COAGULATION MECHANISMS

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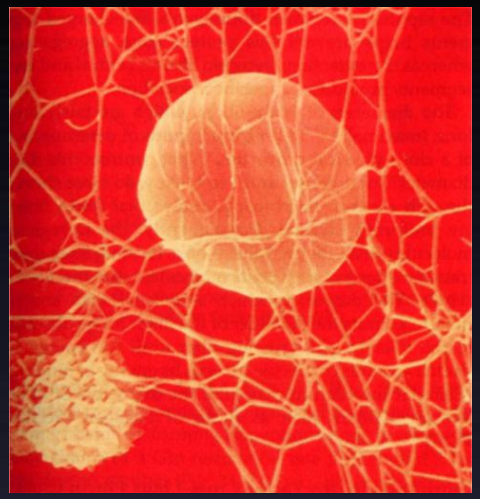


Vessel injury

Antithrombogenic
(Favors fluid blood)

Thrombogenic
(Favors clotting)

HANDOUTS…12/16/2019

OBJECTIVES

At the end of the lecture you should be able to…

- Recognize different stages of haemostasis
- Explain the role of platelets in haemostasis.
- Recognize different clotting factors & cascade of clotting.
- Describe the intrinsic, extrinsic and common pathway.
- Recognize the role of thrombin in coagulation
- The role of anticoagulants and their mechanism of action

HAEMOSTASIS

The spontaneous arrest of bleeding from ruptured blood vessels

FOUR STEPS OF HEMOSTASIS

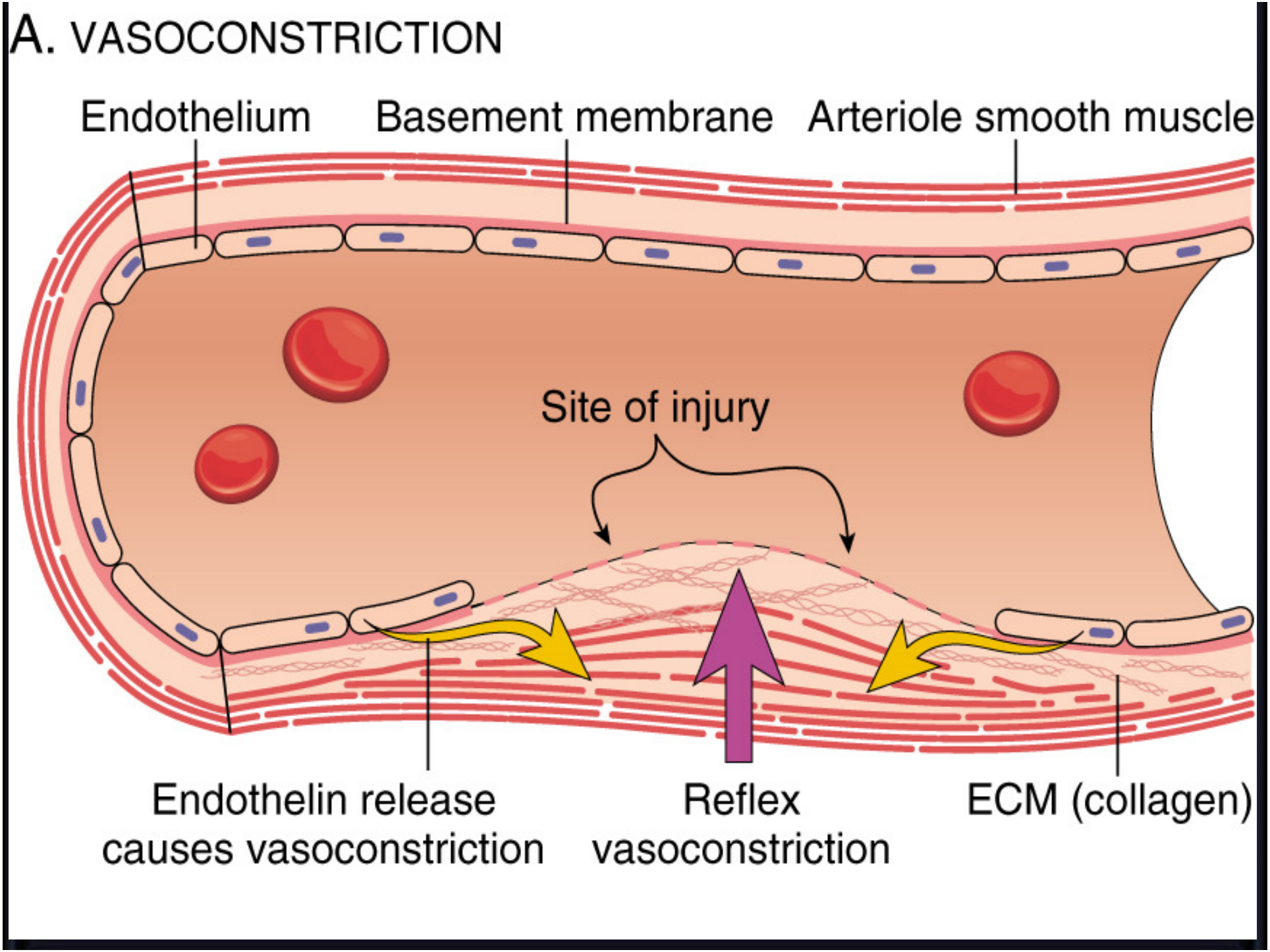
1. **VASCULAR PHASE ► Vascular Spasm**
2. **PLATELET PHASE ► Formation of platelet plug**
3. **COAGULATION PHASE ► Blood Coagulation & Clot Retraction**
4. **FIBRINOLYTIC PHASE ► Fibrinolysis**

1-VASCULAR SPASM (Vascular Constriction)

Immediately After injury there is localized Vasoconstriction.

