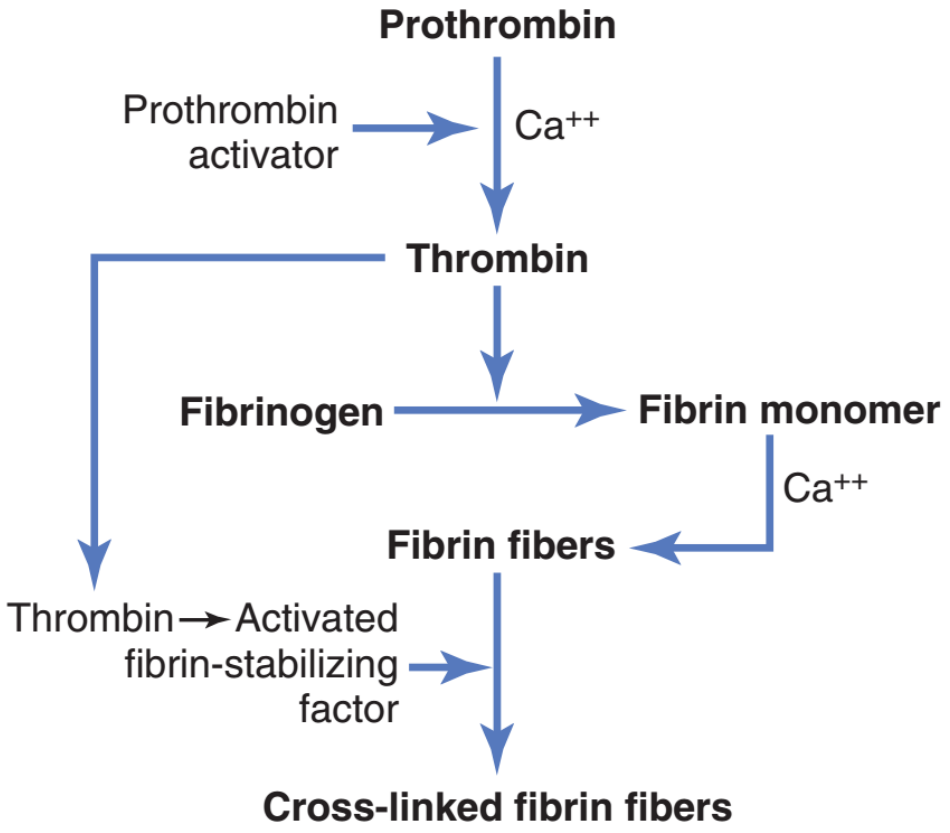
Clot formation

Clotting takes place in three essential steps:

i. Formation of **Prothrombin Activator**

ii. Conversion of prothrombin to **thrombin**

iii. Conversion of fibrinogen to **fibrin**



i. Formation of Protrombin Activator

- As a result of
  1) rupture of a blood vessel damage to special substances in the blood
  2) contact of the blood with damaged endothelial cells or with collagen and other tissue elements outside the blood vessel
- is the rate-limiting factor in causing blood coagulation
- causes conversion of prothrombin to thrombin and all the subsequent clotting steps

