Lecture 17. Types and mechanisms of hemostasis. Physiology of thrombocytes

Blood Coagulation

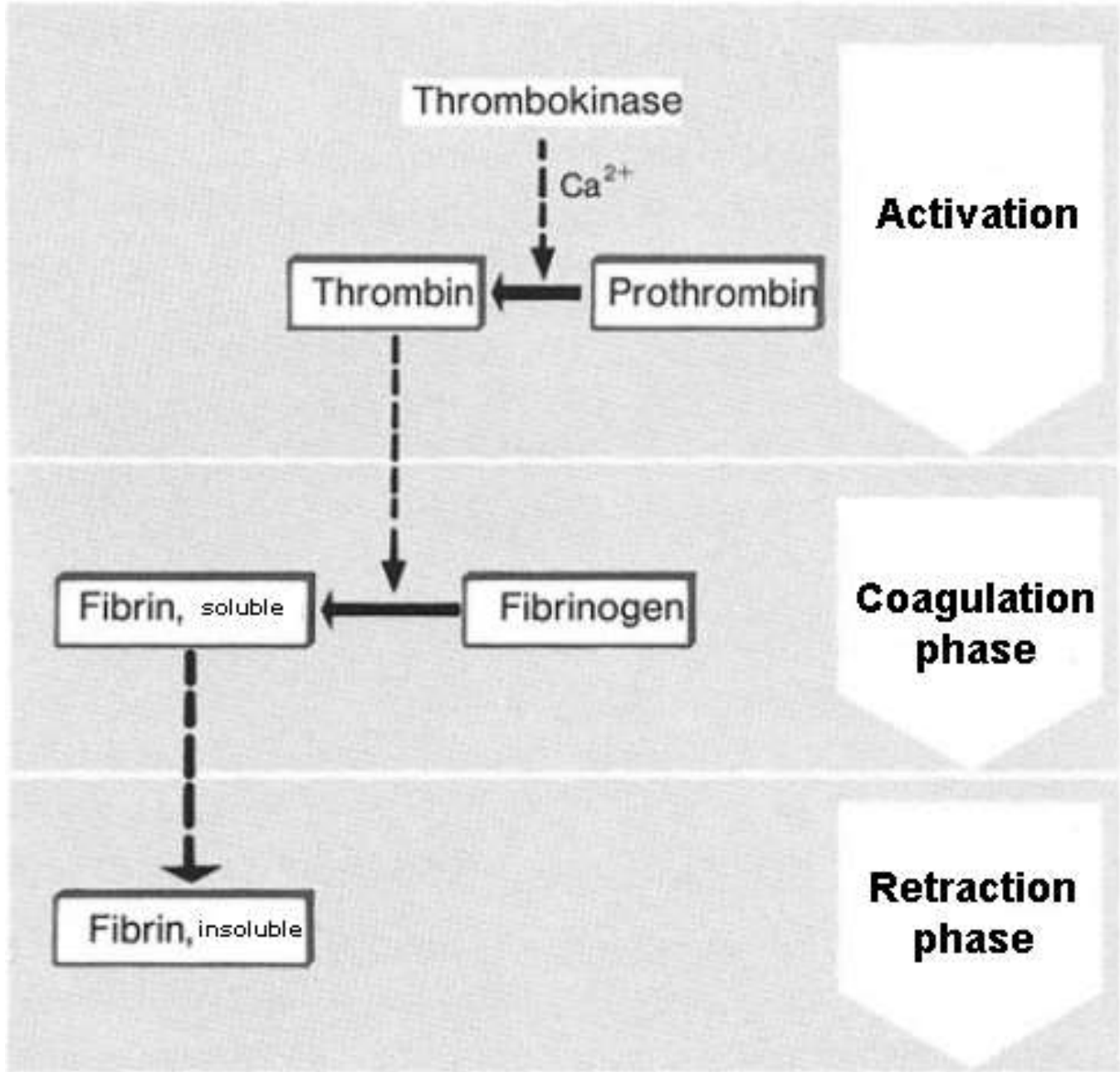
Such definition was introduced by Dr. Moravitz in 1906.

According to it, hemostasis has the following stages:

I. substance or complex of substances called **prothrombin activator** **is formed** by blood in response to rupture of the vessel or damage of the tissue.

II the prothrombin activator catalyzes the conversion of **prothrombin into thrombin.**

III the thrombin acts as an enzyme to convert fibrinogen into fibrin threads that enmesh platelets, blood cells, and plasma to form the clot itself.


Thrombokinase
Ca2+
Thrombin
Prothrombin
Activation
Fibrin, soluble
Fibrinogen
Coagulation phase
Fibrin, insoluble
Retraction phase

Factors of blood coagulation

Scientific Name	Common Name
Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Tissue thromboplastin
Factor IV	Calcium
Factor V	Proaccelerin
Factor VII	Proconvertin
Factor VIII	Antihemophilic factor A
Factor IX	Antihemophilic factor B (Christmas factor)
Factor X	Stuart factor
Factor XI	Plasma thromboplastin antedecent
Factor XII	Hageman factor
Factor XIII	Fibrin stabilizing factor
Factor XIV	Kininogen, Fitzgerald factor
Factor XV	Prekallikrein, Fletcher factor
Factor XVI	Kallikrein

Prothrombin activator's synonyms: thrombokinase, prothrombinase, complete thromboplastin.

Coagulation

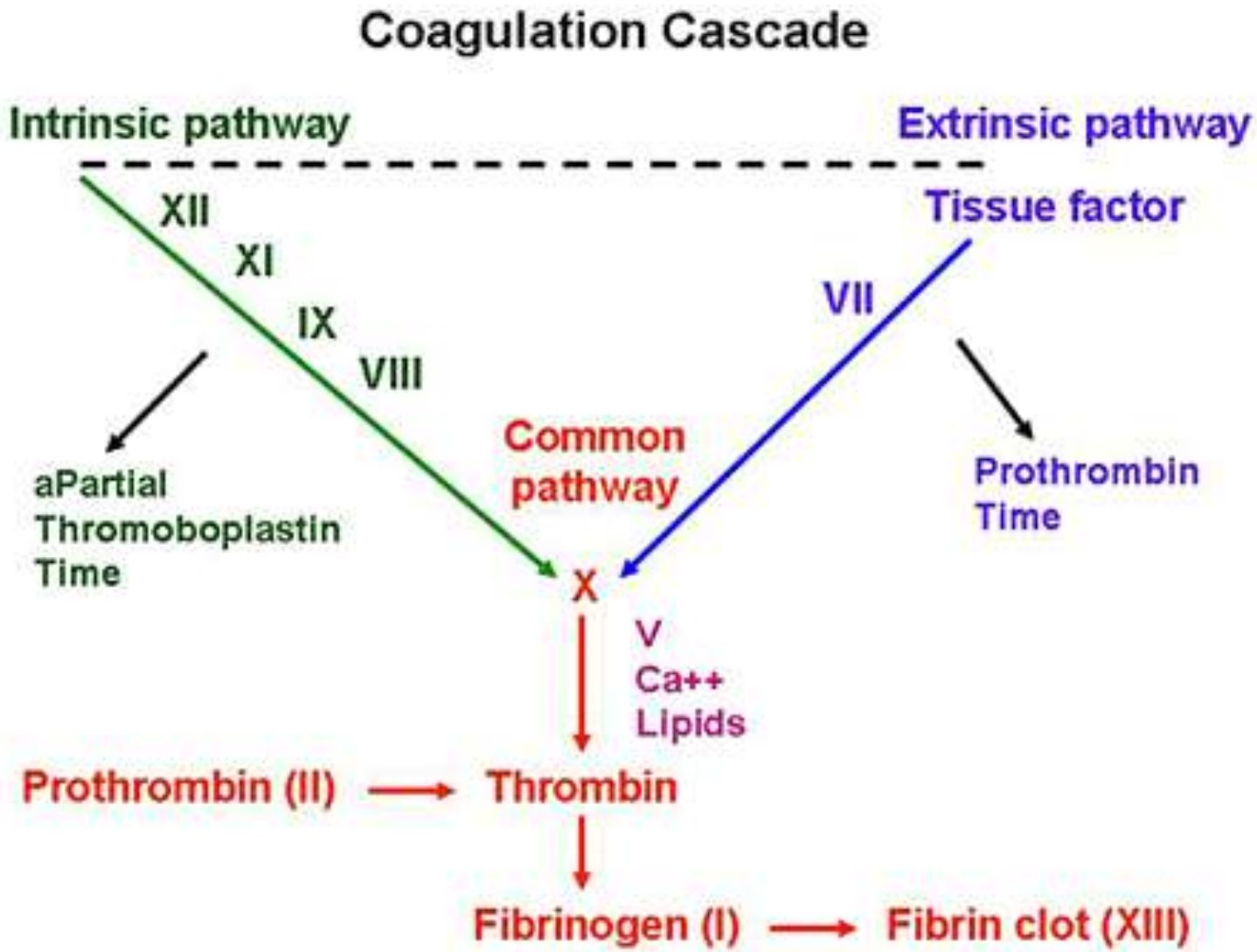
- **Coagulation factors**.
  - **Proteins found in plasma**.
  - Circulate in inactive state until tissues are injured.
  - Damaged tissues and platelets produce chemicals that begin activation of the factors.
- Result: **blood clot**. A network of threadlike **fibrin** fibers, trapped blood cells, platelets and fluid
- **Calcium (Ca^{2+})** and **Vitamin K** are required