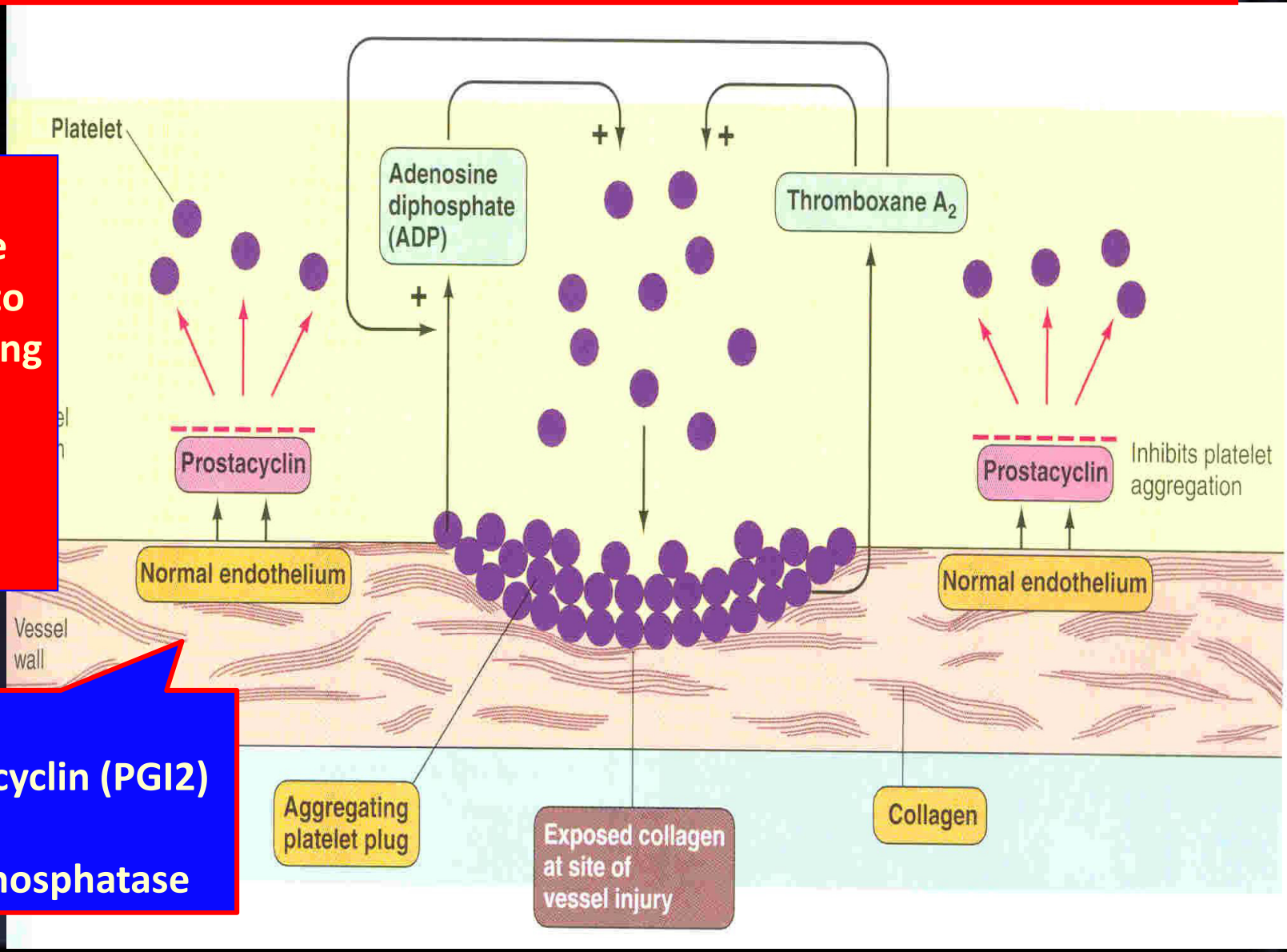
- **Causative Factors are three (3)**
  1. Nervous reflexes
  2. Local myogenic spasm
  3. Local humoral factors….Platelets → Thromboxane A_2 [TXA2] **(Vasoconstrictor)**
- **Importance**
  - Crushing injuries → Intense spasm → No lethal loss of blood

TXA2 is inhibited by aspirin…How?

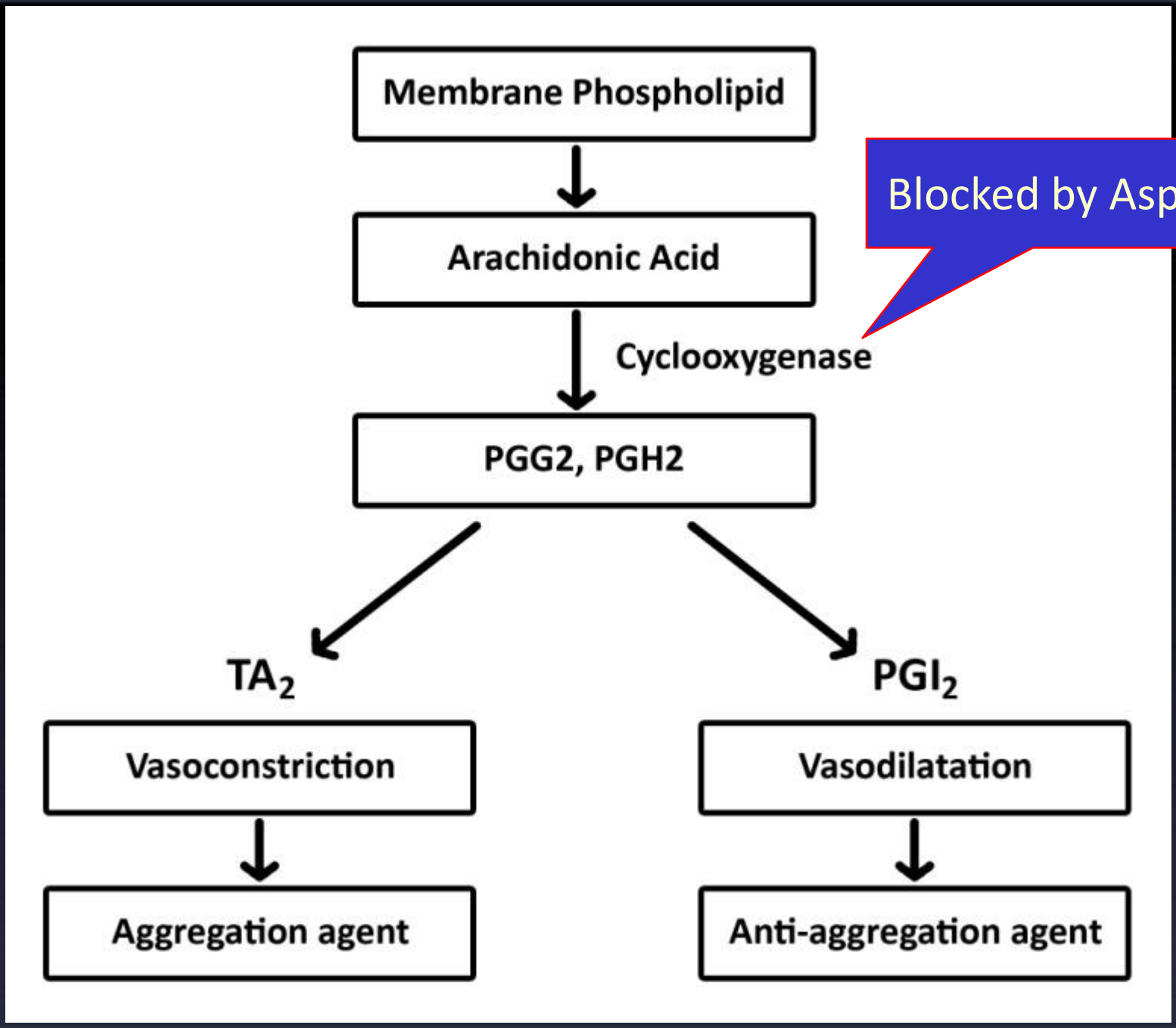

A. VASOCONSTRICTION
Endothelium
Basement membrane
Arteriole smooth muscle
Site of injury
Endothelin release causes vasoconstriction
Reflex vasoconstriction
ECM (collagen)