Prothrombin activator is generally considered to be formed in two ways

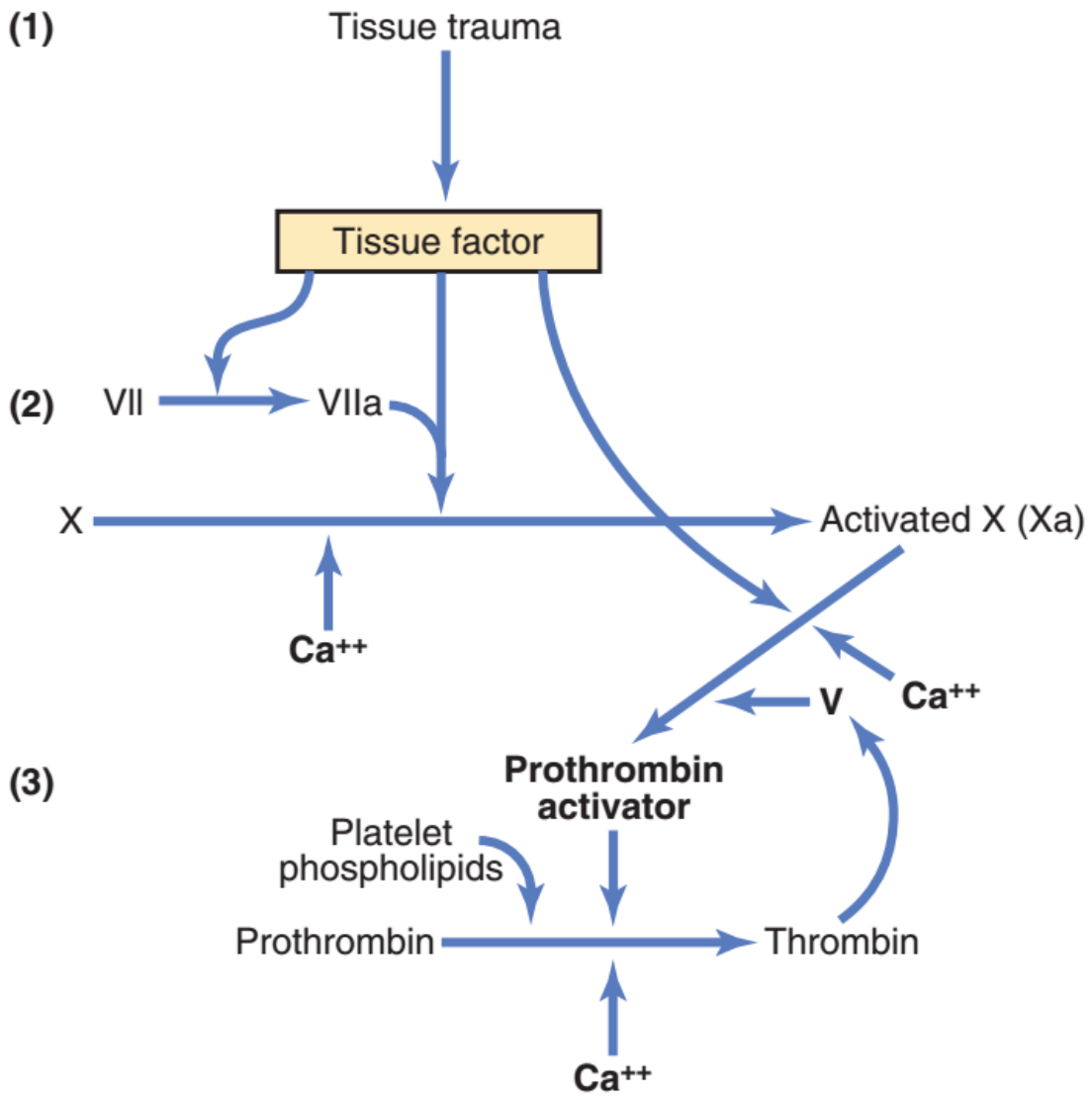
- ***Extrinsic pathway:*** begins with trauma to the vascular wall and surrounding tissues
- ***Intrinsic pathway:*** begins in the blood
- A series of different plasma proteins called *blood-clotting factors* plays a major role
- *inactive* forms of proteolytic enzymes
- active forms cause the successive cascading reactions of the clotting process
- a:indicates an active form

Clotting Factor	Synonyms
Fibrinogen	Factor I
Prothrombin	Factor II
Tissue factor	Factor III; tissue thromboplastin
Calcium	Factor IV
Factor V	Proaccelerin; labile factor; Ac-globulin (Ac-G)
Factor VII	Serum prothrombin conversion accelerator (SPCA); proconvertin; stable factor
Factor VIII	Antihemophilic factor (AHF); antihemophilic globulin (AHG); antihemophilic factor A
Factor IX	Plasma thromboplastin component (PTC); Christmas factor; antihemophilic factor B
Factor X	Stuart factor; Stuart-Prower factor
Factor XI	Plasma thromboplastin antecedent (PTA); antihemophilic factor C
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor
Prekallikrein	Fletcher factor
High-molecular-weight kininogen	Fitzgerald factor; HMWK (high-molecular-weight kininogen)
Platelets	

