L-1: PLATELETS STRUCTURE & FUNCTIONS

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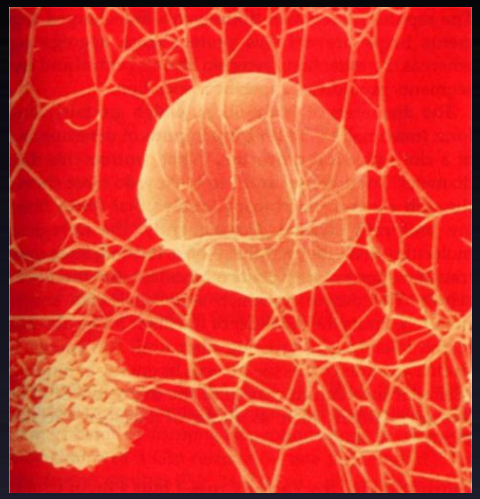
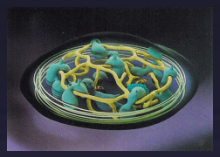


Vessel injury



Antithrombogenic
(Favors fluid blood)

Thrombogenic
(Favors clotting)

HANDOUTS…12/18/2017

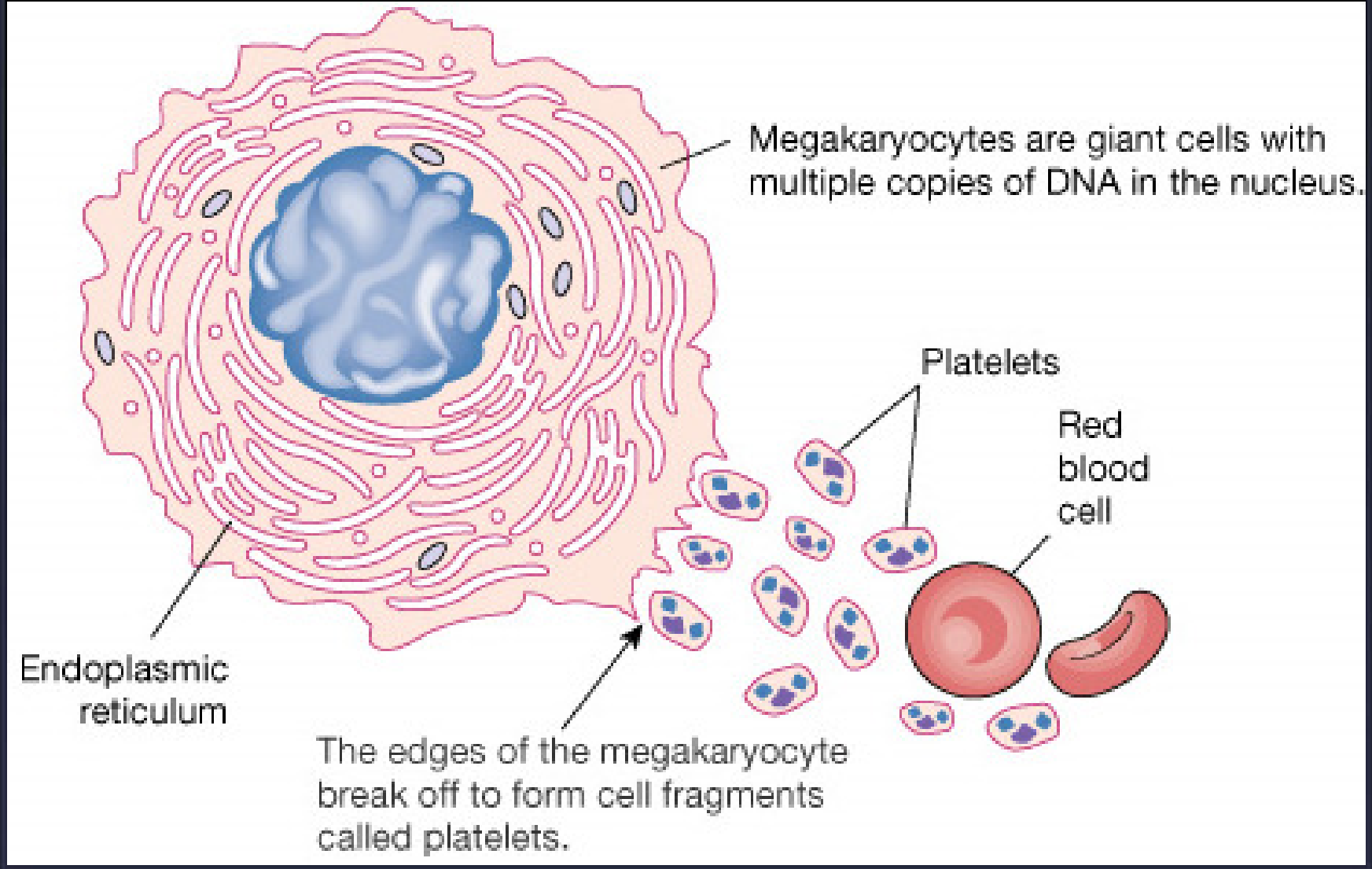
OBJECTIVES

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

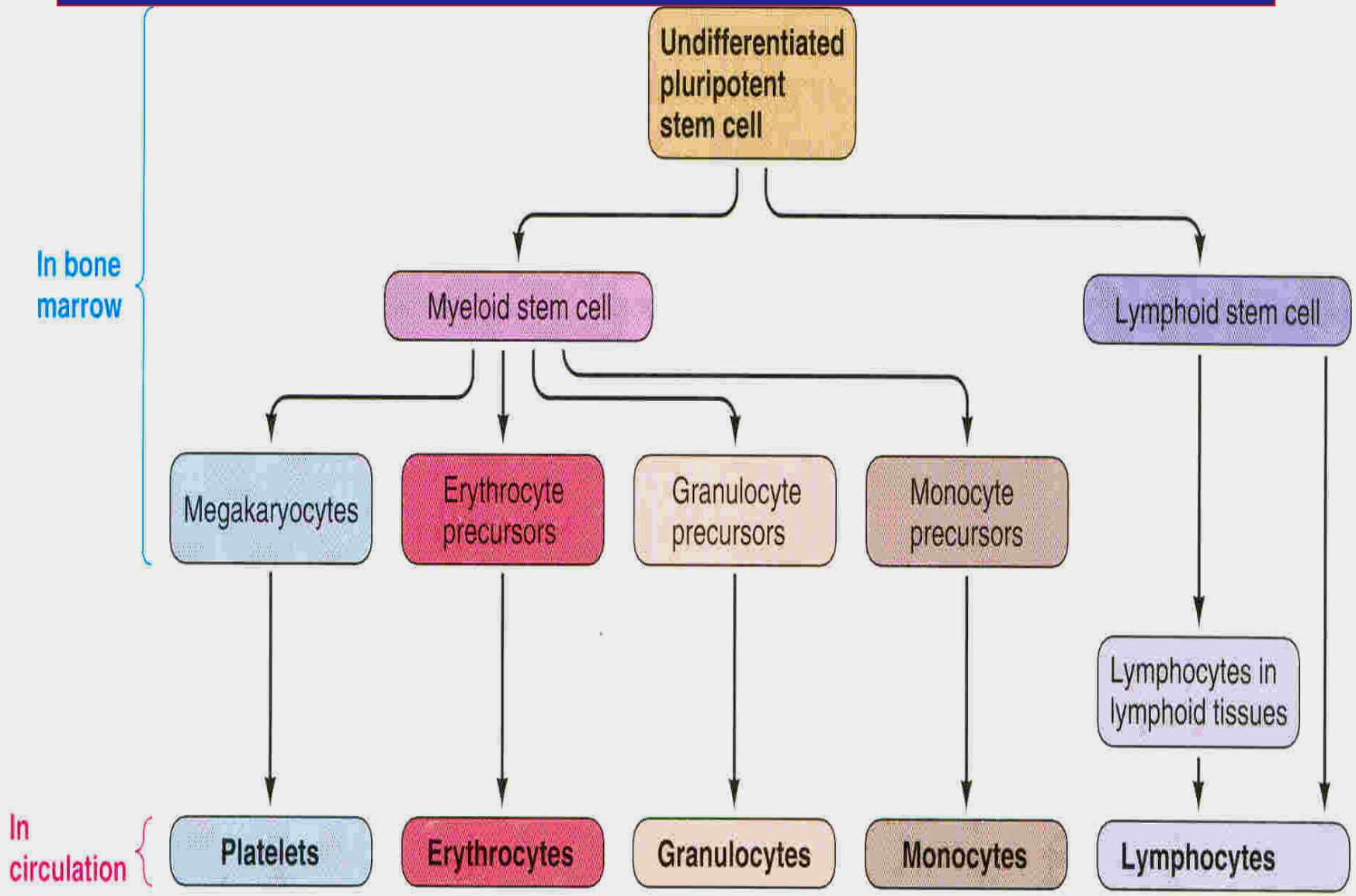
- **Describe formation and development of platelets**
- **Understand platelet normal ultrastructure**
- **Describe the functions of different platelets organelles and surface receptors**
- **Describe the mechanisms of platelet functions**
- **Relate membrane receptors and granule content to normal function in hemostasis and bleeding (platelet) disorders**

