PLATELETS STRUCTURE AND FUNCTIONS
COAGULATION MECHANISMS

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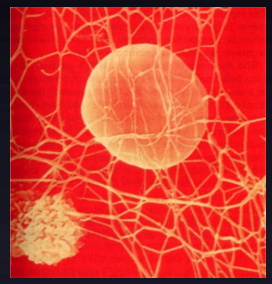


Vessel injury

Antithrombogenic

(Favors fluid blood)

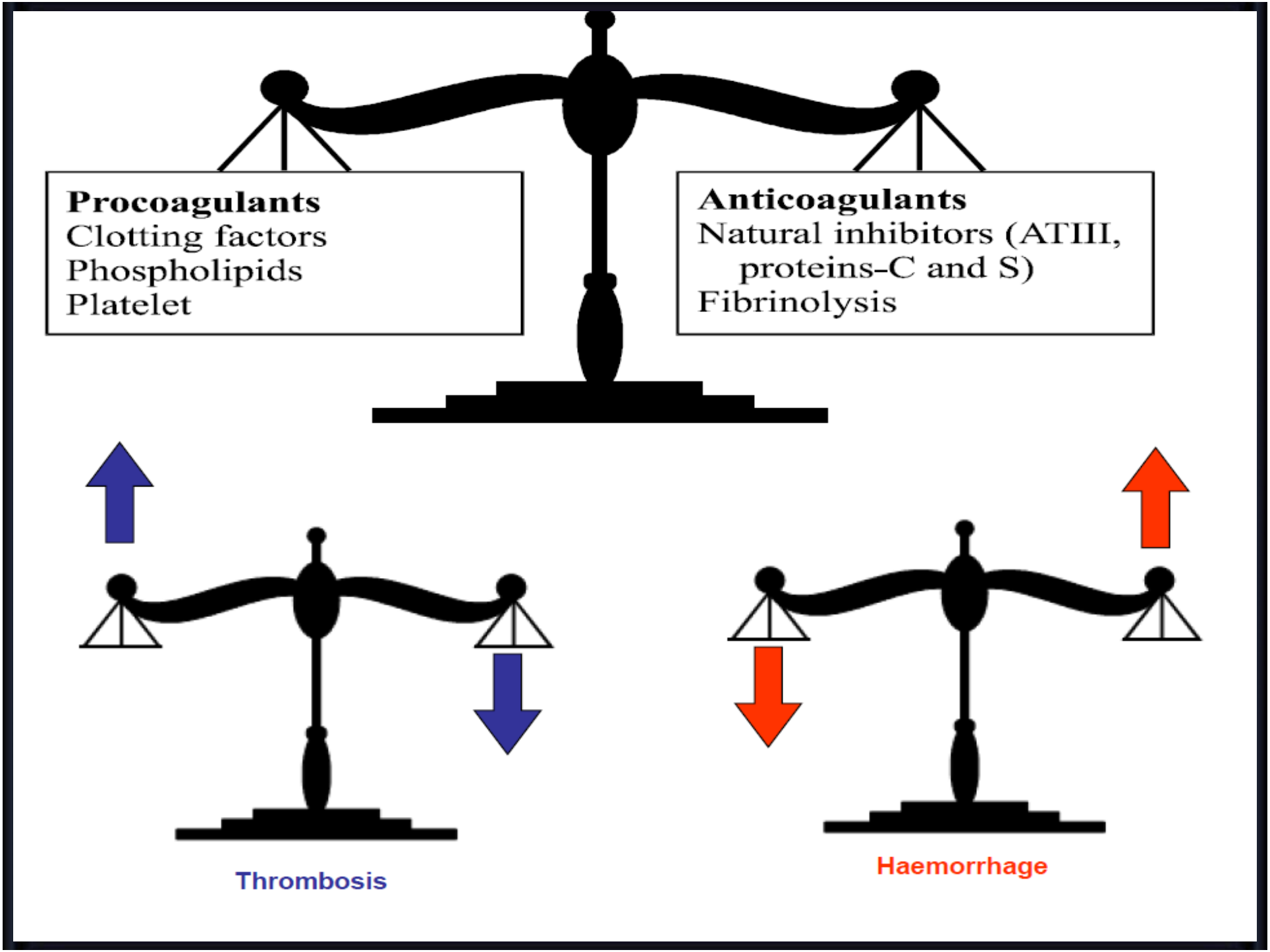
Thrombogenic

(Favors clotting)

OBJECTIVES

- **At the end of the lecture you should be able to describe…..**

1. Describe formation and development of platelet
2. Recognoize different stages of haemostasis
3. Explain the role of platelets in haemostasis.
4. Recognize different clotting factors & cascade of clotting.
5. Describe the intrinsic, extrinsic and common pathway.
7. Recognize the role of thrombin in coagulation
8. Explain process of fibrinolysis and function of plasmin


Procoagulants
Clotting factors
Phospholipids
Platelet
Anticoagulants
Natural inhibitors (ATIII, proteins-C and S)
Fibrinolysis
Thrombosis
Haemorrhage

HEMOSTASIS

The spontaneous arrest of bleeding from ruptured blood vessels

STEPS OF HEMOSTASIS

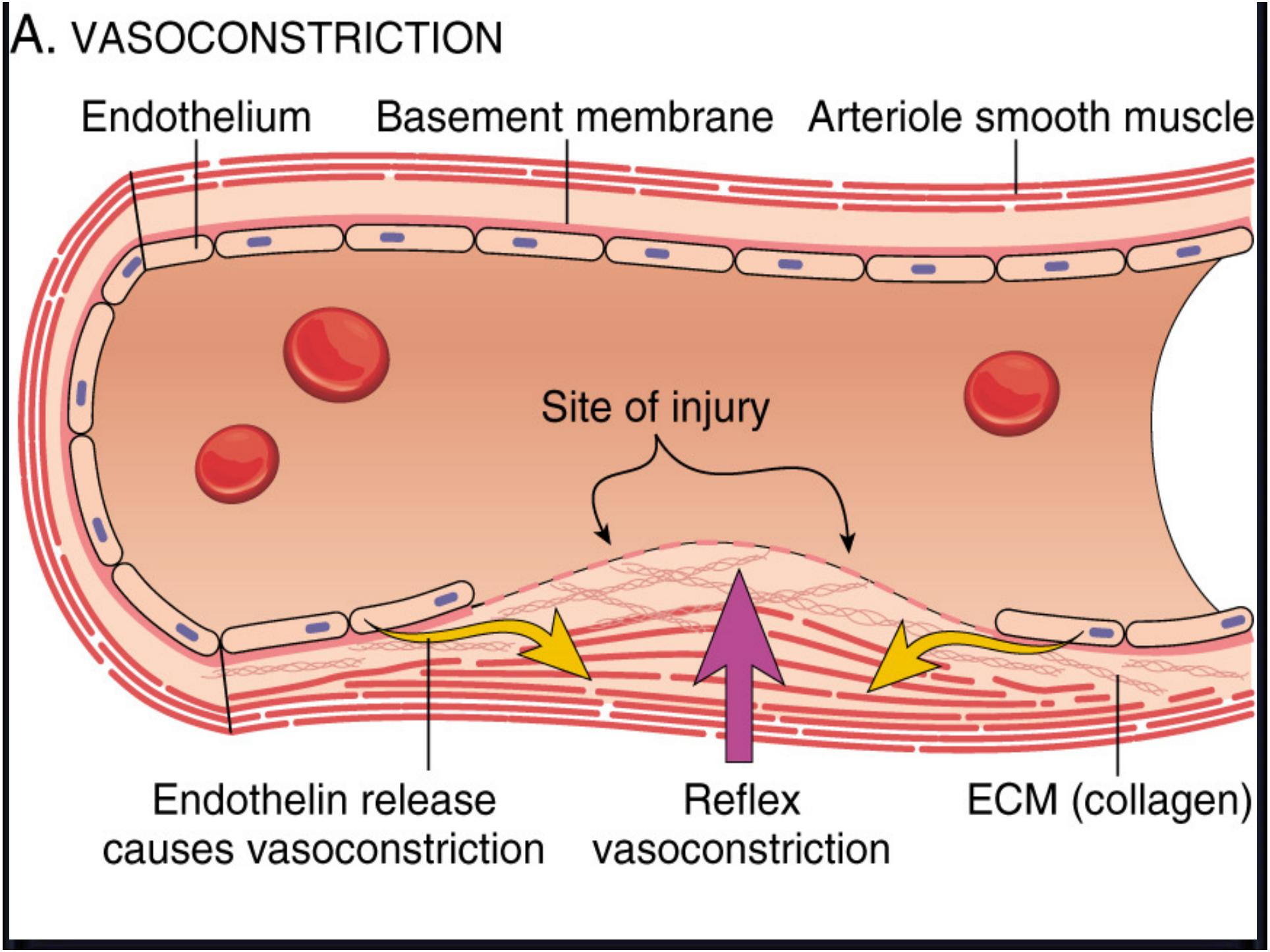
1. **Vascular Spasm**
2. **Formation of platelet plug**
3. **Blood Coagulation & Clot Retraction**
4. **Fibrinolysis**

1-VASCULAR SPASM (Vascular Constriction)

Immediately After injury there is localized Vasoconstriction.

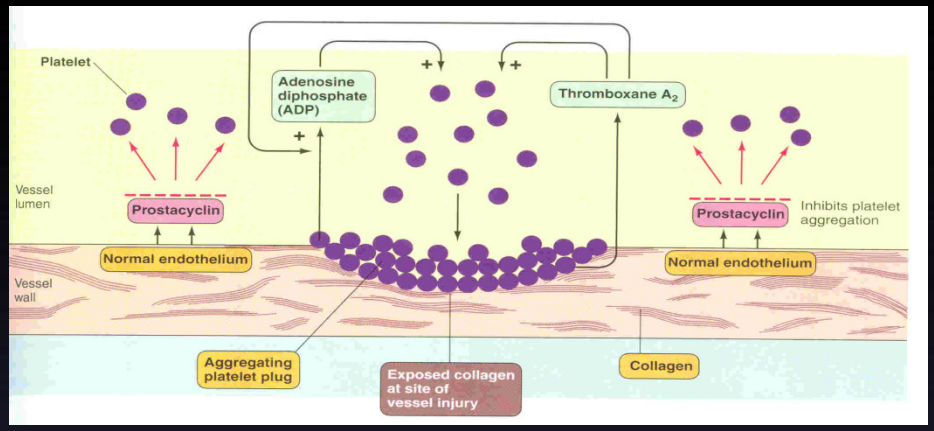
- **Causative Factors are three (3)**
  1. **Nervous reflexes**
  2. **Local myogenic spasm**
  3. **Local humoral factors**
- **For smaller vessels**
  - **Platelets → Thromboxane A_2 (Vasoconstrictor)**
- **Importance**
  - **Crushing injuries → Intense spasm → No lethal loss of blood**

TXA2 is inhibited by aspirin

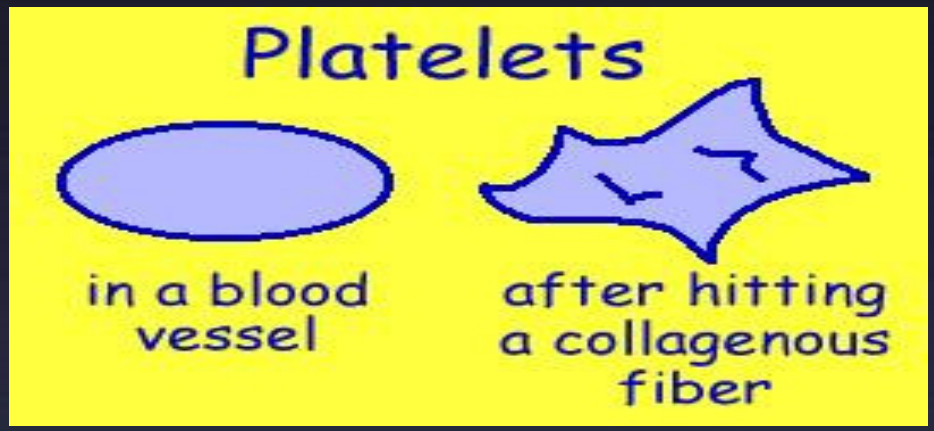

A. VASOCONSTRICTION
Endothelium
Basement membrane
Arteriole smooth muscle
Site of injury
Endothelin release causes vasoconstriction
Reflex vasoconstriction
ECM (collagen)

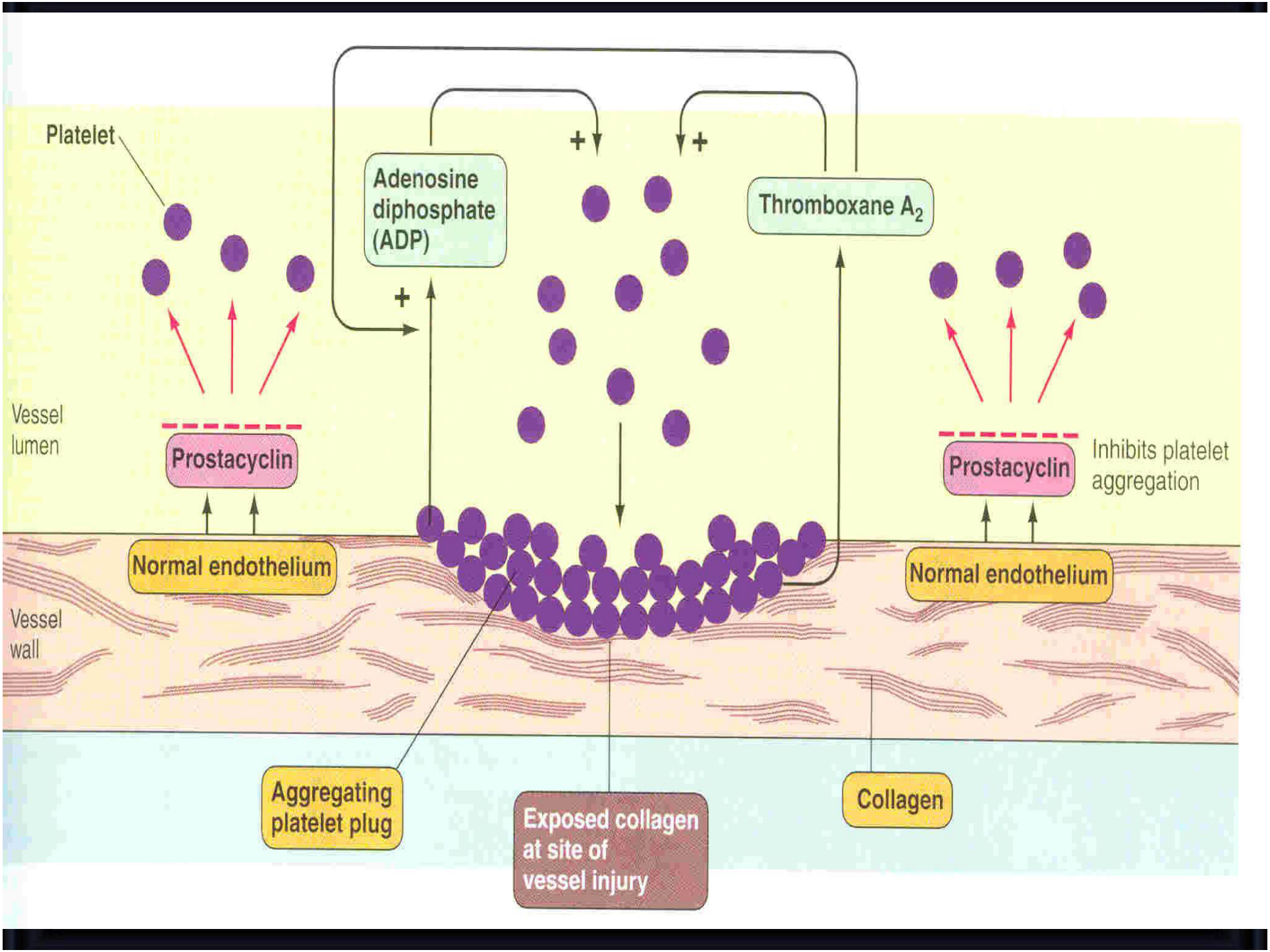
2-FORMATION OF PLATELET PLUG

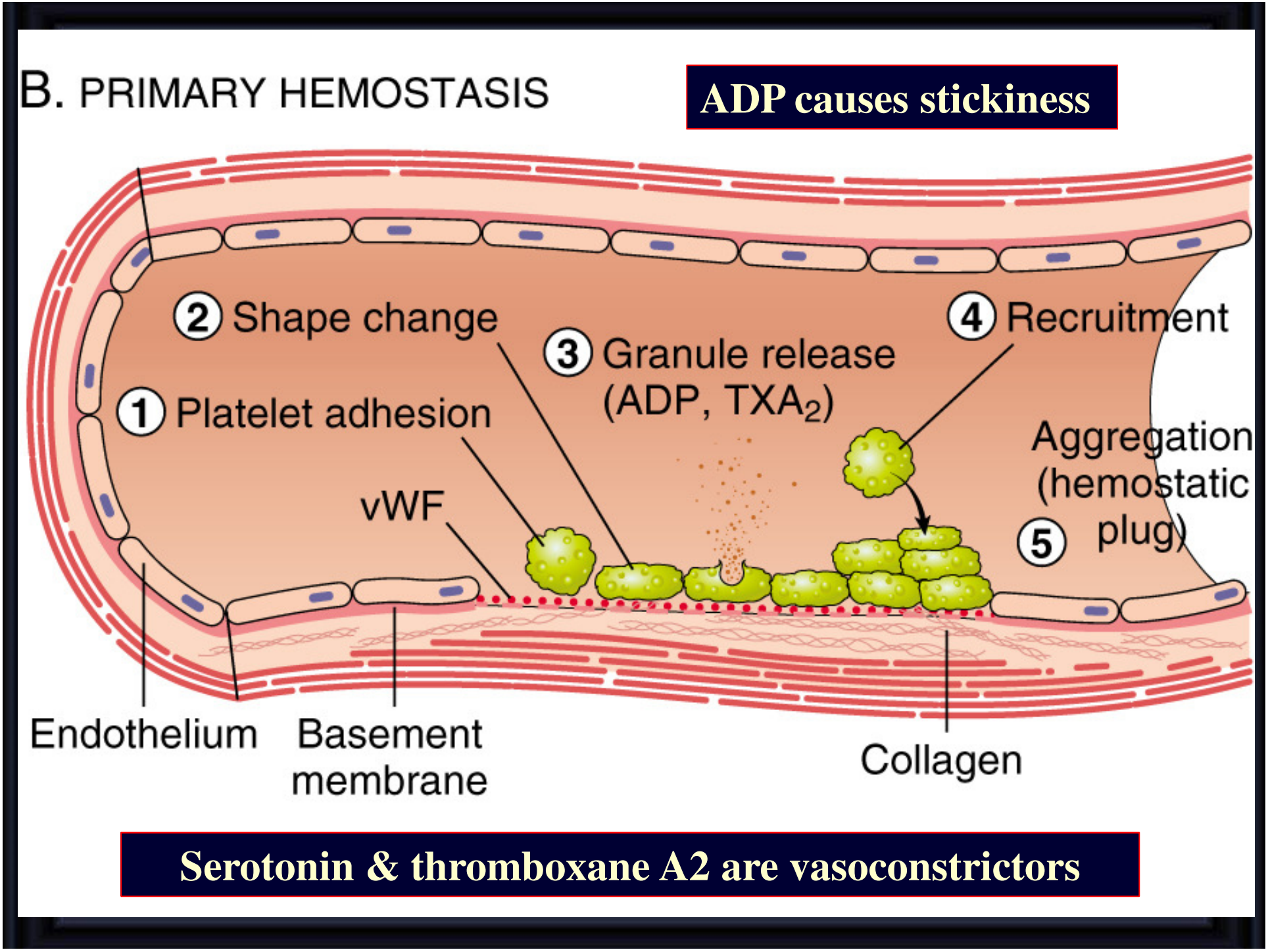
- **Importance of platelet plug → small vascular damage**