Extrinsic Pathway

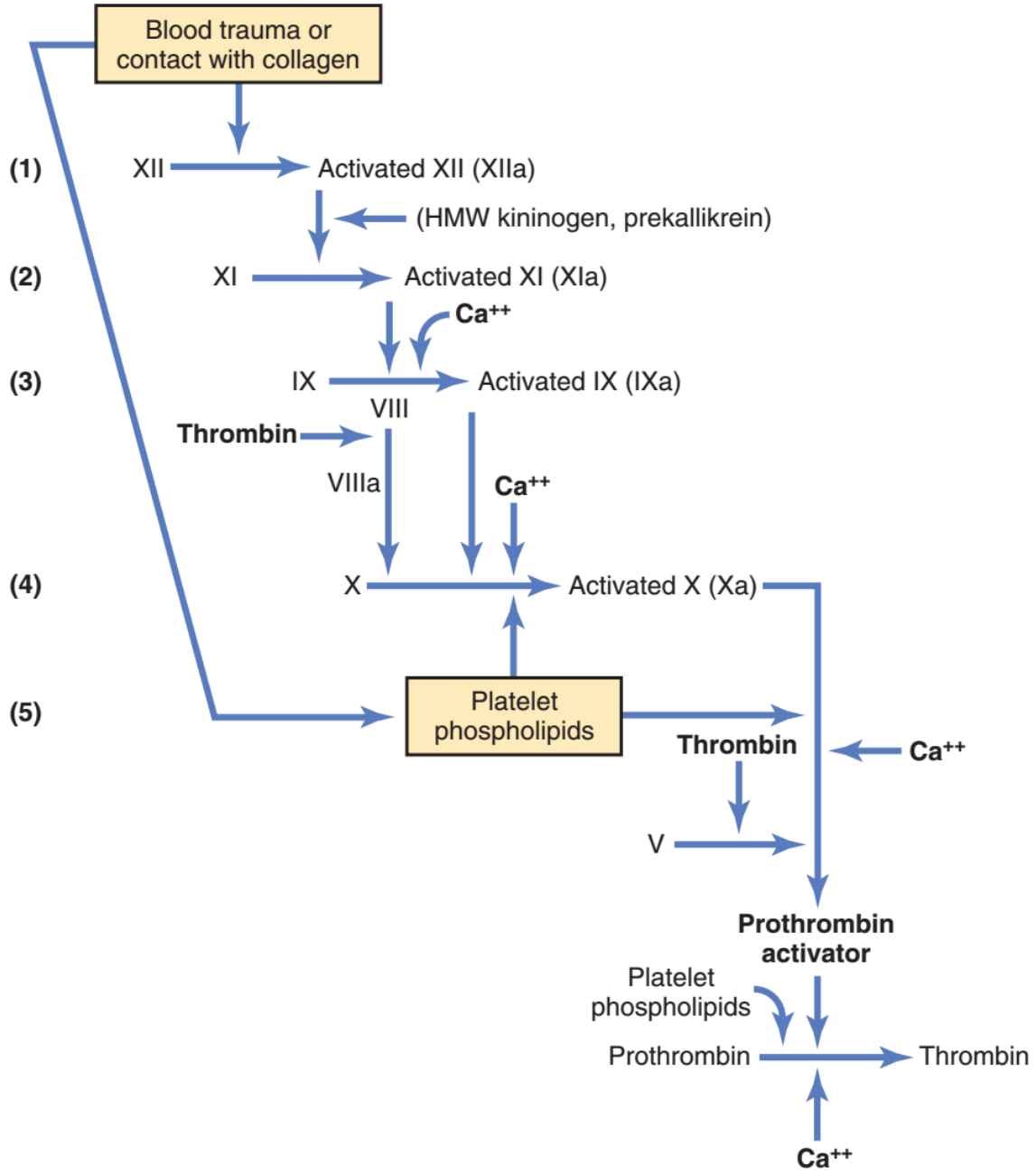
- Begins with a traumatized vascular wall or traumatized extravascular tissues that come in contact with the blood.

1) Release of tissue factor
2) Activation of Factor X
3) Formation of Prothrombin activator