PLATELETS

Formed by fragmentation from megakaryoctyes



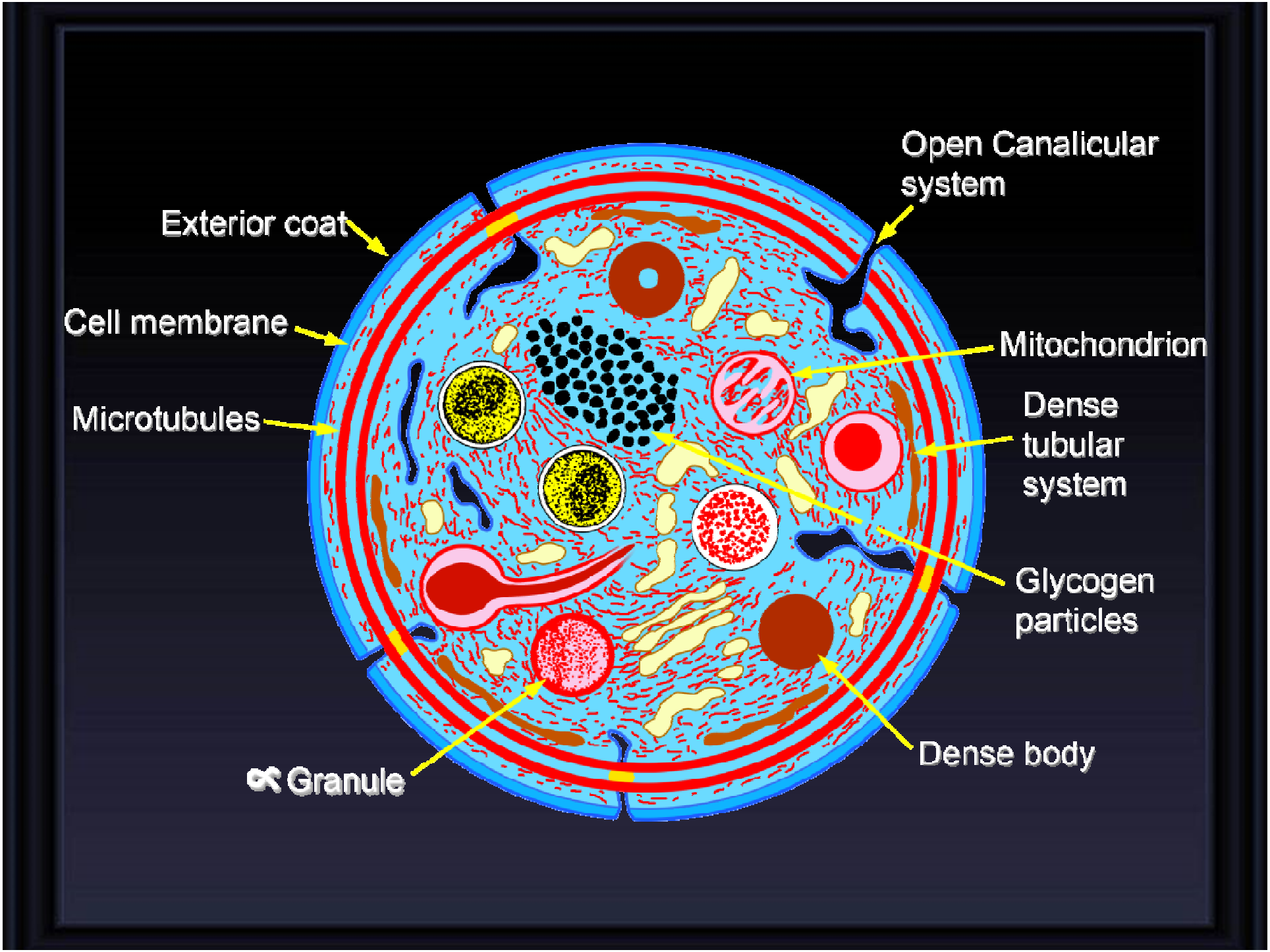
SITE OF FORMATION
Bone-marrow



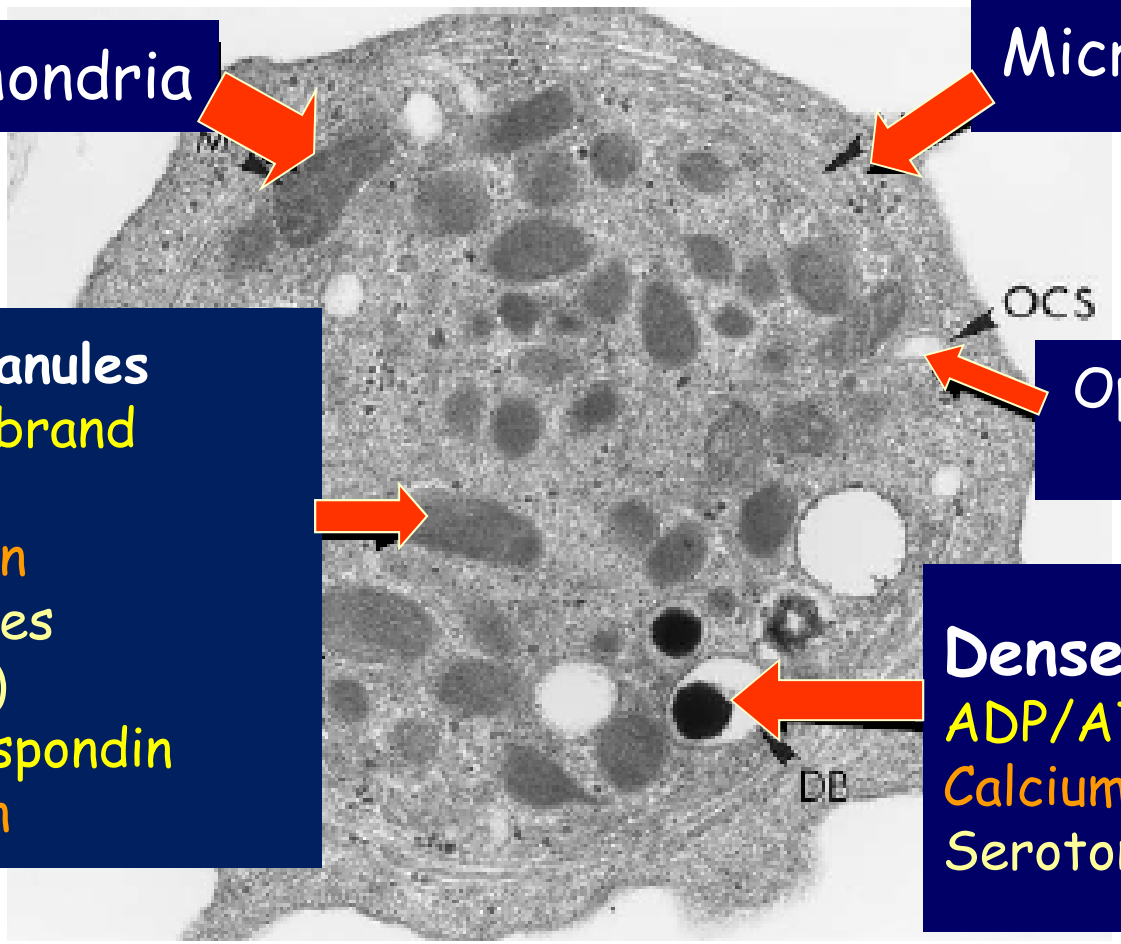
PLATELETS (Characteristics)

SHAPE: MINUTE ROUND OR OVAL DISCS
SIZE: 1.5-3.0 um IN DIAMETER
HALF LIFE: 7-10 DAYS
COUNT: 150,000 – 300,000/ microlitrer
LOCATION: 80% in blood & 20% in spleen

- **Anuclear and discoid cell**
- **Contractile, adhesive, cell fragments.**
- **Store coagulation factors & enzymes**
- **Surface Binding sites Glycoproteins (surface Antigens)**