Intact endothelium secrete prostacyclin



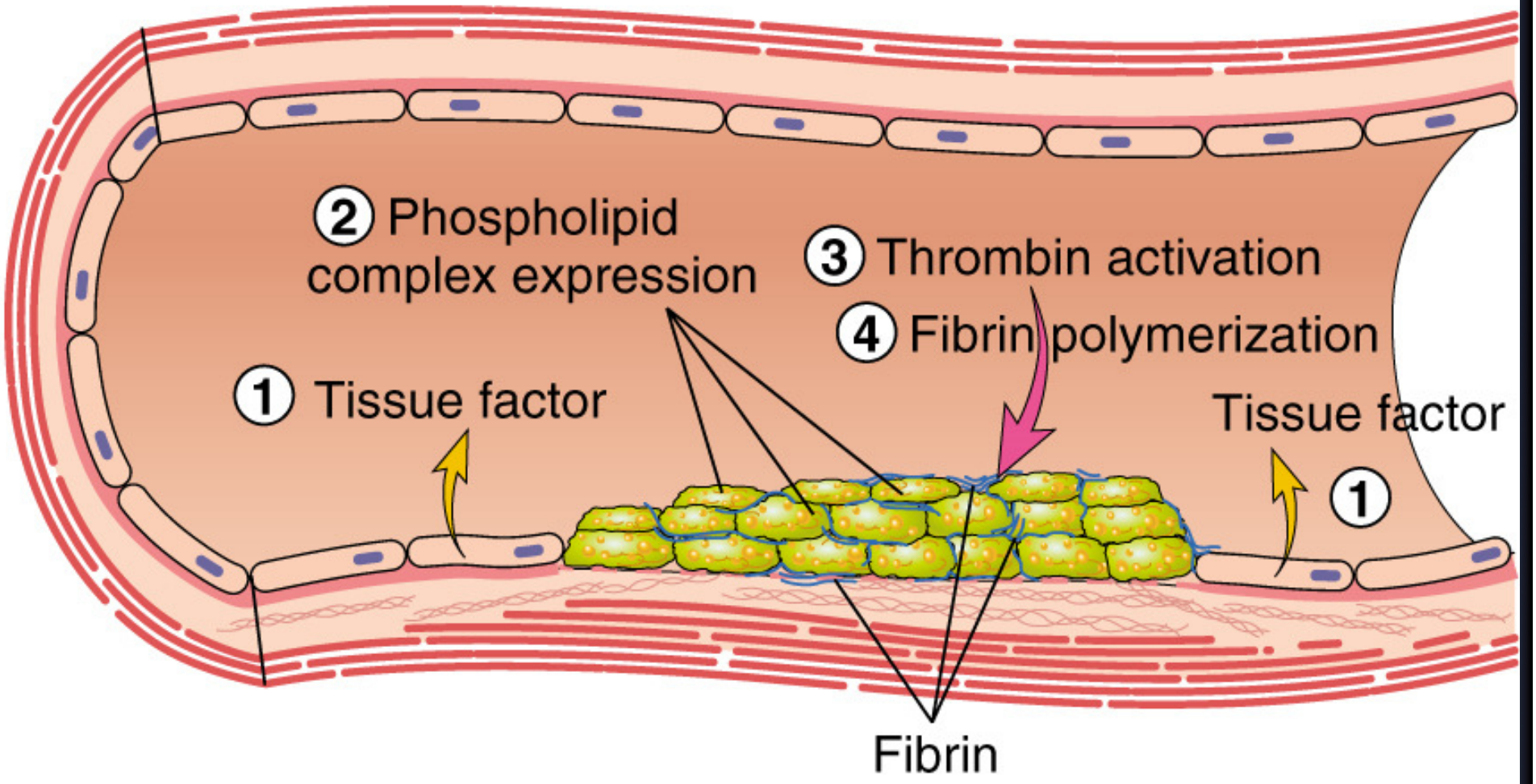

Platelet
Adenosine diphosphate (ADP)
Thromboxane A_2
+
+
+
Vessel lumen
Prostacyclin
Prostacyclin
Inhibits platelet aggregation
Normal endothelium
Normal endothelium
Vessel wall
Aggregating platelet plug
Exposed collagen at site of vessel injury
Collagen


B. PRIMARY HEMOSTASIS
ADP causes stickiness
2 Shape change
3 Granule release (ADP, TXA2)
4 Recruitment
1 Platelet adhesion
vWF
Aggregation (hemostatic plug)
5
Endothelium
Basement membrane
Collagen
Serotonin & thromboxane A2 are vasoconstrictors

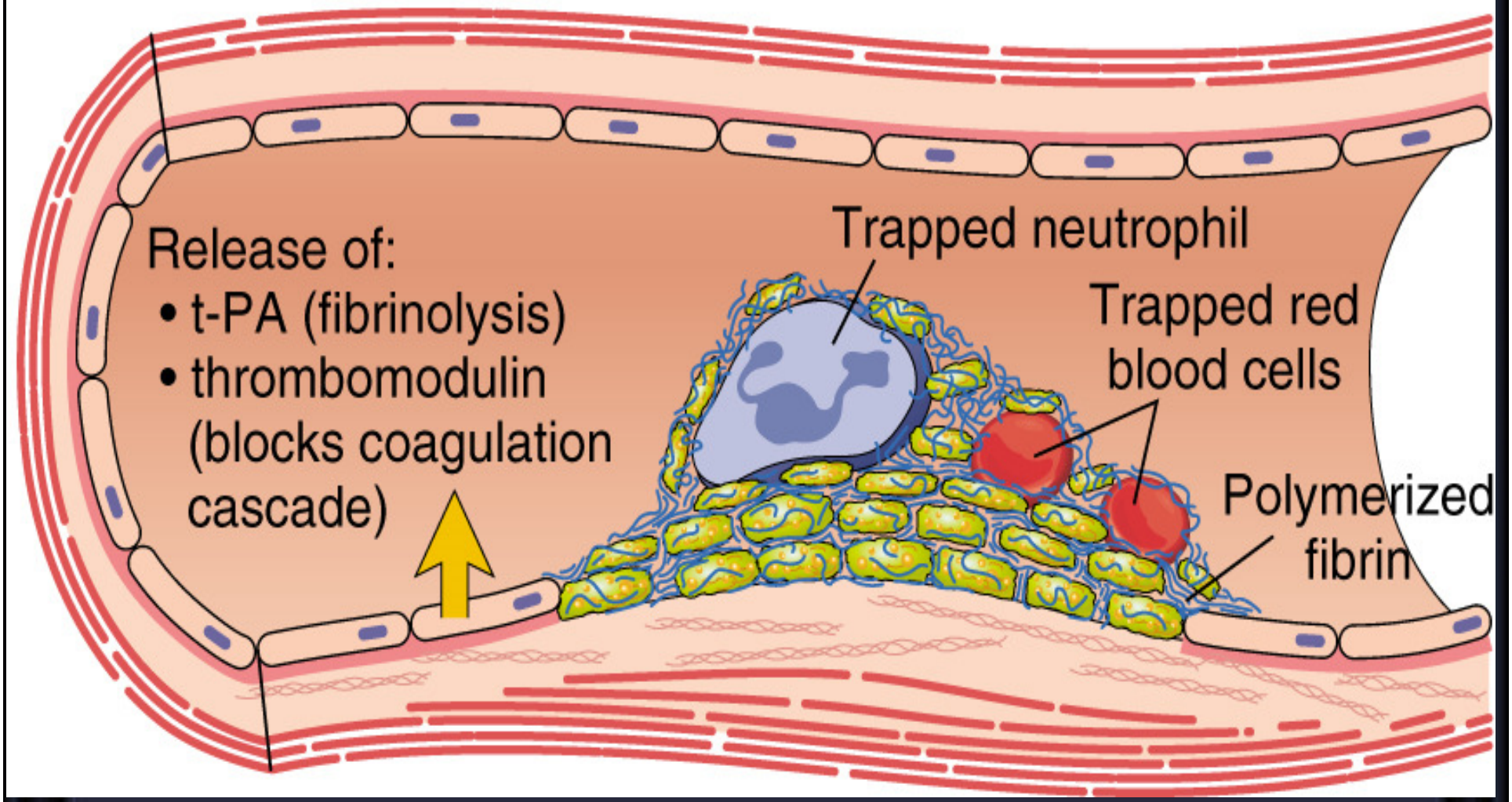
3-BLOOD COAGULATION
Formation of Clot

- **Blood clotting is the transformation of blood from a liquid into a solid gel form**
- **Pathways**
  - **Intrinsic**
  - **Extrinsic**
- **Initiated by: Activator substances from traumatized vascular wall, platelets & blood proteins**
- **Begins to develop in**
  - **15-20 sec → Minor trauma**
  - **1-2 min. → Severe trauma**

C. SECONDARY HEMOSTASIS

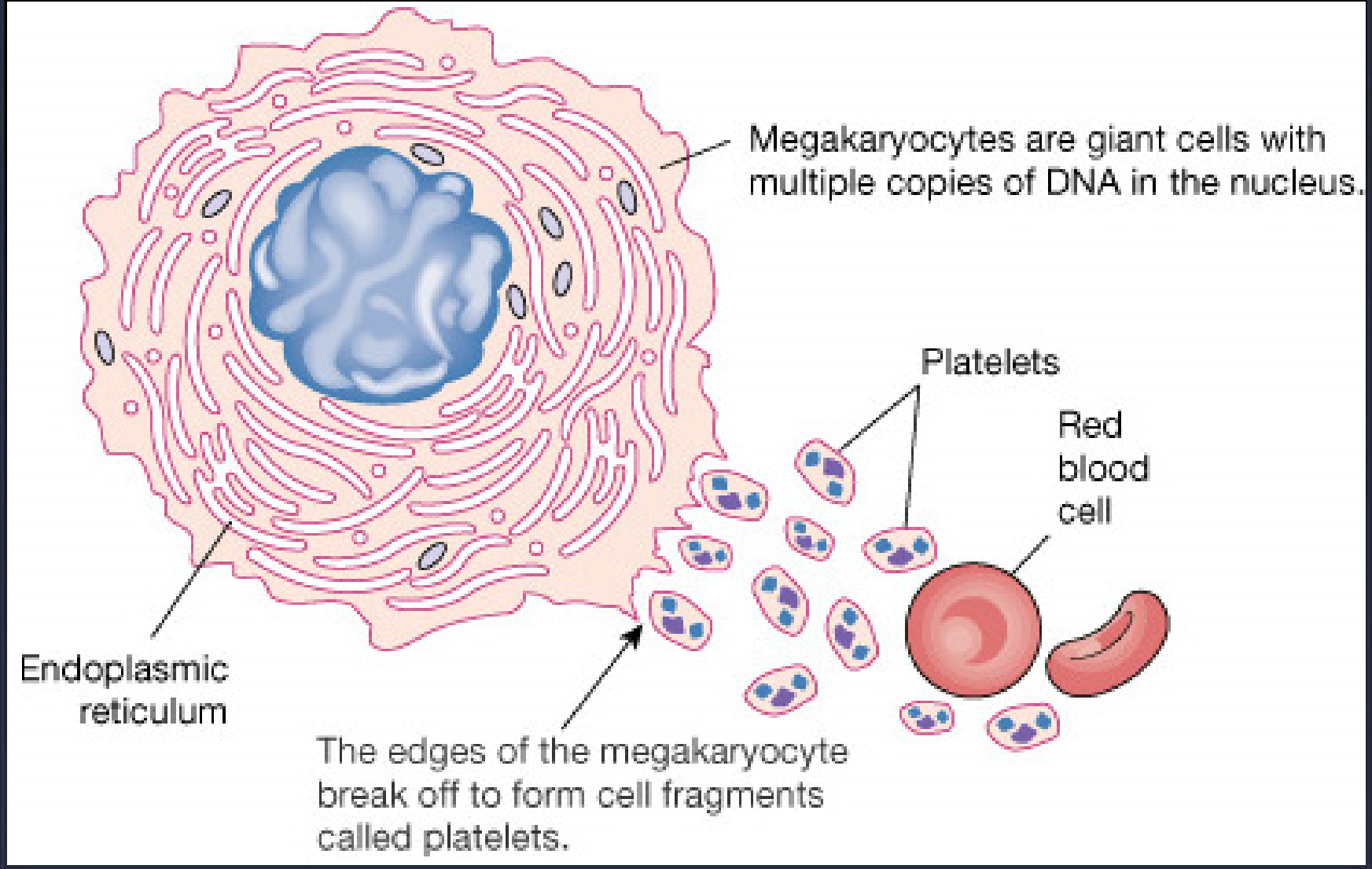


D. THROMBUS AND ANTITHROMBOTIC EVENTS

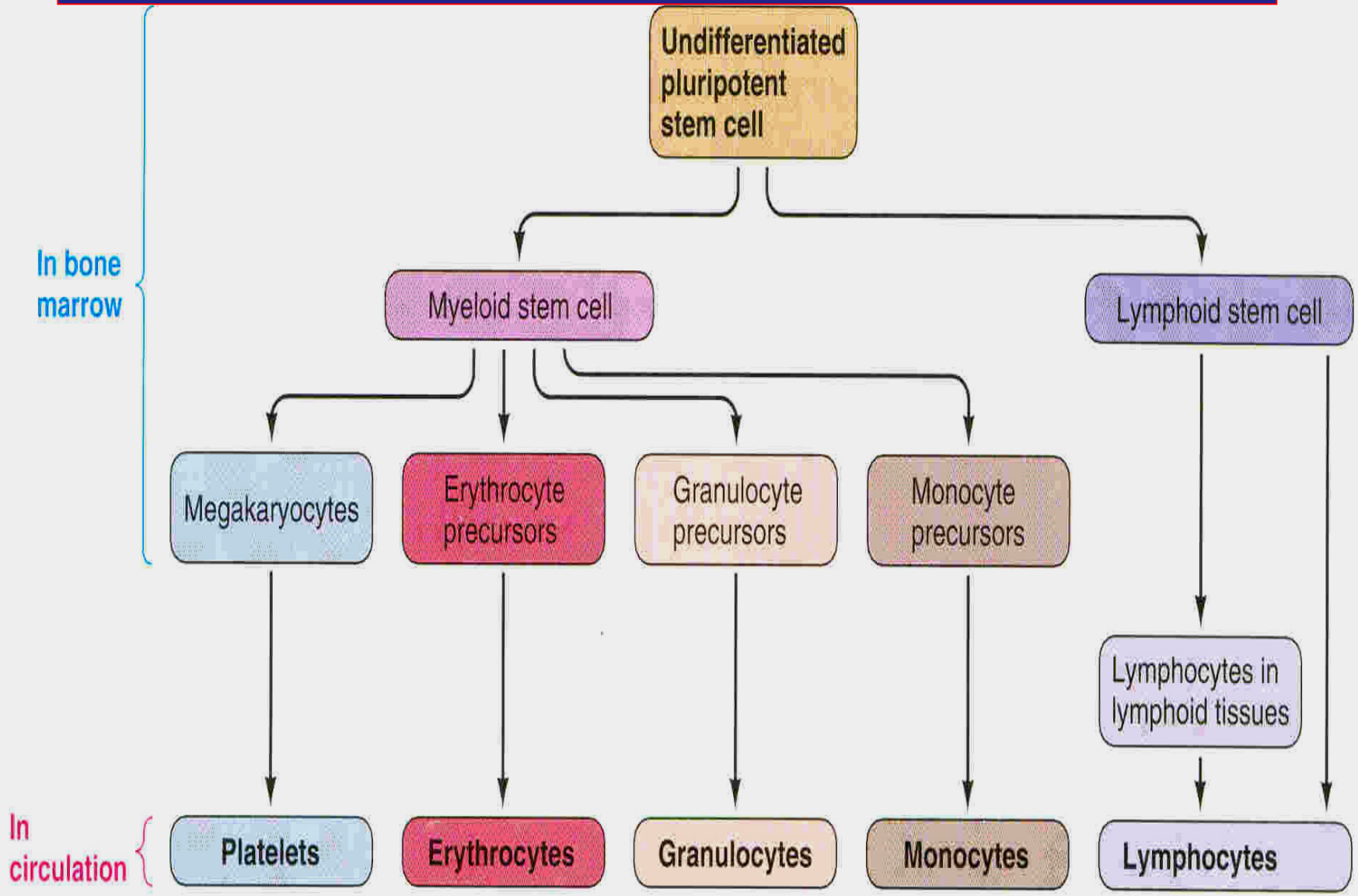


PLATELETS

Formed by fragmentation from megakaryoctyes



SITE OF FORMATION
Bone-marrow



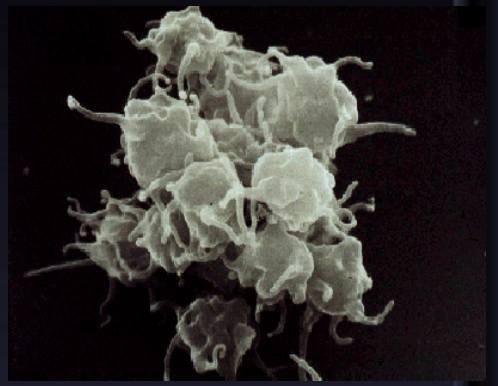
PLATELETS (Characteristics)