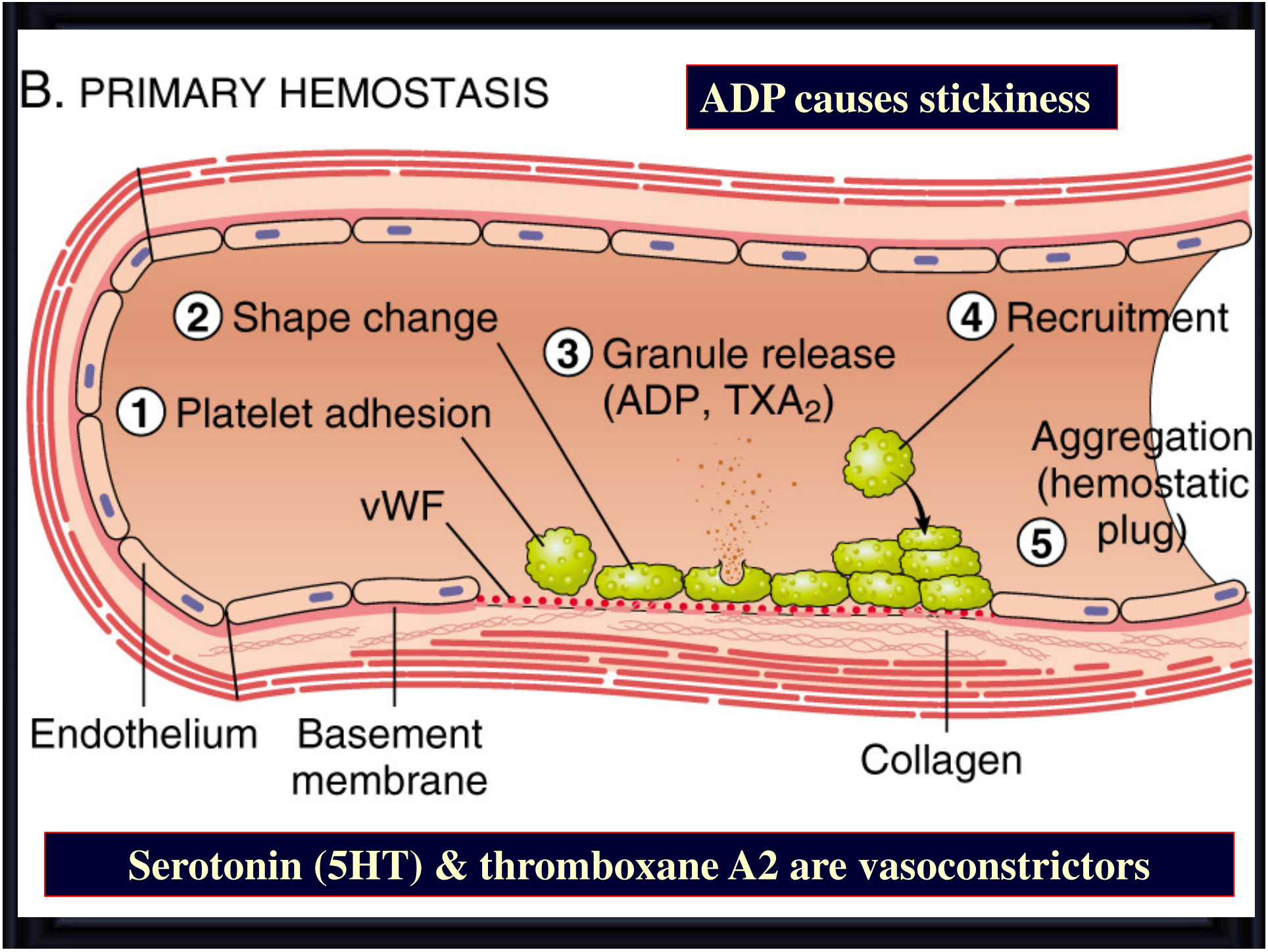
2-FORMATION OF PLATELET PLUG [PRIMARY HEMOSTASIS]

Importance
►enough to stop bleeding from small vascular damage



Secrete
- **prostacyclin (PGI2)**
- **NO**
- **ADP phosphatase**


Membrane Phospholipid
Arachidonic Acid
Blocked by Aspirin
Cyclooxygenase
PGG2, PGH2
TA_2
PGI_2
Vasoconstriction
Vasodilatation
Aggregation agent
Anti-aggregation agent

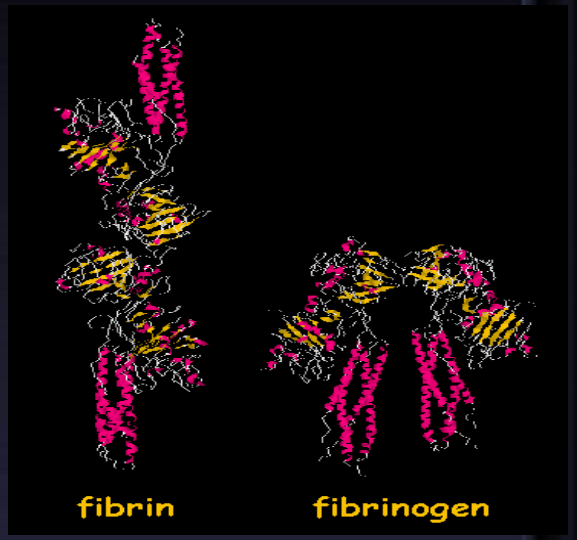

B. PRIMARY HEMOSTASIS
ADP causes stickiness
2 Shape change
3 Granule release (ADP, TXA2)
4 Recruitment
1 Platelet adhesion
vWF
Aggregation (hemostatic plug)
5
Endothelium
Basement membrane
Collagen
Serotonin (5HT) & thromboxane A2 are vasoconstrictors

3-BLOOD COAGULATION

Formation of Clot or Thrombus

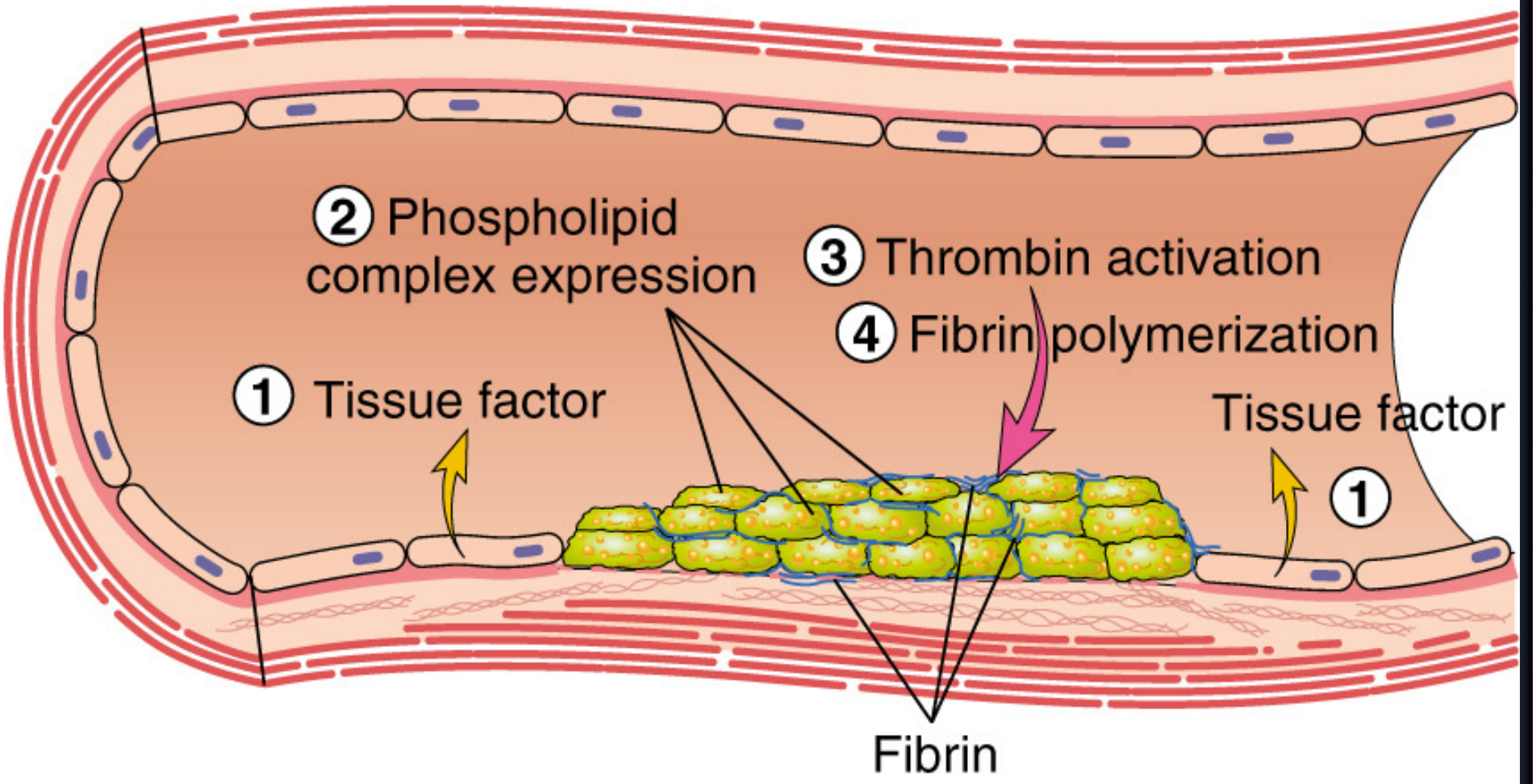
[SECONDARY HEMOSTASIS]