Open Canalicular system
Exterior coat
Cell membrane
Mitochondrion
Microtubules
Dense tubular system
Glycogen particles
Granule
Dense body

Platelet Ultrastructure



Mitochondria

Microtubules

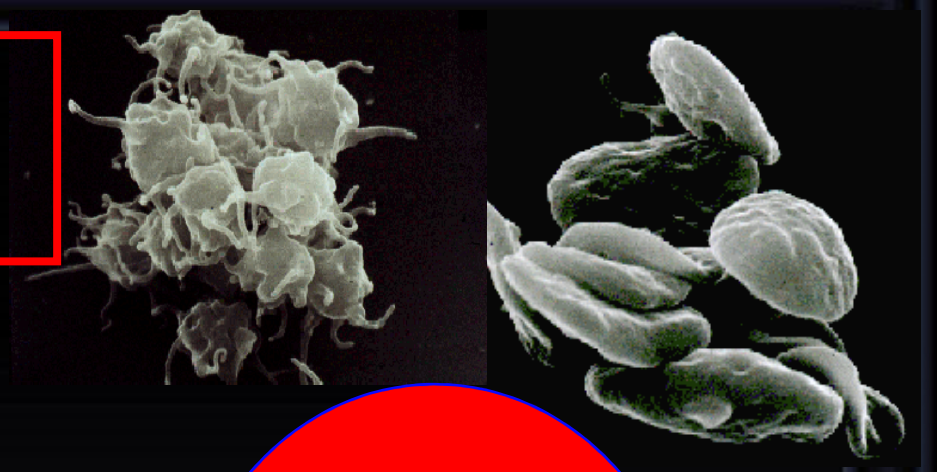
Open canalicular system

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

Dense Granules
ADP/ATP
Calcium
Serotonin

Photo by Dr. James White, in "Methods in Molecular Biology: Platelets and Megakaryocytes, Vol. 1", Gibbins, J.M., and Mahaut-Smith, M.P.,[eds.], 2004, pg. 48.