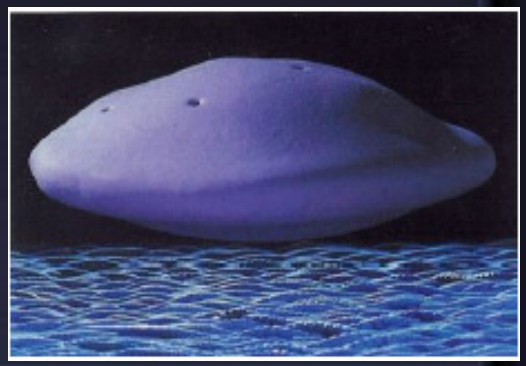
SHAPE: MINUTE ROUND OR OVAL DISCS
SIZE: 1-4 um IN DIAMETER
HALF LIFE: 7-10 DAYS
COUNT: 150,000 – 300,000/ microlitrer
LOCATION: 80% in blood & 20% in spleen

- **Contractile, adhesive, cell fragments.**
- **Store coagulation factors & enzymes**
- **Surface Binding sites for fibrinogen**
- **Surface Glycoprotein Antigens-HPA1.**

FUNCTIONAL CHARACTERISTICS

- Actin and Myosin Molecules
- Endoplasmic Reticulum and Golgi Apparatus
- Mitochondria
- Enzyme Systems For Synthesis Of Prostaglandins
- Fibrin Stabilizing Factor
- Growth Factor

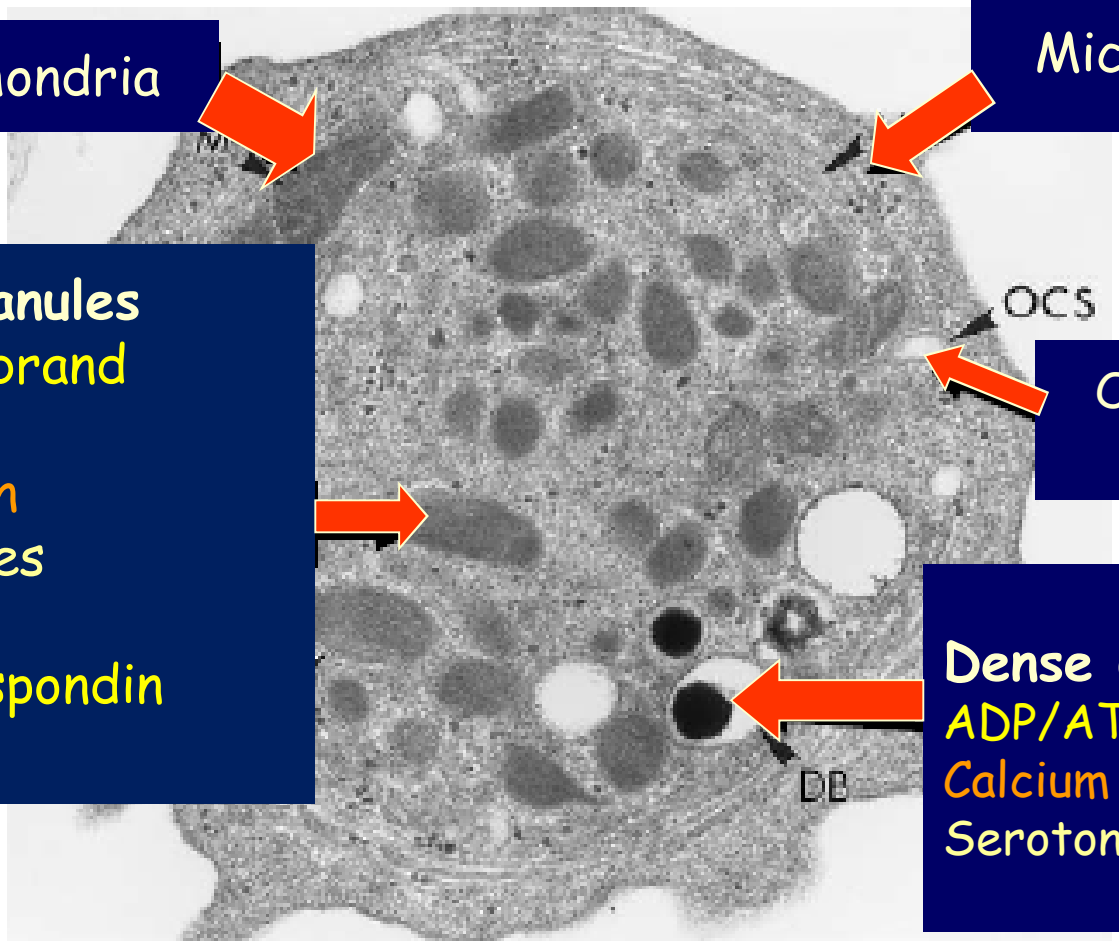




Platelet Ultrastructure

Mitochondria

Microtubules

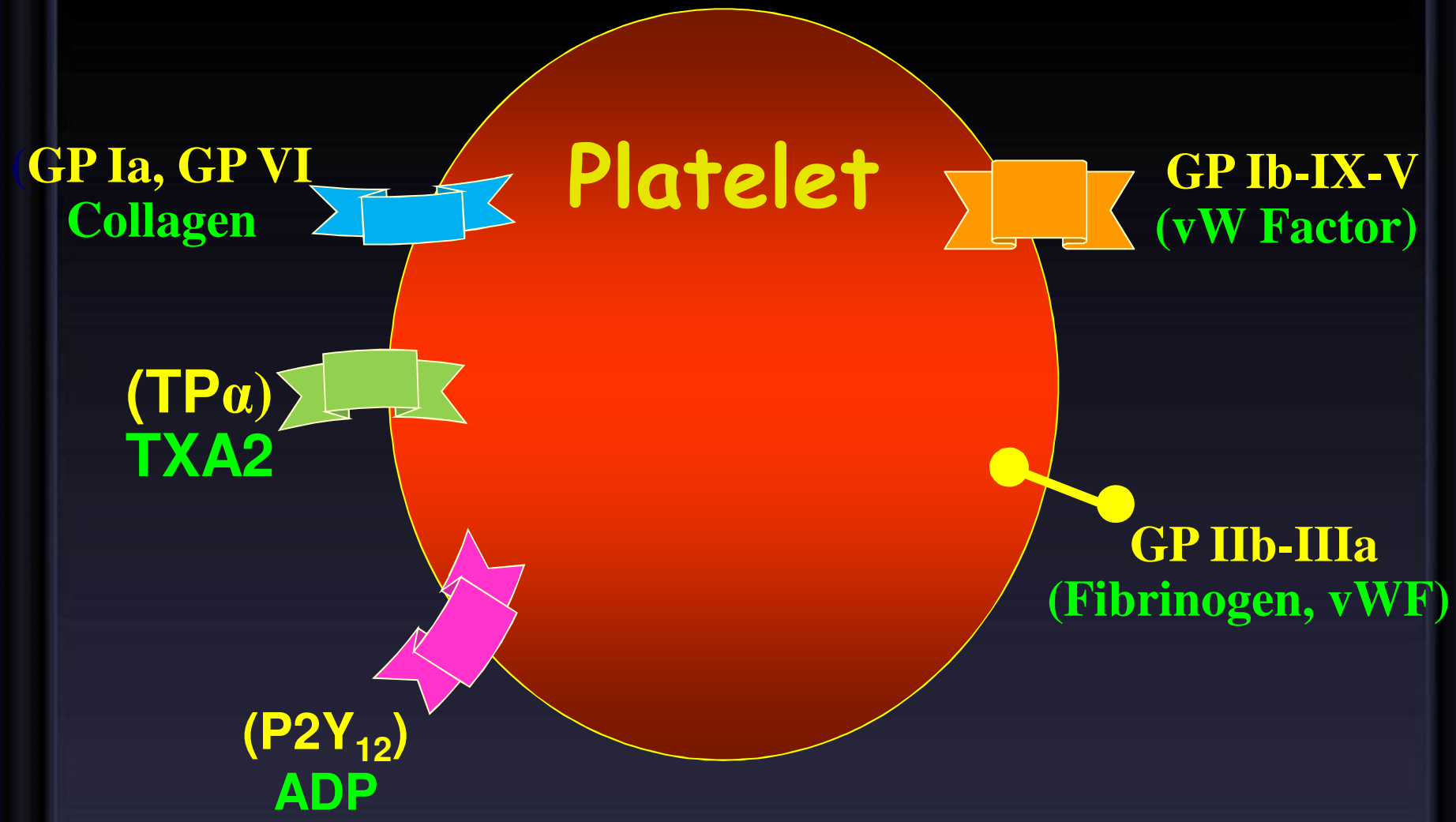


Alpha Granules
von Willebrand Factor
Fibrinogen
Chemokines (PF4,etc.)
Thrombospondin
P-selectin

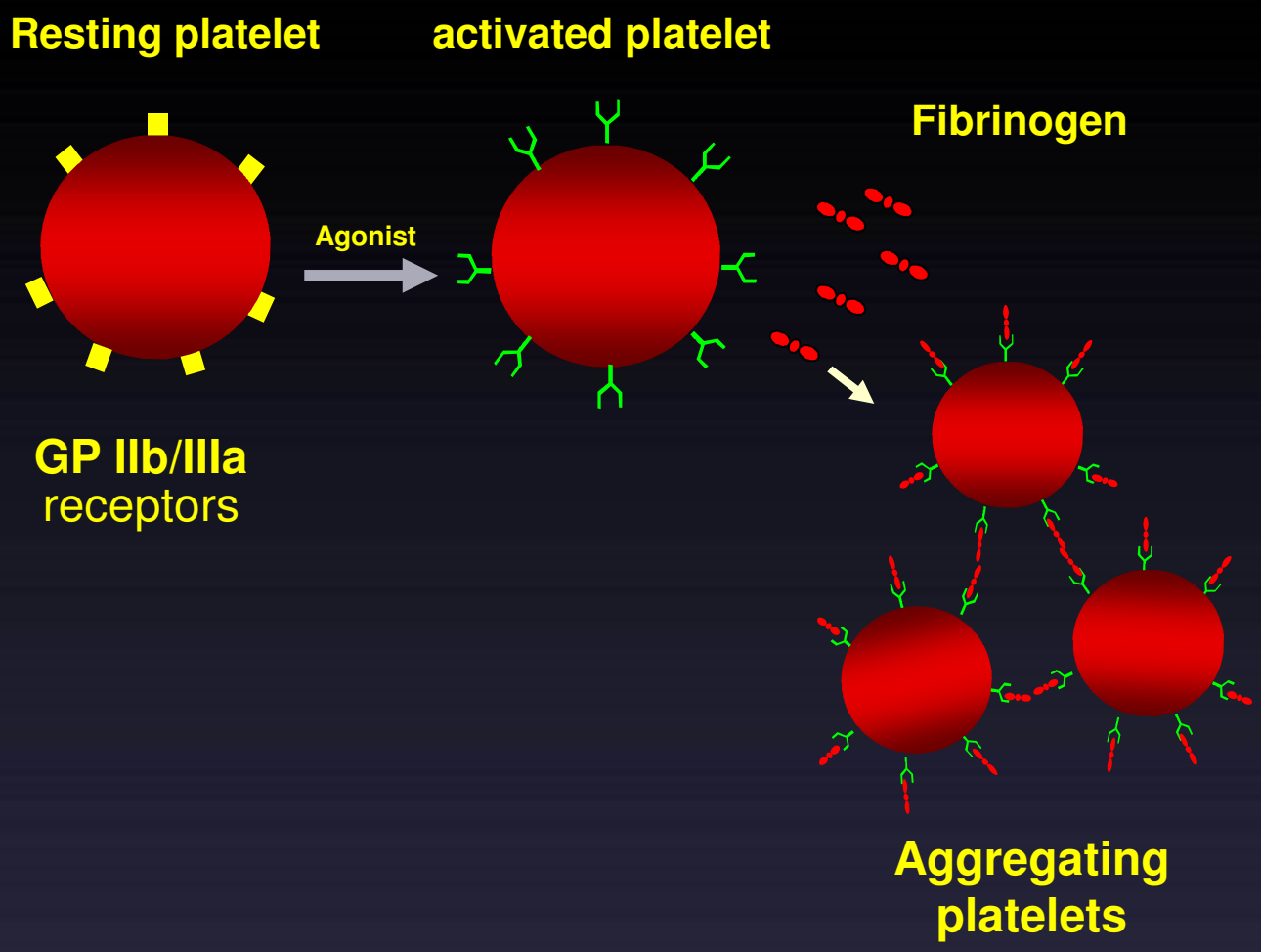
Open canalicular system

Dense Granules
ADP/ATP
Calcium
Serotonin

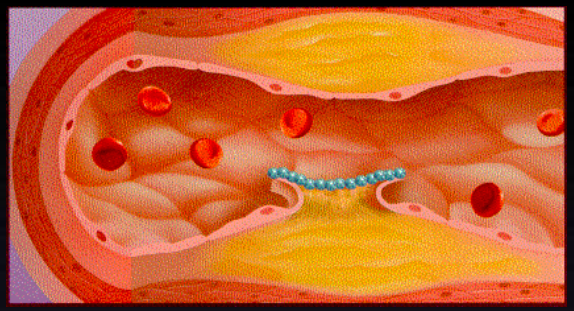
Platelet Receptors



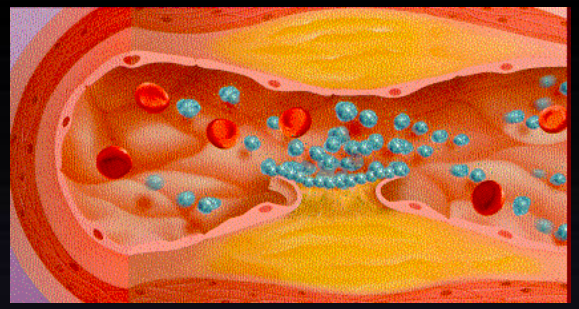
Platelet functions



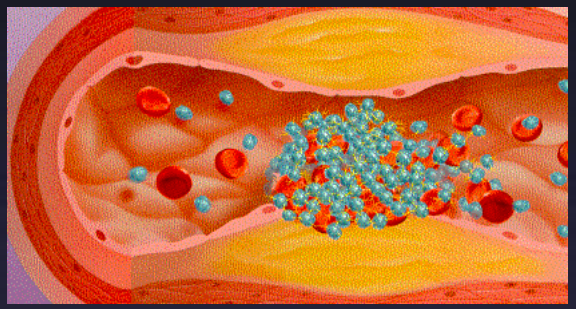
Platelet function



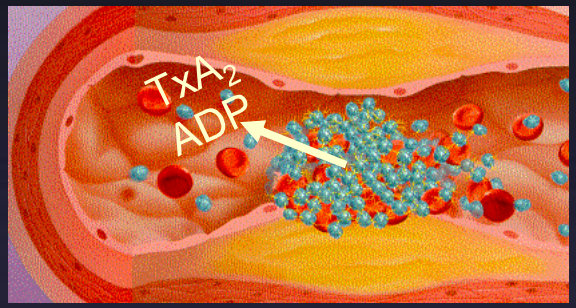
Adhesion



Activation



Aggregation



Secretion

Activated Platelets

Secrete:

1. 5HT → vasoconstriction
2. Platelet phospholipid (PF3) → clot formation
3. Thromboxane A2 (TXA2) is a prostaglandin formed from arachidonic acid

   Function:

- ❖ vasoconstriction
- ❖ Platelet aggregation

   (TXA2 inhibited by aspirin)

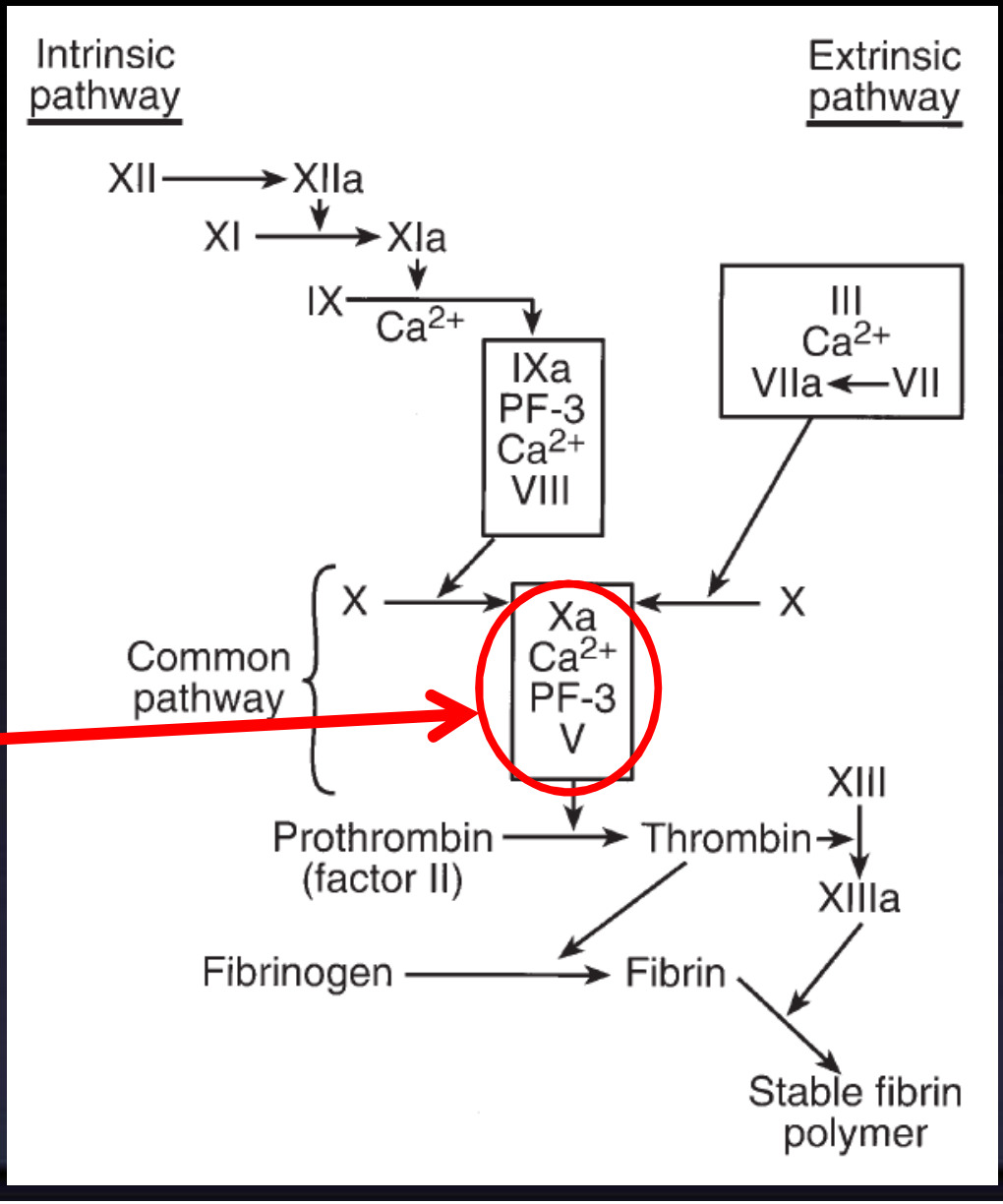
4. ADP causes stickiness and enhances aggregation