- Blood clotting is the transformation of blood (soluble fibrinogen) from a liquid into a solid gel form (insoluble fibrin strands)
- Pathways
  - Intrinsic
  - Extrinsic
- Begins to develop in
  - 1-2 min → Minor trauma
  - 15-20 sec → Severe trauma

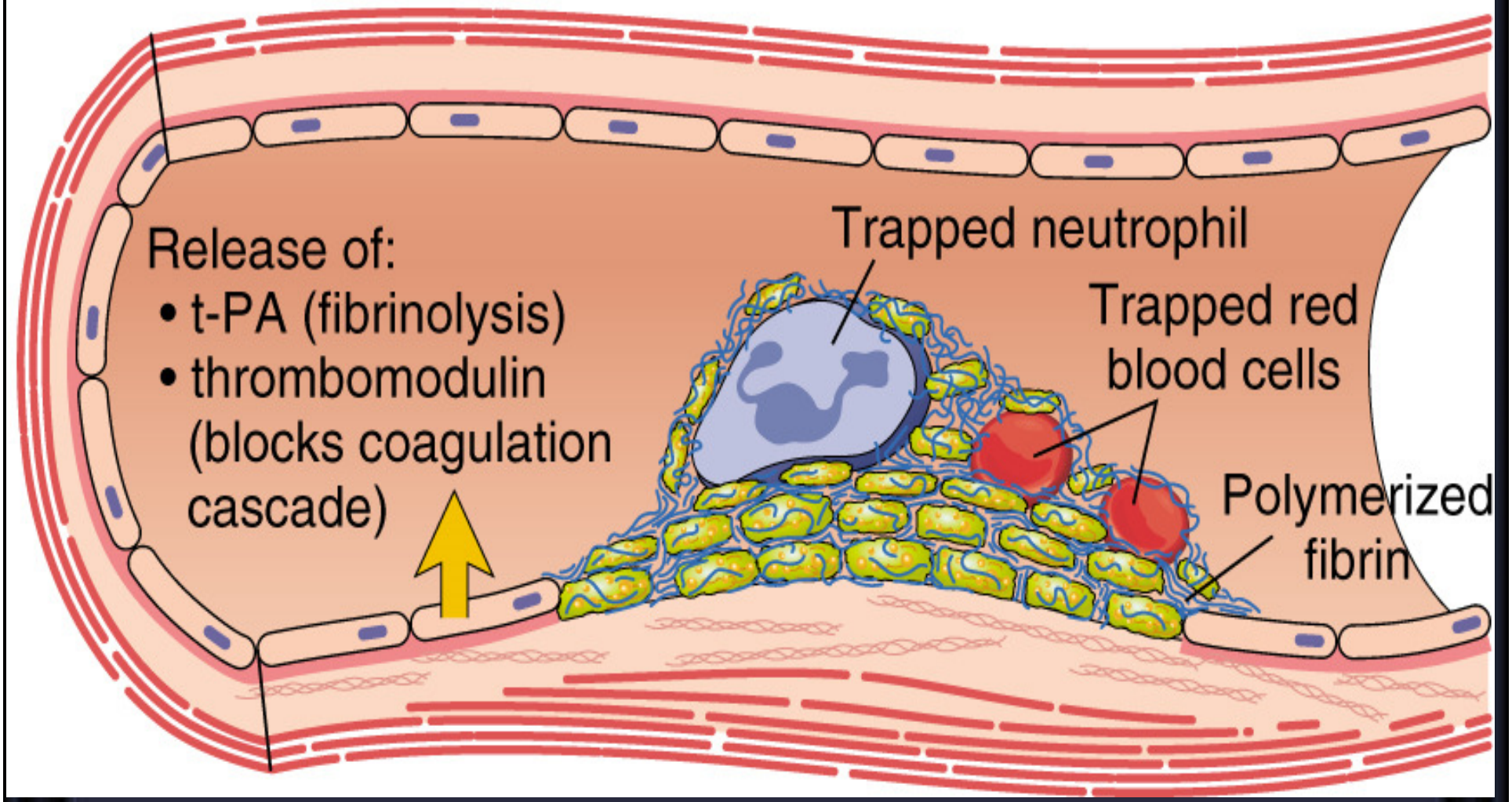


CLOT is a meshwork of fibrin fibres running in all directions entrapping blood cells, platelets and plasma.

C. SECONDARY HEMOSTASIS



D. THROMBUS AND ANTITHROMBOTIC EVENTS



MECHANISM OF CLOTTING - STEPS

1. Formation of Prothrombin activator complex (Xa+Ca+PF-3+V) by Extrinsic & Intrinsic Pathways leading to Common Pathway
2. Conversion of prothrombin into thrombin
3. Conversion of fibrinogen into fibrin
4. Fibrin converts to stable fibrin polymer