FUNCTIONAL CHARACTERISTICS



- **Motile:** Actin And Myosin Molecules
- **Active:** Endoplasmic Reticulum, Golgi Apparatus & Mitochondria
- **Enzymes** Systems For Synthesis Of Prostaglandins
- **Garnules**

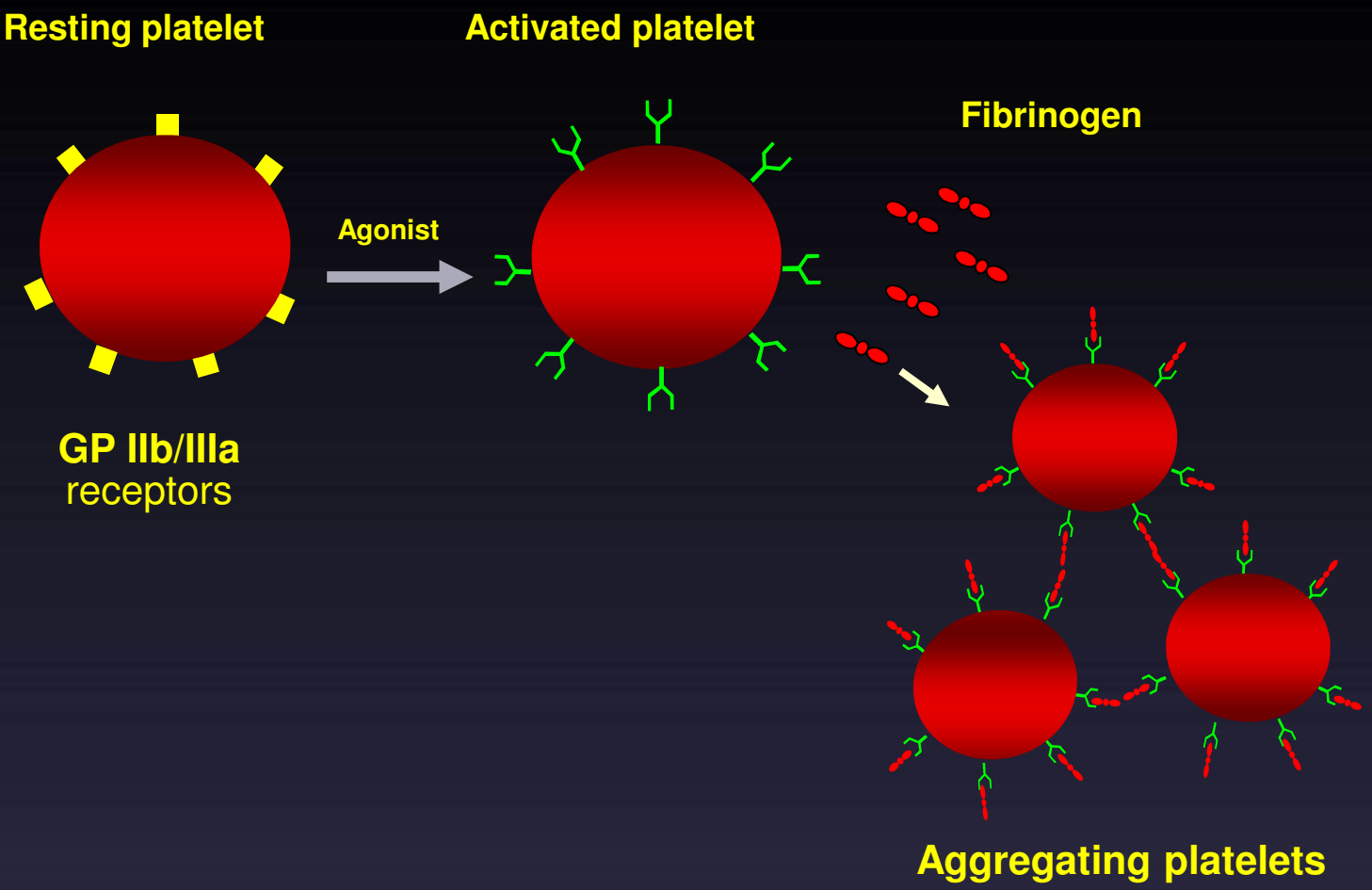
Dense or δ granules

- Serotonin
- ADP
- Ca^{++}

α granules

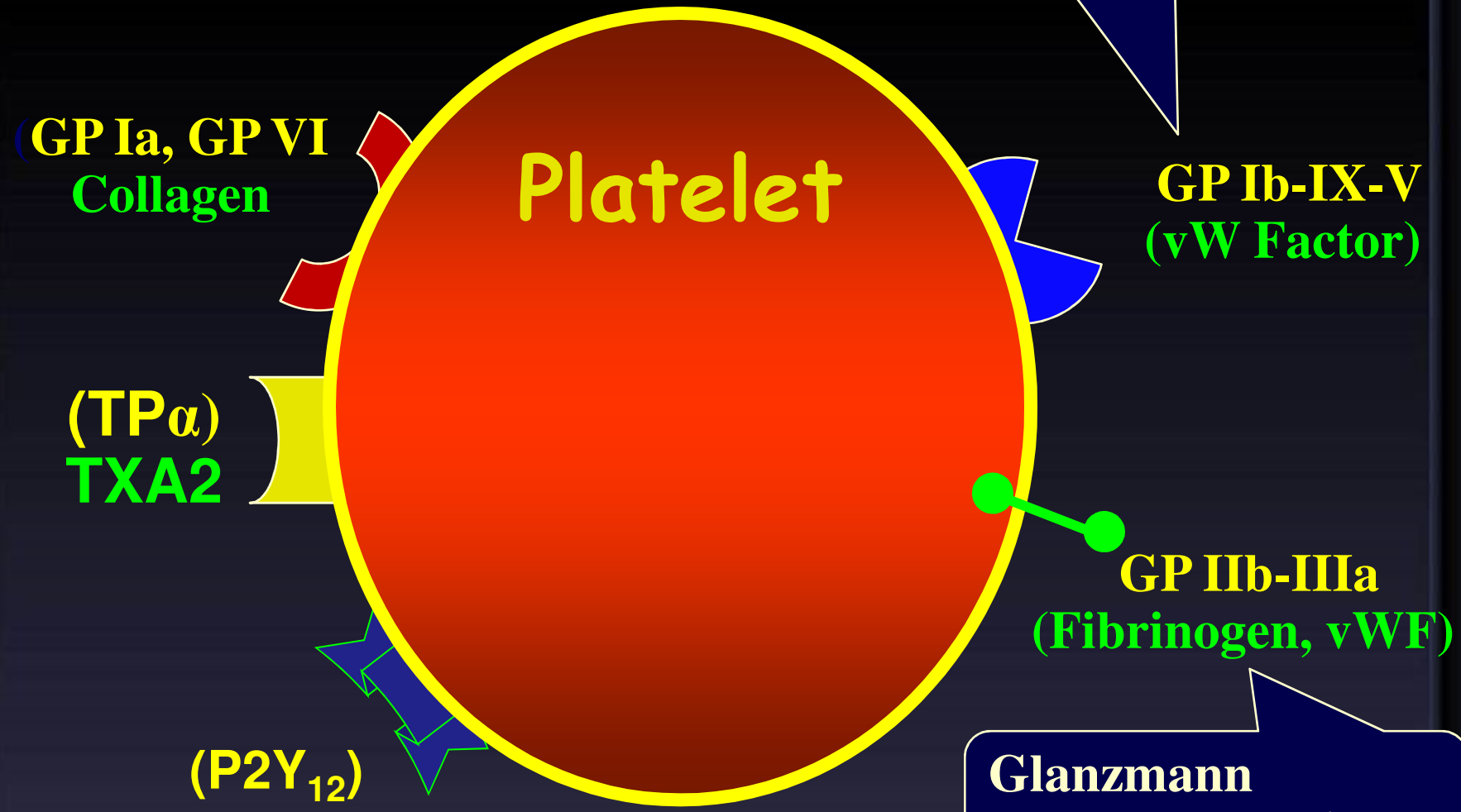
- Coag Factors (eg:Fibrinogen,vWF)
- PDGF
- Chemokines

