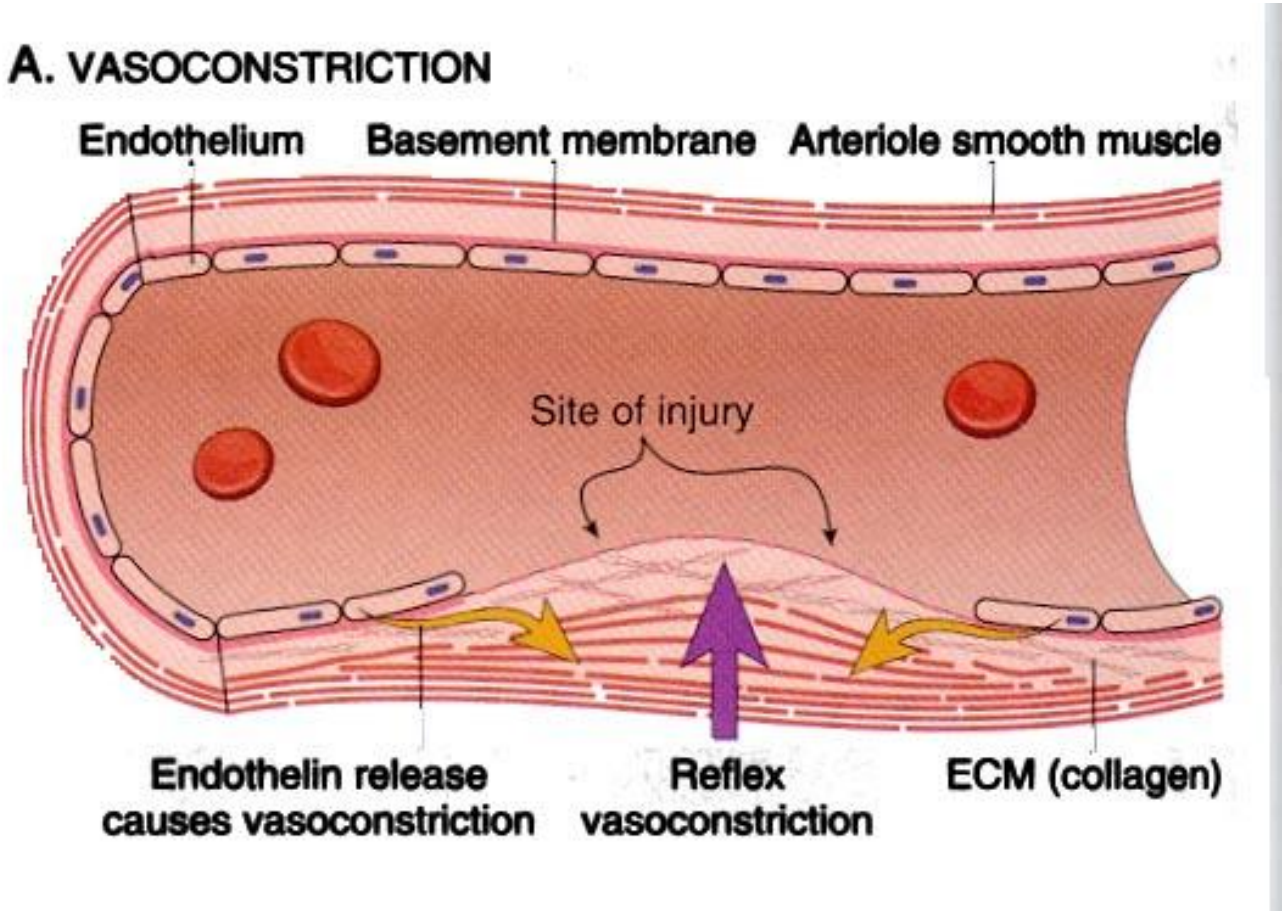
HEMOSTASIS

Nidal M. Almasri, M.D.
Professor and Consultant
King Hussein Cancer Center

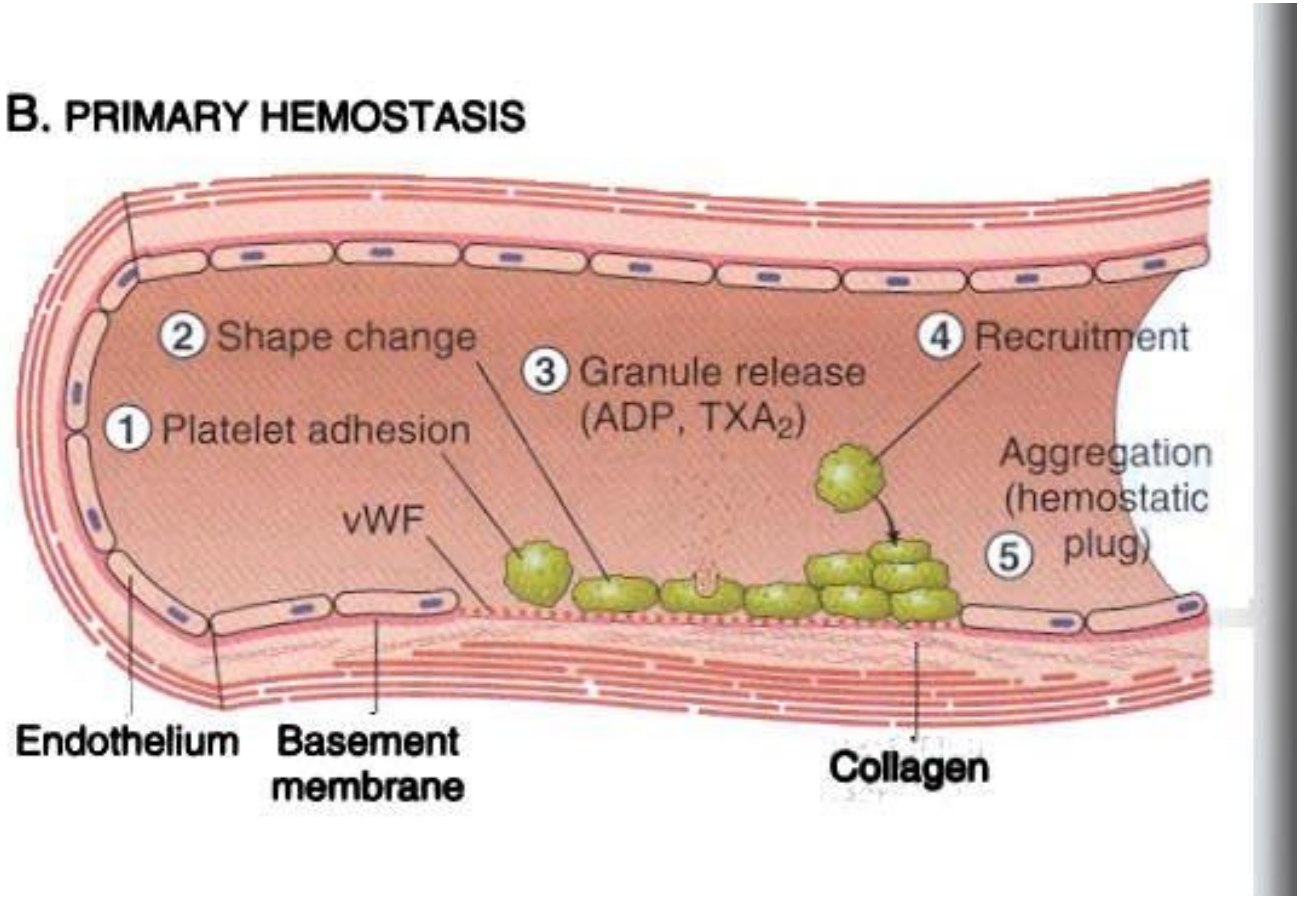
Hemostasis

- **Definition: Arrest of bleeding**
- **Hemostasis depends on the integrity of**
  - **Blood vessels**
  - **Platelets**
  - **Coagulation factors**
  - **Anticoagulation factors**