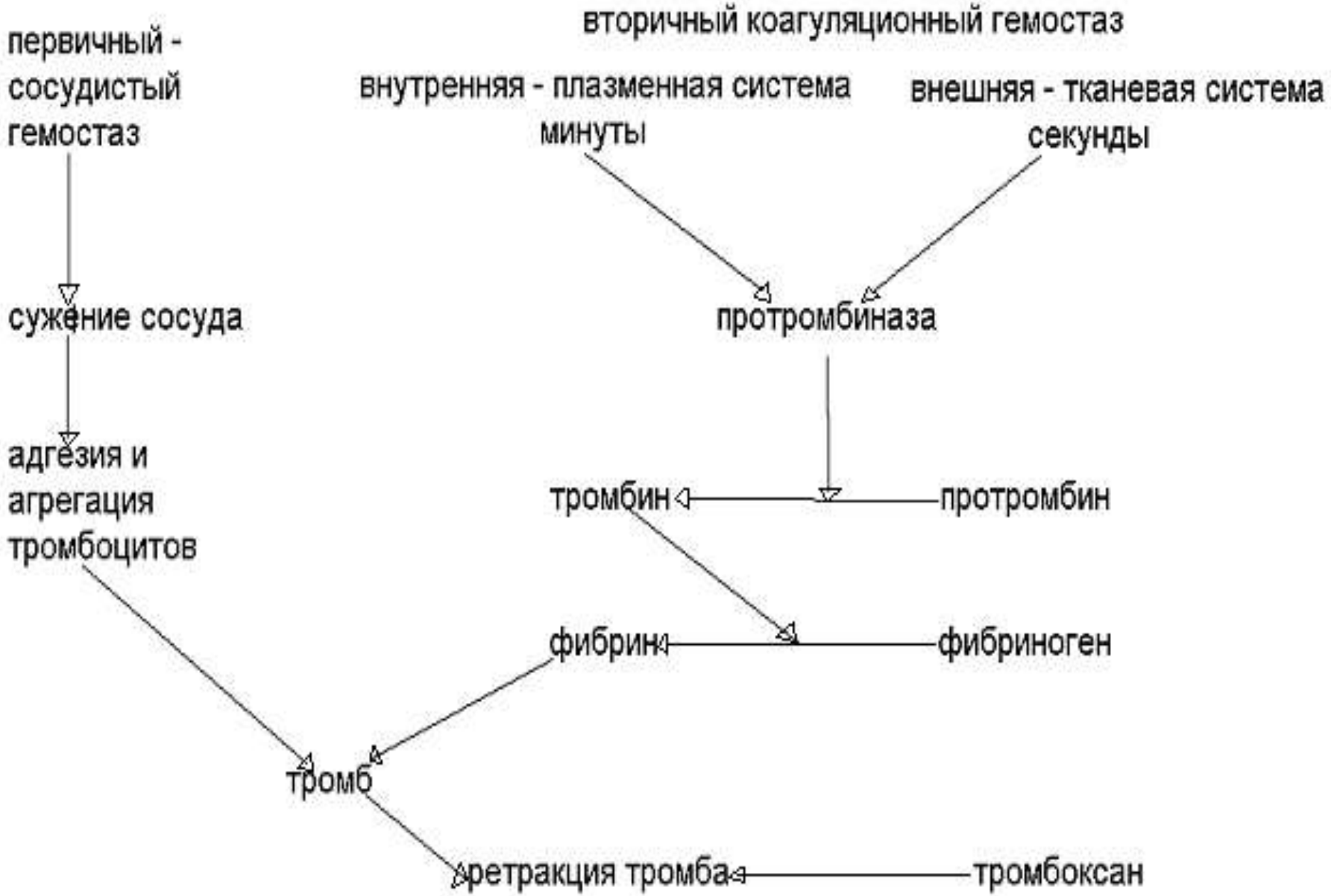
Coagulation or secondary hemostasis

This phase is included almost simultaneously with the primary. This process involves 12 coagulation factors. There are two systems:

Extrinsic pathway (tissue) - activated within a few seconds;

Intrinsic pathway (blood) - is activated within a few minutes.


Coagulation Cascade
Intrinsic pathway
Extrinsic pathway
XII
XI
IX
VIII
Tissue factor
VII
aPartial
Thromoboplastin
Time
Common
pathway
Prothrombin
Time
X
V
Ca++
Lipids
Prothrombin (II)
Thrombin
Fibrinogen (I)
Fibrin clot (XIII)


первичный - сосудистый гемостаз
вторичный коагуляционный гемостаз
внутренняя - плазменная система
минуты
внешняя - тканевая система
секунды
сужение сосуда
протромбиназа
адгезия и агрегация тромбоцитов
тромбин
протромбин
фибрин
фибриноген
тромб
ретракция тромба
тромбоксан

The extrinsic mechanism: (duration is 3-5 s)

1. Release of tissue factor and tissue phospholipids. The traumatized tissue releases two factors that set the clotting process into motions. They are:

a). **tissue factor**, which is a proteolytic enzyme, and

b). **Tissue phospholipids**, which are mainly phospholipids of the tissue cell membranes.