Platelets Activation



Platelet Receptors

https://www.youtube.com/watch?v=0pnpoEy0eYE

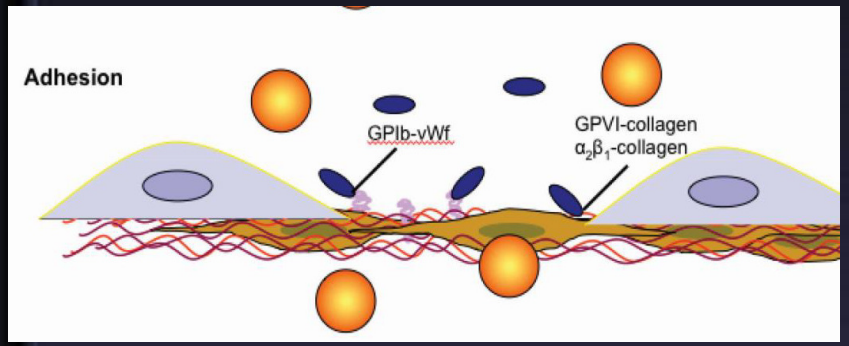


Congenital Platelet Disorders

Disorders of Adhesion:
- Bernard-Soulier

Disorder of Aggregation:
- Glanzmann thrombosthenia

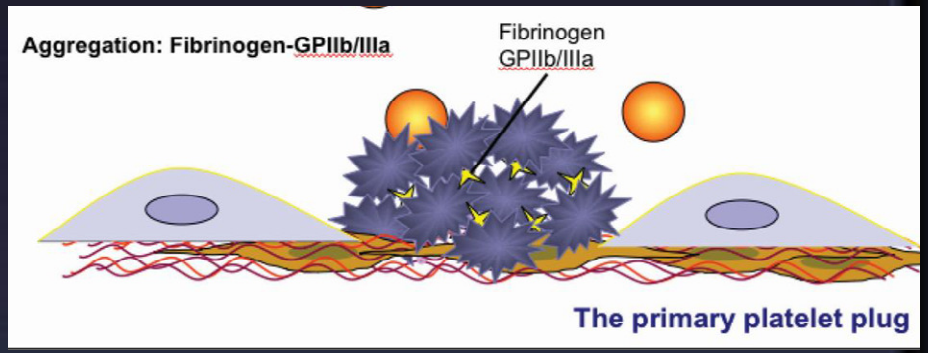
Disorders of Granules:
- Grey Platelet Syndrome
- Storage Pool deficiency
- Hermansky-Pudlak Synd
- Chediak-Higashi Synd



Disorders of Cytoskeleton:
- Wiskott-Aldrich syndrome

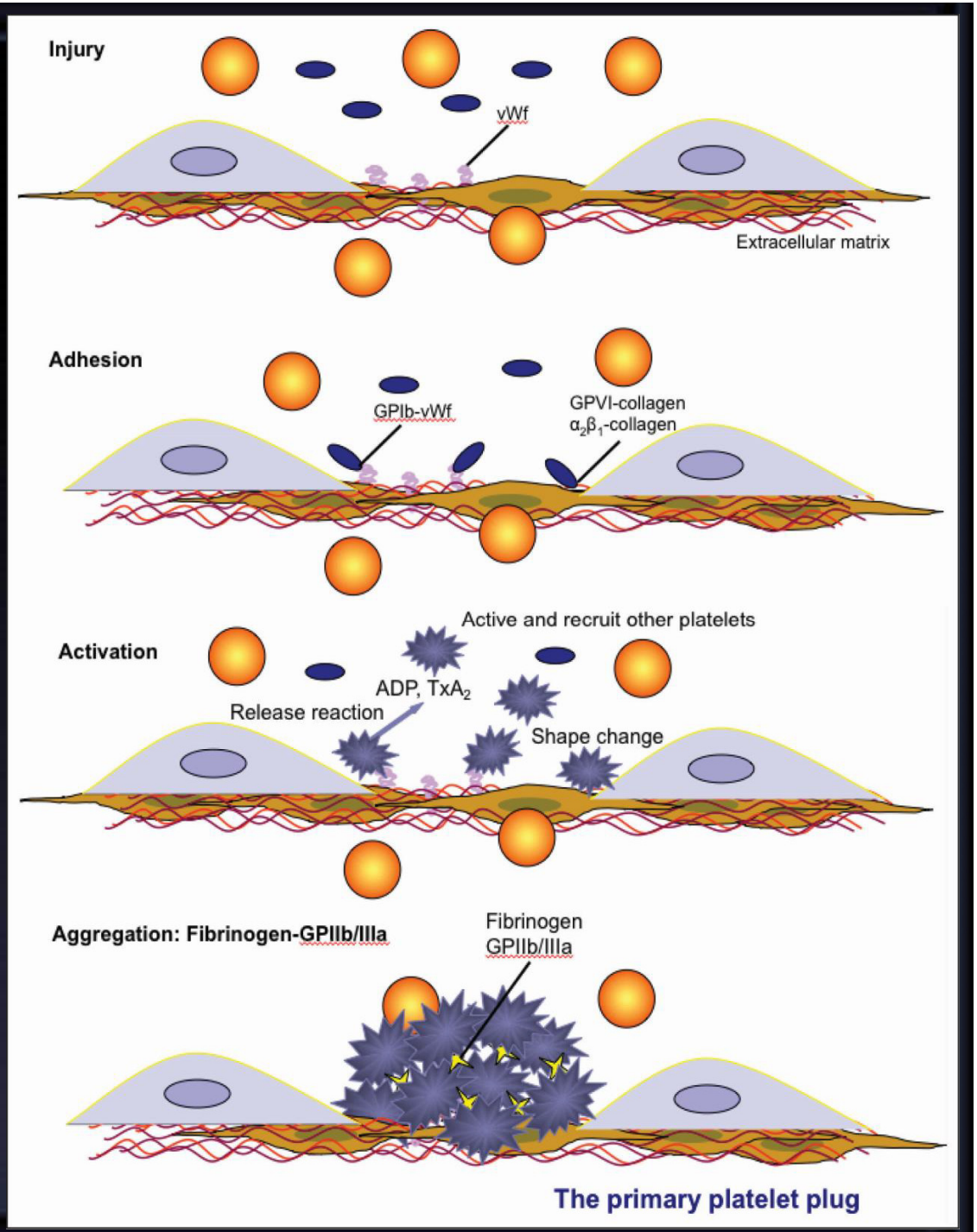
Disorders of Primary Secretion:
- Receptor defects (TXA2, collagen ADP, epinephrine)