MECHANISM OF CLOTTING

1.Formation of Prothrombin activator complex (by Extrinsic and Intrinsic Pathways)

2.Conversion of prothrombin into thrombin by PAComplex

3.Conversion of fibrinogen into fibrin



2-CONVERISON OF PRTHROMBIN TO THROMBIN

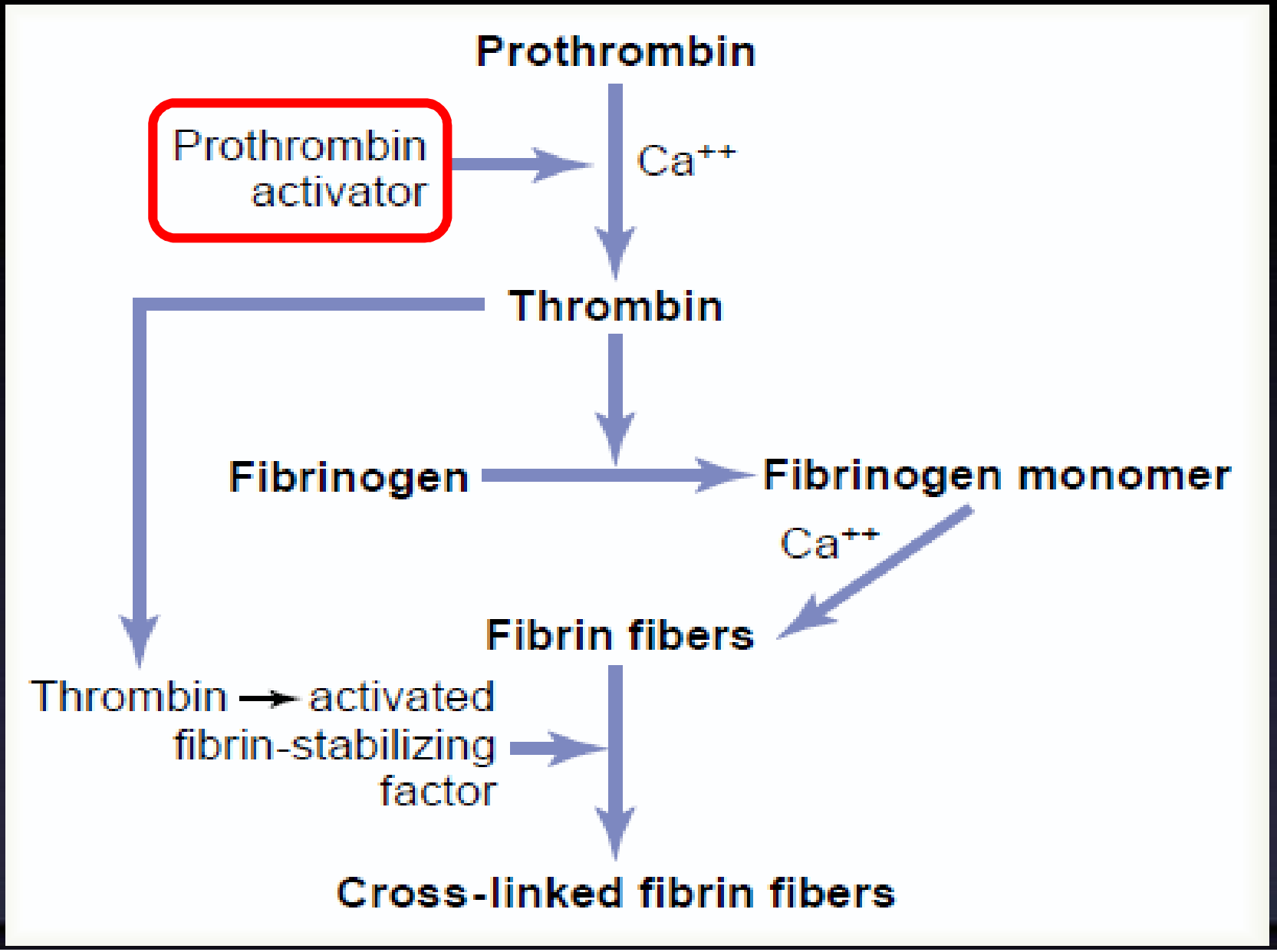
By Prothrombin Activator Complex

- **Prothrombin**
  - Plasma protein (Alpha$_2$ globulin)
  - Mol. Wt. - 68,700
  - Plasma conc. - 15 mg/dl
  - Unstable protein
  - Synthesized by liver
  - Vitamin-K is required for synthesis

3-CONVERSION OF FIBRINOGEN TO FIBRIN
Formation Of Clot

- **Fibrinogen**
  - **Mol. Wt. – 340,000**
  - **Plasma conc. – 100 – 700 mg/dl**
  - **Synthesized in liver**

ACTION OF THROMBIN ON FIBRONOGEN TO FORM FIBRIN



BLOOD CLOT

- A meshwork of fibrin fibres running in all directions entrapping blood cells, platelets and plasma

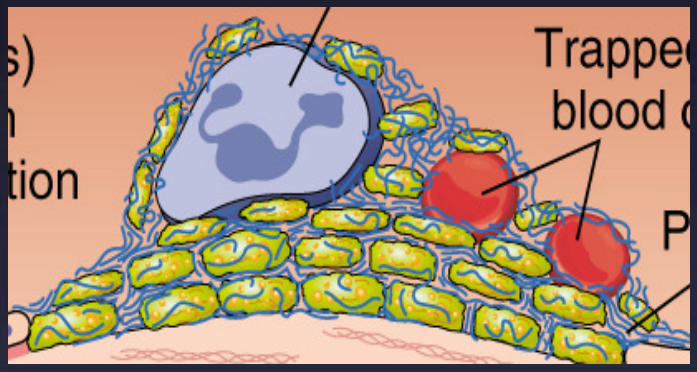


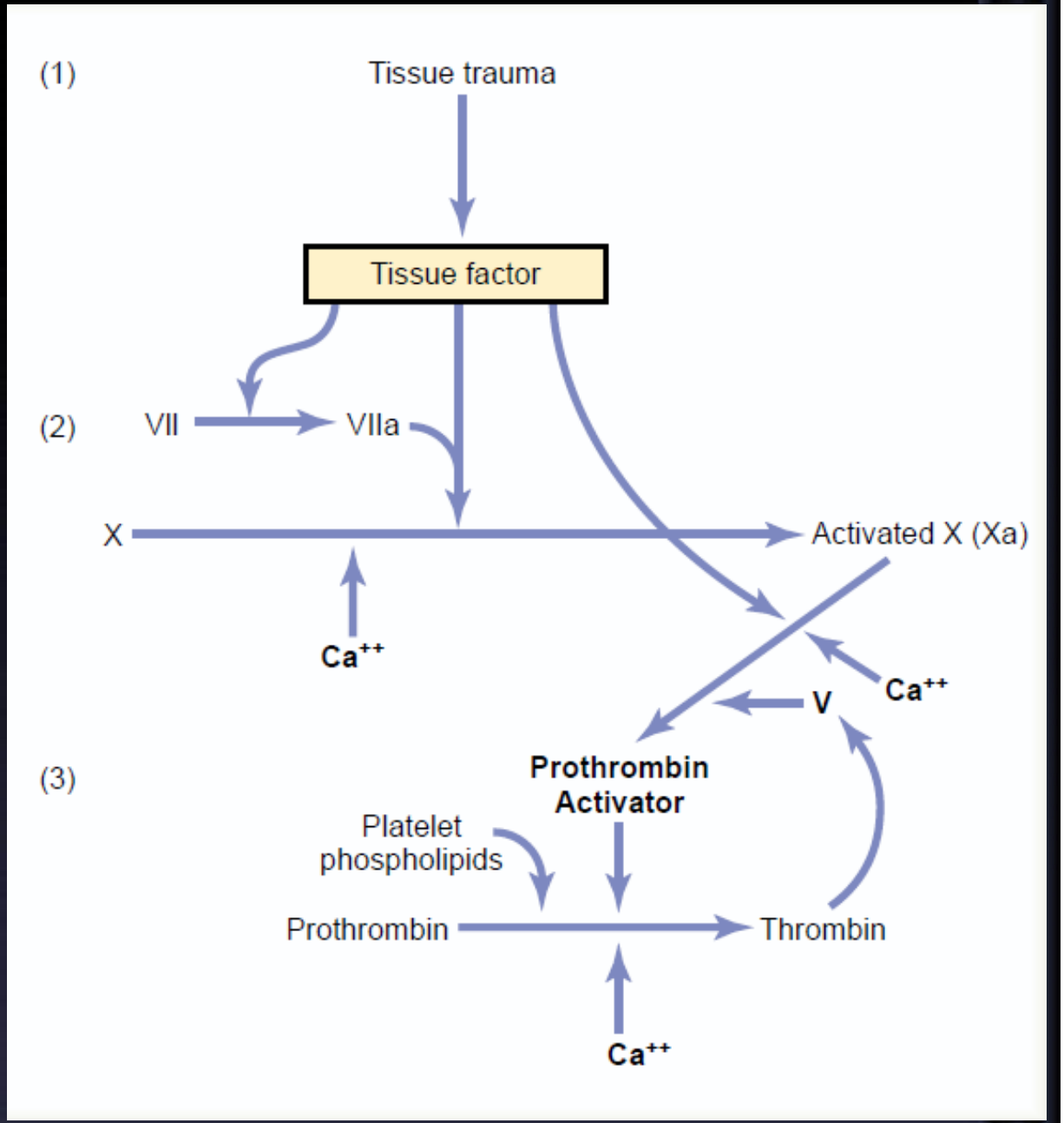
TABLE 31–5 System for naming blood-clotting factors.

Factor[a]	Names
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Proaccelerin, labile factor, accelerator globulin
VII	Proconvertin, SPCA, stable factor
VIII	Antihemophilic factor (AHF), antihemophilic factor A, antihemophilic globulin (AHG)
IX	Plasma thromboplastic component (PTC), Christmas factor, antihemophilic factor B
X	Stuart–Prower factor
XI	Plasma thromboplastin antecedent (PTA), antihemophilic factor C
XII	Hageman factor, glass factor
XIII	Fibrin-stabilizing factor, Laki–Lorand factor
HMW-K	High-molecular-weight kininogen, Fitzgerald factor
Pre-Ka	Prekallikrein, Fletcher factor
Ka	Kallikrein
PL	Platelet phospholipid

[a]Factor VI is not a separate entity and has been dropped.

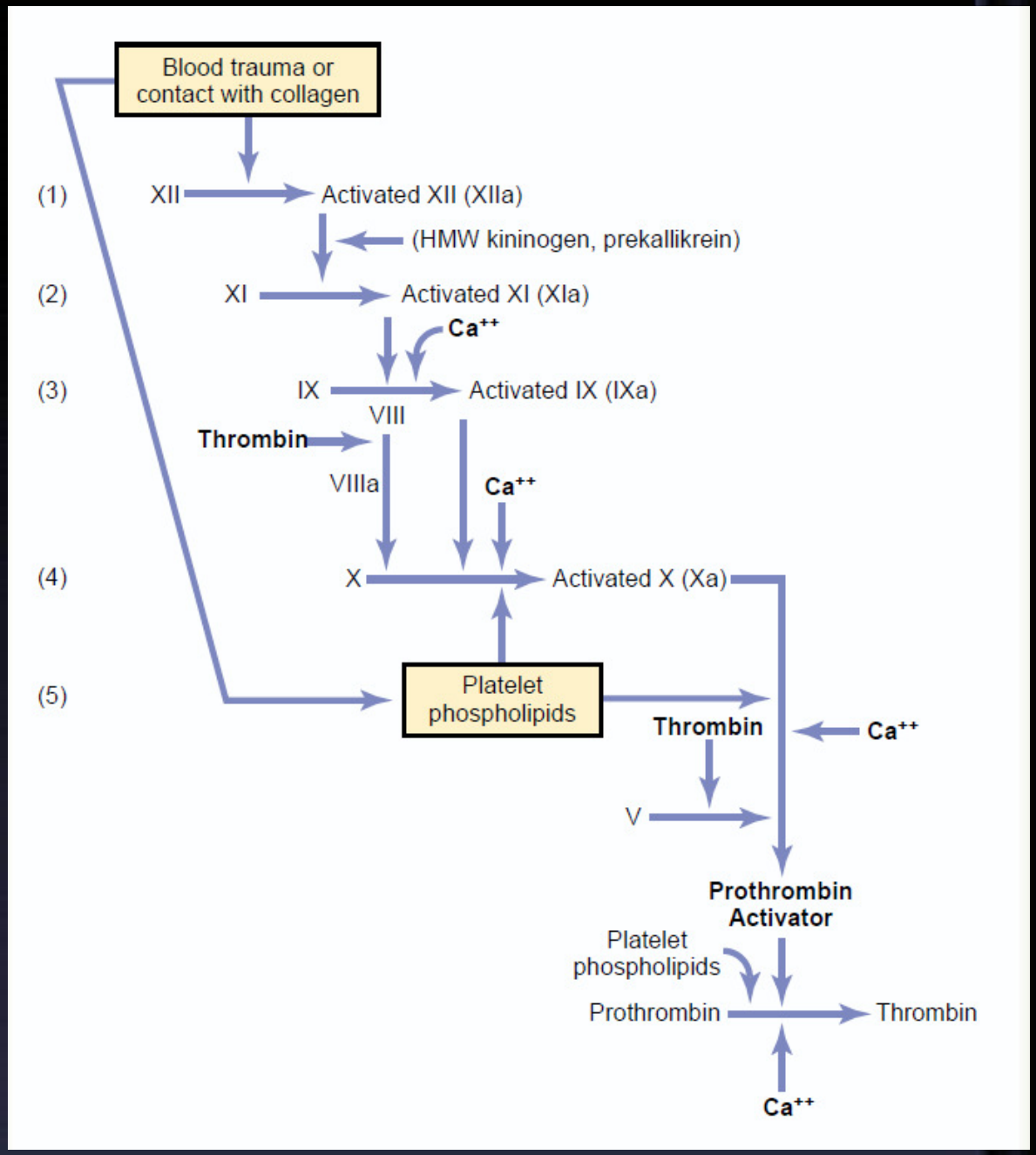
EXTRINSIC MECHNANISM FOR INITIATING CLOTTING

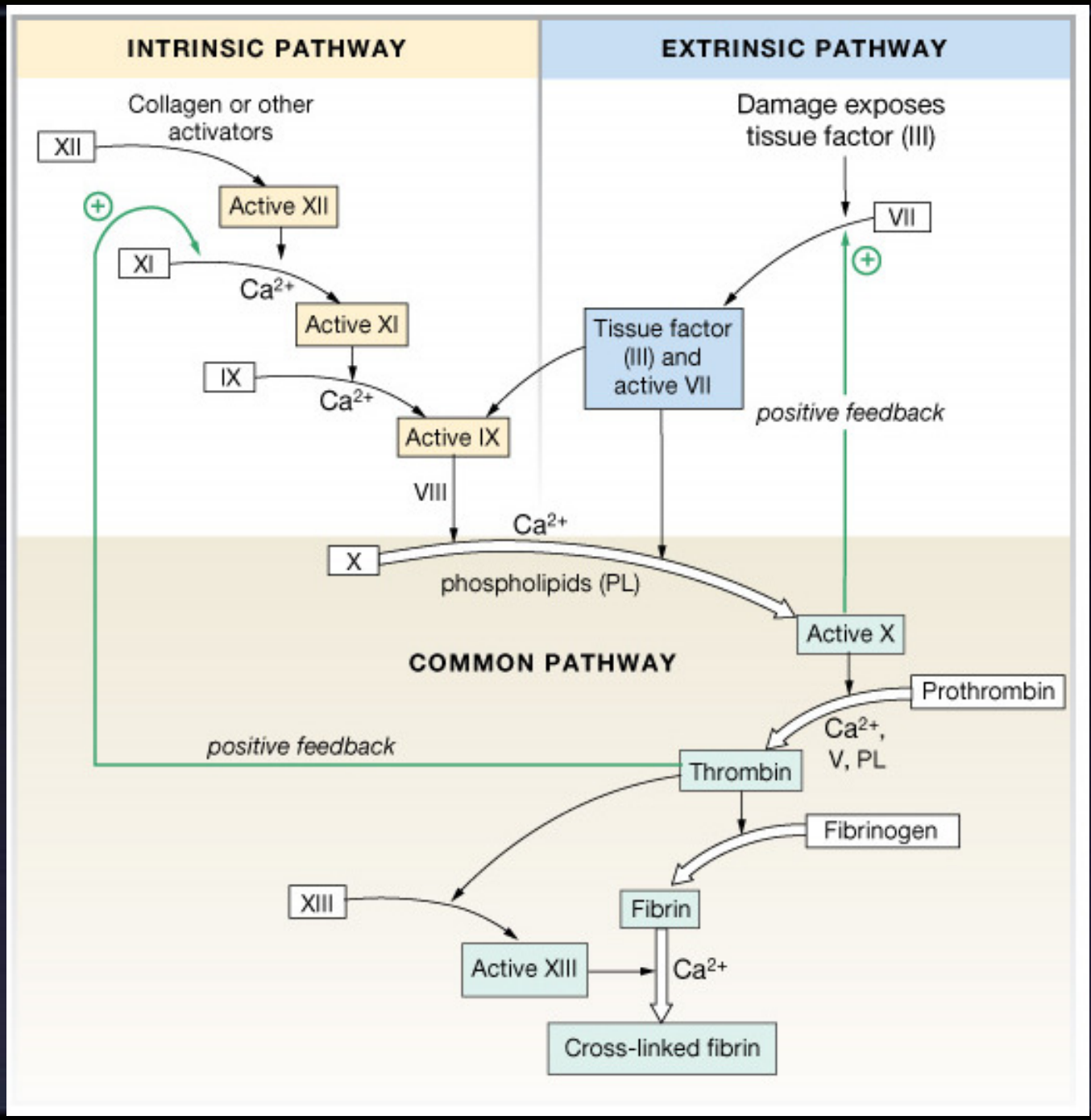
TF or tissue thromboplastin; includes phospholipids from the membranes of the tissue plus a lipoprotein complex that functions mainly as a proteolytic enzyme.