2. Activation of factor X to form activated factor X – role of factor VII and tissue factor.

3. Effect of activated factor X to form prothrombin activator – role of factor V.

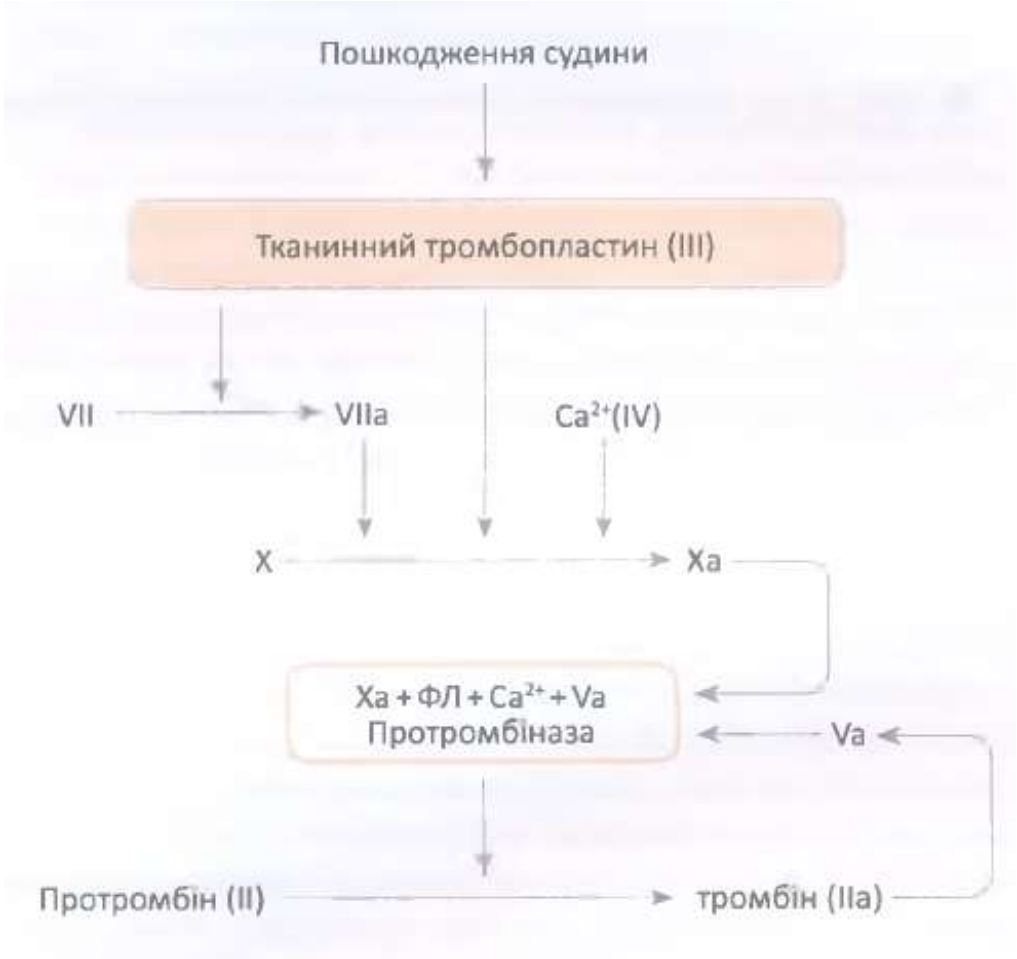
The intrinsic mechanism: (duration 3-5 min).

1. *Activation of factor XII and platelets by contact with collagen fibers and release of platelet phospholipids by blood trauma;*
2. *Activation of factor XI;*
3. *Activation of factor IX by activated factor XI;*
4. *Activation of factor X – role of factor VIII;*
5. *Action of activated factor X to form prothrombin activator –role of factor V.*

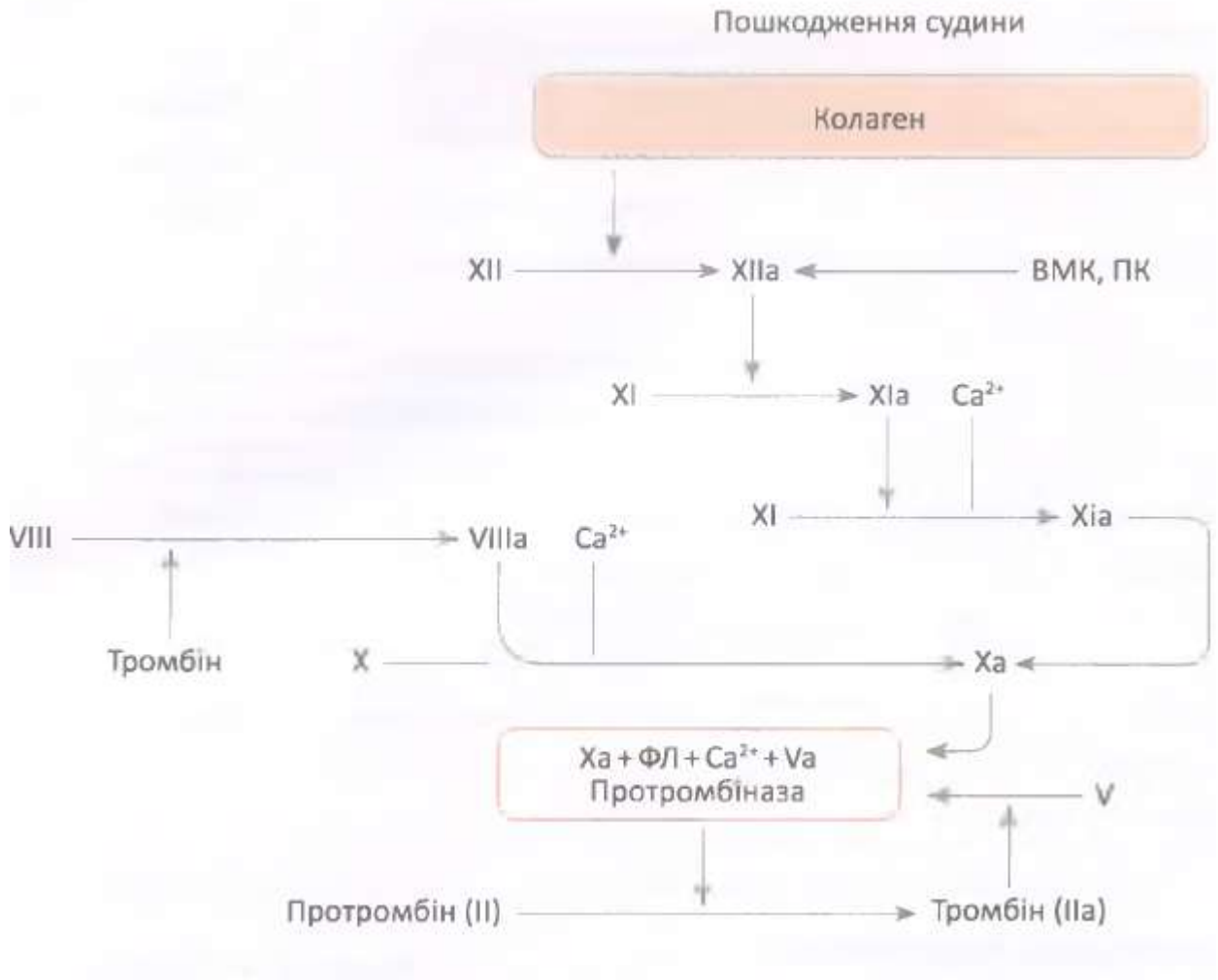
Prothrombin activator

- This is complex of activated factor X combines with factor V and platelet phospholipids

Утворення тканинної протромбінази (зовнішній шлях)



Каскад реакцій утворення кров’яної протромбінази



1. The extrinsic pathway of clotting starts with thromboplastin, which is released outside of the plasma in damaged tissue.

2. The intrinsic pathway of clotting starts when inactive factor XII, which is in the plasma, is activated by coming into contact with a damaged blood vessel.