Disorders of Production:
- Congenital amegakaryocytic thrombocytopenia
- MYH9 related disorders
- Thrombocytopenia with absent radii (TAR)
- Paris-Trousseau/Jacobsen



Adapted from Balduini et al, Haematologica 88[5]: 582-592, May 2003

Aggregation:
Fibrinogen is needed to join platelets to each other via platelet fibrinogen receptors



LAB TESTS IN BLEEDING AND CLOTTING

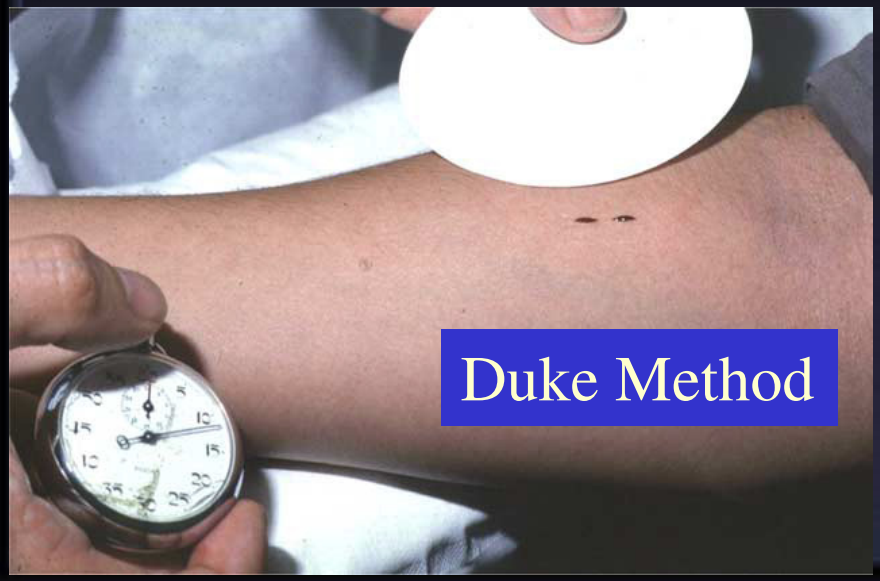
Test	Normal Value	Importance
PLATELET COUNT	100,000 - 400,000 CELLS/MM^3	Thrombocytopenia
PLATELET FUNCTIONS	Normal Aggregation	Thrombocytopathy (normal count) [Congenital or Acquired…Aspirin]
BLEEDING TIME (BT)	2-8 MINUTES	Bleeding disorders
PROTHROMBIN TIME (PT)	10-15 SECS	Measures Effectiveness of the Extrinsic Pathway
PARTIAL THROMBOPLASTIN TIME (PTT)	25-40 SECS	Measures Effectiveness of the Intrinsic Pathway
THROMBIN TIME (TT) $INR = \left(\frac{PT_{test}}{PT_{normal}}\right)^{ISI}$	9-13 SECS	A Measure of Fibrinolytic Pathway Time for Thrombin To Convert Fibrinogen ► Fibrin

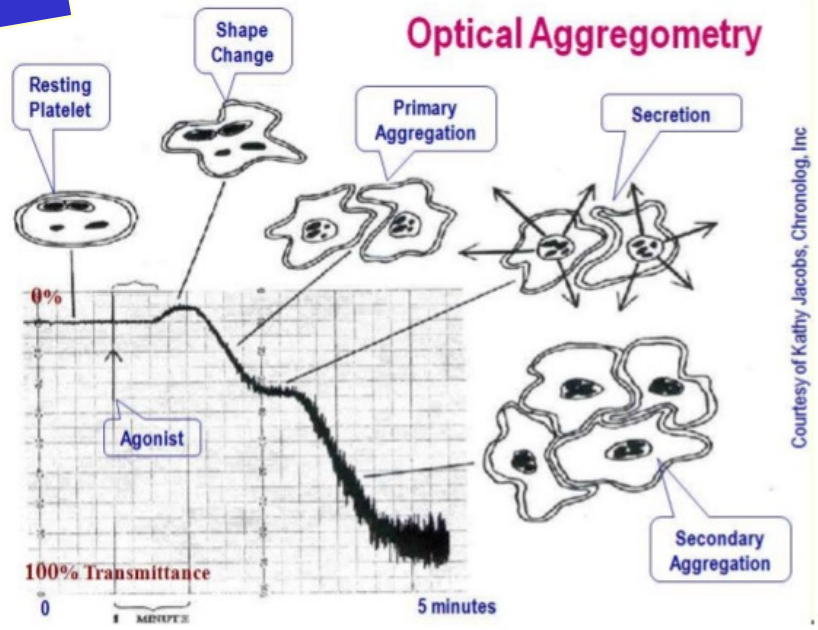
Testing Platelet Functions

- Peripheral smear and Platelet count
- Bleeding time (duke Method)
- Platelet Function Analyzer (PFA-100)
- Platelet Aggregation
- Flow-cytometry
- Electron-microscopy
- Granule release products