Clotting Factors Guyton

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

Fibrinogen

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

Table 36–1

Clotting Factors in Blood and Their Synonyms

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	

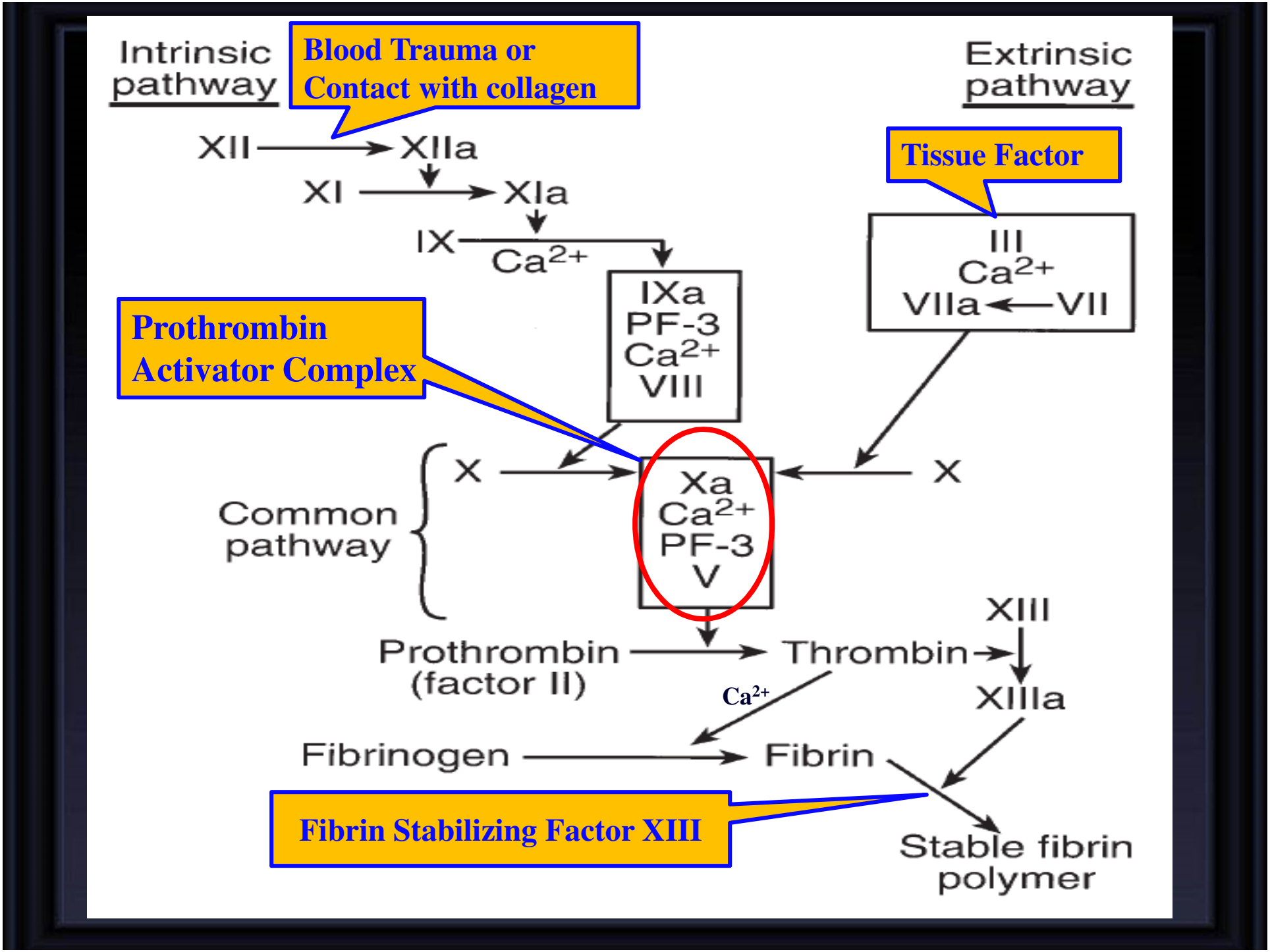
Clotting Factors

Ganong

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

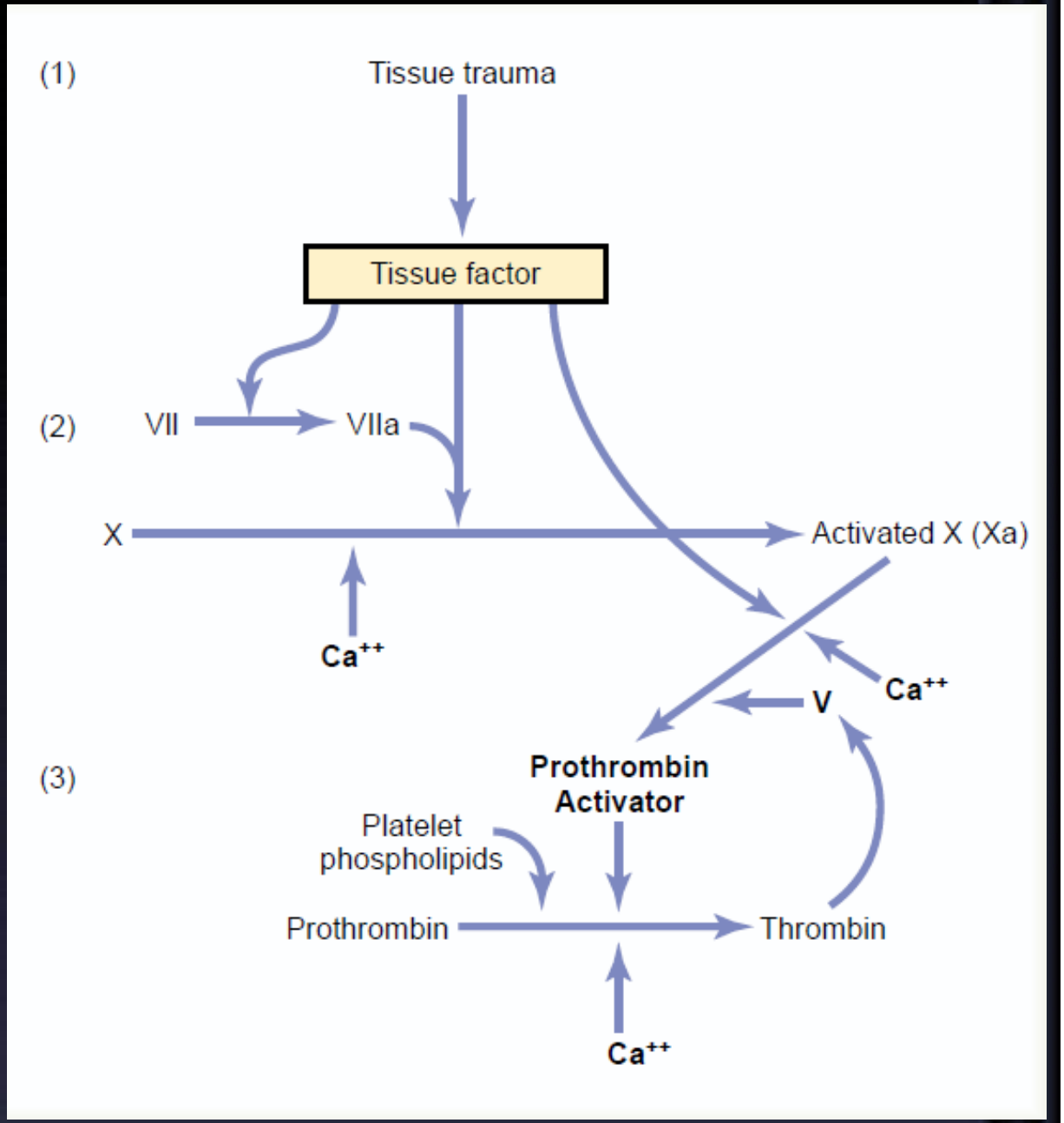
Factor[a]	Names
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Proaccelerin, labile factor, accelerator globulin
VII	Proconvertin, SPCA, stable factor
VIII	Antihemophilic factor (AHF), antihemophilic factor A, antihemophilic globulin (AHG)
IX	Plasma thromboplastic component (PTC), Christmas 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.


Intrinsic pathway
Blood Trauma or Contact with collagen
Extrinsic pathway
XII → XIIa
XI → XIa
IX
Ca2+
IXa PF-3 Ca2+ VIII
Tissue Factor
III Ca2+ VIIa ← VII
Prothrombin Activator Complex
Common pathway
X
Xa Ca2+ PF-3 V
X
Prothrombin (factor II)
Thrombin
XIII
XIIIa
Ca2+
Fibrinogen
Fibrin
Fibrin Stabilizing Factor XIII
Stable fibrin polymer