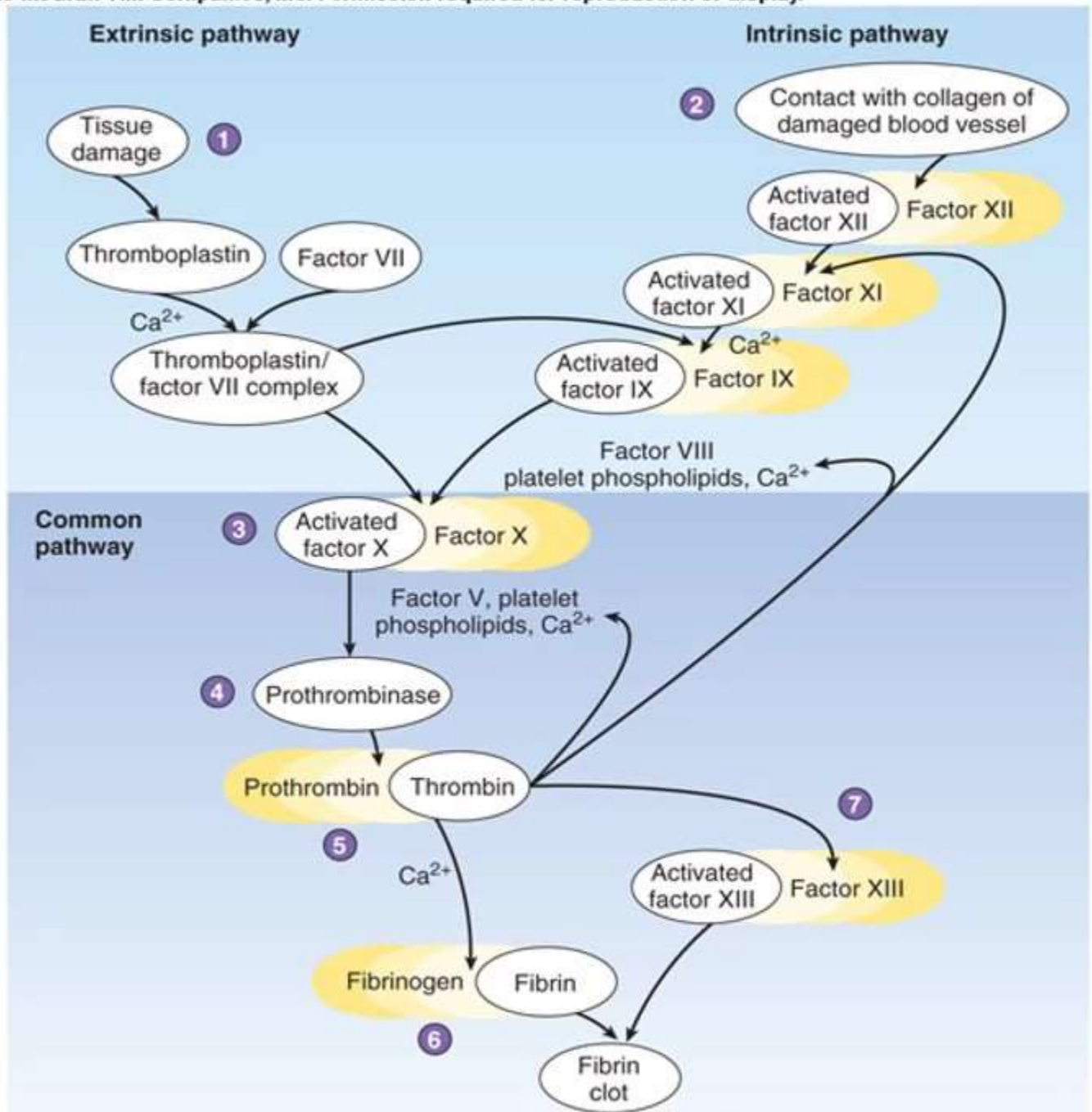
3. Activation of the extrinsic or intrinsic clotting pathway results in the production of activated factor X.

4. Activated factor X, factor V, phospholipids, and Ca^{2+} form prothrombinase.

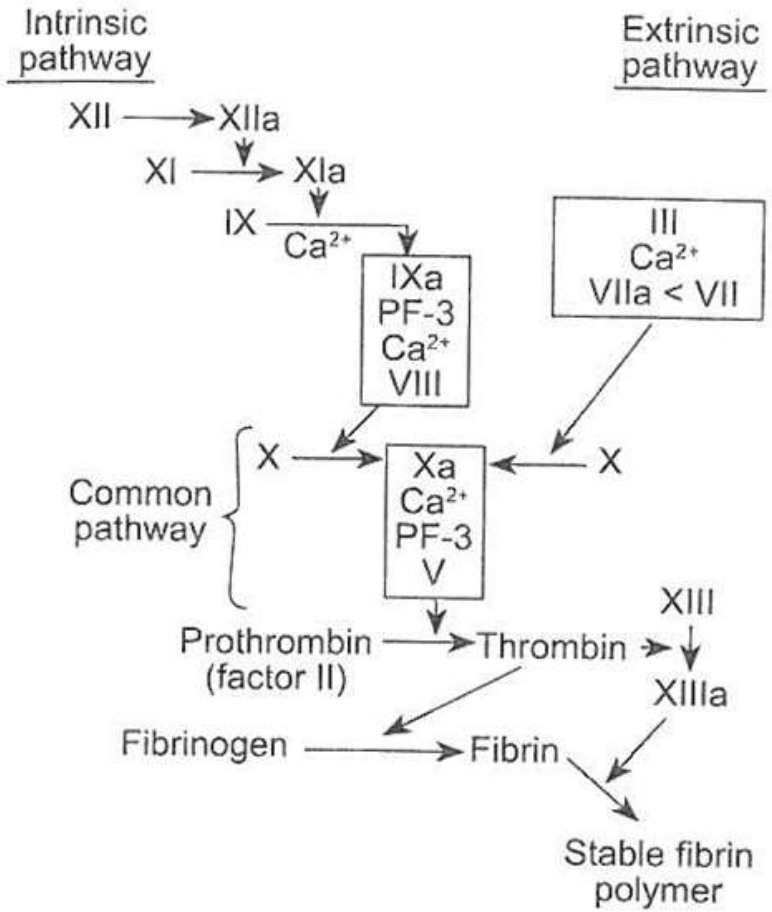
5. Prothrombin is converted to thrombin by prothrombinase.

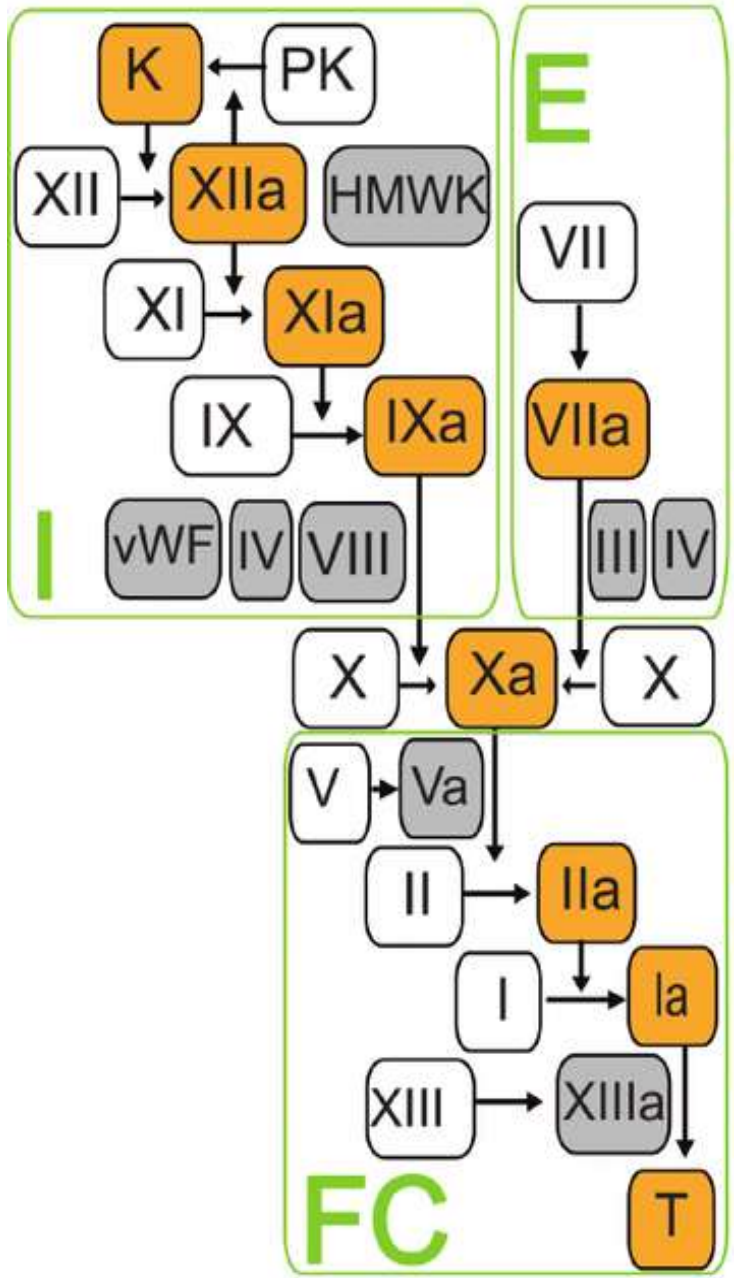
6. Fibrinogen is converted to fibrin (the clot) by thrombin.