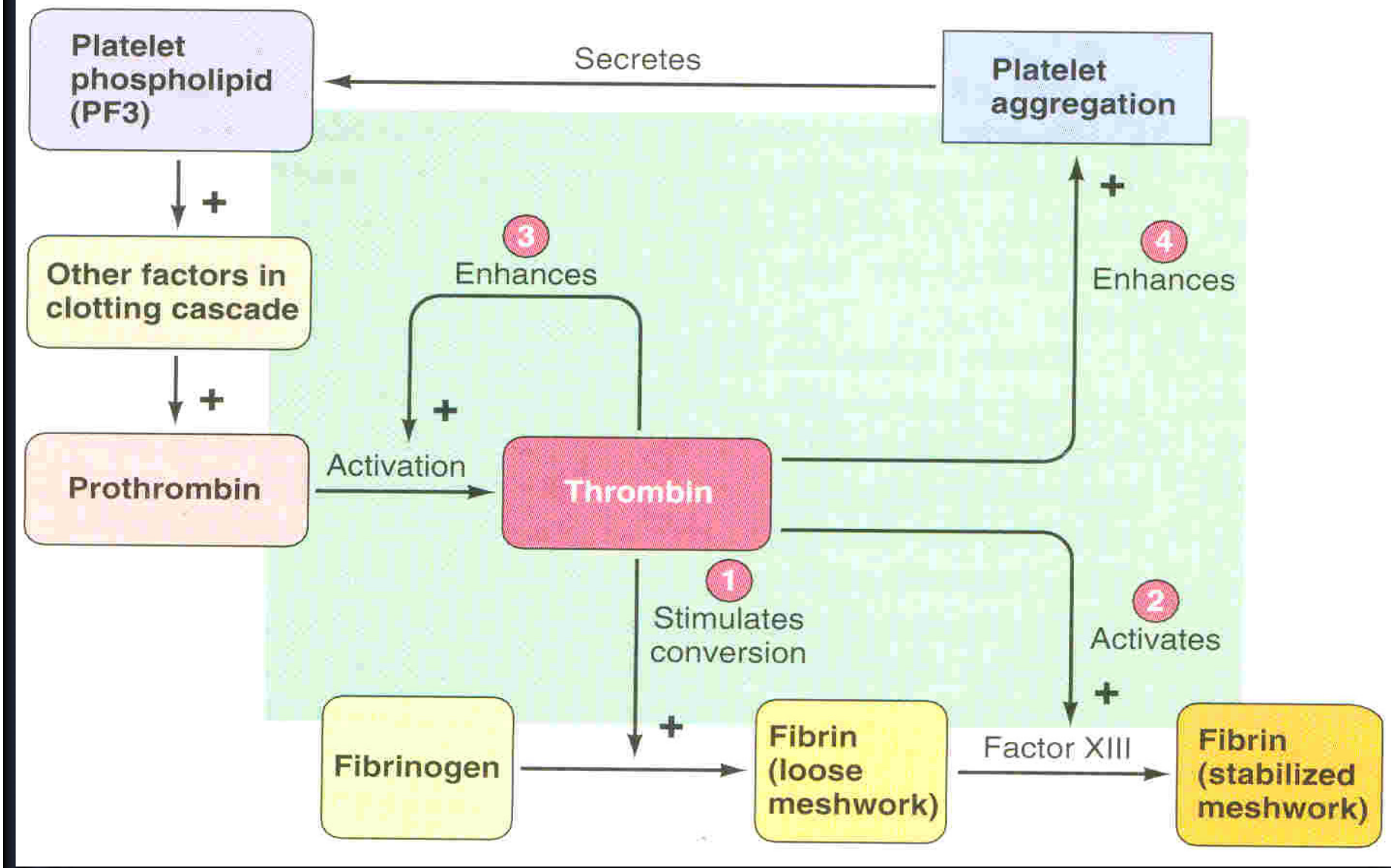
INTRINSIC MECHNANISM FOR INITIATING CLOTTING

Trauma to the blood itself or exposure of the blood to collagen (from a traumatized blood vessel wall), foreign surface/glass




INTRINSIC PATHWAY
EXTRINSIC PATHWAY
Collagen or other activators
XII
Active XII
XI
Ca2+
Active XI
IX
Ca2+
Active IX
VIII
Damage exposes tissue factor (III)
VII
Tissue factor (III) and active VII
positive feedback
Ca2+
X
phospholipids (PL)
Active X
COMMON PATHWAY
Prothrombin
Ca2+, V, PL
positive feedback
Thrombin
Fibrinogen
XIII
Fibrin
Active XIII
Ca2+
Cross-linked fibrin

ROLES OF THROMBIN IN HEMOSTASIS



CLOT RETRACTION

- When clot contracts, it expresses most of the fluid from the clot within 20-60 min called → Serum
- Serum cannot clot
- Role of platelets in clot formation & retraction
- Vicious circle of clot formation

ROLE OF CALCIUM IONS IN CLOTTING

- **No Ca^{++} → No Clotting**

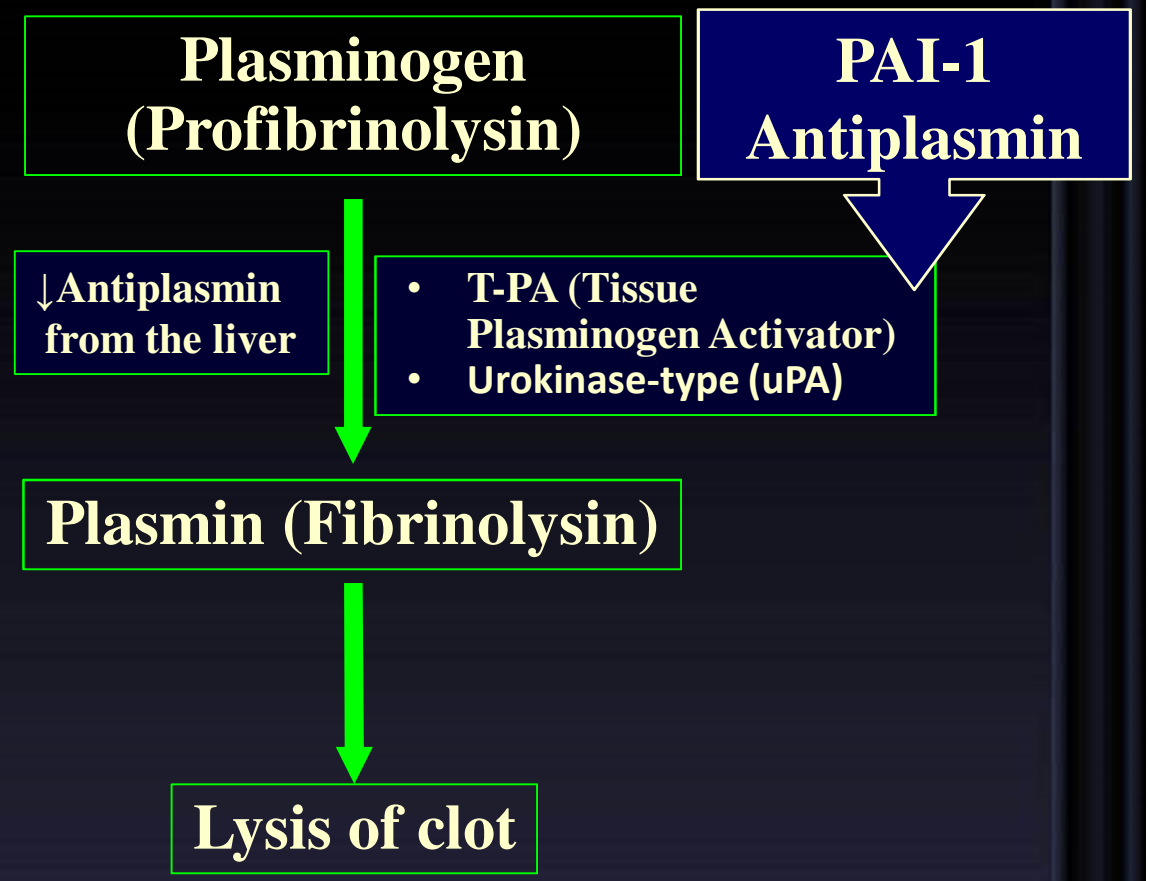
Blood samples are prevented from clotting by:

- **Citrate ions** → **Deionization of Ca^{++}**
- **Oxalate ions** → **Precipitate the Ca^{++}**
- **Heparin** → **combines with antithrombin effectiveness increases by 100-1000 fold, Also remove Factors XII, XI, X, and IX**
- **Warfarin: ↓ production of Factors VII, IX and X by liver.**
- **EDTA** → "sequester" metal ions such as Ca^{2+}

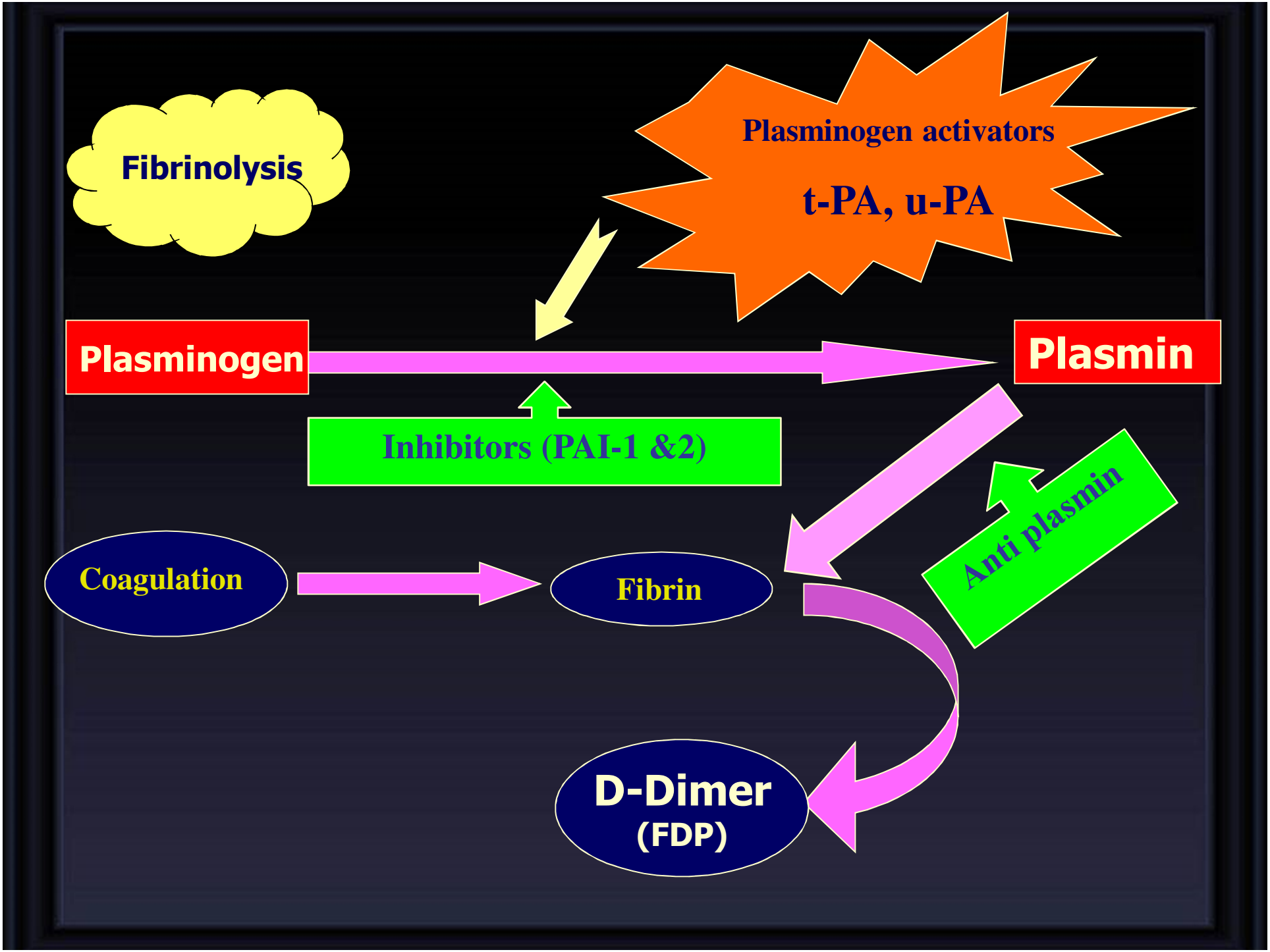
LYSIS OF BLOOD CLOTS BY PLASMIN

Formed blood clot can either become fibrous or dissolve.

- **Fibrinolysis (dissolving) = Break down of fibrin by naturally occurring enzyme plasmin therefore prevent intravascular blocking.**



Tissue Plasminogen Activator (TPA) used to activate plasminogen to dissolve coronary and cerebral clots.


Fibrinolysis
Plasminogen activators
t-PA, u-PA
Plasminogen
Plasmin
Inhibitors (PAI-1 &2)
Anti plasmin
Coagulation
Fibrin
D-Dimer
(FDP)

NATURAL INTRAVASCULAR ANTICOAGULANTS

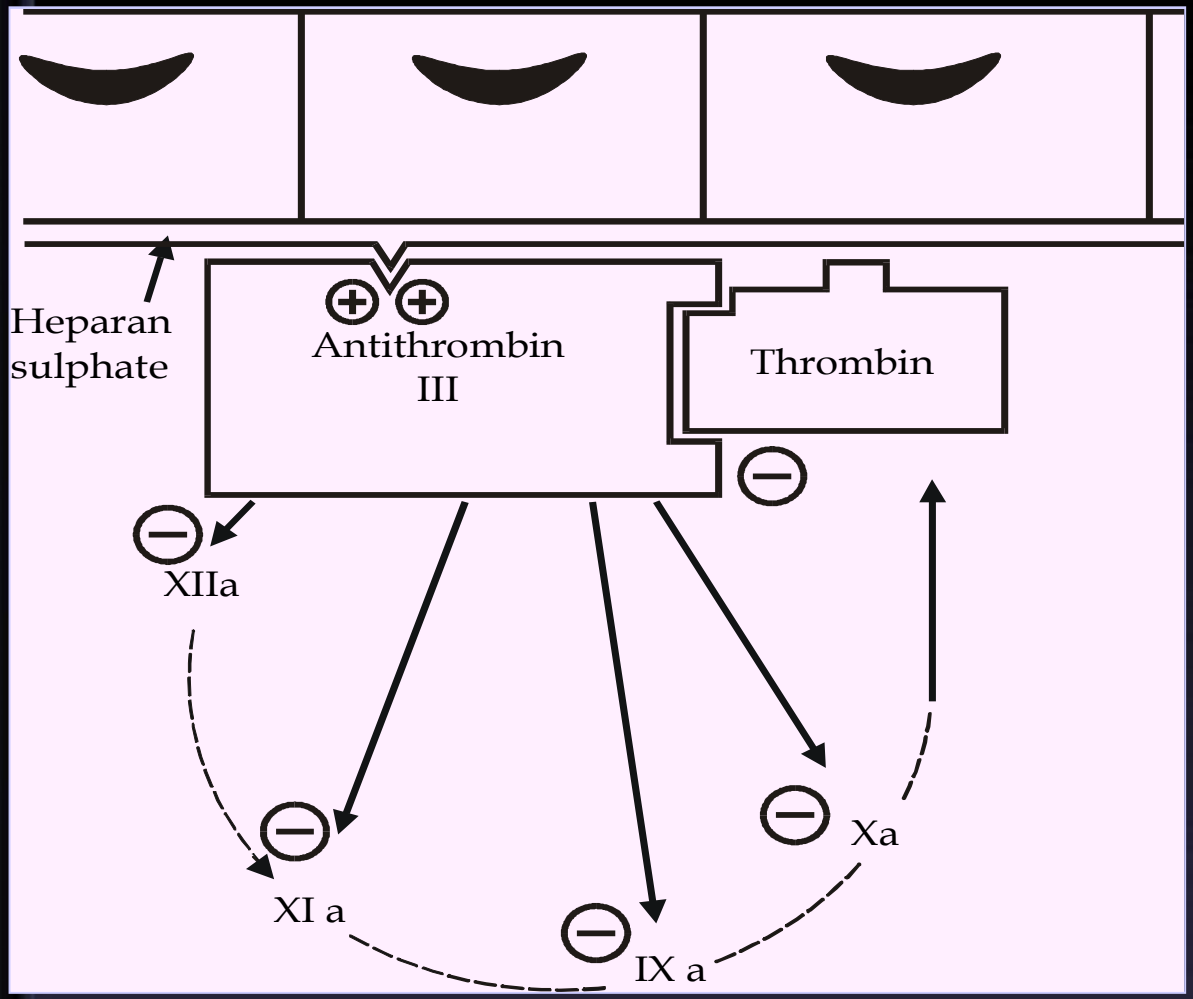
1. **Endothelial Surface Factors**
   - Smoothness of Endothelium
   - Glycocalyx Layers
   - Thrombomodulin Protein binds to thrombin→Activates Protein C (with ProtS)→ inactivates factors V & VIII and inactivates an inhibitor of tPA → increasing the formation of plasmin.
2. **Antithrombin III**
   - 85-90 % Thrombin binds with Fibrin
   - 10-15 % Thrombin binds with Antithrombin III
3. **Protein C** (inhibits Va & VIIIa) & **Protein S** (Cofactor)