Automated

Aggregation





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

L-2: COAGULATION MECHANISMS

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Vessel injury

Antithrombogenic
(Favors fluid blood)

Thrombogenic
(Favors clotting)

HANDOUTS…12/18/2017

OBJECTIVES

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

- **Recognoize 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**
- **Explain process of fibrinolysis and function of plasmin**

HEMOSTASIS

The spontaneous arrest of bleeding from ruptured blood vessels

FOUR STEPS OF HEMOSTASIS

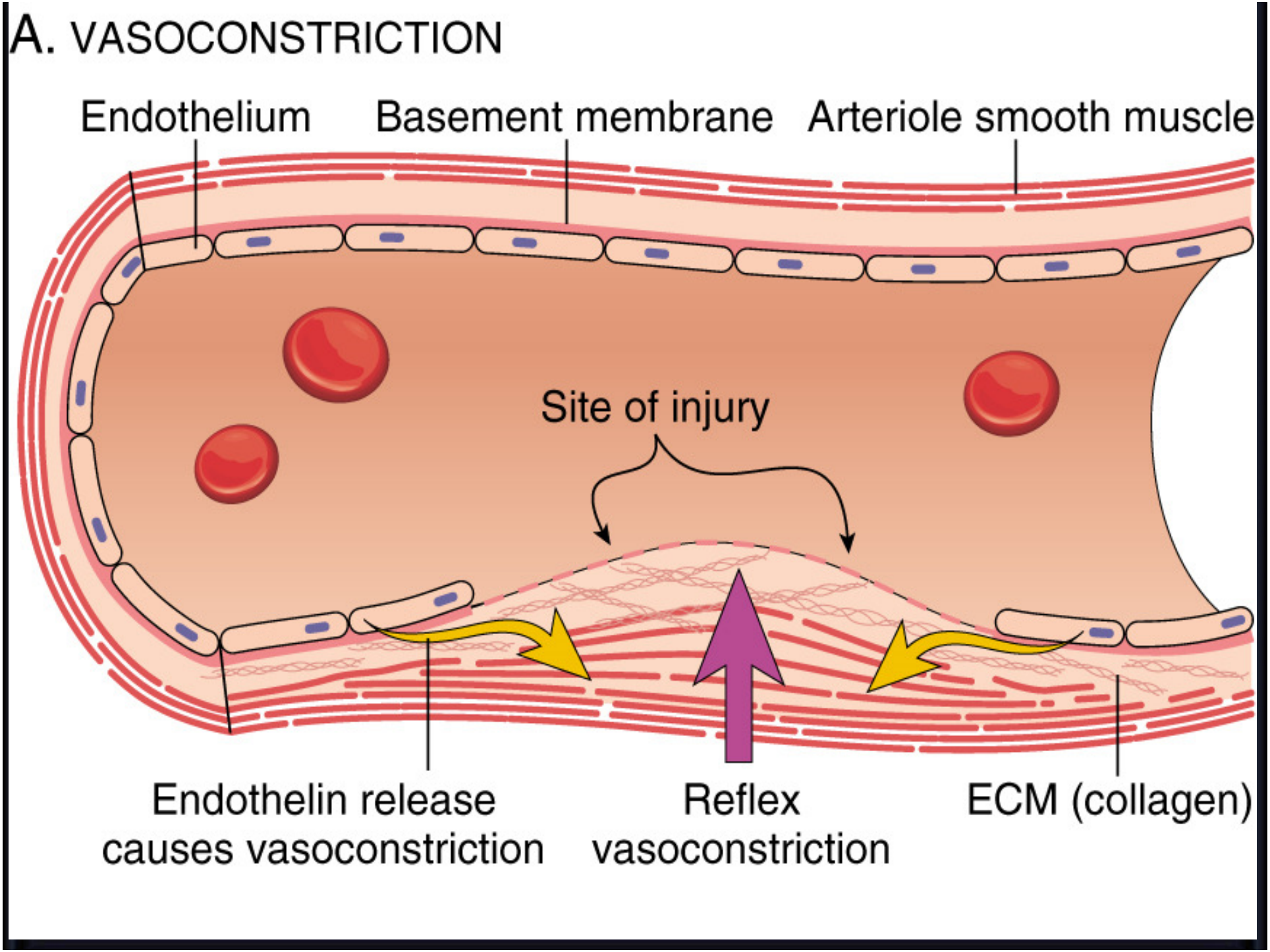
1. VASCULAR PHASE ► **Vascular Spasm**
2. PLATELET PHASE ► **Formation of platelet plug**
3. COAGULATION PHASE ► **Blood Coagulation & Clot Retraction**
4. FIBRINOLYTIC PHASE ► **Fibrinolysis**

1-VASCULAR SPASM (Vascular Constriction)

Immediately After injury there is localized Vasoconstriction.