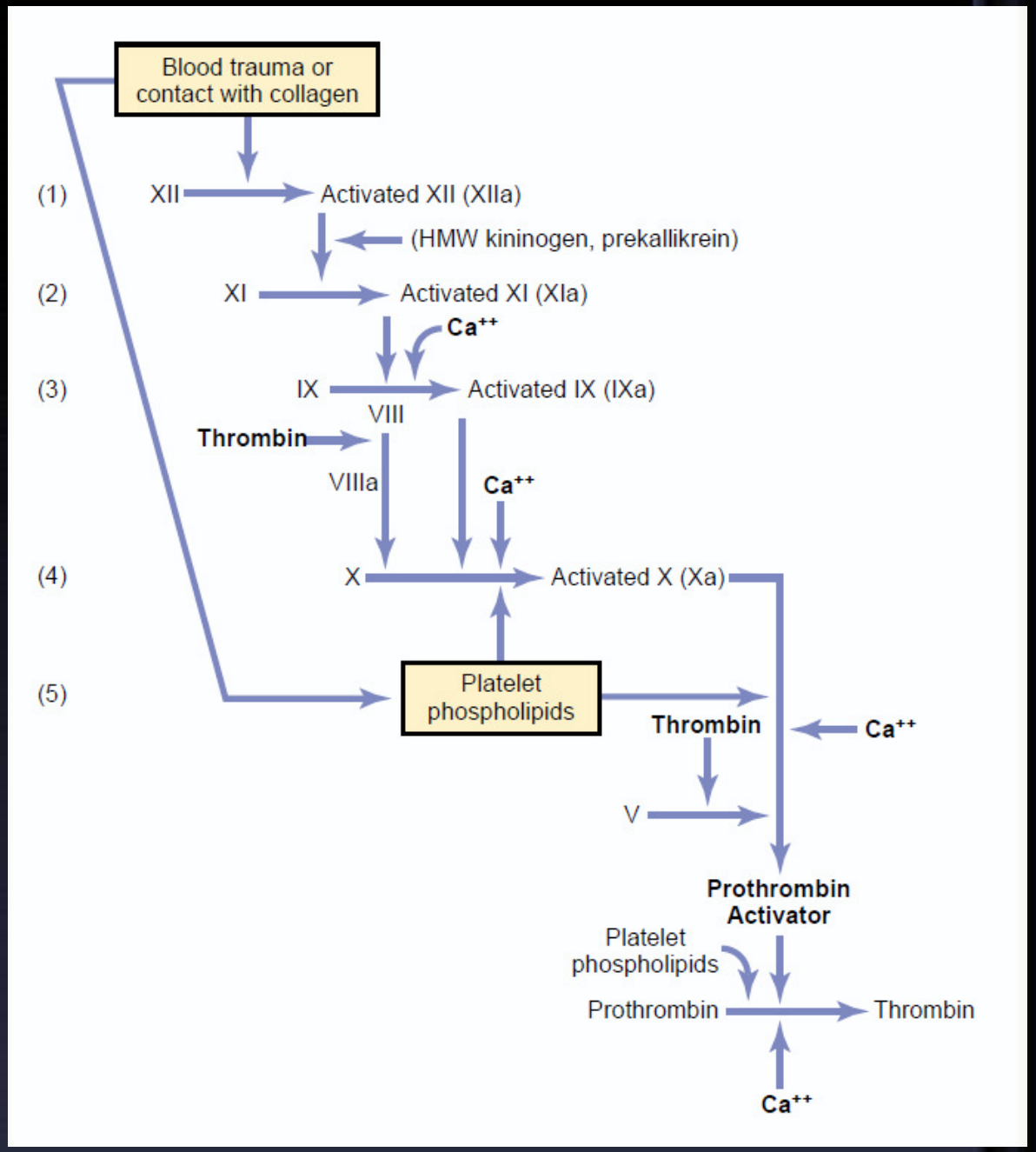
EXTRINSIC MECHNANISM FOR INITIATING CLOTTING