Antithrombin III is a circulating protease blocking clot factors

NATURAL INTRAVASCULAR ANTICOAGULANTS

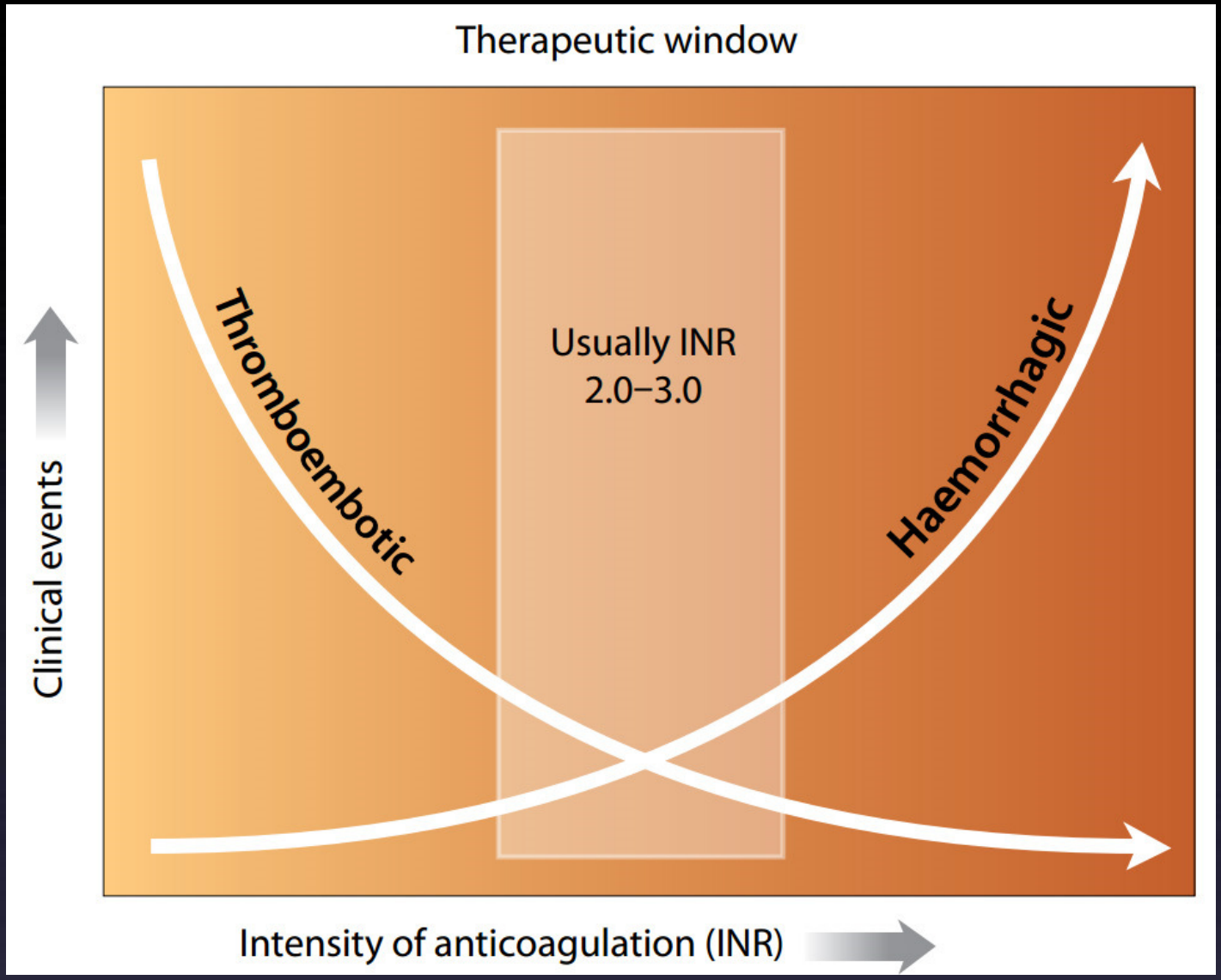
3. **Heparin**
   - **- vely charged conjugated polysaccharide**
     - **Increase the effectiveness of Antithrombin III (100-1000 fold)**
     - **Produced by**
       - **Mast cells**
       - **Basophil cells**
   - **Most widely used anticoagulant clinically e.g. in stroke**
4. **Alpha$_2$ – Macrogobulin**
   - **Acts as a binding agent for several coagulation factors**

Inactivation of coagulation by antithrombin III



- **Synthesis:** hepatocytes & endothelial cells.
- **Action:** ATIII + thrombin → thrombin-ATIII complex.
- Heparin dramatically enhances this action .

PT of Patient/PT of Normal



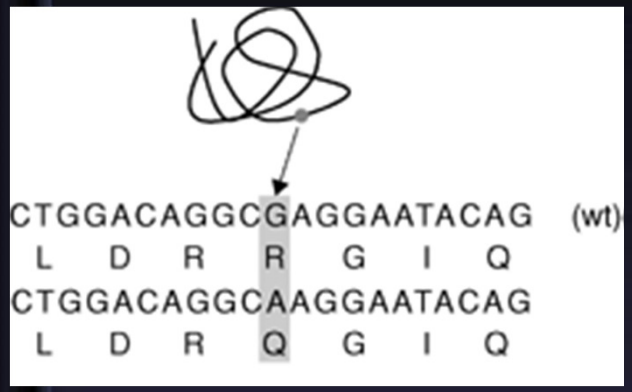
Protein C:

- Vitamin K-dependent.
- Synthesized by the hepatocytes

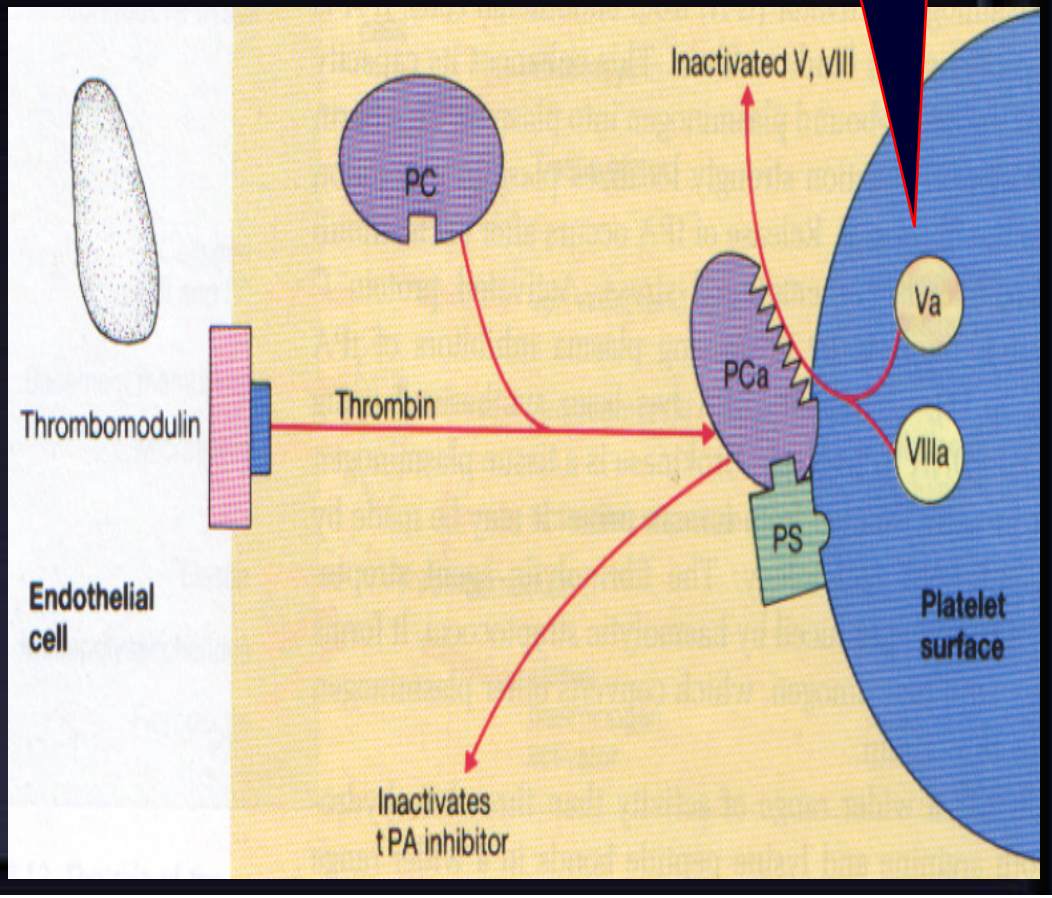
Protein C
(inhibits Va & VIIIa)
& **Protein S** (Cofactor)

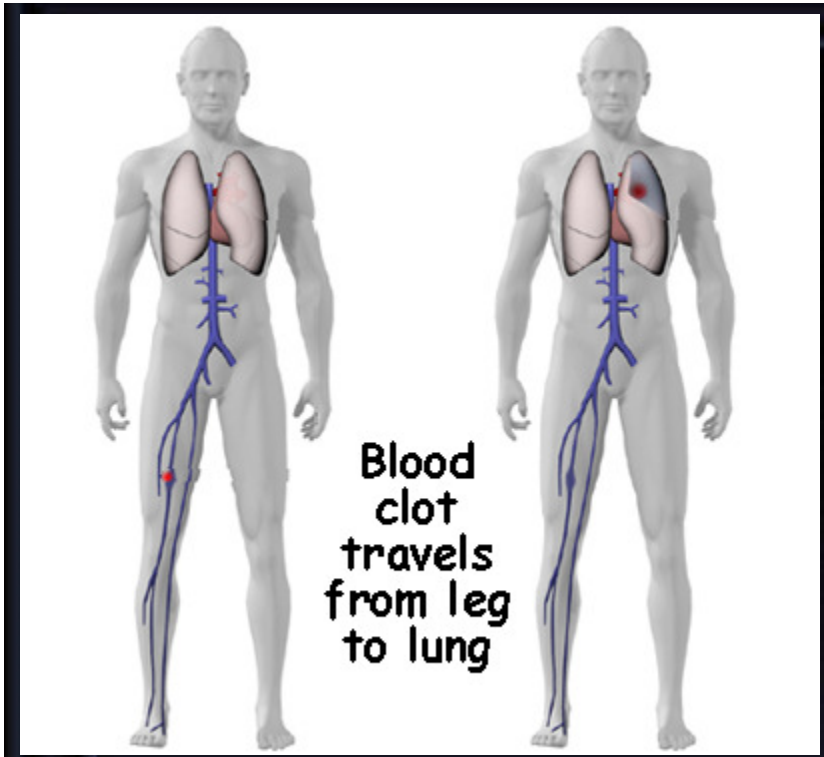
Activated protein C resistance (APC-R):

is mainly due to a genetic abnormality of clotting factor V called (factor V Leiden mutation).

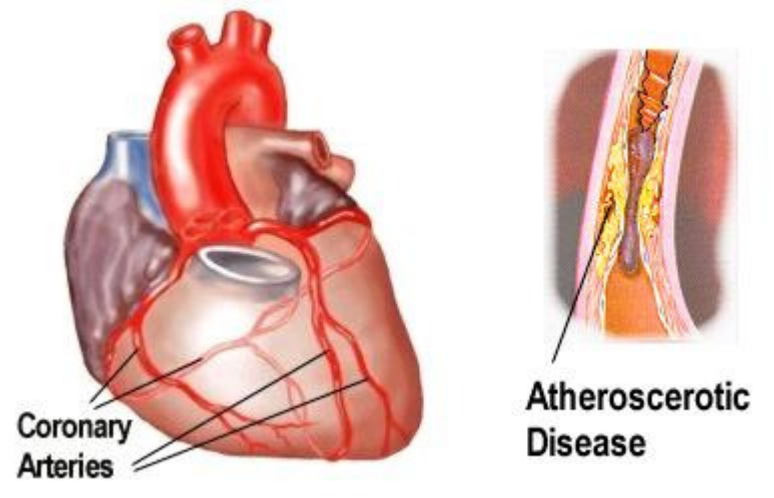


point mutation in the factor V gene, G1691A in exon 10, leading to Arg506Gln.



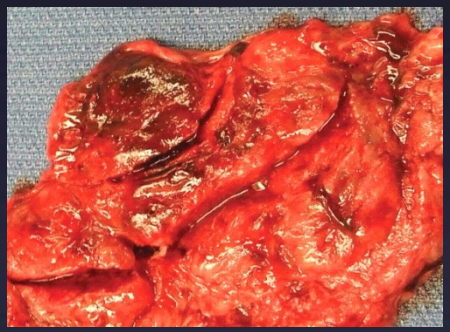


DVT & PE

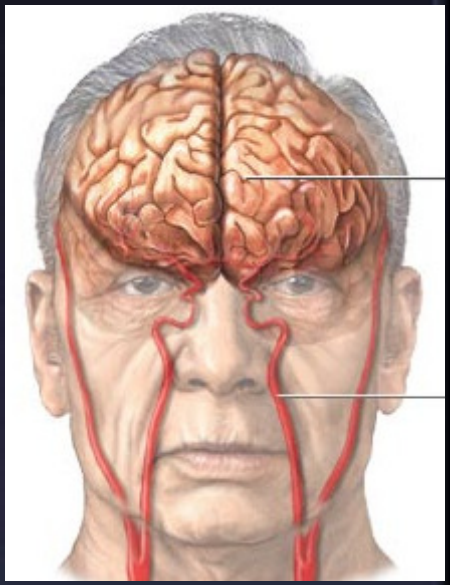


MI

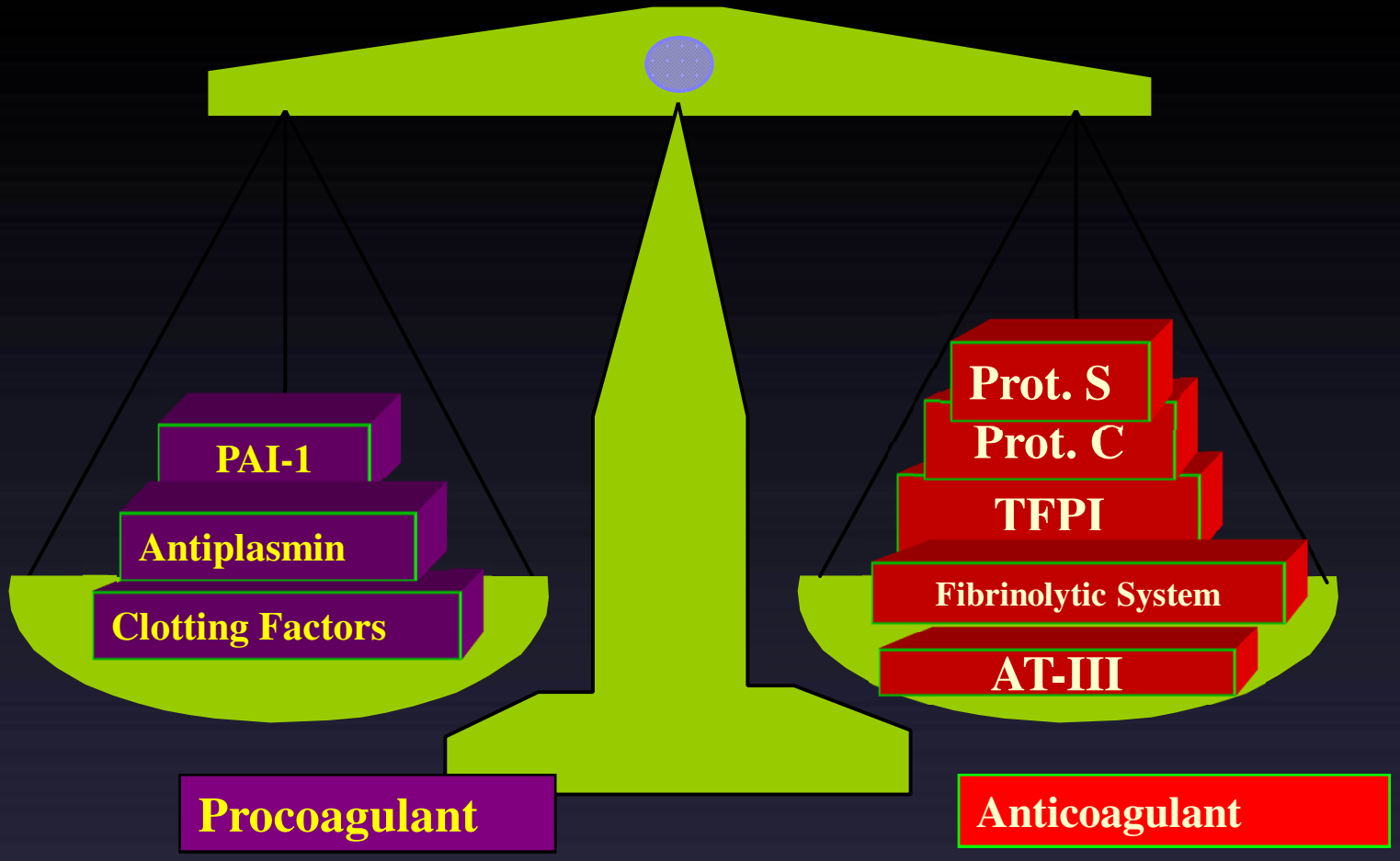
Placental infraction



Stroke



Hemostatic Balance



Laboratory tests of hypercoagulability

Natural anticoagulants

- ATIII
- Protein C
- Protein S

Fibrinolysis

- PAI-1
- FDPs (D-Dimer)

Laboratory tests of hypercoagulability *cont.*

Coagulation activation markers

- Thrombin-Antithrombin complexes (TAT)
- Prothrombin fraction 1+2
- D-Dimer

Activated protein C Resistance (APCR)

- Functional Assay
- Genetic assay (Factor V Leiden)

Genotyping:

- Factor V Leiden
- Prothrombin G20210A
- Hyperhomocysteinaemia (MTHFR)

BLEEDING & CLOTTING DISORDERS

A. Liver diseases & Vitamin-K deficiency
B. Hemophilia
C. Thrombocytopenia

BLEEDING DISORDERS

A. **Liver diseases & Vitamin-K deficiency**

- **e.g. Hepatitis, Cirrhosis**
  - **Decreased formation of clotting factors**
  - **Icnreased clotting time**
- **Vitamin K dependent factors**
  - **Prothrombin, Factor VII, IX, X**

BLEEDING DISORDERS

A. **Vitamin-K**

- Fat soluble vitamin
- Required by liver for formation 4 clotting factors
- Sources
  - Diet
  - Sythesized in the intestinal tract by bacteria
- Deficiency
  - Malabsorption syndromes
  - Biliary obstruction
  - Broad spectrum antibiotics
  - Dietary def (in Neonates)
  - Rx.: Treat the underlying cause Vit K injections

THROMBOCYTOPENIA

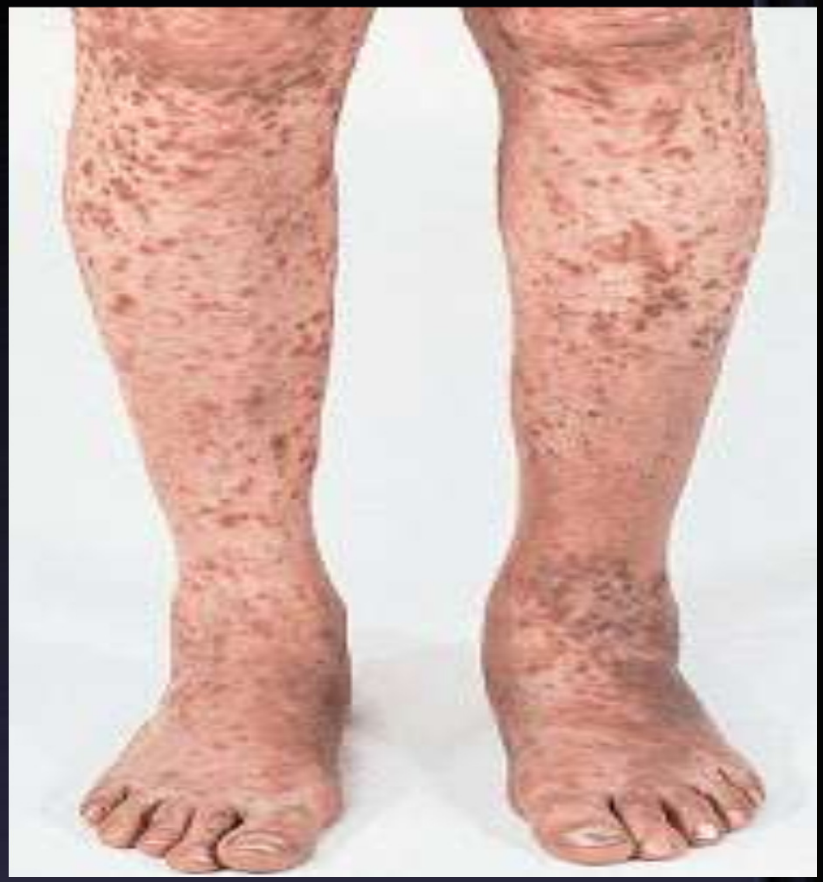
- Platelet count upto 50,000 ul
- Less than 10,000 ------ Fatal
- ETIOLOGY
- Decreased production
  - Aplastic anemia
  - Leukemia
  - Drugs
  - Infections (HIV, Measles)

THROMBOCYTOPENIA (cont.)