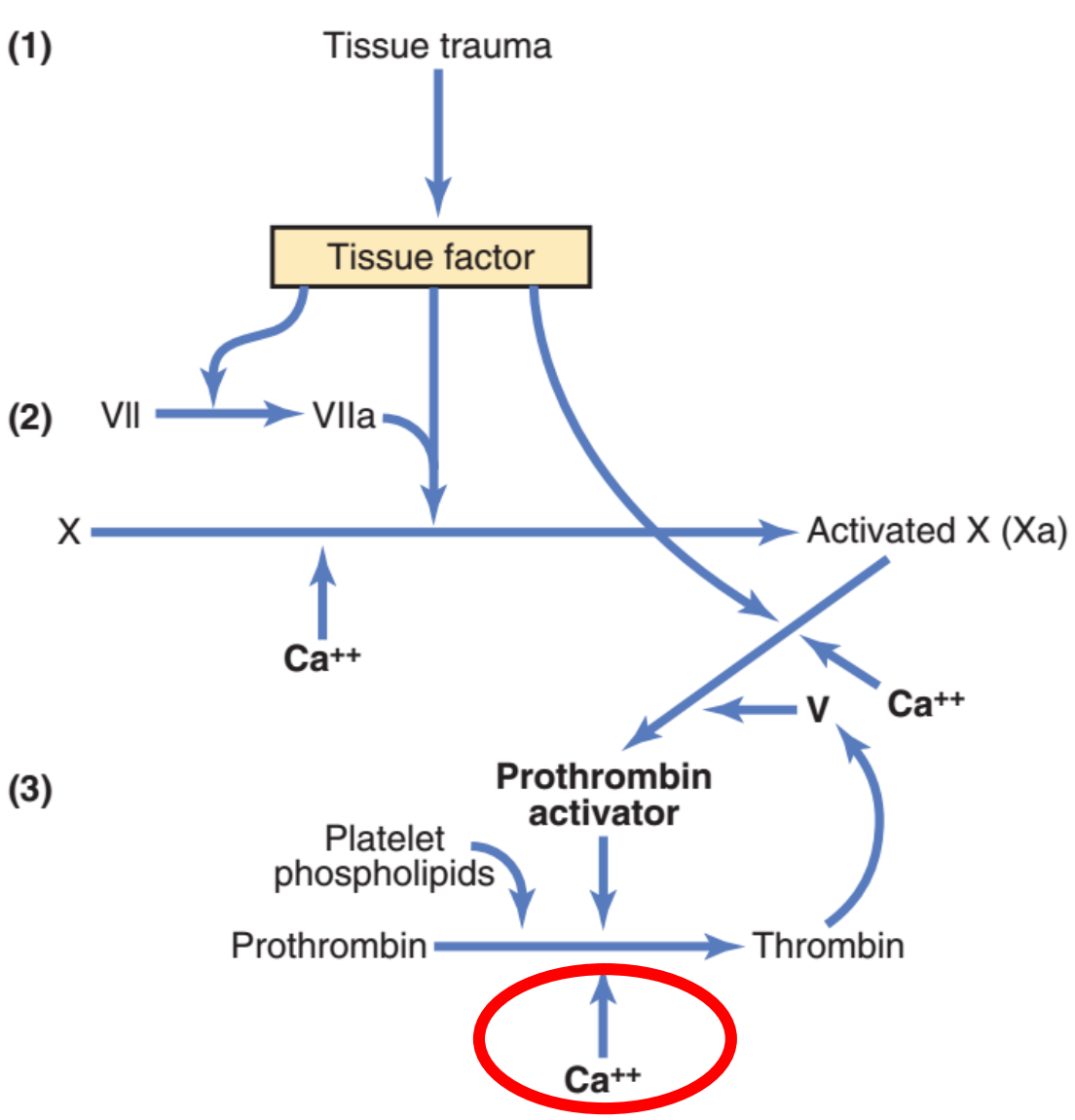


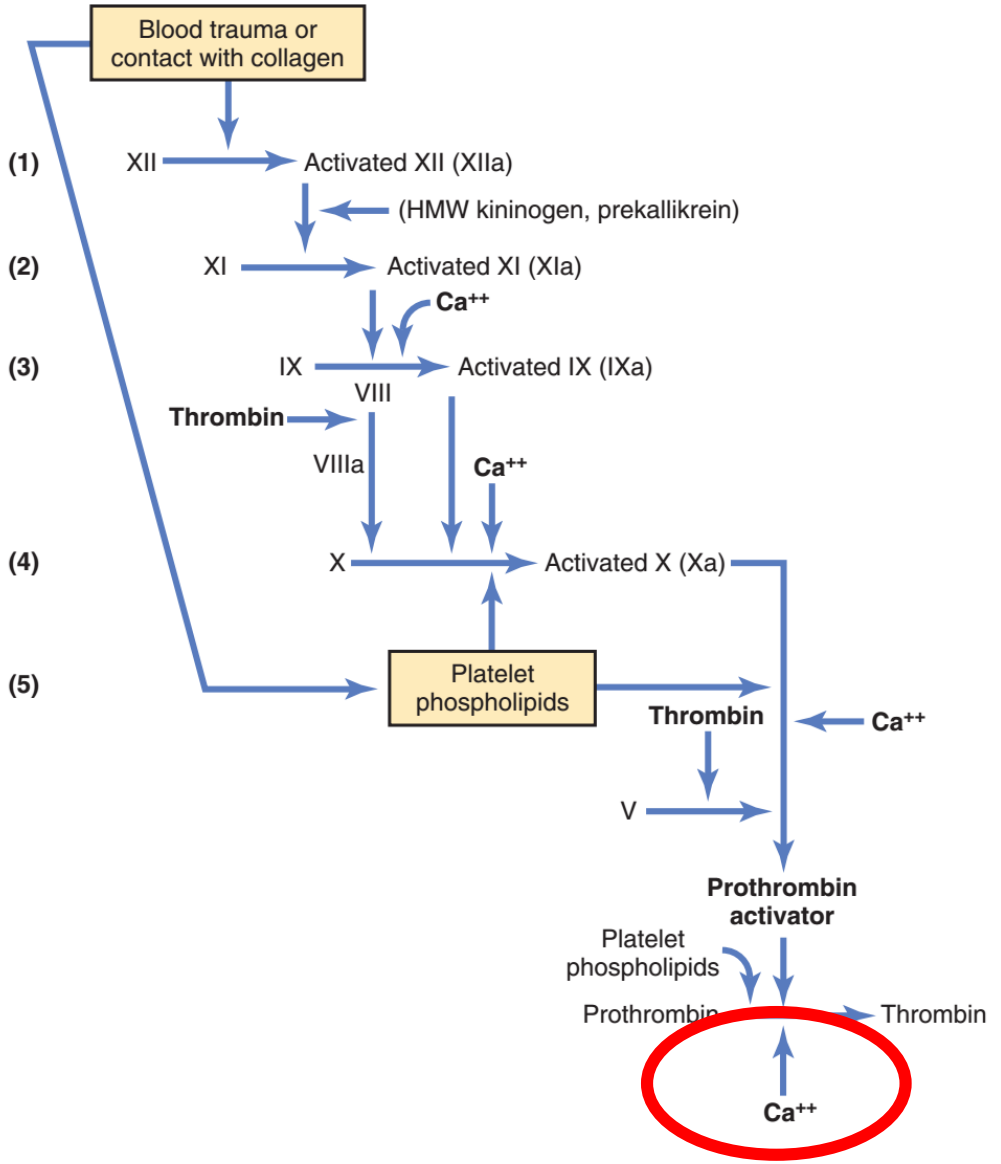
Intrinsic Pathway

- Begins with trauma to the blood or exposure of the blood to collagen from a traumatized blood vessel wall.
- Blood trauma or contact with collagen causes
  1) *activation of Factor XII*
  2) *release of platelet phospholipids.*