7. Thrombin activates factor XIII, which stabilizes the fabrin clot.



Stages of coagulation





#	Name	Features	Pathway
I	Fibrinogen		E, I
II	Prothrombin	VKD, SP	E, I
III	Tissue factor, thromboplastin	NEC	E
IV	Ca++	NEC	E, I
V	Proaccelerin	NEC	E, I
VII	Proconvertin	VKD, SP	E
VIII	Anti-hemophilic factor	NEC	I
IX	Christmas factor	VKD, SP	I
X	Stuart factor	VKD, SP	E, I
XI	Plasma thromboplastin antecedent	SP	I
XII	Hageman factor	SP	I
XIII	Fibrin-stabilizing factor	TG	E, I
vWF	Von Willebrand factor	NEC	I
PK	Prekallikrein, Fletcher factor	SP	E, I
HMWK	High Molecular Weight Kininogen, Fitzgerald factor	NEC	I

General scheme of blood clotting:

I. Formation of the platelet plug.
II. Blood coagulation (formation of the fibrin' clot).

1.Initiation of coagulation: formation of **prothrombin activator's (thrombokinase, prothrombinase):**

a). Extrinsic mechanism (forming of **tissue prothrombinase) (5-7 s)**;

Factors: **tissue phospholipids, X, Ca^{2+}, VII, V.**

b). Intrinsic mechanism (forming of **blood prothrombinase**) (5-7 min);

Factors: **contact with collagen, XII, XI, IX, Ca^{2+}, VIII, X, platelet phospholipids.**

2. Prothrombin (Ca^{2+}, V**,** prothrombinase) → **thrombin (2-4 s).**

3. Fibrinogen (thrombin**)** → **fibrin monomer**

Ca^{2+}, XIII (fibrin stabilizing factor)

fibrin threads

4. Clot retraction

Factor-6 platelets (thrombostenin) (30-60 min).

III. Fibrinolysis

Plasminogen (profibrinolysin) XII Plasmin(fibrinolysin)

Factors: **thrombin, activated XII, lysosomal enzymes from damaged tissues, factors from vascular endothelium**