- **Increased destruction**
  - **ITP**
  - **Drugs**
  - **Infections**
- **Clinical Features**
  - **Easy brusability**
  - **Epistaxis**
  - **Gum bleeding**
  - **Hemorrhage after minor trauma**
  - **Petechiae/Ecchumosis**

THROMBOCYTOPENIA (cont.)

- Diagnosis
  - PLT decreased
  - B.T increased
- Rx
  - Rx of the underlying cause
  - PLT concentrates
  - Fresh whole blood transfusion
  - Splenectomy



INHERITED COAGULATION DISORDERS

HEMOPHILIA

- **HEMOPHILIA A**
  - Classic Hemophilia
  - 85 % cases
  - Def. Of factor VIII
- **HEMOPHILIA B**
  - 15 % cases
  - Def. Of factor IX

- **Transmitted by female X chromosome as recessive trait**
- **Occurs in males & Females are carriers**

Laboratory diagnosis :

The following tests are abnormal:

PTT

Factor VIII or IX or both.

BT & PT are normal.

HEMOPHILIA

- **Clinical Features**
  - **Easy bruising, massive bleeding after trauma or operation, hemorrhages in joints**
  - **Factor VIII**
    - **Small Comp. → Hemophilia A**
    - **Large Comp. → Von-Willebrand's disease**
  - **Rx**
    - **Injection of factor VIII (Hemophilia A)**
    - **Injection of factor IX (Hemophilia B)**

ACQUIRED COAGULATION DISORDERS

Disseminated Intravscular Coagulation

- Wide spread activation of coagulation with formation of microthrombi in small blood vessels.
- Bleeding diathesis or tendency secondary to depletion of coagulation factors & platelets.

Laboratory diagnosis of DIC:

Low platelet count

Prolonged PT &PTT.

Low fibrinogen or falling fibrinogen on repeated testing.

Fragmented RBCs in blood smear.

Increased FDPs & D-dimer.

VITAMIN K DEFICIENCY

- Vit. k is required for gamma carboxylation of glutamic acid residues (that is important for calcium binding) of factors II,VII, IX, X, protein C & protein S.
- In absence of vit k. , gamma carboxylation fail to occur & non functional forms of vit k. dependant factors circulate in the blood.

Laboratory diagnosis:

Both PT&PTT are prolonged.

Platelet count & fibrinogen are normal with absent FDPs.

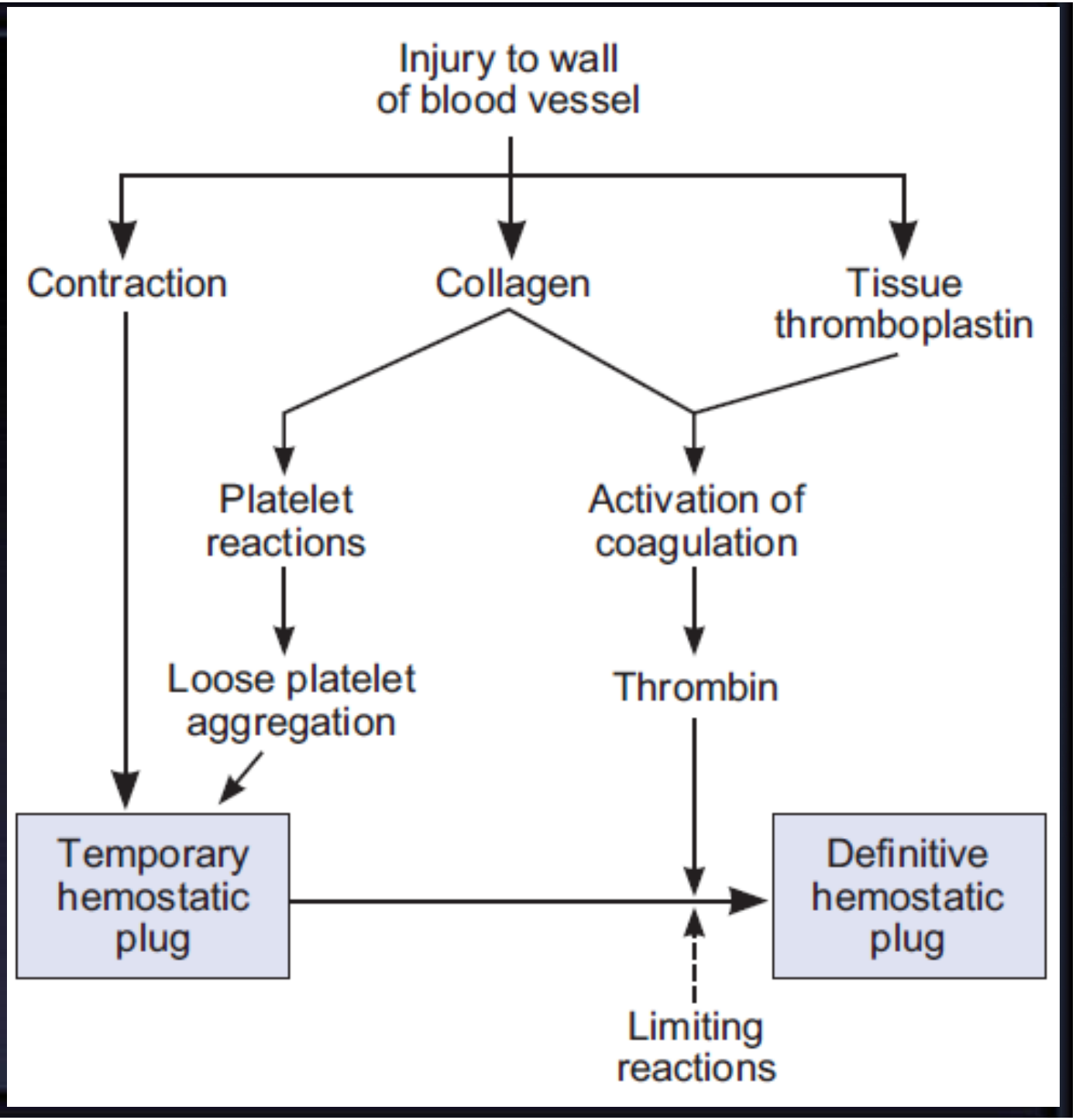
CHRONIC LIVER DISEASE

- Decreased clearance of activated clotting factors & increased level of fibrinogen /fibrin degradation products.
- Liver disease with portal hypertension & splenomegaly --- thrombocytopenia.
- Hepatoma & cirrhosis--- dysfibrinogenemia which is better to be diagnosed by thrombin time & functional fibrinogen assay.

LIVER DISEASE

- The Liver is the major site of clotting factor synthesis
- Hemorrhagic diathesis occur in acute hepatitis or cirrhosis.
- In these conditions, vitamin K- dependant factors II,VII, IX, X, protien C & S are reduced.
- Factors V,VIII are not synthesized by hepatic parenchymal cells but they are increased as acute phase reactants.
- vWF synthesized by endothelial cells is increased as an acute phase reactant.

Summary of reactions involved in hemostasis.



FACTORS AFFECTING BLOOD PLATELET COUNT

- AGE : ↓ in newborn
- Menstrual cycle:
  - ↓ prior to menstruation
  - ↑ After menstruation
- Pregnancy: ↓
- Injury: ↑
- Adrenaline: ↑
- Hypoxia: ↑
- Smoking: ↓
- Nutritional deficiencies: ↓ eg; vitamin b12, folic acid and iron

The fibrinolytic system and its regulation by Protein C

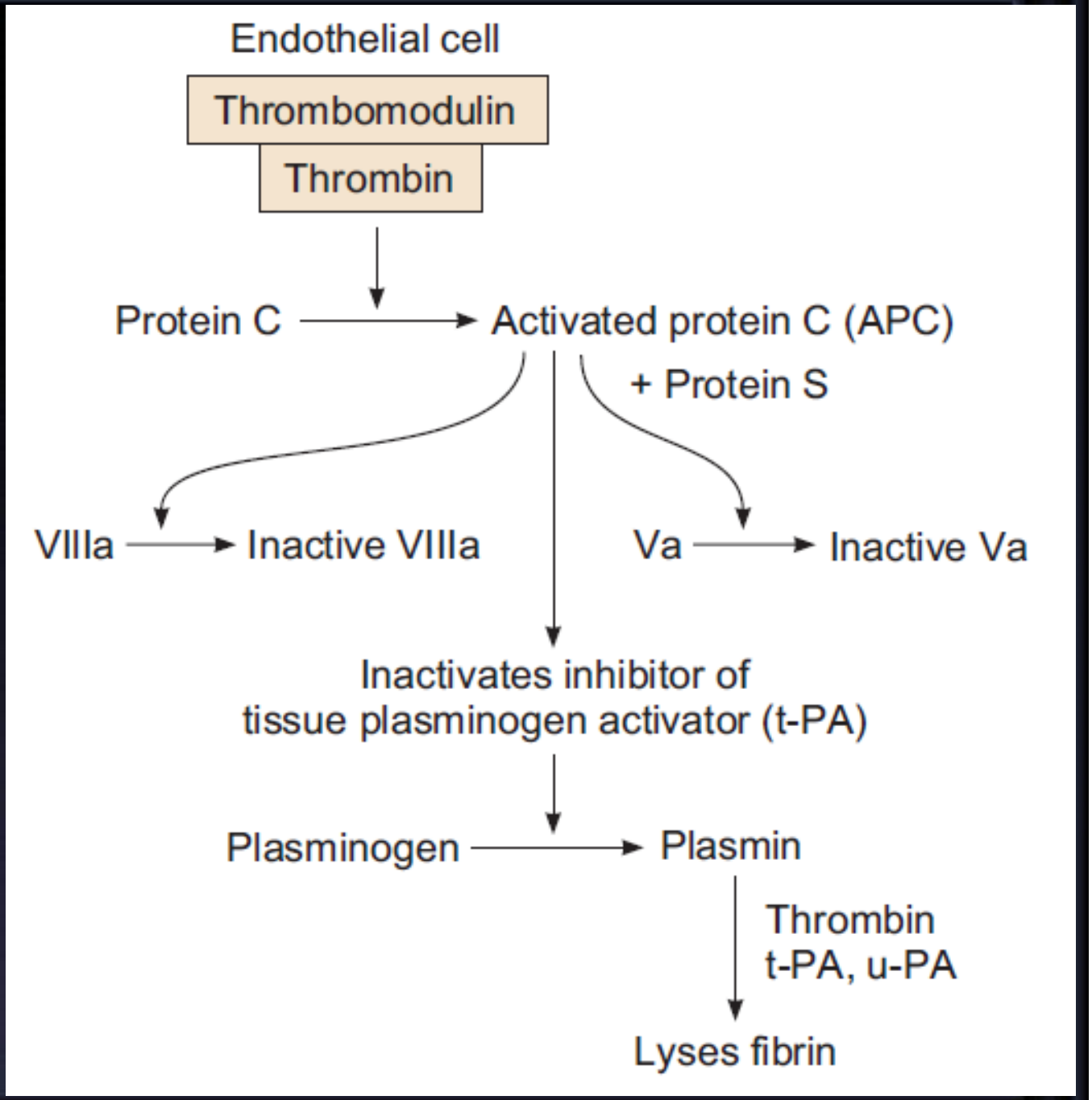
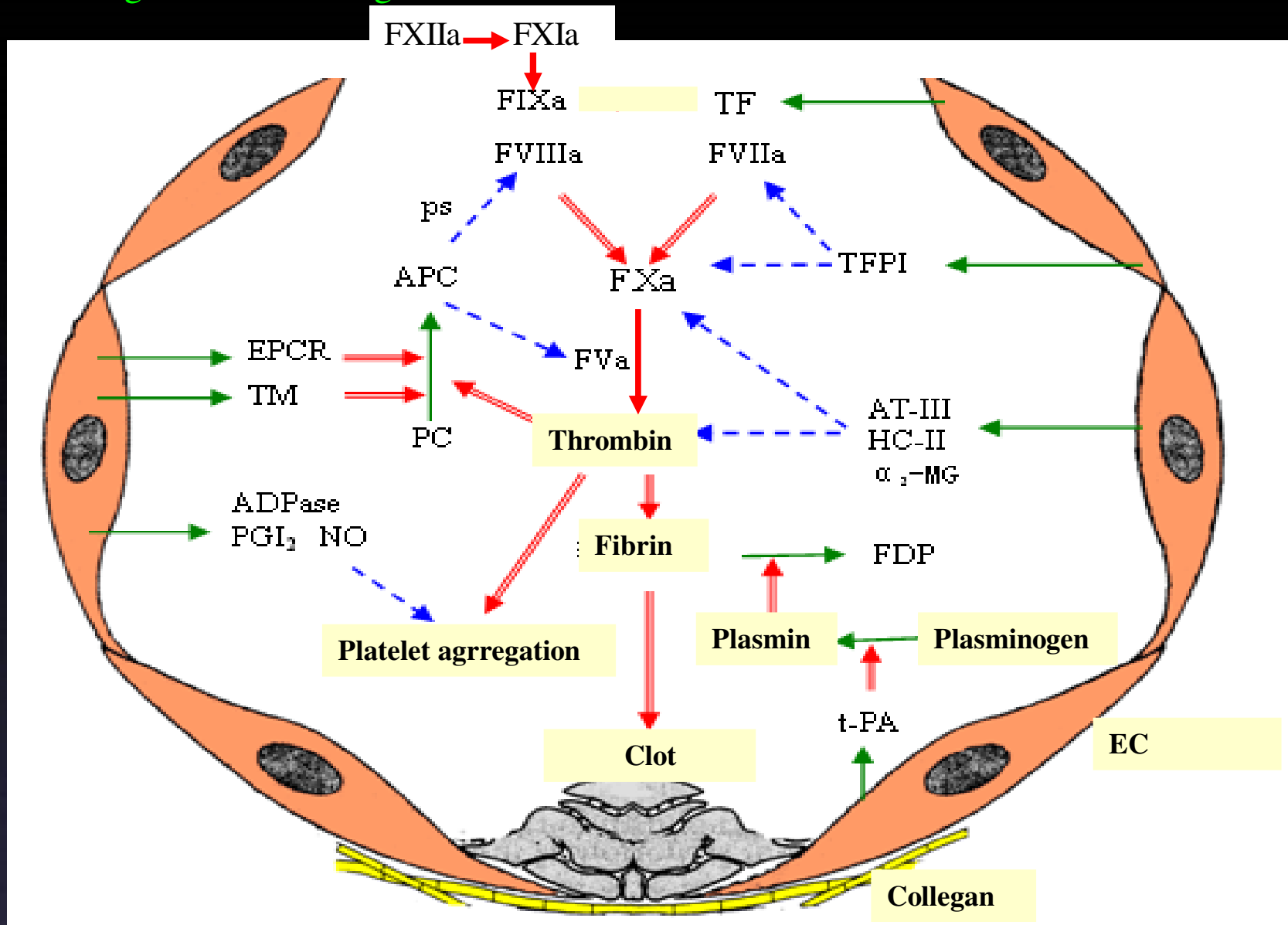


Fig 9-3 Coagultion and its regulation



“ ” activate; “ ” inactivate; “ ” source / transforming

Coagulation Tests

The process of hemostasis occurs in three phases :

1. The vascular platelet phase, which assures primary hemostasis ;
2. Activation of the coagulation cascade, which assures formation of the clot ;
3. And activation of a series of control mechanisms, which stop propagation of the clot and limit activation of the coagulation cascade to the region of endothelial rupture.

Coagulation Tests

1. **Tests of the Vascular Platelet Phase of Hemostasis:**

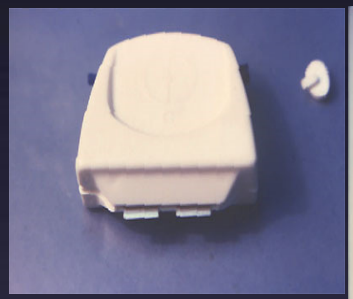
- Bleeding Time (BT)

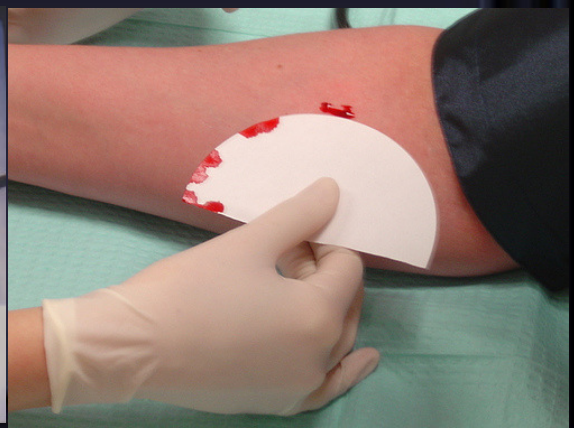
2. **Tests of the Coagulation Cascade:**

- Clotting Time (CT) or Coagulation time
- Activated Partial Thromboplastin Time (APTT).
- Prothrombin Time (PT).