ii. Conversion of prothrombin to thrombin

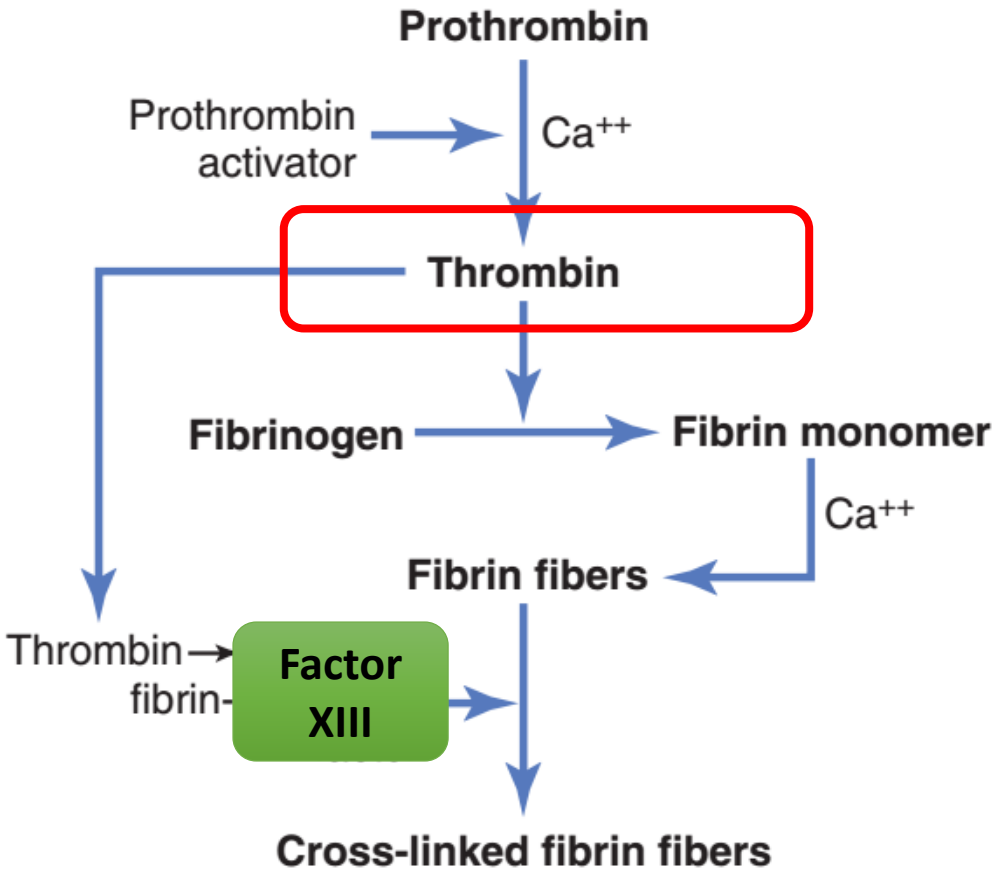


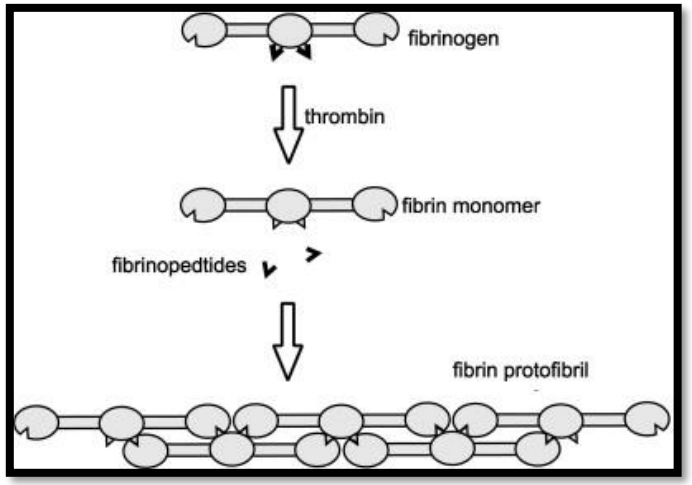


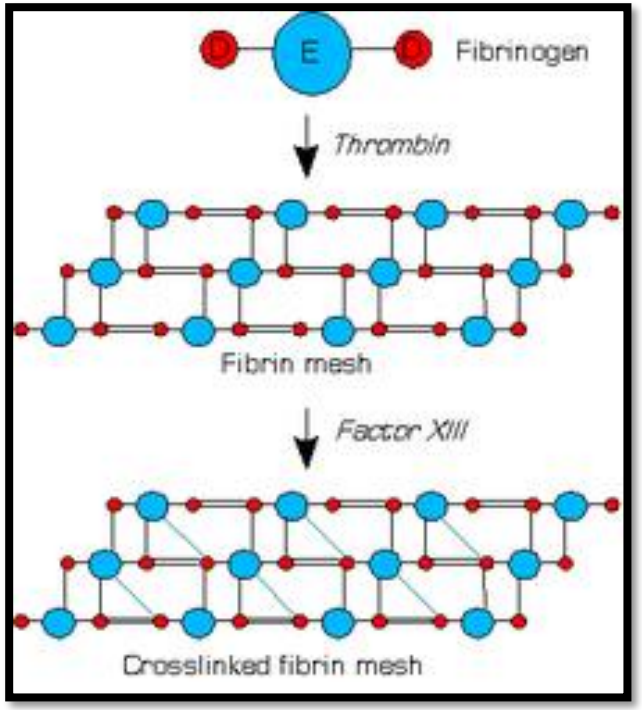
Positive Feedback of Clot Formation

- Once a blood clot has started to develop, it normally extends within minutes into the surrounding blood
- The clot initiates a positive feedback to promote more clotting.
- **the proteolytic action of thrombin:** Activates many of the clotting factors and fibrinogen