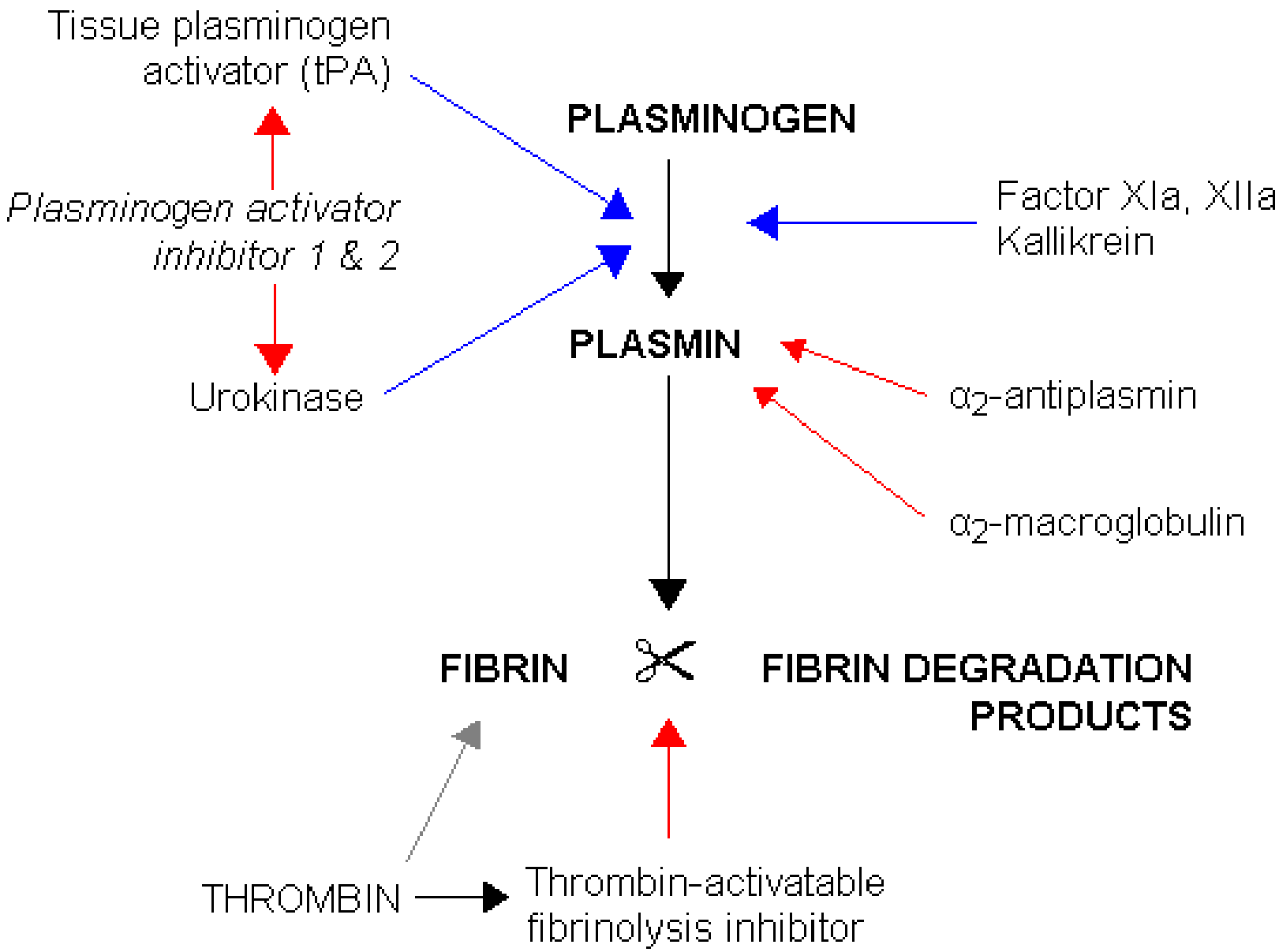
Fibrinolysis

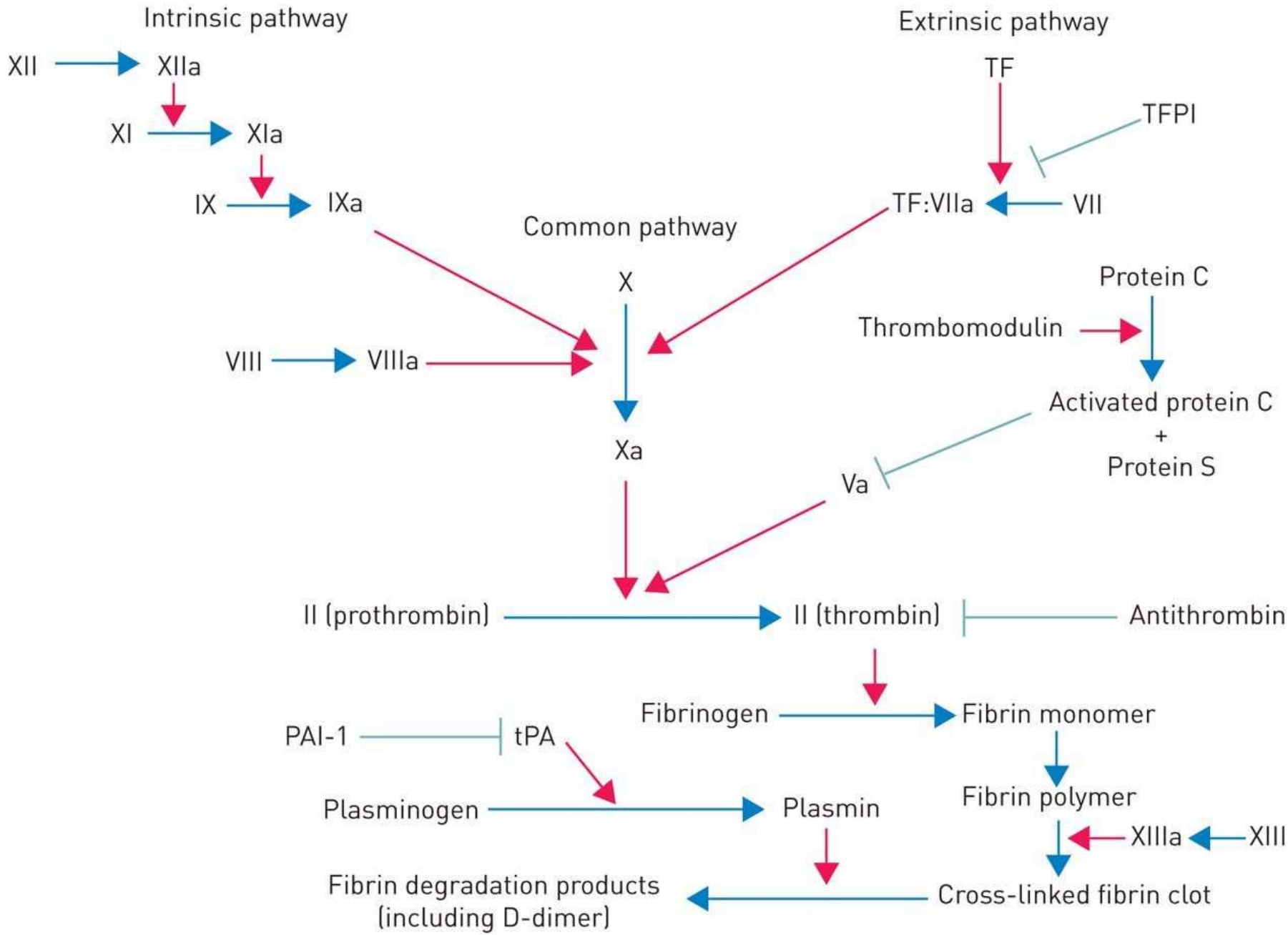
- **<u>Removal of unwanted clots when healing has occurred</u>**

- Enzyme ***plasmin*** digests fibrin

- **Heparin** – a natural anticoagulant found in basophils and mast cells;

- **Anticoagulants**: prevent coagulation factors from initiating clot formation
  - aspirin / coumadin


Tissue plasminogen activator (tPA)
PLASMINOGEN
Plasminogen activator inhibitor 1 & 2
Factor XIa, XIIa Kallikrein
PLASMIN
Urokinase
α2-antiplasmin
α2-macroglobulin
FIBRIN
FIBRIN DEGRADATION PRODUCTS
THROMBIN
Thrombin-activatable fibrinolysis inhibitor


Intrinsic pathway
XII
XIIa
XI
XIa
IX
IXa
VIII
VIIIa
Extrinsic pathway
TF
TFPI
TF:VIIa
VII
Common pathway
X
Xa
Protein C
Thrombomodulin
Activated protein C
+
Protein S
Va
II (prothrombin)
II (thrombin)
Antithrombin
Fibrinogen
Fibrin monomer
PAI-1
tPA
Plasminogen
Plasmin
Fibrin polymer
XIIIa
XIII
Fibrin degradation products
(including D-dimer)
Cross-linked fibrin clot

Fibrinolysis - Process by which fibrin clot is degraded to prevent excessive clot formation

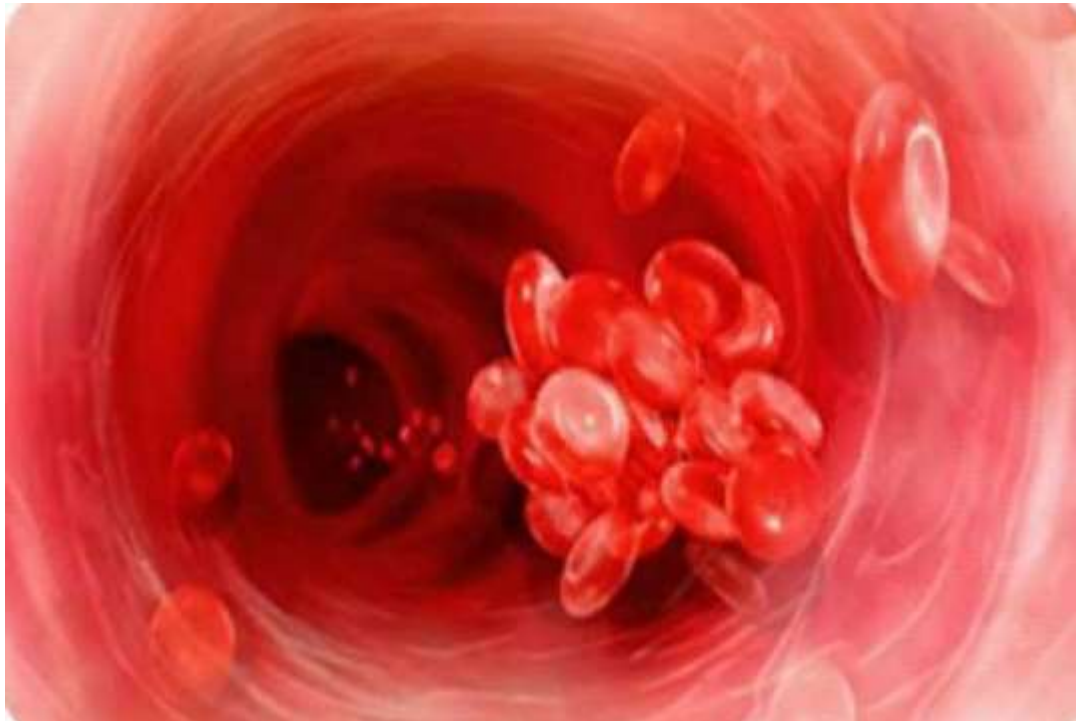
- Plasminogen incorported into clot
- - Injured tissues and vascular endothelium gradually produce Tissue Plasminogen Activator (t-PA) in a delayed manner.
- - Urokinase is produced by monocytes, macrophages and urinary epithelium
- - Factor XII (Hageman factor) acts as a weak activator of plasminogen
- - Plasminogen activated by t-PA, u-PA and factor XII to plasmin, a serine protease
- - Plasmin cleaves fibrin into fibrin degradation products

 reducing clot stability Clot breakdown

- Regulation of fibrinolysis
- - Plasmin Activator Inhibitor (PAI) 1 & 2 – produced by vascular endothelium and inhibits tPA and uPA - Protein C inactivates PAI,
- TAFI - Thrombomodulin – expressed on in tact endothelium – binds thrombin and subsequently activates protein C - Thrombin Activatable Fibrinolysis Inhibitor – produced by liver and cleaves plasmin binding sites on fibrin