3. **Tests of Fibrinolysis and the Mechanisms That Control Hemostasis:**

- Fibrin Degradation Products (FDP)

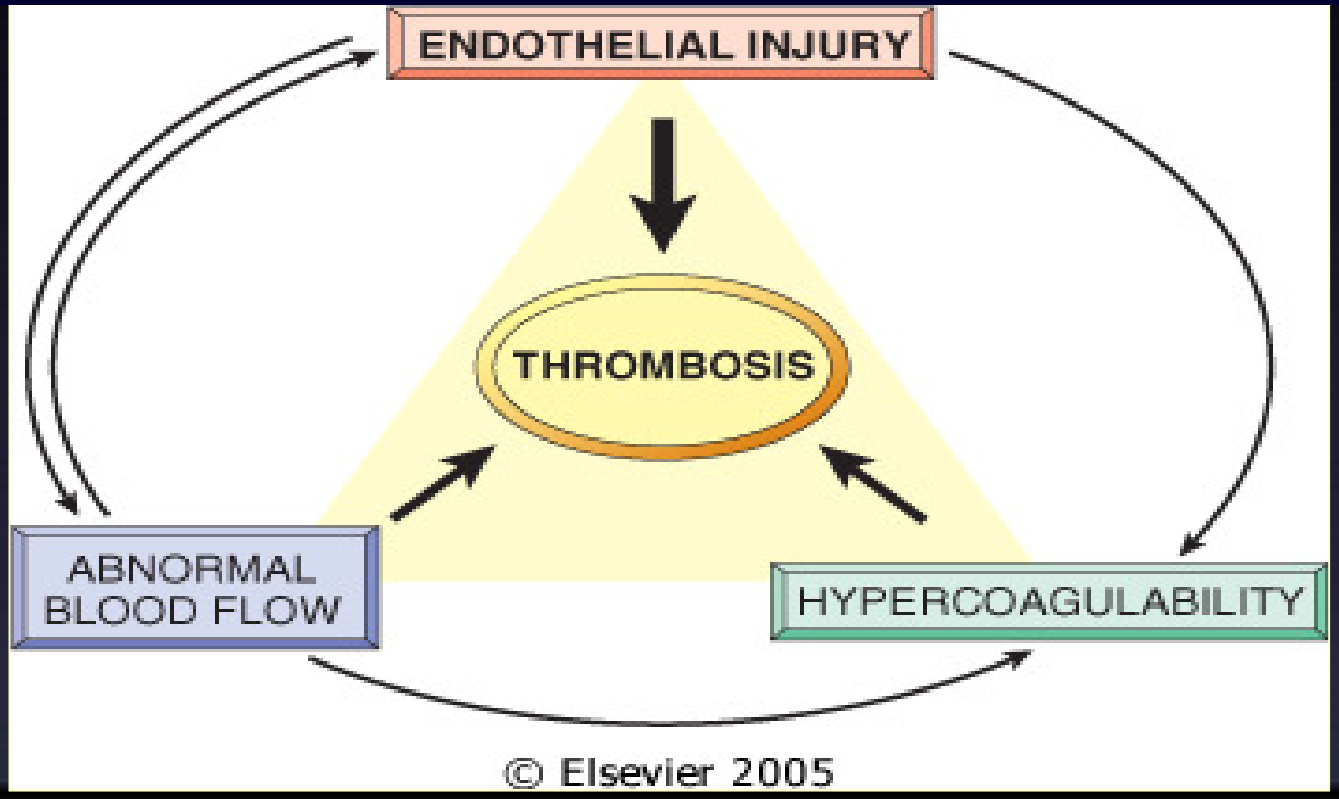





Hypercoagulability:

- Is a laboratory phenotype whereby activation of the of clotting, fibrinolysis, endothelial cells and platelets are identified.

Thrombus formation: Virchow's triad



Hypercougulability/Prothrombotic States

Hereditory Hemostatic disorders:

- Factor V Leidin
- Prothrombin G20210A
- Hyperhomocysteinaema
- Deficiencies of AT III, Proteins C & S
- Increased FVIII

Acquired Hemostatic disorders:

- Raised Levels of fibrinogen & FVII
- Antiphosphlipid Antibodies (LA & ACAs)
- Oestrogen therapy
- Pregnancy and its complications
- Surgery and prolonged immobility
- Major Trauma
- Malignancy
- Nephrotic Syndrome

Hypercougulability/Prothrombotic States *cont.*

Acquired Hemostatic disorders : cont.

Dehydration, Hyperviscosity, Polycycaemia, Thrombocytosis

Sepsis

Smoking

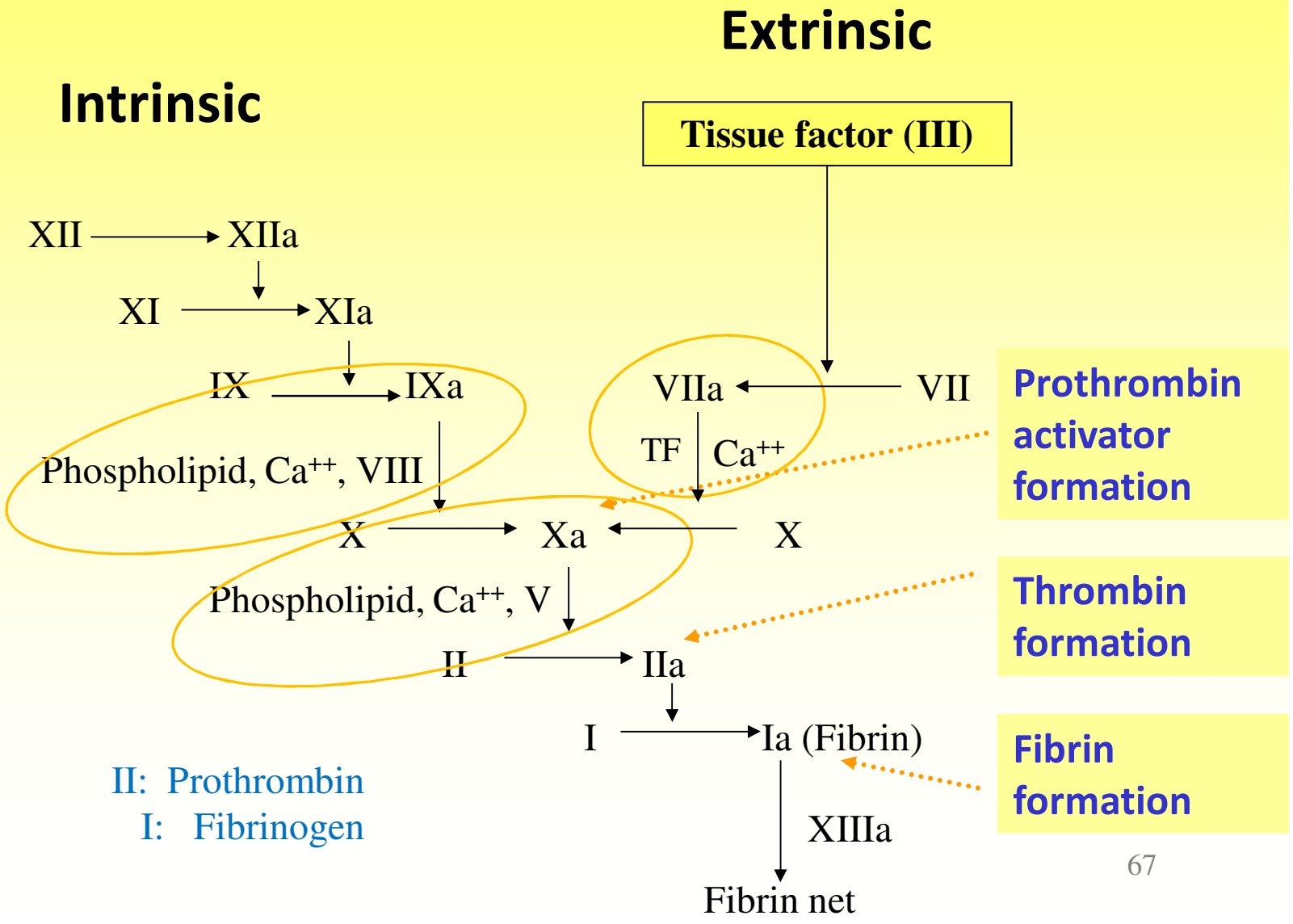
Obesity

Age

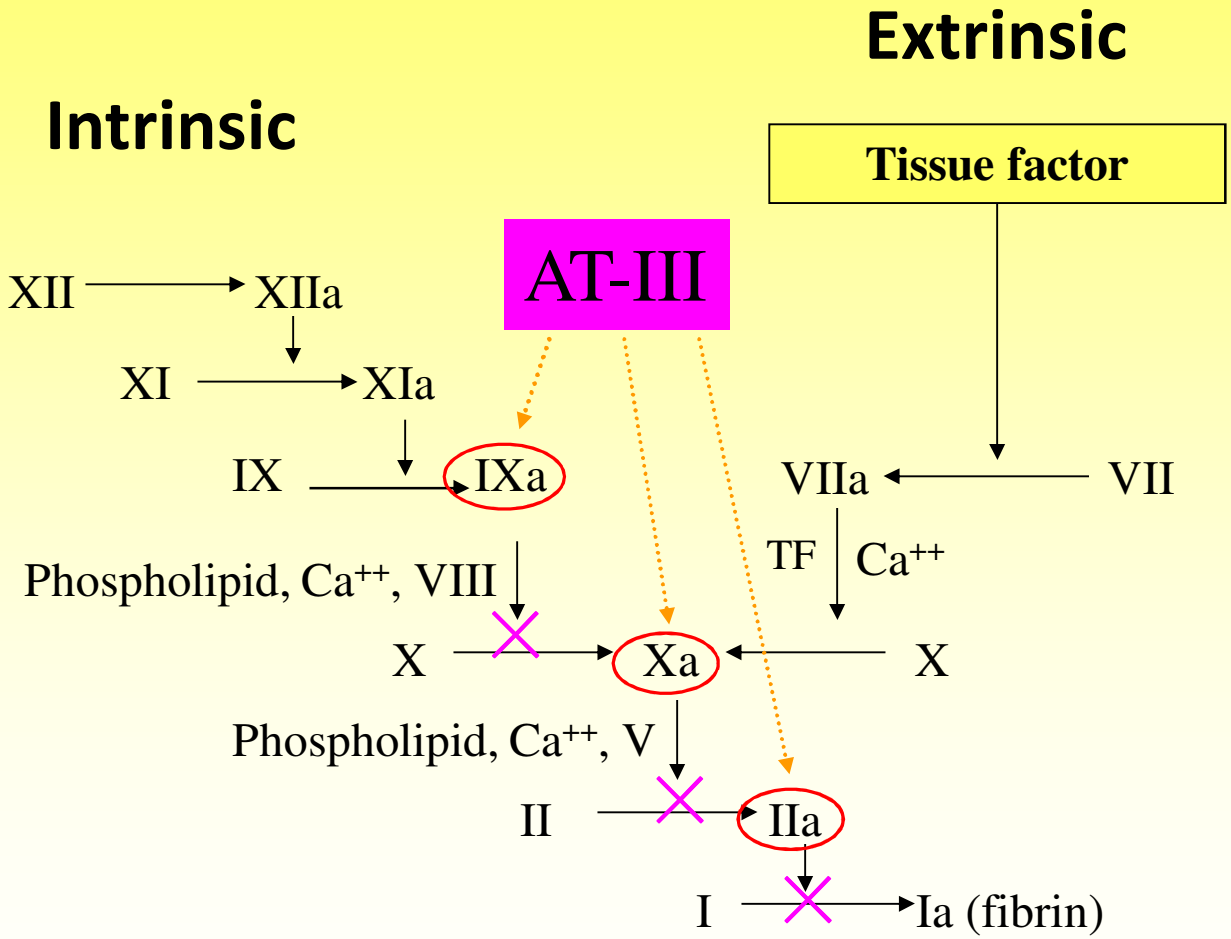
Varicose veins


THANKS

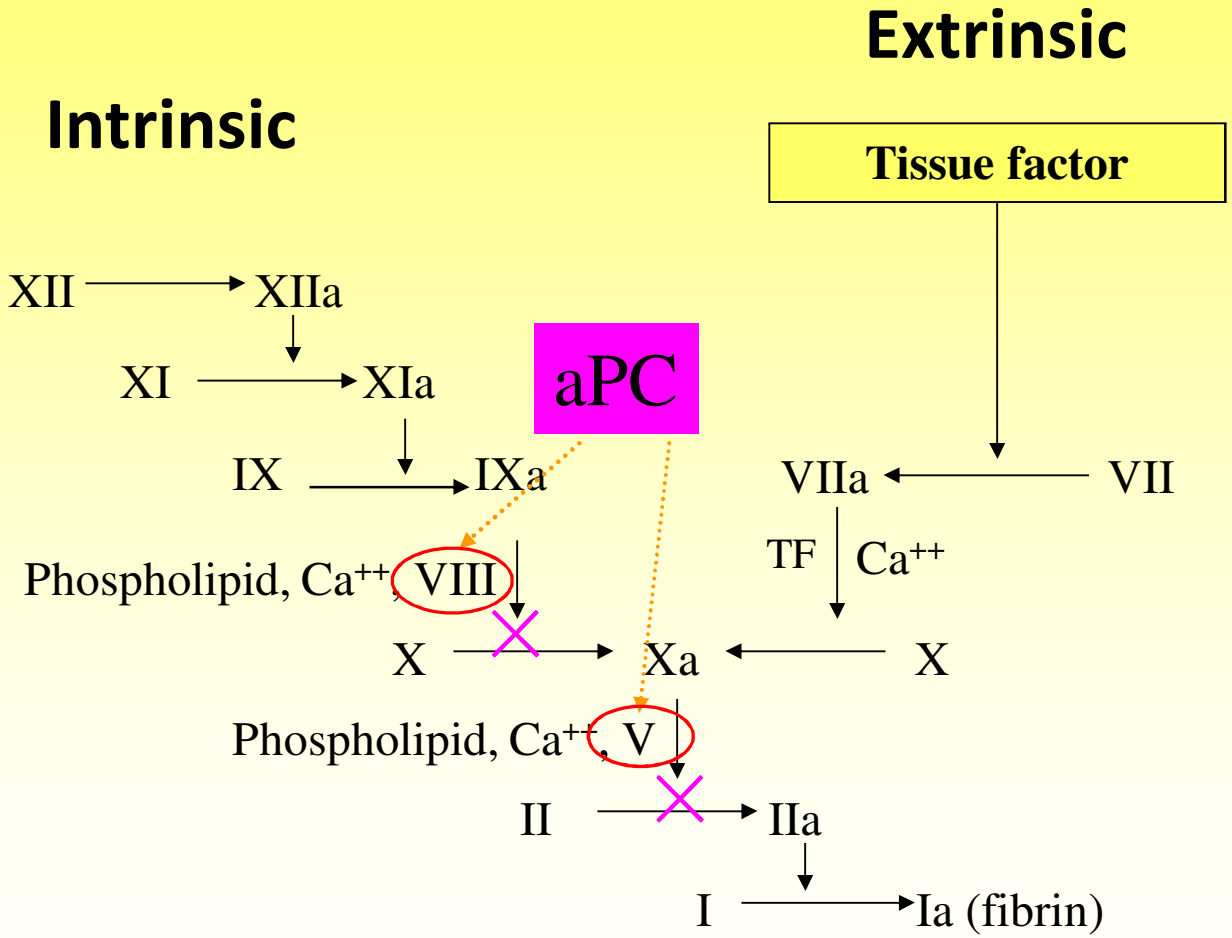
The "Classic" Coagulation System



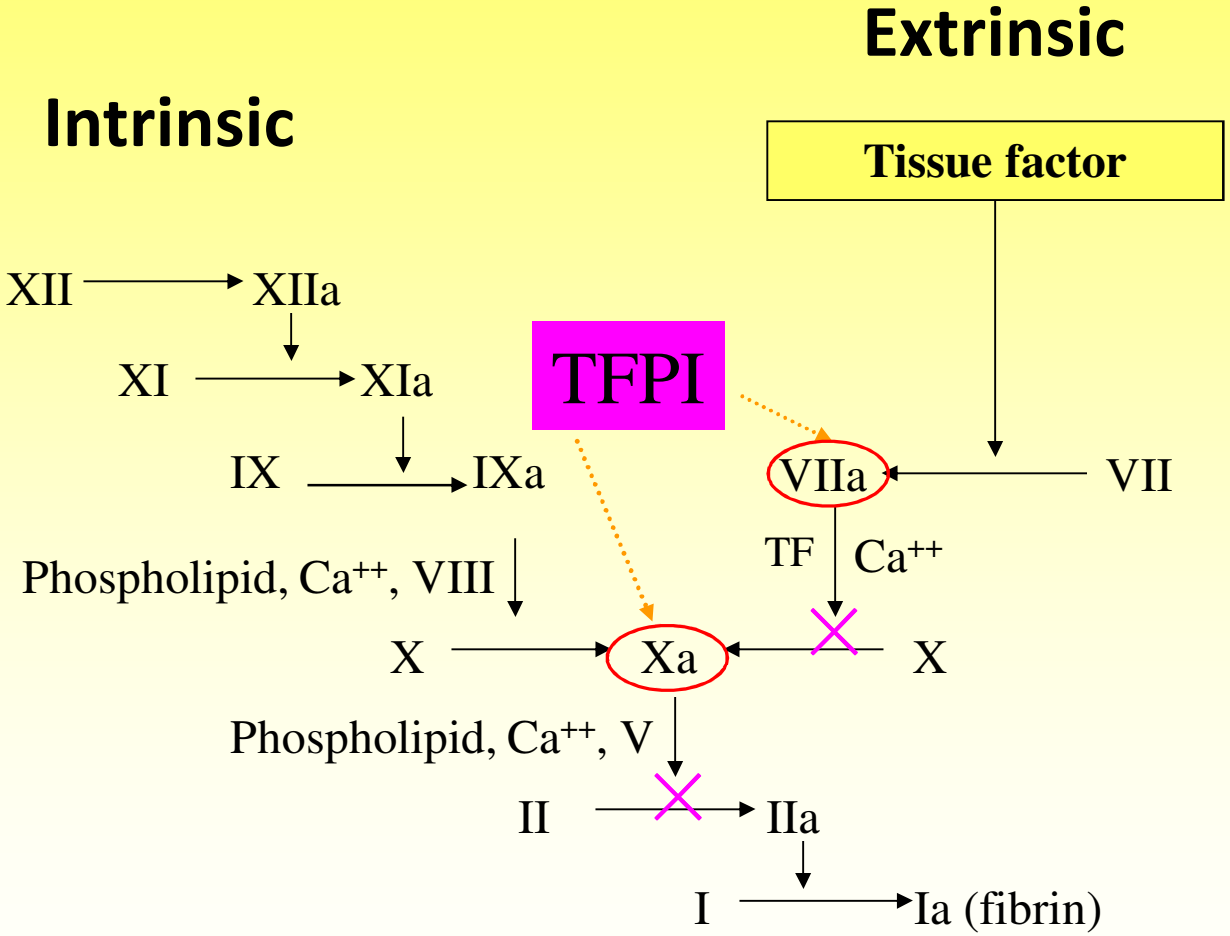
The Effect of Antithrombin III



The Effect of Protein C



The Effect of TFPI



THE ”CLASSIC” COAGULATION SYSTEM