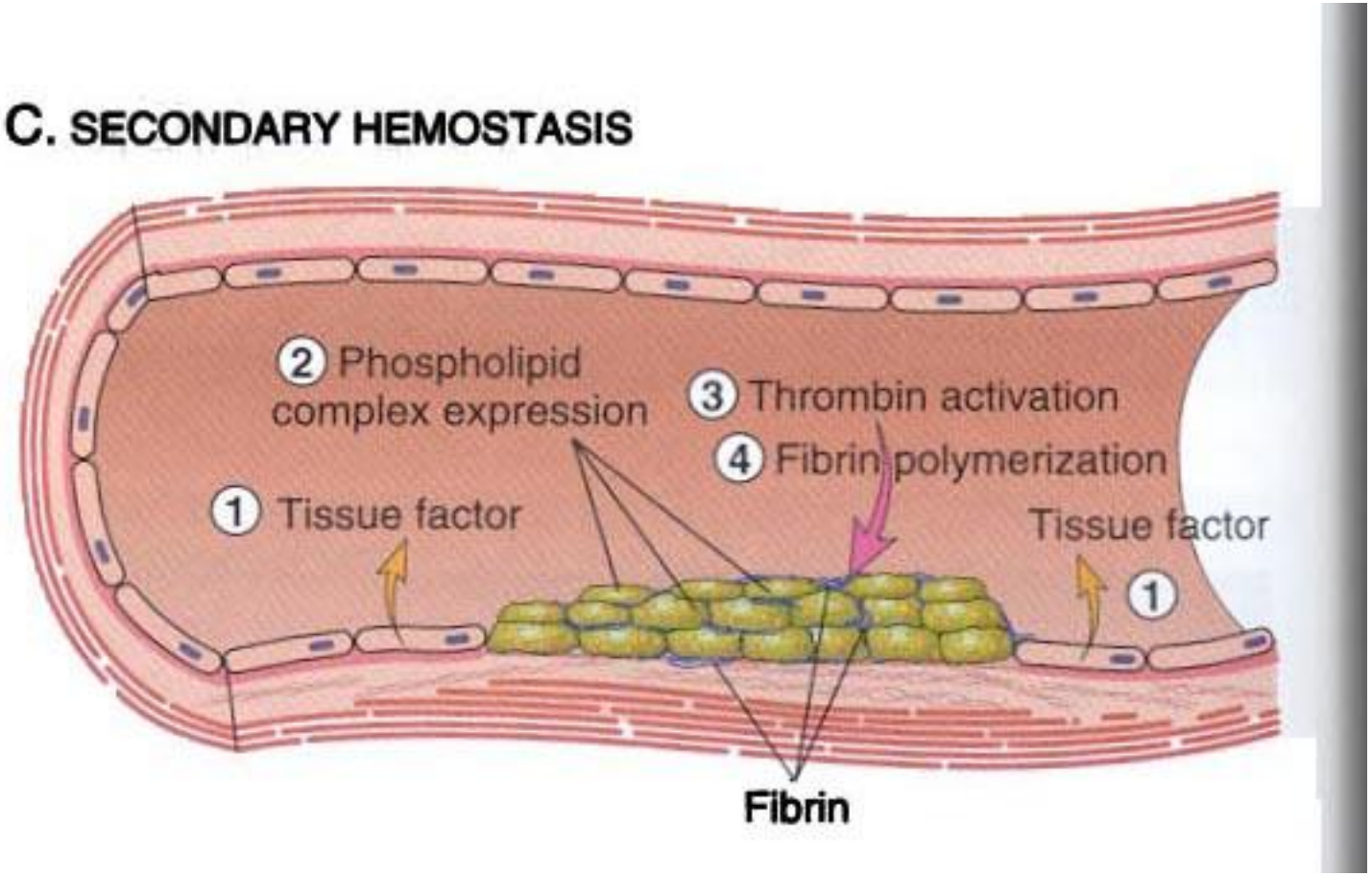
Steps of Hemostasis (1)



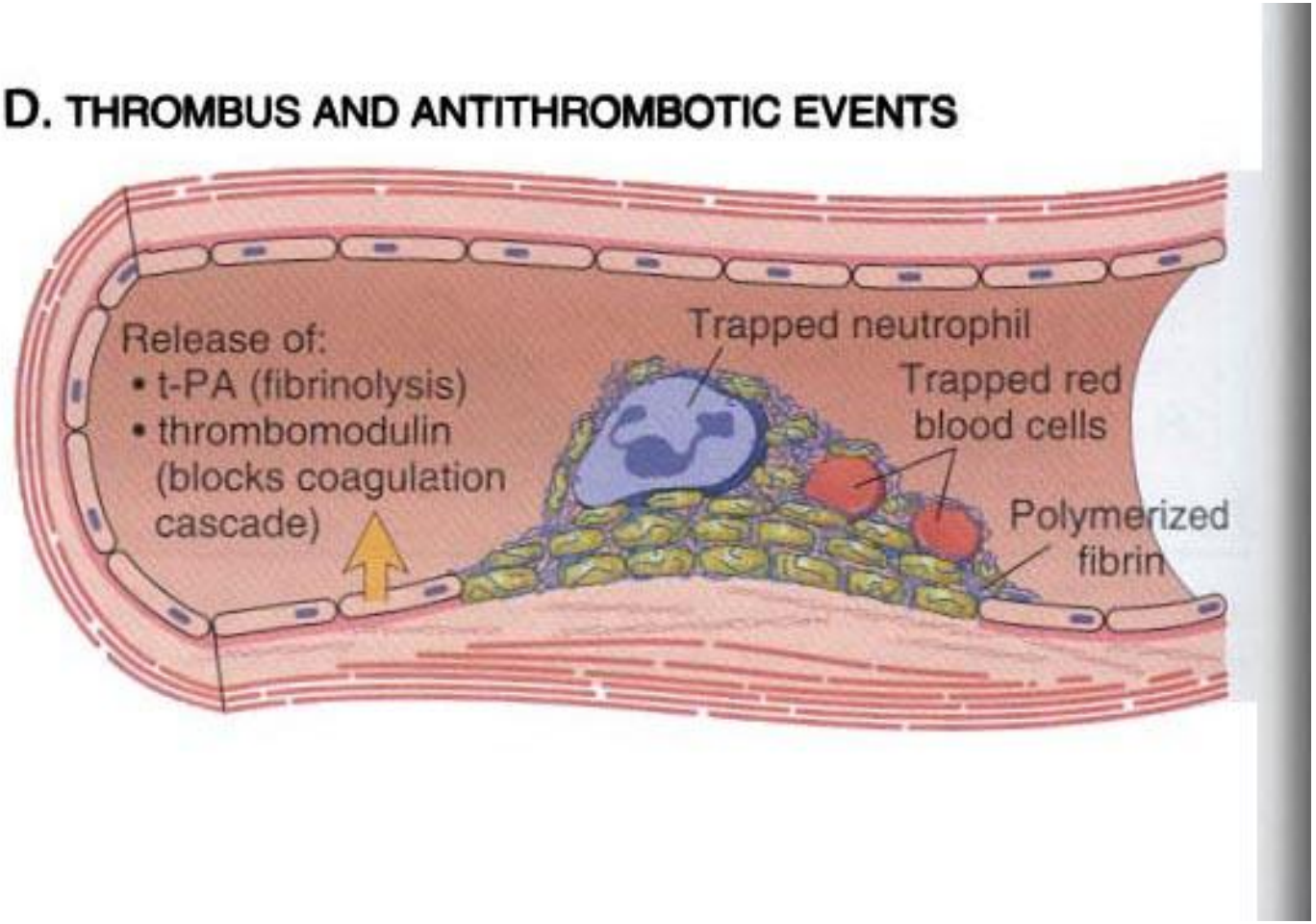
Steps of Hemostasis (2)



Steps of Hemostasis (3)



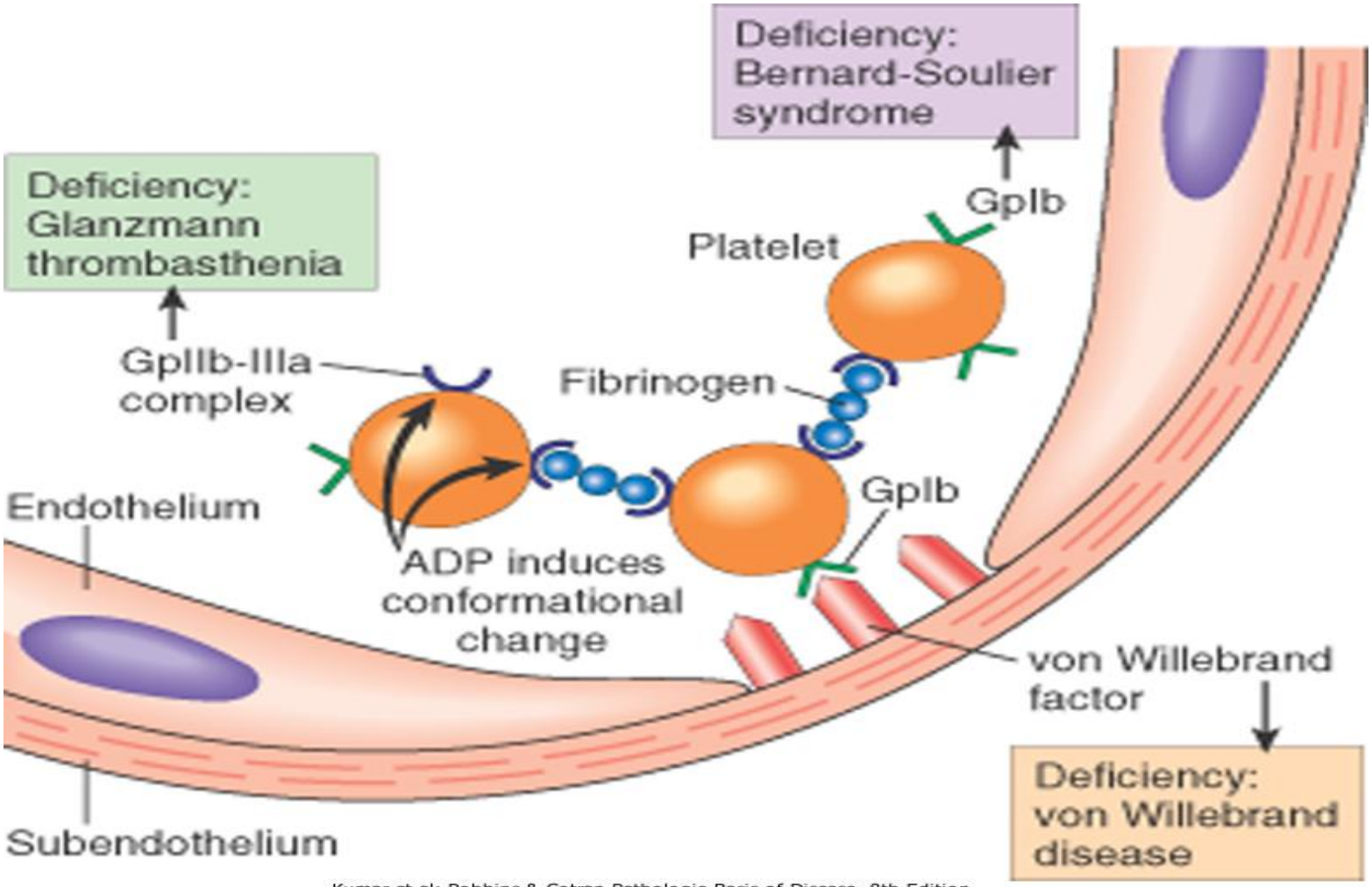
Steps of Hemostasis (4)