- □ decreased degradation - α2 anti-plasmin – produced by liver, inhibits circulating plasmin, not fibrin bound plasmin

HEMOSTASIS - BLOOD COAGULATION

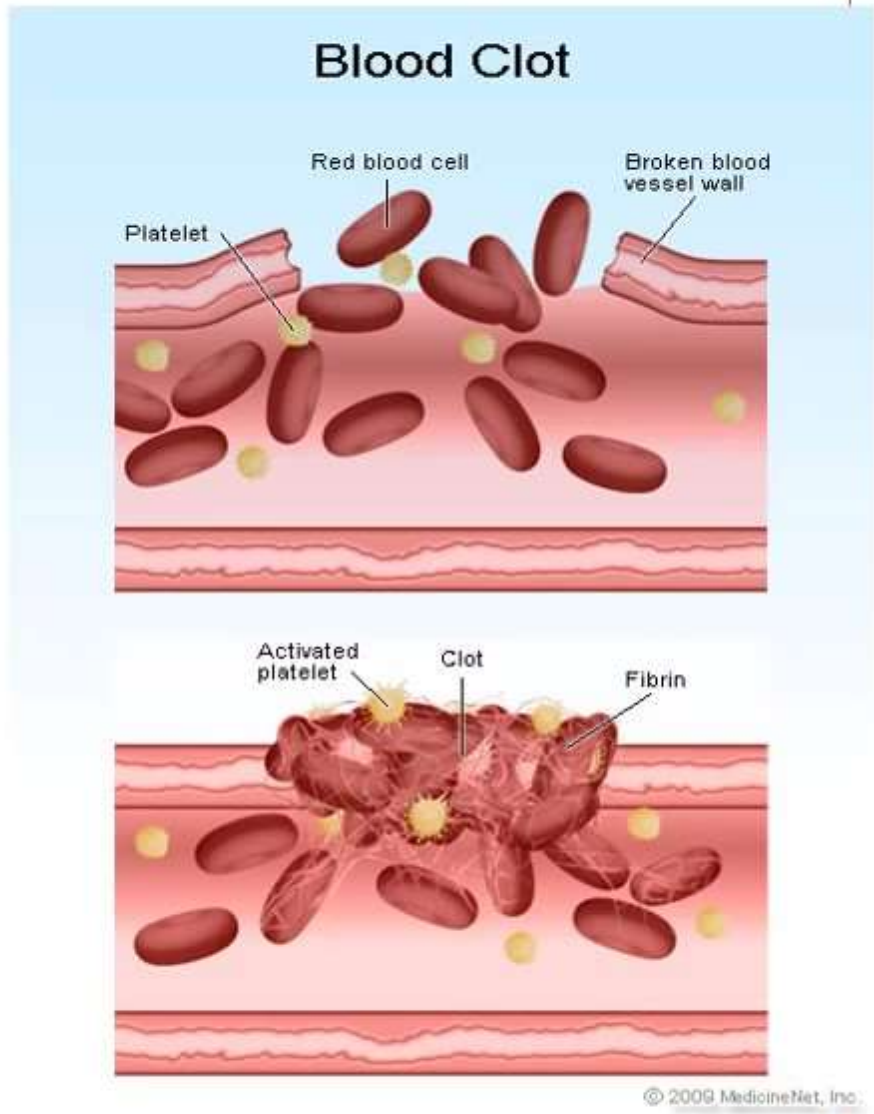


Hemostasis

- **Stoppage of bleeding**

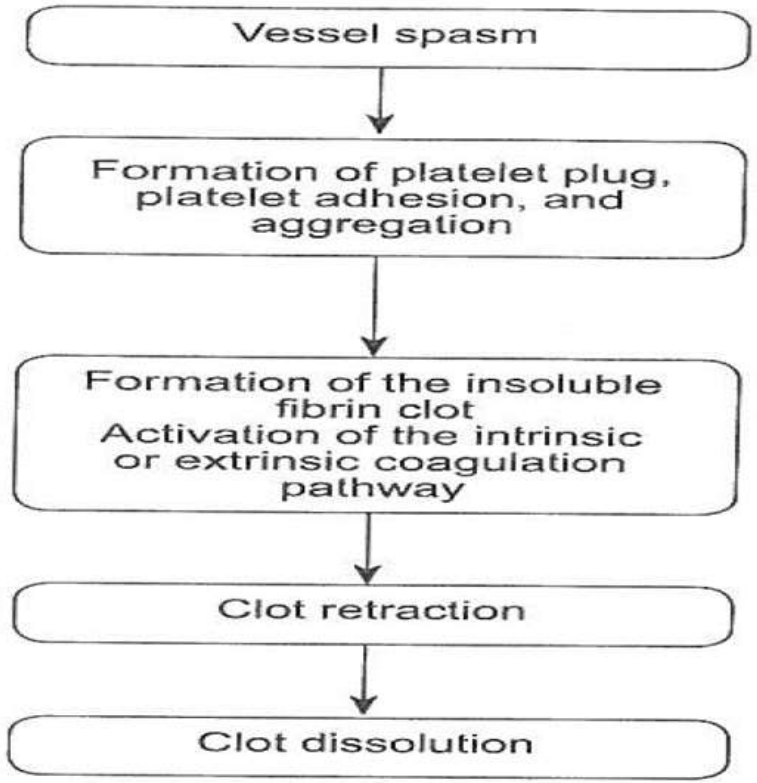
- **3 Phases:**

  - **Vasospasm**
    - vasoconstriction of damaged blood vessels
  - **Platelet plug formation**
    - platelets stick together
  - **Coagulation or blood clotting**
    - reinforces platelet plug with fibrin threads (created from fibrinogen) creating a fibrin mesh



Hemostasis is achieved by several mechanisms, including:

(1) vascular spasm;
(2) formation of a platelet plug;
(3) blood coagulation;
(4) growth of fibrous tissue into the blood clot to close the hole in the vessel permanently.



hemostasis

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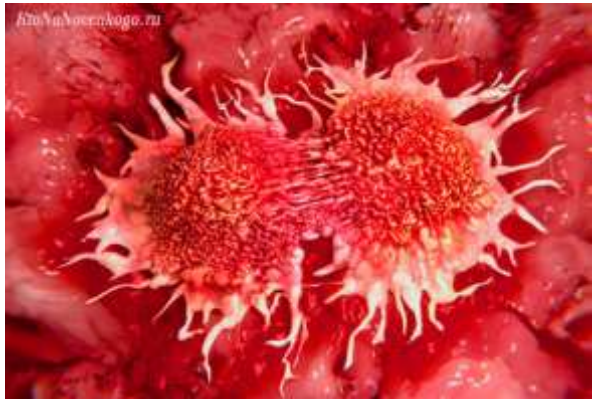
- (1) **vascular spasm;**
- **(2) formation of a platelet plug;**
- **(3) blood coagulation;**
- **(4) growth of fibrous tissue into the blood clot to close the hole in the vessel** permanently.

Blood platelets (thrombocytes)

- They have no nuclei.
- The number of platelets in human blood is **(200-400)x10^9/l**. Blood platelets are formed by megakaryocytes, giant cells present in the red bone marrow and in the spleen.
- Platelets live two to five days

- **Functions:**
- Blood platelets are rapidly destroyed in bleeding and factors that take part in **coagulation** and retract zymes are liberated into the plasma.