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

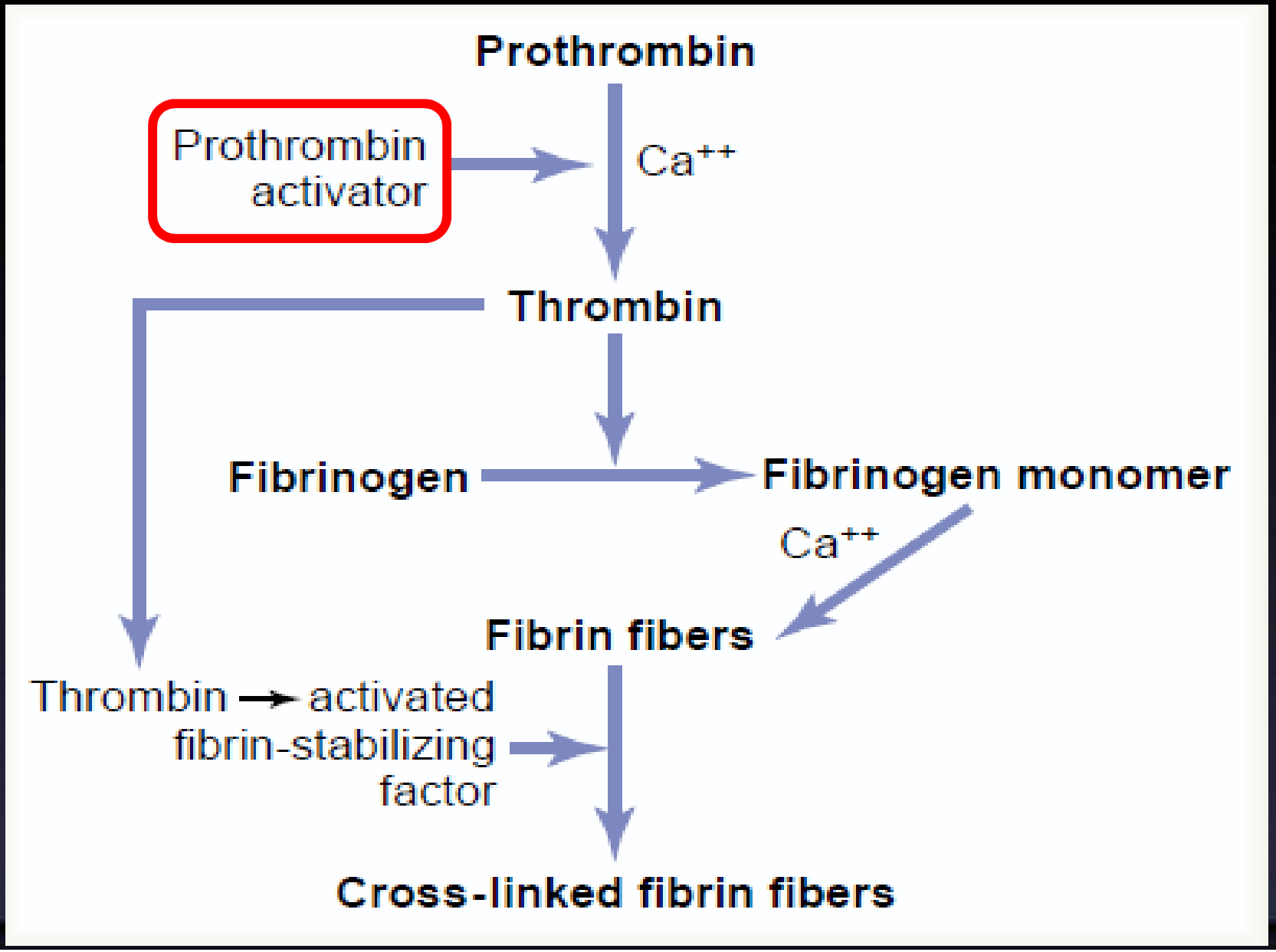


INTRINSIC MECHNANISM FOR INITIATING CLOTTING

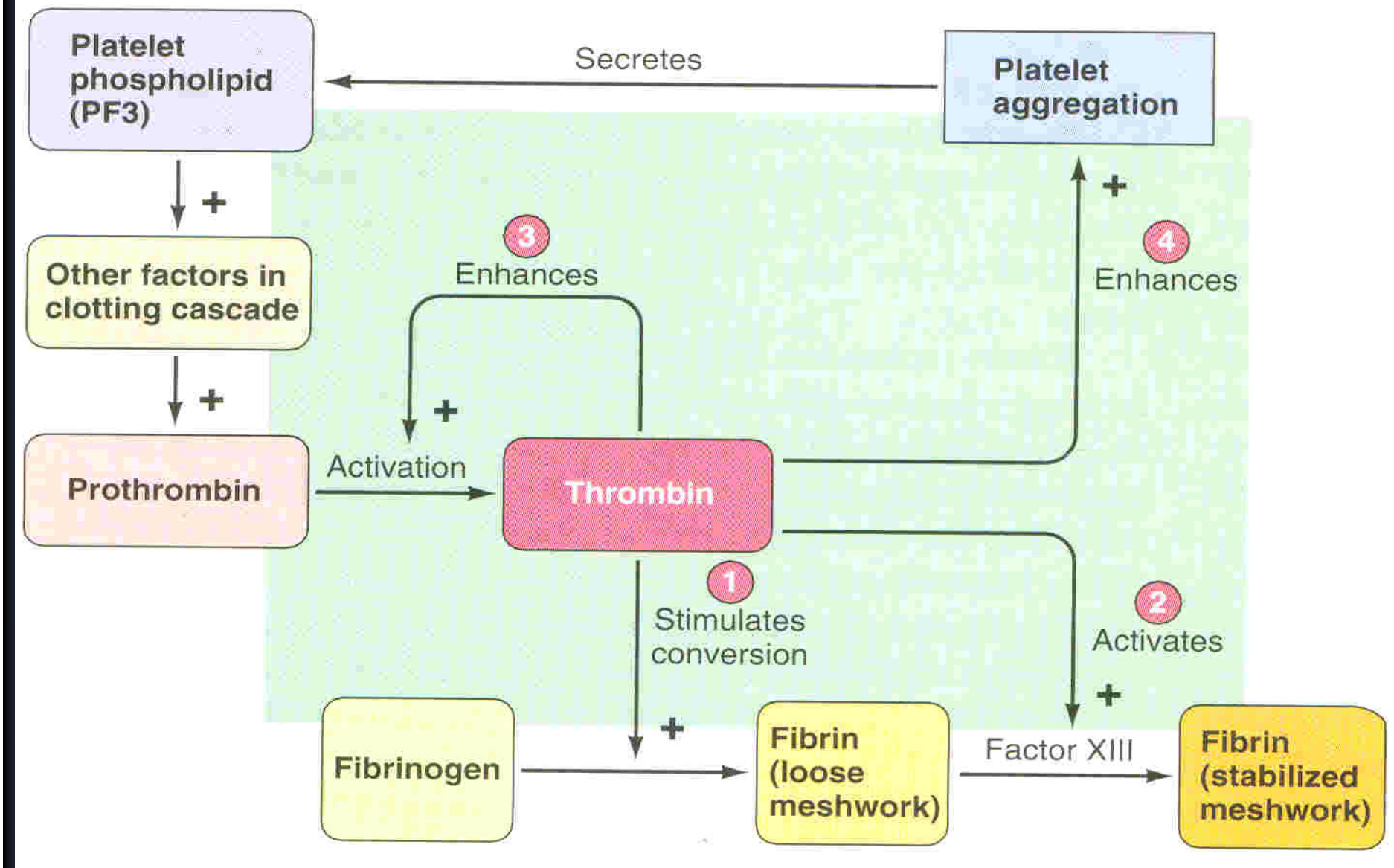
Trauma to the blood itself or exposure of the blood to collagen (from a traumatized blood vessel wall), foreign surface/glass



ACTION OF THROMBIN ON FIBRONOGEN TO FORM FIBRIN



ROLES OF THROMBIN IN HEMOSTASIS



CLOT RETRACTION

- When clot retracts (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……they are contractile.

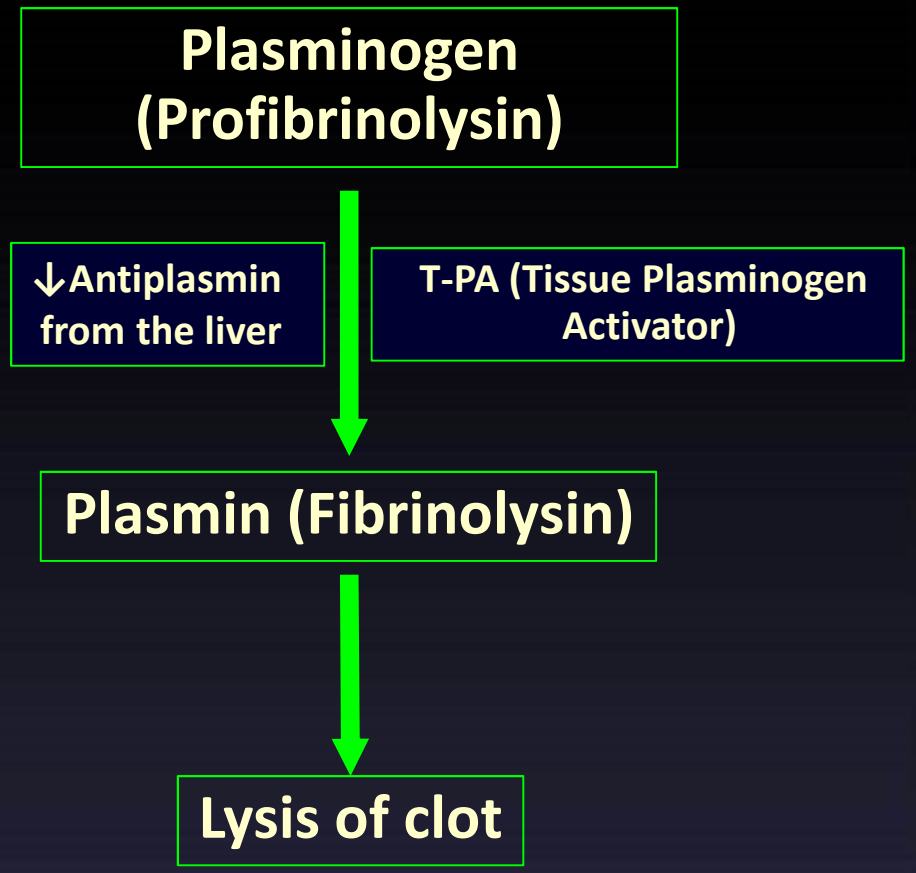
Fate of Clot:

Lysis or Fibrous tissue Formation (platelet derived growth factor)

LYSIS OF BLOOD CLOTS BY PLASMIN

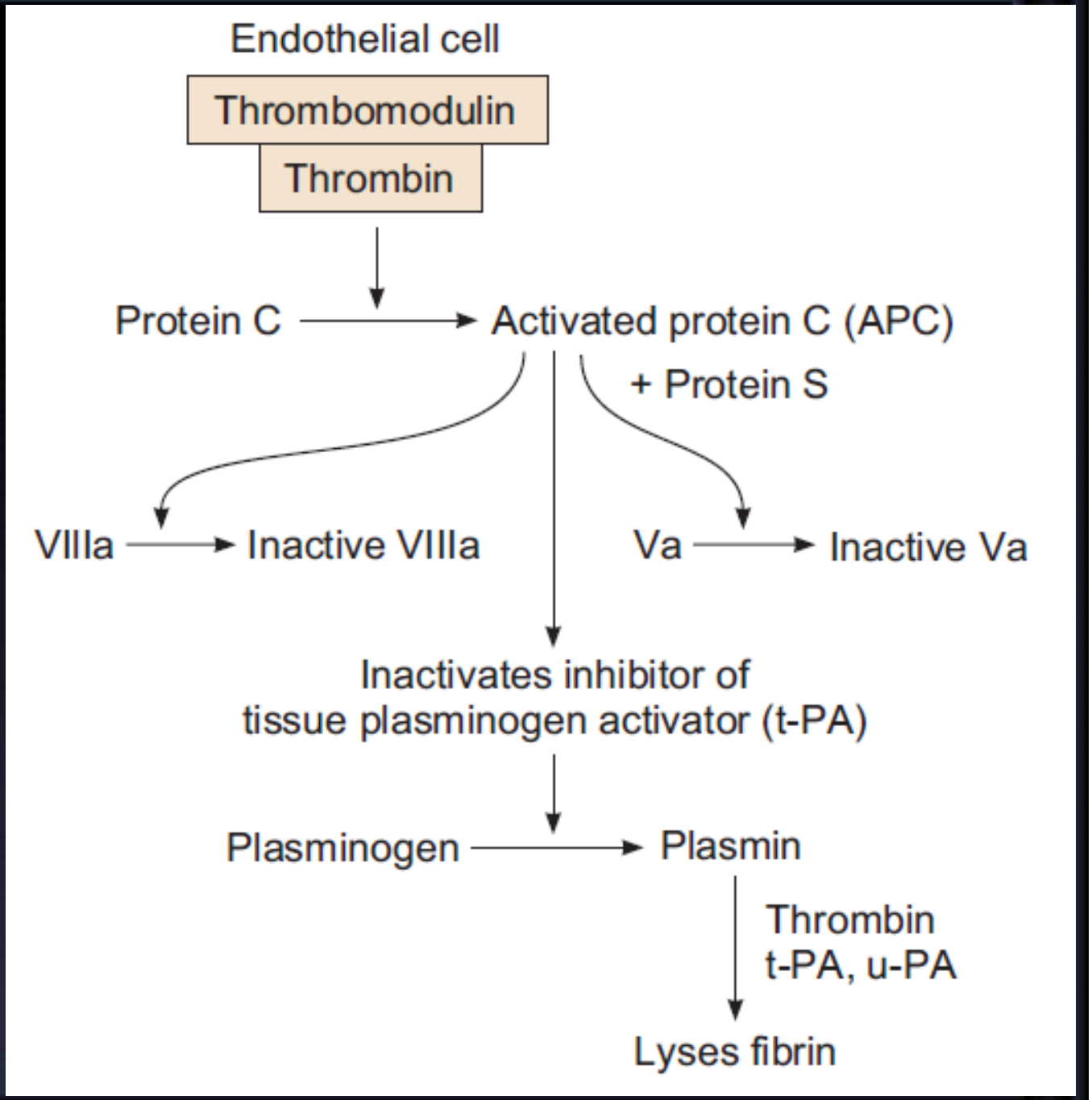
Formed blood clot can either become fibrous or dissolve.