Steps in Hemostasis

- **Vasoconstriction**
- **Formation of primary platelet plug due to adhesion of platelets to collagen and traces of thrombin.**
- **Conversion into permanent plug supported by fibrin clot.**
- **Lysis of fibrin and confinement of clot to the site of injury.**

Platelets Adhesion and Aggregation



Platelets

- **Normal number: 140,000-400,000/μl.**
- **Redundancy in number.**
- **Life span: 8-10 days.**
- **Volume: 6-12 fL.**
- **Diameter: 2-4 μl.**

Platelet Structure

- Outer membrane rich in glycoproteins.
  - GP Ia: adhesion to collagen.
  - GP Ib: adhesion to subendothelial tissue via vWF.
  - GP IIb/IIIa: binding to fibrinogen.
- Open canalicular system.
- Microtubules.
- Dense tubular system.
- Electron dense, α granules and lysosomes.
- Glycogen.

Membrane Lipids

- 35% of the membrane. Proteins constitute 57%. Glycolipids and glycoproteins form the rest.
- Phospholipis (PL) to cholesterol ratio is 2:1.
- PL arranged in a bilayer; polar heads peripheral and acyl chains internal.
- Neutral PL (phosphatidyl choline and sphingomyelin) on the outer surface.
- Anionic PL (phosphatidylinositol, phosphatidylethanolamine and phosphatidylserine) on the inner surface.

Platelet Granules

Electron dense granules

- ADP
- ATP
- Ca^{++}
- 5 hydroxytryptamine

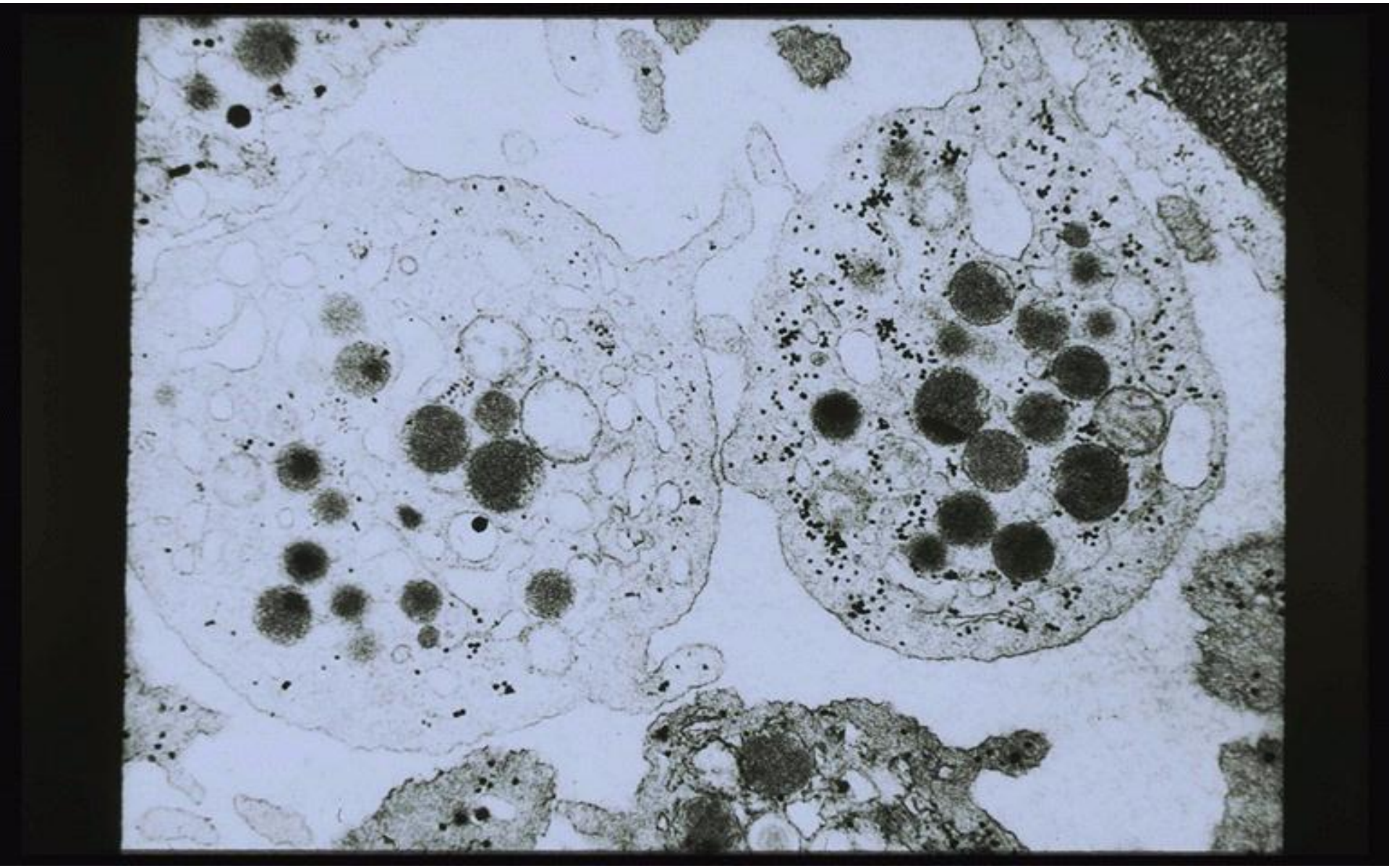
Alpha granules

- Fibrinogen
- PDGF
- vWF
- Thromboglobulin
- PF4 (heparin neutralizing)
- Factor V
- P selectin