- They play the main role in the platelet plug ' hemostasis. Cytoplasm of the thrombocytes contains numerous **granules with a biological active matters** likes serotonin, ATP, ADP, angiotrophic factor and many others.
- Blood platelets release serotonin (to vasoconstriction).
- Thus, they counter hemorrhage not only by facilitating
- coagulation of the blood, but also through this vasoconstrictor,
- and that constitutes their defense role in the body.



Blood platelets

- Their amounts is regulated by thrombopoietin which controls platelet production. It is released from the liver, kidney, smooth muscle, and bone marrow.

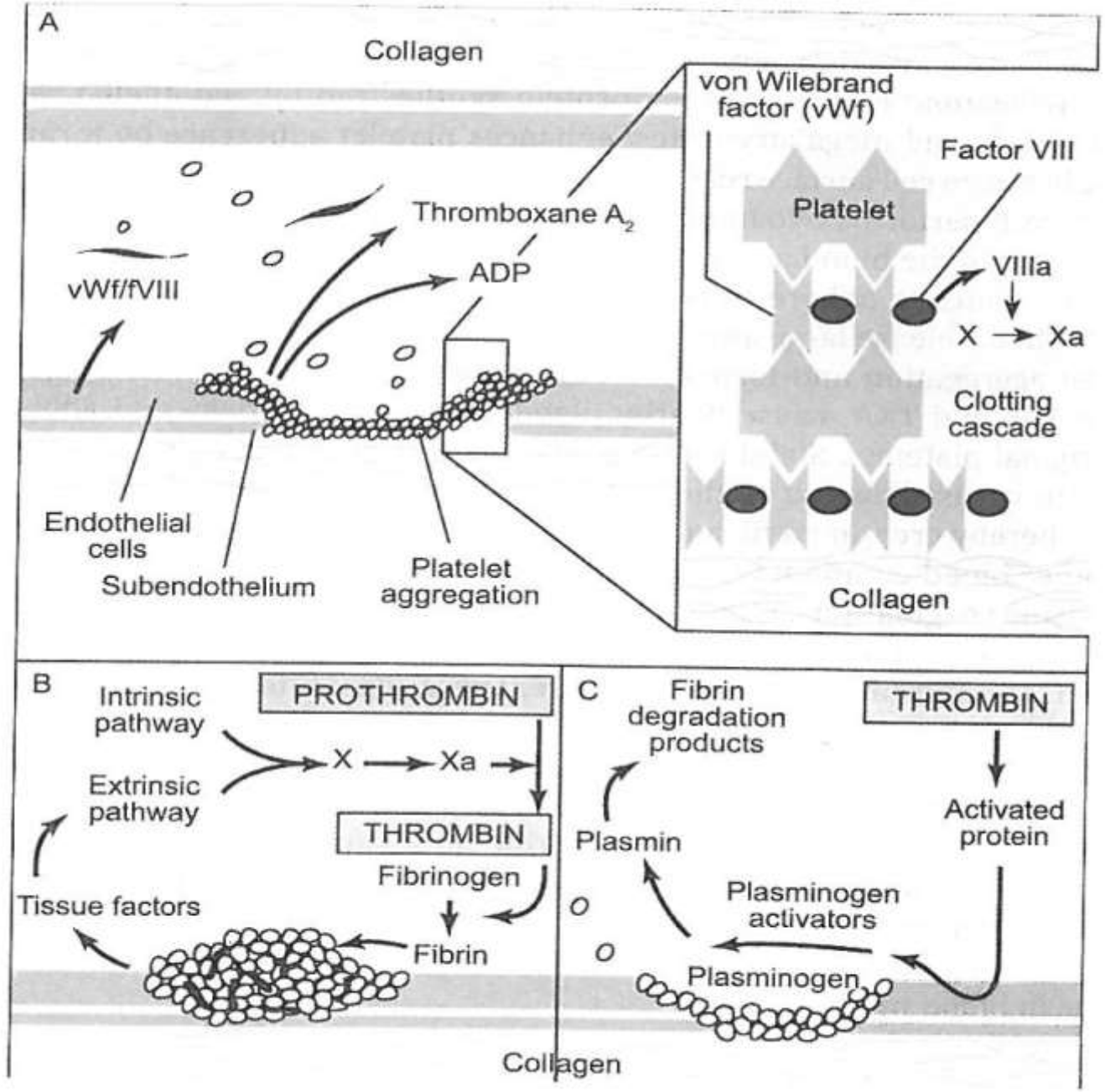
- Platelets also produce a growth factor that causes vascular endothelial cells, smooth muscle cells, and fibroblasts to proliferate and grow

Platelet plug formation

Platelet activation results in the sequential responses of *adherence*, *aggregation* and *secretion.*

The main stages of the Platelet Plug forming are:

1. Vascular spasm;
2. Adhesion of platelets to the damaged vascular wall;
3. Changeable aggregation of the platelets;
4. Unchangeable aggregation of the platelets;
5. Retraction of the plug.


A
Collagen
von Wilebrand factor (vWf)
Factor VIII
Platelet
Thromboxane A_2
ADP
vWf/fVIII
VIIIa
X → Xa
Clotting cascade
Endothelial cells
Subendothelium
Platelet aggregation
Collagen
B
Intrinsic pathway
PROTHROMBIN
X → Xa
Extrinsic pathway
THROMBIN
Fibrinogen
Tissue factors
Fibrin
Collagen
C
Fibrin degradation products
THROMBIN
Activated protein
Plasmin
Plasminogen activators
Plasminogen

Vascular hemostasis or primary 1-3 minutes

- **Tissue damage - pain - adrenaline, vasopressine - vasoconstriction;**
- **Tissue damage - collagen fibers are exposed - adhesion (adhesion) of platelets to the damaged surface;**

- **Releasing from platelets granules of serotonin, catecholamines, ADP - increased vasoconstriction;**

❑ **Changeable aggregation of clots and blood cells is formed;**

There will be a small amount of thrombin - fibrinogen in fibrin - the clot is insolvable.

ANTICOAGULATIVE SYSTEM

Prevention of blood clotting in the normal vascular system.

1. The smoothness of the endothelium which prevents contact activation of the clotting system.

2. Monomolecular layer of *negatively charged protein* absorbed to the inner surface of the endothelium that repels the clotting factors and platelets, thereby preventing activation of clotting.

3. Negative charge of endothelium and the same of formed elements prevent their connection.

4. Velocity of the blood flow.

5. Certain substances known as **anticoagulants** prevent coagulation.

- **Primary** include alpha globulin called *antithrombin III* or also *antithrombin-heparin cofactor*. They include *heparin,* which occurs in the tissues of the liver and lungs Heparin interferes with the action of thrombin on fibrinogen and suppresses the activity of thromboplastin.

- **Secondary anticoagulants**. Among the most important anticoagulants in the blood itself are those that remove thrombin from the blood. The most powerful of them are the *fibrin threads* that are formed during the process of clotting.

The serum proteins include yet another substance, namely *fibrinolysin,* an enzyme that occurs in an inactive form in plasma, and dissolves fibrin being formed. Its precursor, *profibrinolysin* is activated by the fibrinokinase encountered in many body tissues.

Disorders of Hemostasis

- ***Thromboembolic disorders***
  - *Undesirable clot formation*
  - **thrombus (clot)formation** – can dislodge to become an ***embolus***
  - *can block a blood vessel (stroke /heart attack)*

✓ ***Causes of thrombus;***

- ✓ anything that roughens endothelial surface –increases clinging of platelets
- ✓ **inflammation/ atherosclerosis /stasis in blood vessels**

- ***Bleeding disorders***

✓ **hemophilia** due to deficient clotting factors

Conditions that cause excessive bleeding

1. Bleeding caused by vitamin K deficiency;
2. Hemophilia;
3. Thrombocytopenia (platelet deficiency).

Vitamin K is necessary for some of the intermediate stages in the formation of all the factors for blood coagulation.

Thank you!