- Fibrinolysis (dissolving) means Breaking down of fibrin by naturally occurring enzyme plasmin → prevent intravascular blocking.



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

The fibrinolytic system and its regulation by Protein C



ANTICOAGULANTS

USED IN VIVO

Parenteral

Heparin → Combines with antithrombin III and ↑ its effectiveness by 100-1000 fold, Also remove Factors XII, XI, X, and IX (Monitored by PTT time) → **CANNOT BE TAKEN ORALLY; WHY?**

Oral

- **Warfarin**: ↓ production of Vit K dependent clotting factors (II, VII, IX and X) by liver (Monitored by PT time) →
- **IS ALWAYS TAKEN ORALLY**

USED IN VITRO

No Ca^{++} → No Clotting (Needed in many steps)

Citrate ions → Deionization of Ca^{++}

Oxalate ions → Precipitate the Ca^{++}

EDTA → chelates (binds) calcium ions

Heparin → Binds to AT III

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 action of Fibrin and Antithrombin III**
   - 85-90 % Thrombin binds with Fibrin
   - 10-15 % Thrombin binds with Antithrombin III

Antithrombin III is a circulating protease blocking clot factors

NATURAL INTRAVASCULAR ANTICOAGULANTS

3. Heparin

- - vely charged conjugated polysaccharide
  - Increase the effectiveness of Antithrombin III
  - Produced by
    - Mast cells
    - Basophil cells
- Most widely used anticoagulant clinically e.g. in stroke

4. Alpha$_2$ – Macrogobulin

- Synthesized mainly in liver and acts as a binding agent for several coagulation factors and inhibits thrombin.

BLEEDING & CLOTTING DISORDERS

A. Hemophilia

B. Thrombocytopenia

C. Liver diseases & Vitamin-K deficiency

HEMOPHILIA

- Genetic disorders
- Hem A & B are inherited in X linked recessive pattern
- Occurs exclusively in males Females are carriers
- Hem C is autosomal recessive
- VWD autosomal dominant

- **HEMOPHILIA A**
  - Classic Hemophilia
  - 85 % cases
  - Def. Of factor VIII
- **HEMOPHILIA B** (Christmas disease)
  - 15 % cases
  - Def. Of factor IX
- **HEMOPHILIA C** (Rosenthal syndrome)
  - Def of factor XI (both sexes)

- Small Comp. → Hemophilia A ► ↑PTT
- Large Comp. → Von-Willebrand's disease ► ↑PTT & BT

Clinical Features: Easy bruising, massive bleeding after trauma or operation, hemorrhages in joints

THROMBOCYTOPENIA

- Count < 50,000 ul may cause spontaneous bleeding
- Less than 10,000 ------ Fatal
- ETIOLOGY

Decreased production

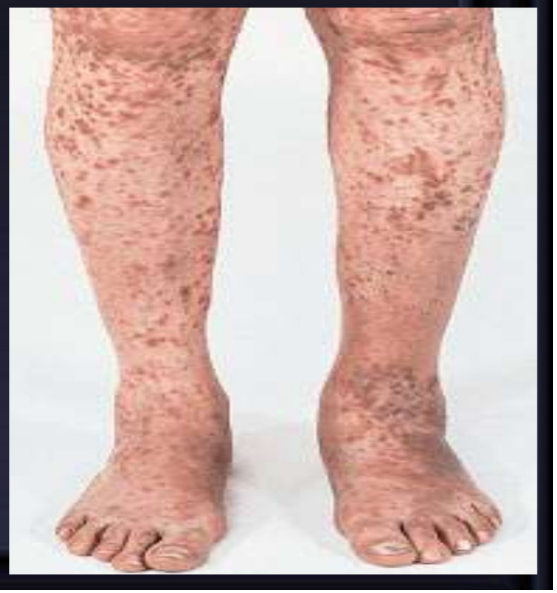
- Aplastic anemia
- Leukemia
- Drugs
- Infections (HIV, Measles)

Increased destruction

- ITP
- Drugs
- Infections (HIV)

Clinical Features

- Easy brusability
- Epistaxis
- Gum bleeding
- Hemorrhage after minor trauma
- Petechiae/Ecchymosis



THROMBOCYTOPENIA (cont.)

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

PSEUDOTHROMBOCYTOPENIA

- Partial clotting of specimen
- EDTA-platelet clumping
- Platelet satellitism around WBCs
- Cold agglutinins
- Giant platelets

BLEEDING DISORDERS

Liver diseases & Vitamin-K deficiency

- e.g. Hepatitis, Cirrhosis
  - Decreased formation of clotting factors
  - Increased clotting time
- Vitamin K dependent factors
  - Factors….II , VII, IX & X

BLEEDING DISORDERS

A. Vitamin-K

- Fat soluble vitamin
- Required by liver for formation 4 clotting factors

Factors: II, VII, XI and X

- Sources
  - Diet
  - Synthesized 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

2) SCREENING TEST

Test	Mechanism Tested	Normal Value	Disorder
Bleeding time (BT)	Hemostasis, capillary & platelet function	3-7 min beyond neonate	Thrombocytopenia , von Willebrand disease
Platelet count	Platelet number	150 000 – 450 000 / mm^3	Thrombocytopenia
Prothrombin time (PT)	Extrinsic & common pathway	< 12 sec beyond neonate; 12-18 sec in term neonate	Defect in Vit K-dependent factor, liver disease, DIC
Activated partial thromboplastin time (APTT)	Intrinsic & common pathway	25-40 sec beyond neonate; 70 sec in term neonate	Hemophilia, von Willebrand disease, DIC

Source from : Nelson Essential of Pediatrics 5th edition

Haemostasis tests in hereditary coagulation disorders

	Haemophilia A	**Haemophilia B**	**VW disease**
Bleeding time	Normal	Normal	Prolonged
Prothrombin time	Normal	Normal	Normal
APTT	Prolonged	Prolonged	Prolonged
Factor VIII	Low	Normal	Low or normal
Factor IX	Normal	Low	Normal
VWF	Normal	Normal	Low