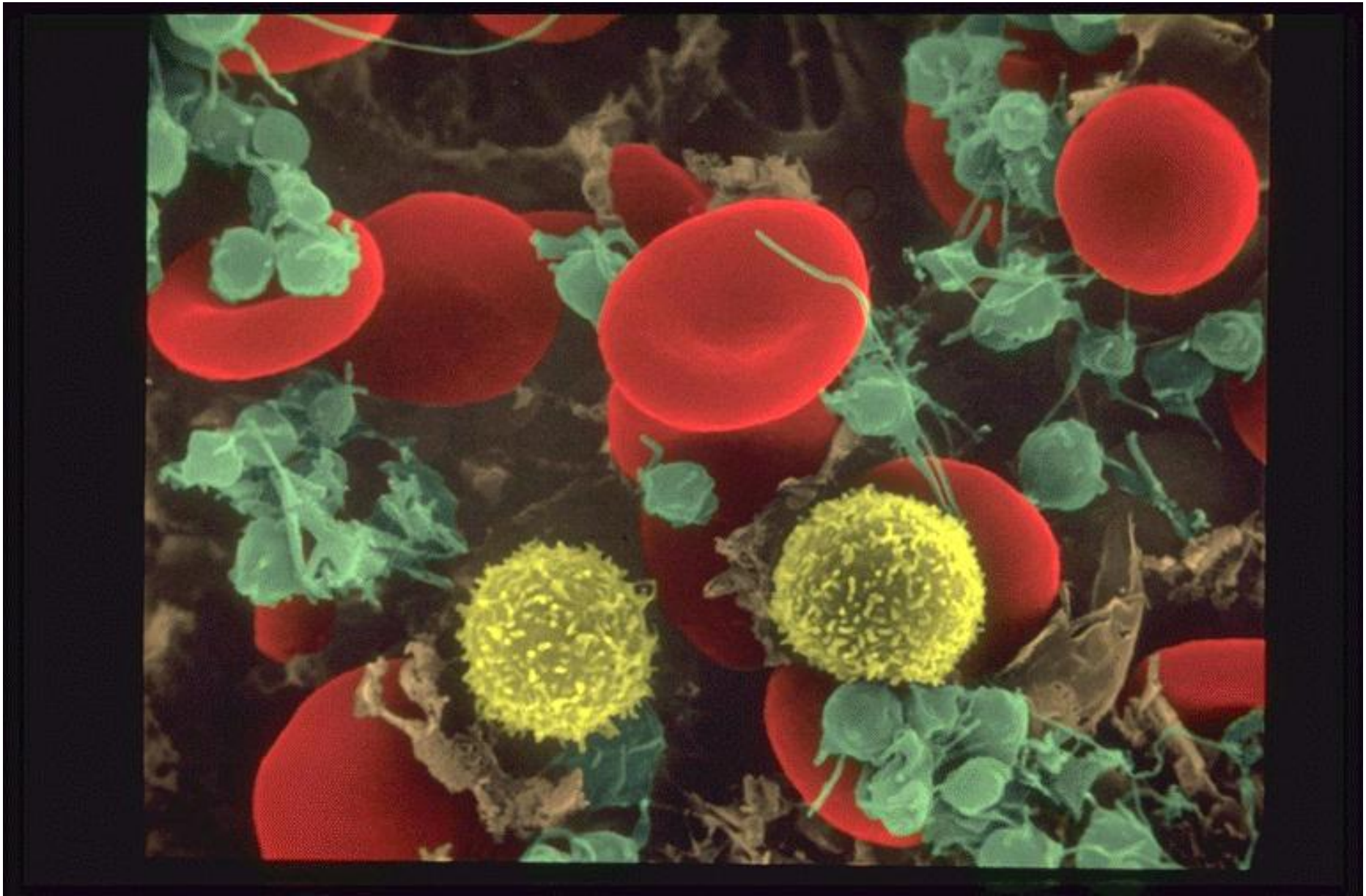
Platelet Membrane Glycoproteins

- GP Ia-IIa: adhesion to collagen.
- GP Ic-IIa: laminin receptor.
- GP IIb-IIIa: binding to fibrinogen.
- GP Ib-IX: adhesion to subendothelial tissue via vWF.