iii. Conversion of fibrinogen to fibrin



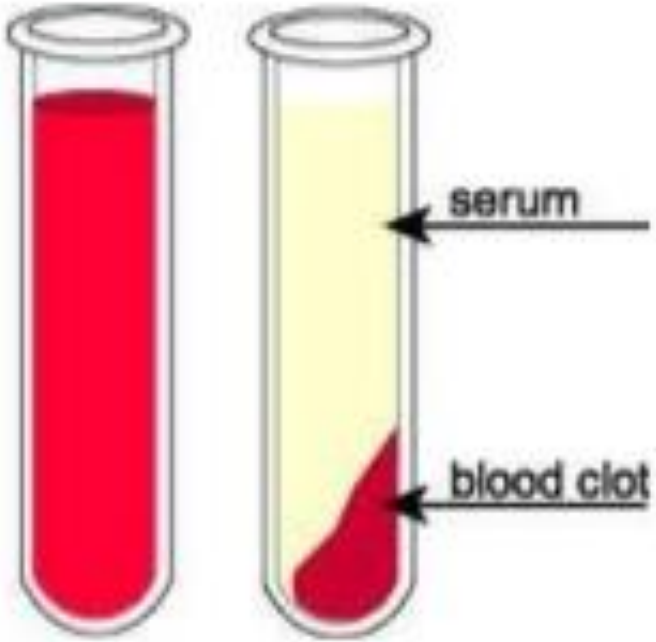


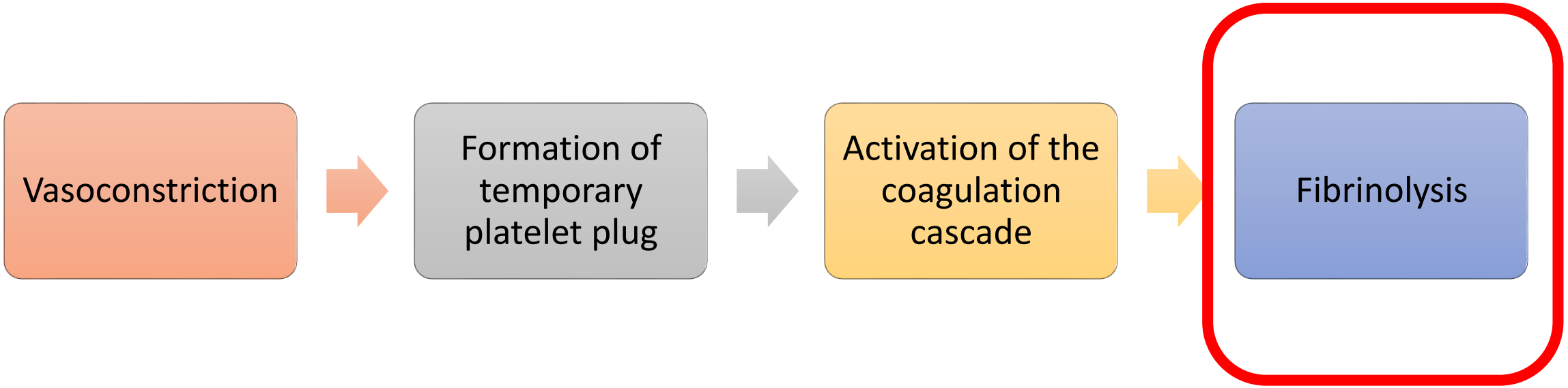


Stabilization of fibrin is maintained by formation of covalent cross-bridges, which is catalyzed by Factor XIII and Calcium

Clot retraction

- Contraction of platelets trapped within the clot shrinks the fibrin meshwork pulling the edges of the damaged vessel closer together: Facilitation of wound healing
- Contractil proteins of the platelets
- Release of fibrin stabilizing factor (**Factor XIII**)
- Serum is squuezed from the clot

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4) Fibrinolysis

- ✓ Dissolution of the clot
- ✓ Conversion of plasminogen to **plasmin** by lysosomal enzymes and Plasminogen Activators (endothelial cells / blood /kidney)
- ✓ Fibrin polymers → Fibrin dimers
- ✓ Plasmin also degrades Factor Va, VIIIa, and GPIIb

Plasminogen activators:

- Adrenaline
- Urokinase
- Thrombomodulin-Thrombin complex
- Kallikrein
- t-PAs

Why blood does not clot in circulation?