Platelet morphology

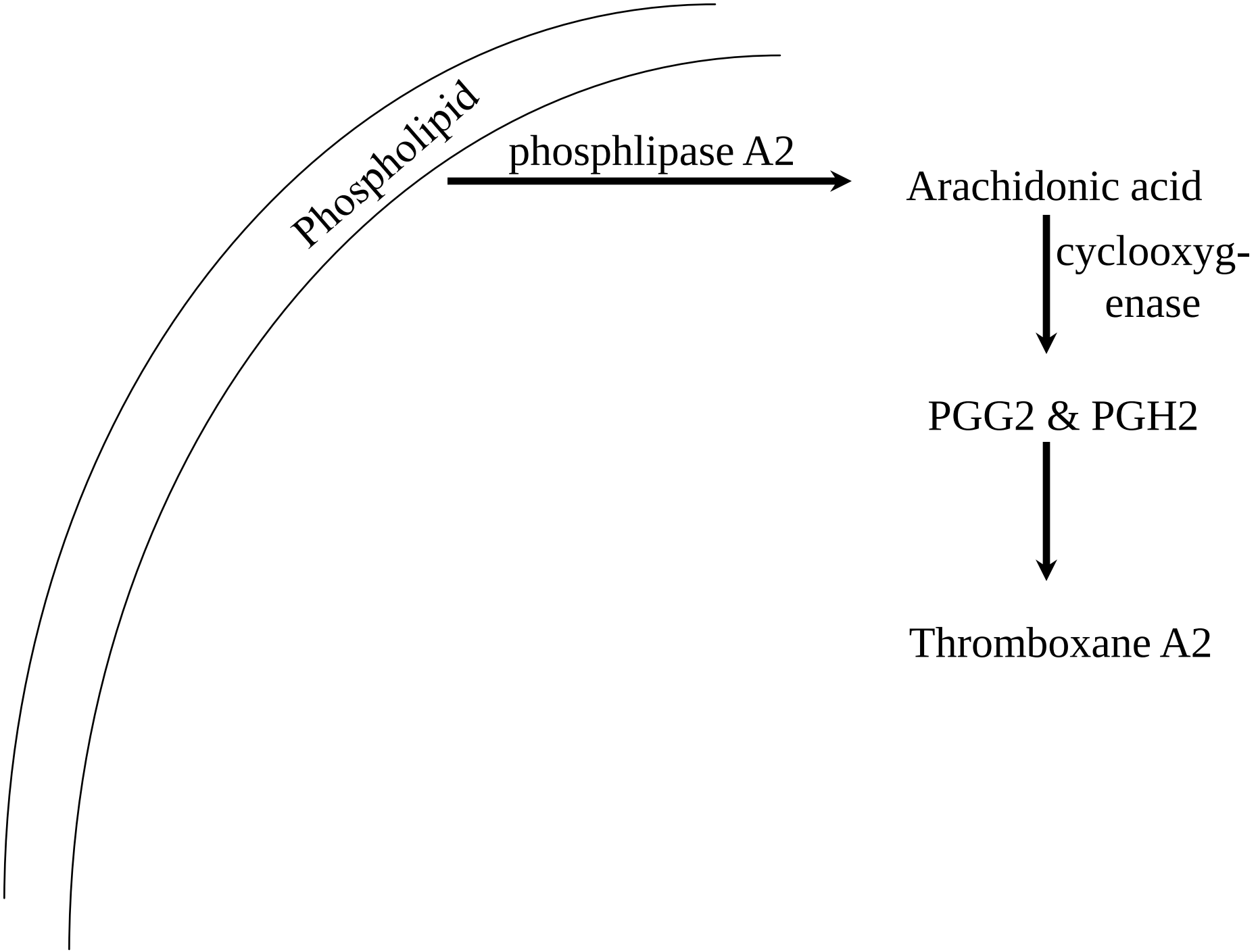


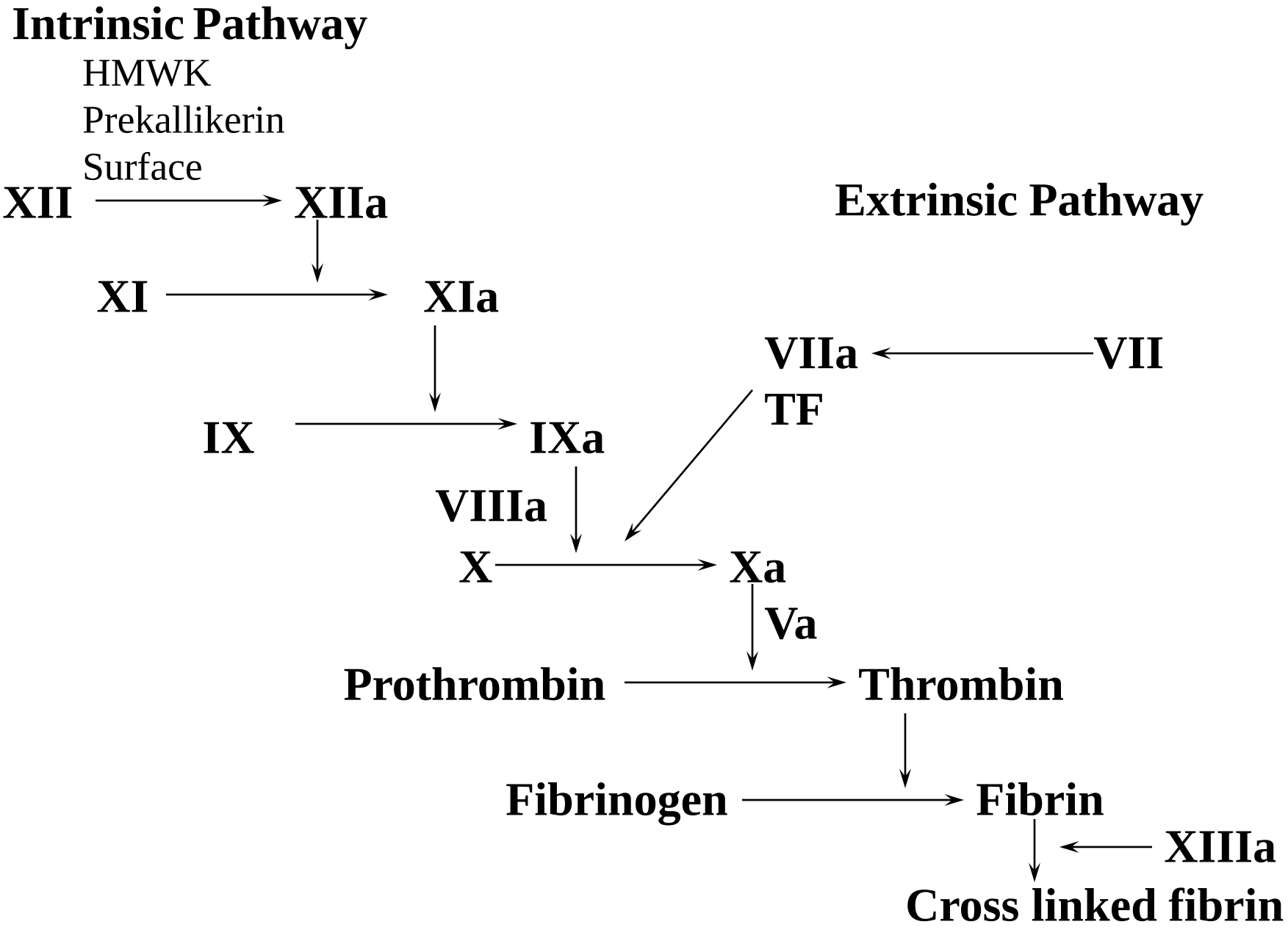
Platelets, RBCs and lymphocytes



Platelet Response to Stimuli

- **Undergo shape changes.**
- **Become adhesive.**
- **Aggregate.**
- **Secrete contents of granules.**

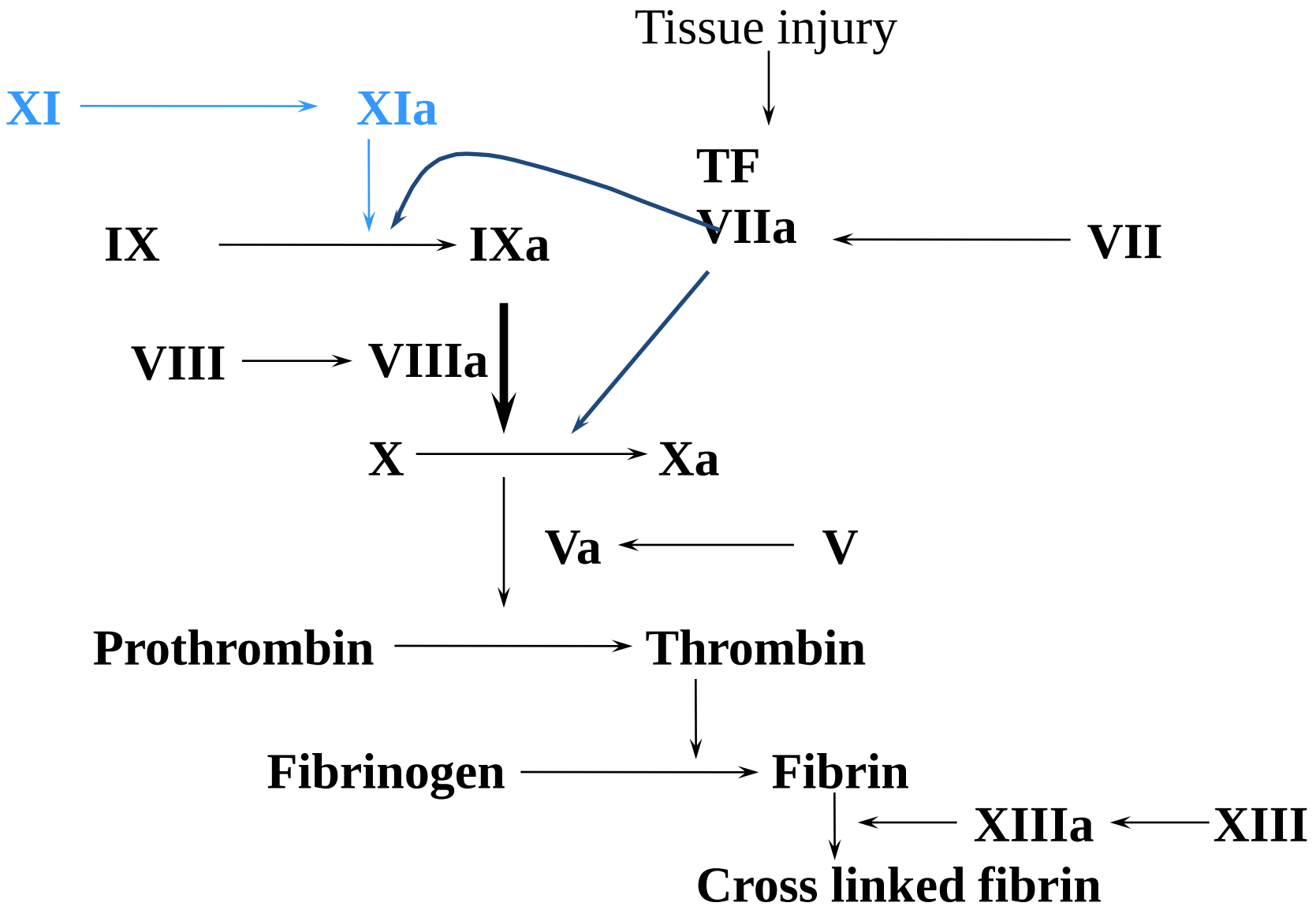

Phospholipid
phosphlipase A2
Arachidonic acid
cyclooxyg-
enase
PGG2 & PGH2
Thromboxane A2


Intrinsic Pathway
HMWK
Prekallikerin
Surface
XII
XIIa
XI
XIa
IX
IXa
VIIIa
X
Xa
Va
Prothrombin
Thrombin
Fibrinogen
Fibrin
XIIIa
Cross linked fibrin
Extrinsic Pathway
VIIa
VII
TF

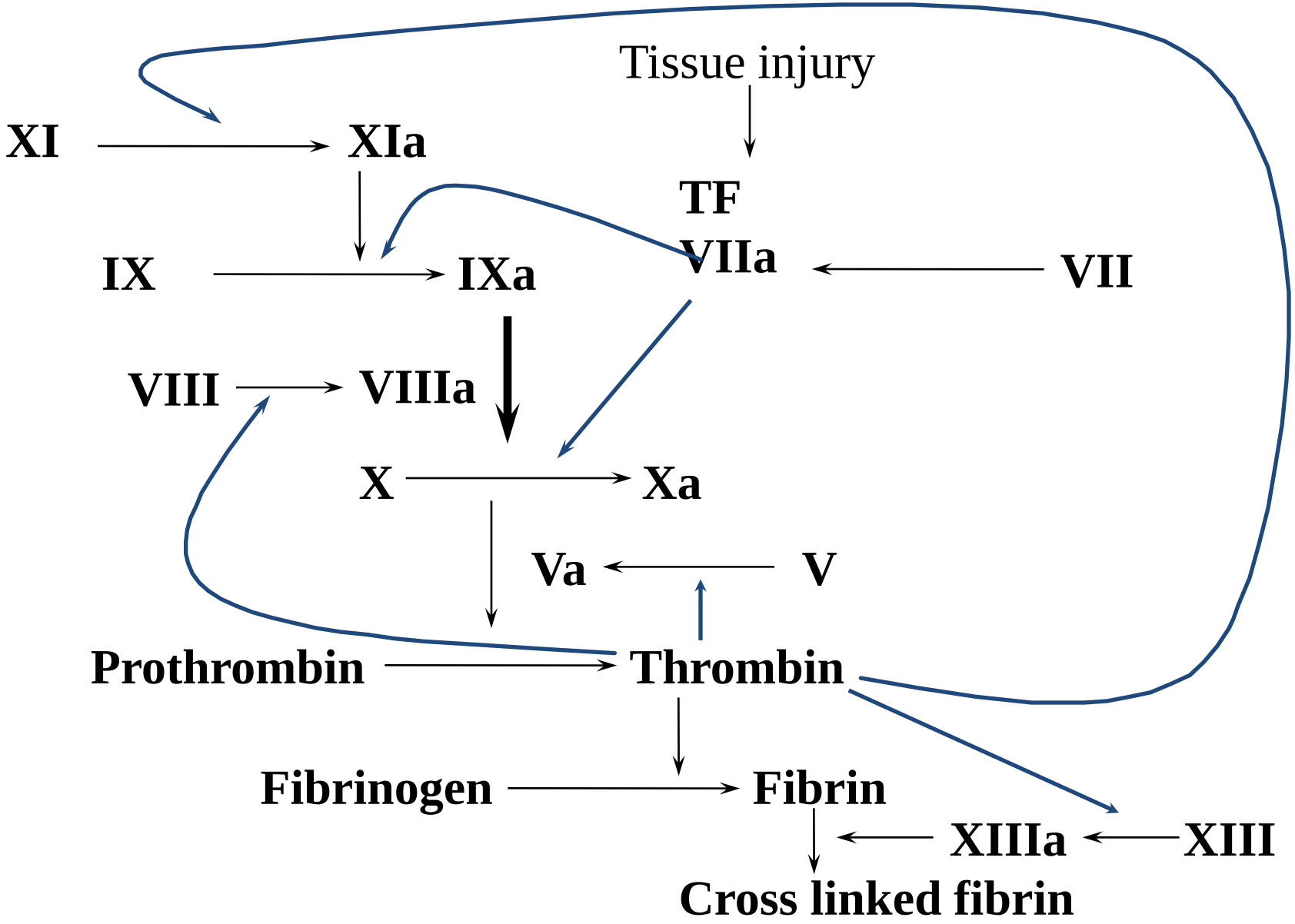
Coagulation factors and related substances

Number and/or name	Function
I (fibrinogen)	Forms clot (fibrin)
II (prothrombin)	Its active form (IIa) activates I, V, VIII, XI, XIII, protein C, platelets
III (Tissue factor or thromboplastin	Co-factor of VIIa
IV (Calcium)	Required for coagulation factors to bind to phospholipid
V (proaccelerin, labile factor)	Co-factor of X with which it forms the prothrombinase complex
VI	Unassigned – old name of Factor Va
VII (stable factor)	Activates IX, X
VIII (antihemophilic factor)	Co-factor of IX with which it forms the tenase complex
IX (Christmas factor)	Activates X: forms tenase complex with factor VIII
X (Stuart-Prower factor)	Activates II: forms prothrombinase complex with factor V
XI (plasma thromboplastin antecedent)	Activates IX
XII (Hageman factor)	Activates factor XI and prekallikrein
XIII (fibrin-stabilizing factor)	Crosslinks fibrin
von Willebrand factor	Binds to VIII, mediates platelet adhesion

The Physiologic Coagulation Cascade



The Physiologic Coagulation Cascade



The Physiologic Coagulation Cascade

- Initiation of coagulation is not dependent on the contact factors (factor XII, prekellikerin or HMWK).
- Coagulation is initiated by exposure of blood to TF.
- TF-VIIa complex activates factors X and IX.
- Factor IXa accelerates factor X acivation by more than 50 folds.

Role of vtamin K

Clotting factors

- II
- VII
- IX
- X

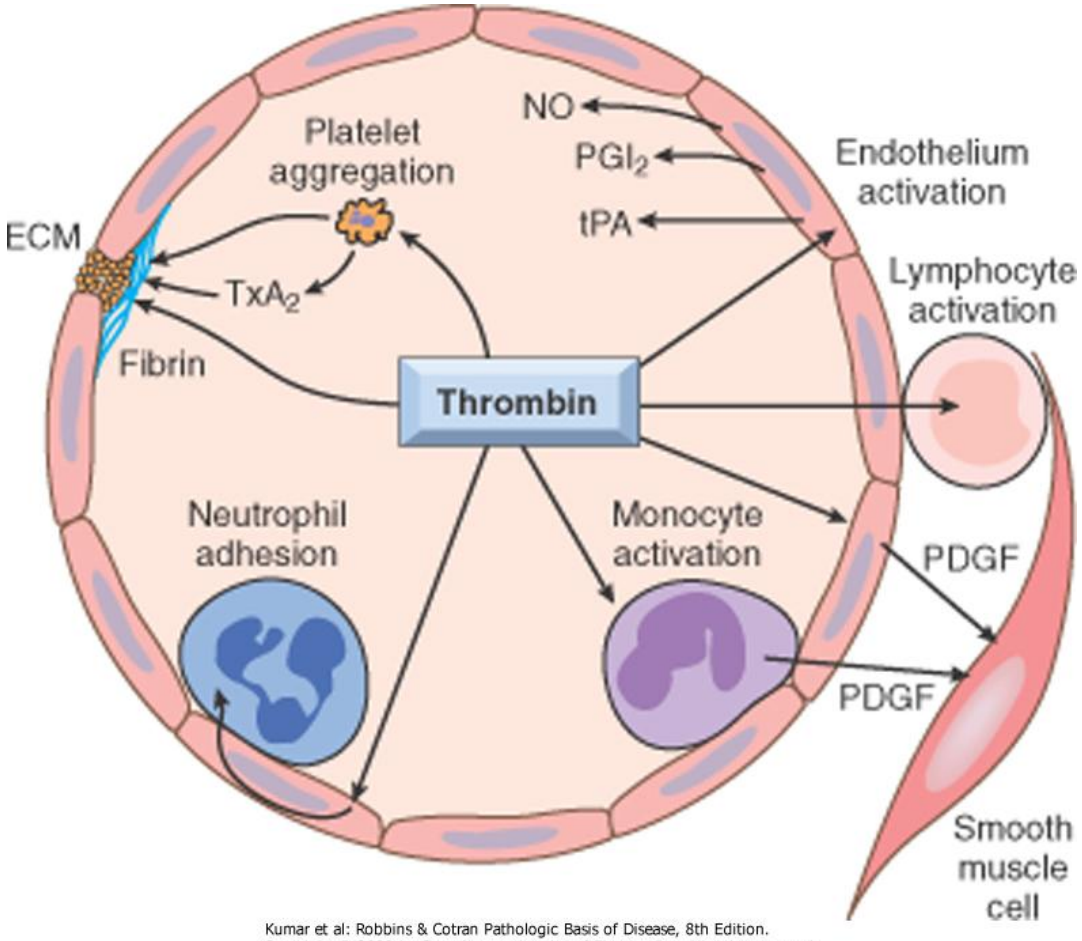
Anticoagulants

- Protein C
- Protein S

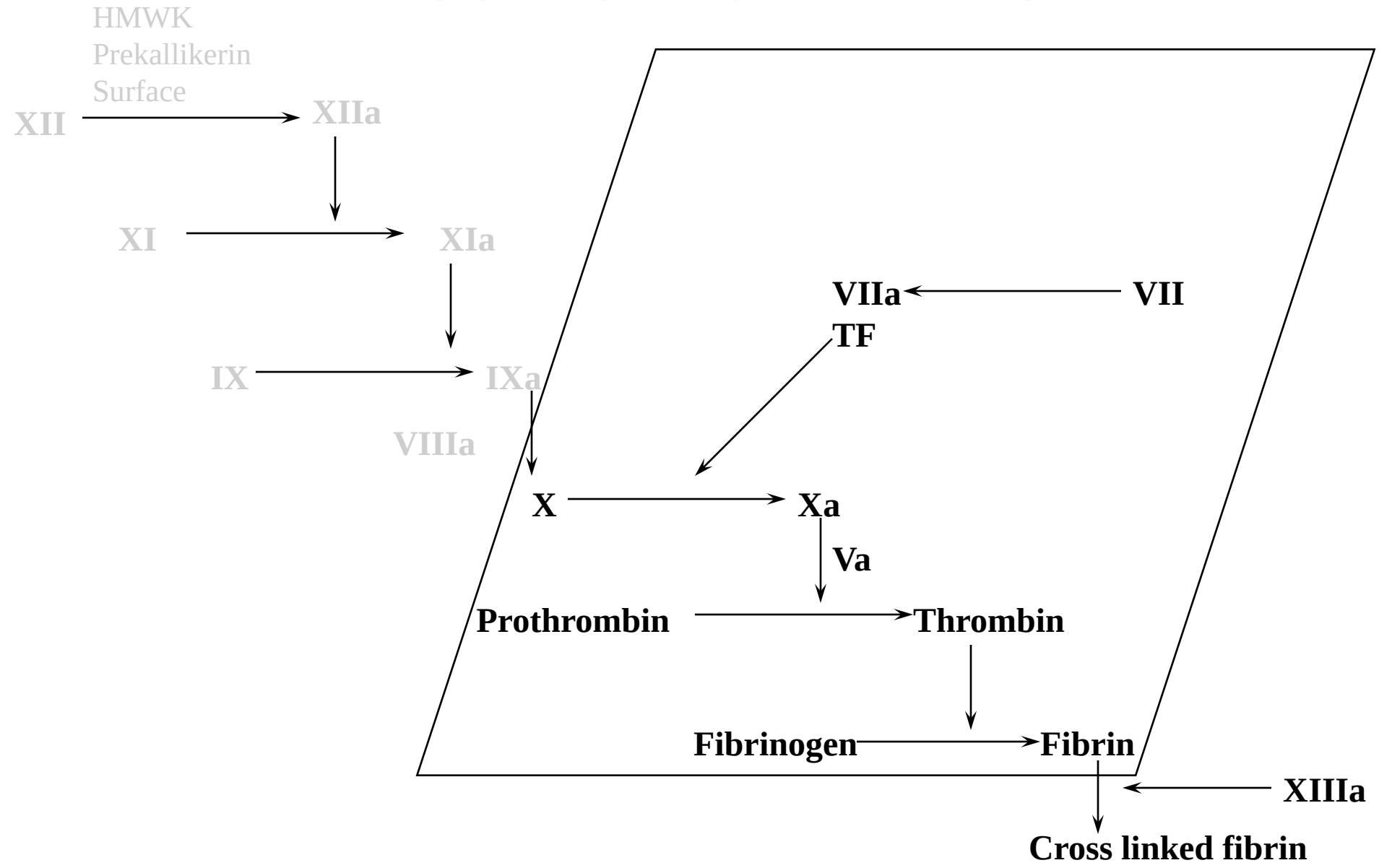
Actions of Thrombin

- Conversion of fibrinogen to fibrin.
- Activation of factor V.
- Activation of factor VIII.
- Activation of factor XIII.
- Activation of protein C.
- Activation of platelets.