- Endothelial surface
  - Smoothness
  - Layer of glycocalyx
- Velocity of circulation
- Natural anticoagulants
- Activation of fibrinolytic sytem
- Liver removes activated clotting factors

Natural anticoagulant mechanisms

Thrombomodulin

Vascular endothelium secretes **thrombomodulin**, the protein that binds thrombin

Formation of **thrombomodulin-thrombin complex**

Activates **Protein C**

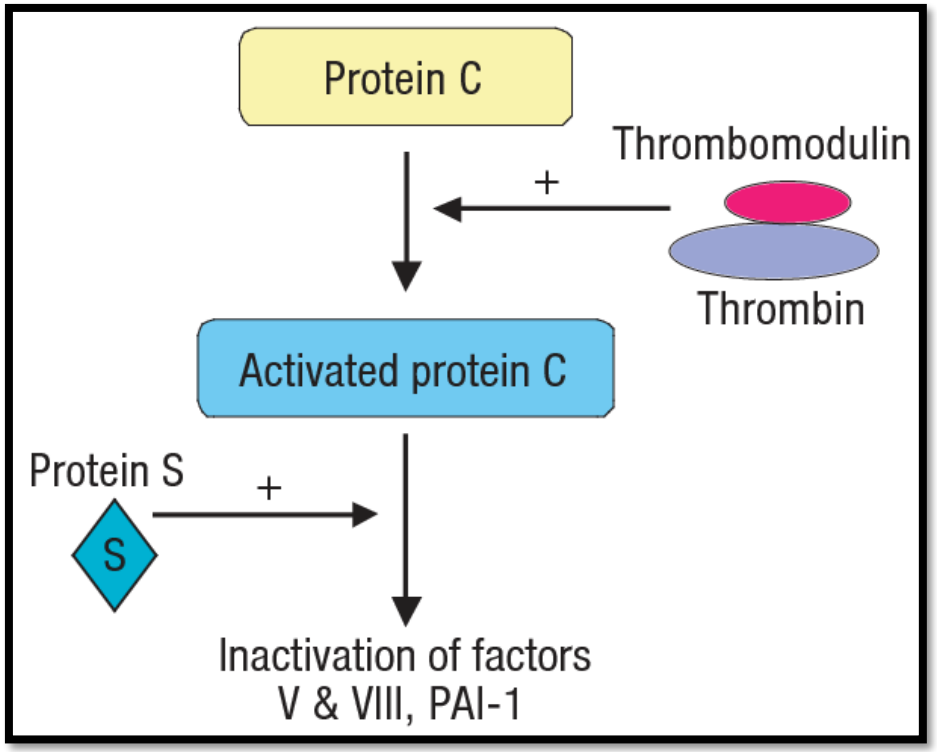
1) Inactivates **TPA-I**

2) Together with **Cofactor S,** Protein C inhibits the Factor V and Factor VIII

Antithrombin III

Heparin secreted by basophils and mast cells activates **Antithrombin III**

Dissolution of the intravascular clot



Antrithrombin III, Plasminogen, Protein C and Protein S deficiencies or abnormalities causes to therombotic disorders.

Anticoagulants		Procoagulants	
Heparin (Natural)	Activates antithrombin III	**Thrombin**	
	Inactivates Factors IX, X, XI, and XII	**Sodium and Calcium alginat**	Activates Factor XII
Antithrombin or heparin cofactors (Natural)		**Oxidized sellulose**	Activates Factor XII
Protein C (Natural)			
Coumarin derivatives Dicumarol and Warfarin	Inhibitor of Vit K*		
EDTA	Sequester Ca2+		
Oxalate	Precipitate Ca2+		
Citrate	Converts Ca2+ to insoluble calcium citrate		
Cold, Smooth surfaces, Silicon**			

*Vit K is esential for the formation of Factor II, VIII, IX, X, protein C, and Protein S. Synthesized by the bacteria in the column

** Prevents activation of Factor XII.

Hemostatic Function Tests

- Bleeding time:
  - The interval between the skin puncture and spontaneous, unassisted stoppage of bleeding.
  - Duke's method
  - Normal: **1-5 min**
- Clotting time:
  - The interval between entry of blood into capillary tube and formation of fibrin threads
  - Wright's capillary glass tube
  - Normal: **3-6 min**
- Prothrombin time:
  - Normal: **15-20 sec**