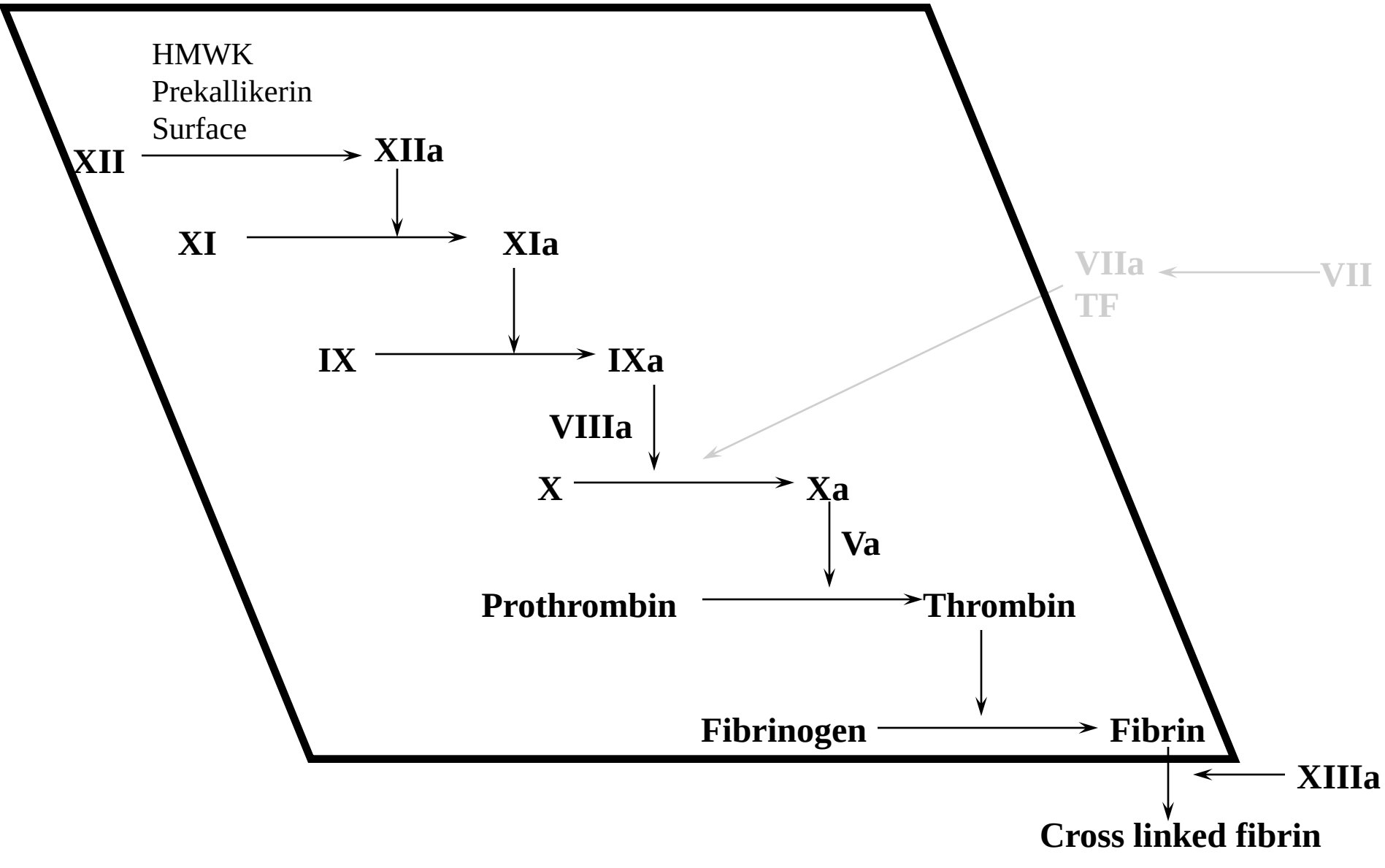
Actions of Thrombin



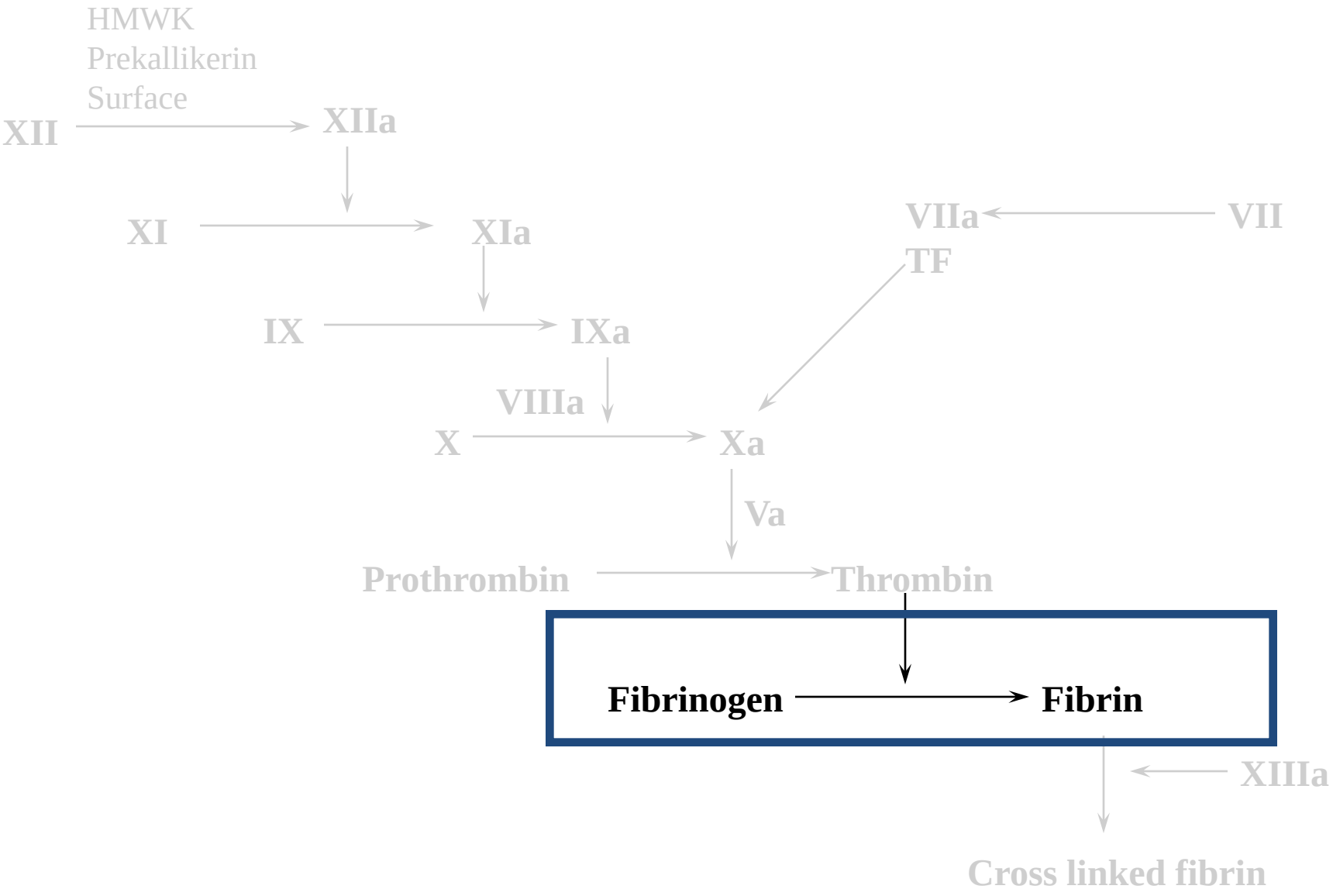
Prothrombin Time



Partial Thromboplastin Time



Thrombin Time



Initial Laboratory Tests For Bleeding Abnormalities

- **Platelet count.**
- **Bleeding time.**
- **Partial thromboplastin time (PTT).**
- **Prothrombin time (PT).**
- **Thrombin time.**

PTT and APTT

- Purpose:
  - Screen for deficiencies of the coagulation factors of the intrinsic and common pathways. All factors except VII and XIII.
  - Detect circulating anticoagulants.
- Principle of test: Phospholipid is incubated with platelet poor plasma; Then Ca++ is added and time for clot formation is measured.
- In APTT: the plasma is incubated with activating agent such as kaolin.

PTT and APTT Interpretation

Prolongation

- Deficiencies in one or more of factors XII, XI, X, IX, VIII, V and II.
- Inhibitors to one of the above factors.
- High concentrations of fibrinogen and fibrin split products, heparin and protamine sulfate.

Shortening

- Poor venipuncture.
- If plasma contains platelets.
- High factor VIII.
- DIC.

Prothrombin Time

- **Purpose**:
  - Screen for deficiencies of the coagulation factors of the exrinsic and common pathways. Factors II, V, VII X and fibrinogen are measured.
  - Monitor oral anticoagulant therapy.
- **Principle**: Ca++ and tissue extract such as brain are added to plasma leading to activation of VII
- **Prolongation**:
  - Deficiency of Factors II, V, VII and X.
  - Fibrinogen level below 100mg/dl.
  - Heparin and fibrin split products

International Randomized Ratio

- INR= [PT ratio]^{ISI}

Thrombin Time

- **Purpose of test:** screen for reduction of fibrinogen concentration and presence of fibrin split products.
- **Principle:** thrombin is added to plasma. Time needed to clot is measured as TT.
- **Interpretation:** prolongation is seen in:
  - Low fibrinogen level below 100 mg/dl.
  - Qualitative change in fibrinogen.
  - Heparin therapy.
  - Fibrin or fibrinogen split products.

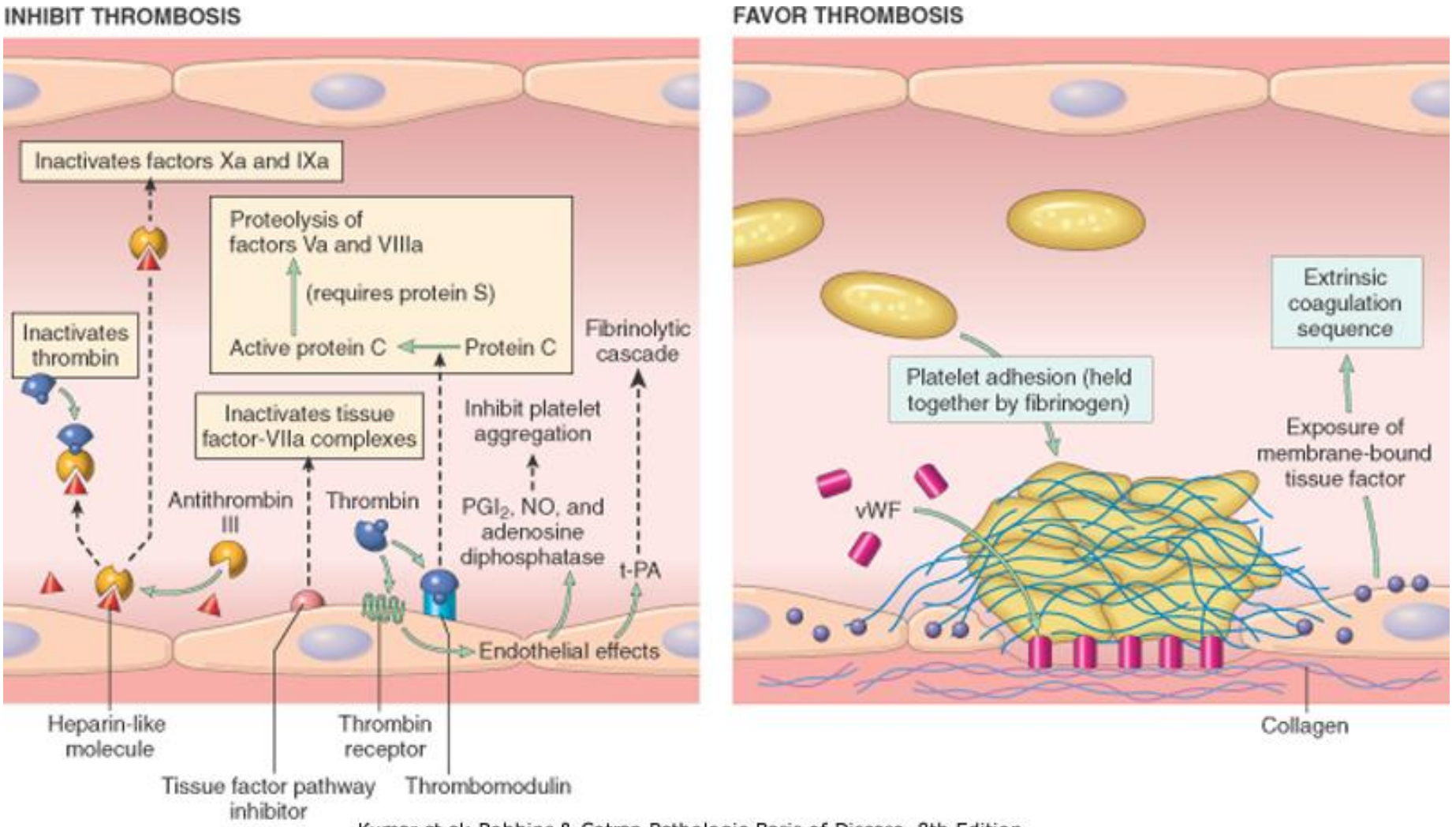
Bleeding Time

- **Definition:** time taken for bleeding from a standardized skin wound to stop.
- **Principle of test:** measurement of platelet function.
- **Purpose of test:** screening test for disorders of platelets (congenital or acquired), and for von Willebrand disease.
- **Normal range:** 2.5 to 7.5 min. Aspirin and other anti-inflammatory drugs prolong bleeding time.

Initial Laboratory Tests For Bleeding Abnormalities

- Platelet count.
- Bleeding time: prolongation is seen in platelet diseases or in patients receiving drugs interfering with platelet function.
- Partial thromboplastin time (PTT).
  - Prolongation is seen in hemophiliacs and in patients with lupus anti-coagulant.
- Prothrombin time (PT).
  - Prolongation is seen in factor VII deficiency.
  - ***Factors V, X, prothrombin and fibrinogen affect APTT and PT.***
- Thrombin time.

Endothelial Cells and Hemostasis



ANTITHROMBOTIC FUNCTION OF NORMAL ENDOTHELIUM

- Separation of blood from subendothelium that activates platelets and initiates blood coagulation
- Release of PGI2 that diminishes platelet response to activating stimuli
- Binding sites for anticoagulants
  - Thrombomodulin binds thrombin altering its enzymatic activity with activation of protein C.
  - Antithrombin III binding sites

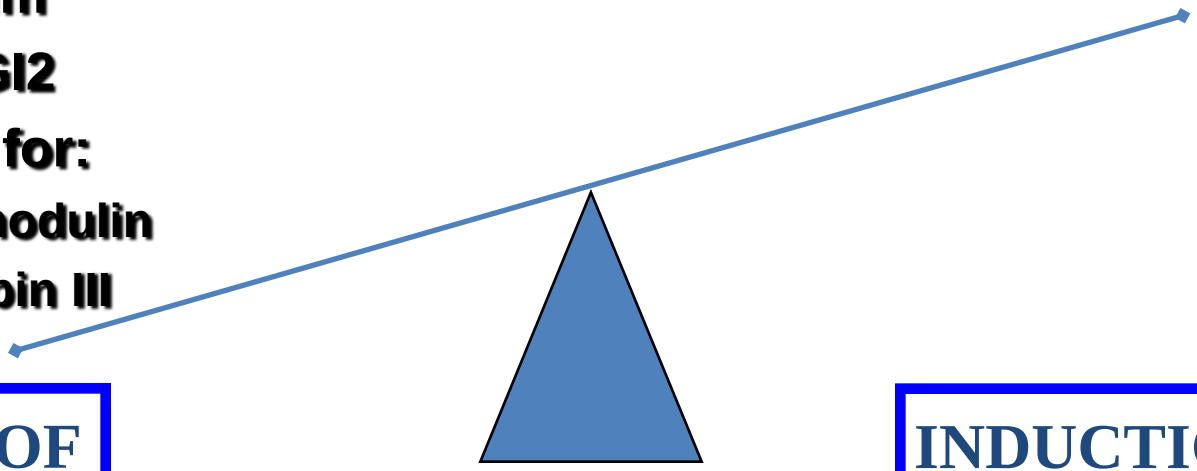
Prothrombotic Role of Endothelium

- Production of von Willebrand factor.
- Synthesis of TF (induced by TNF, IL-1).
- Binding of IXa and Xa on their surfaces.
- Secretion of plasminogen activator inhibitors (PAIs).

Dual Role of Endothelium

- Production of VWF
- Synthesis of TF
- Binding of IXa and Xa
- Secretion of (PAIs).

- Separation of blood from subendothelium
- Release of PGI2
- Binding sites for:
  - –Thrombomodulin
  - –Antithrombin III



INHIBITION OF CLOTTING

INDUCTION OF CLOTTING