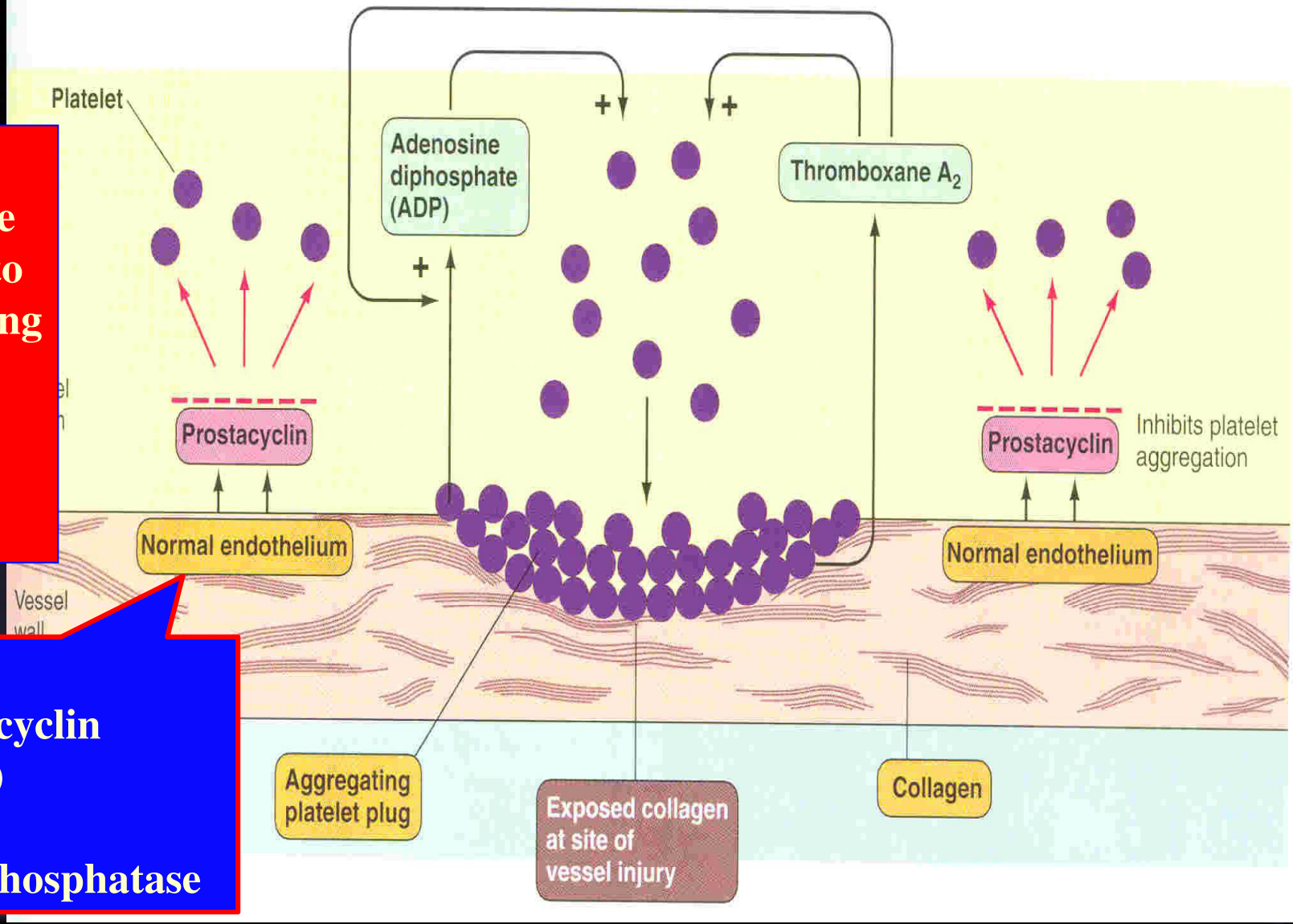
- **Causative Factors are three (3)**
  1. **Nervous reflexes**
  2. **Local myogenic spasm**
  3. **Local humoral factors….Platelets → Thromboxane A_2 [TXA2] (Vasoconstrictor)**
- **Importance**
  - **Crushing injuries → Intense spasm → No lethal loss of blood**

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)

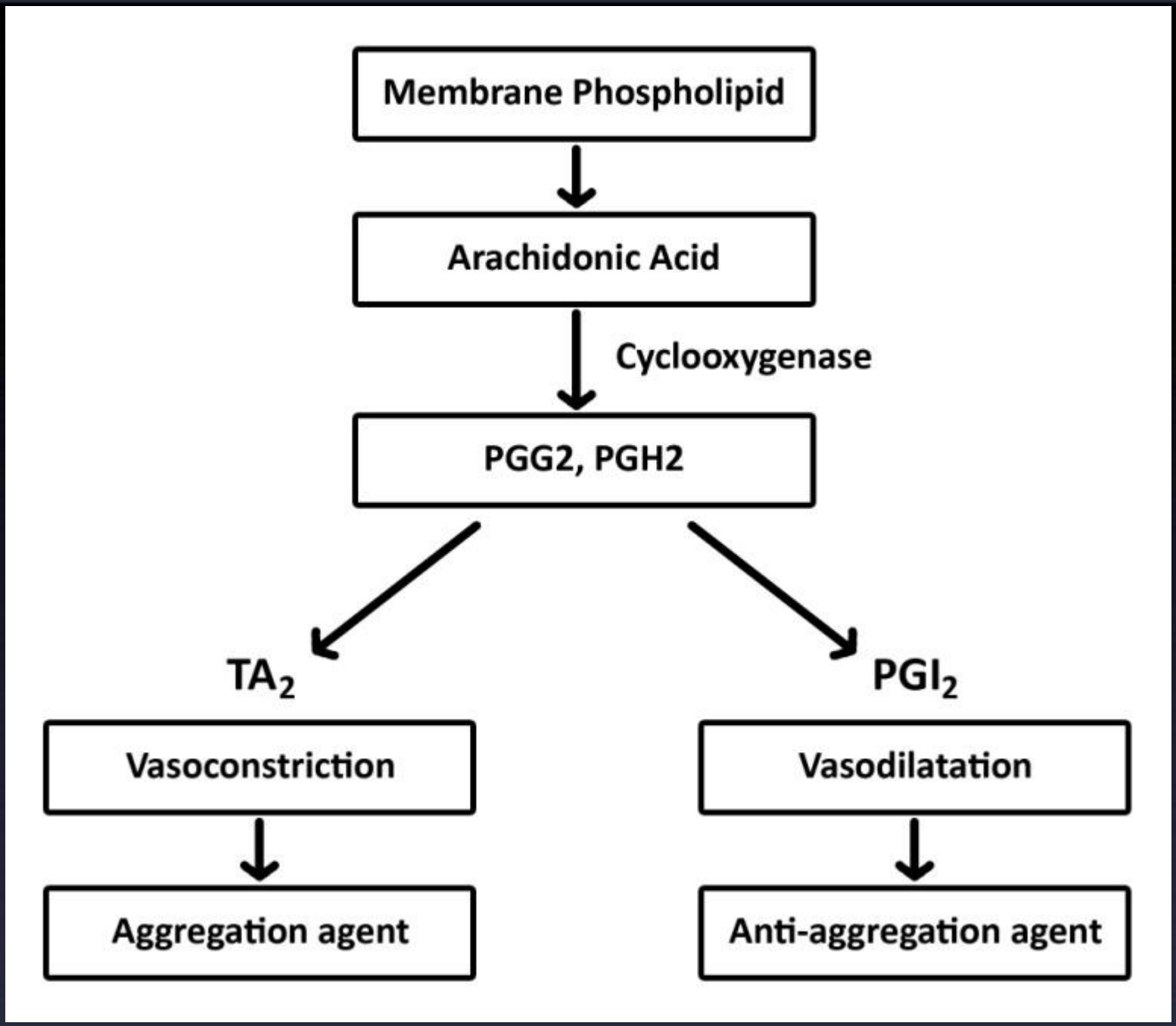
2-FORMATION OF PLATELET PLUG [PRIMARY HEMOSTASIS]

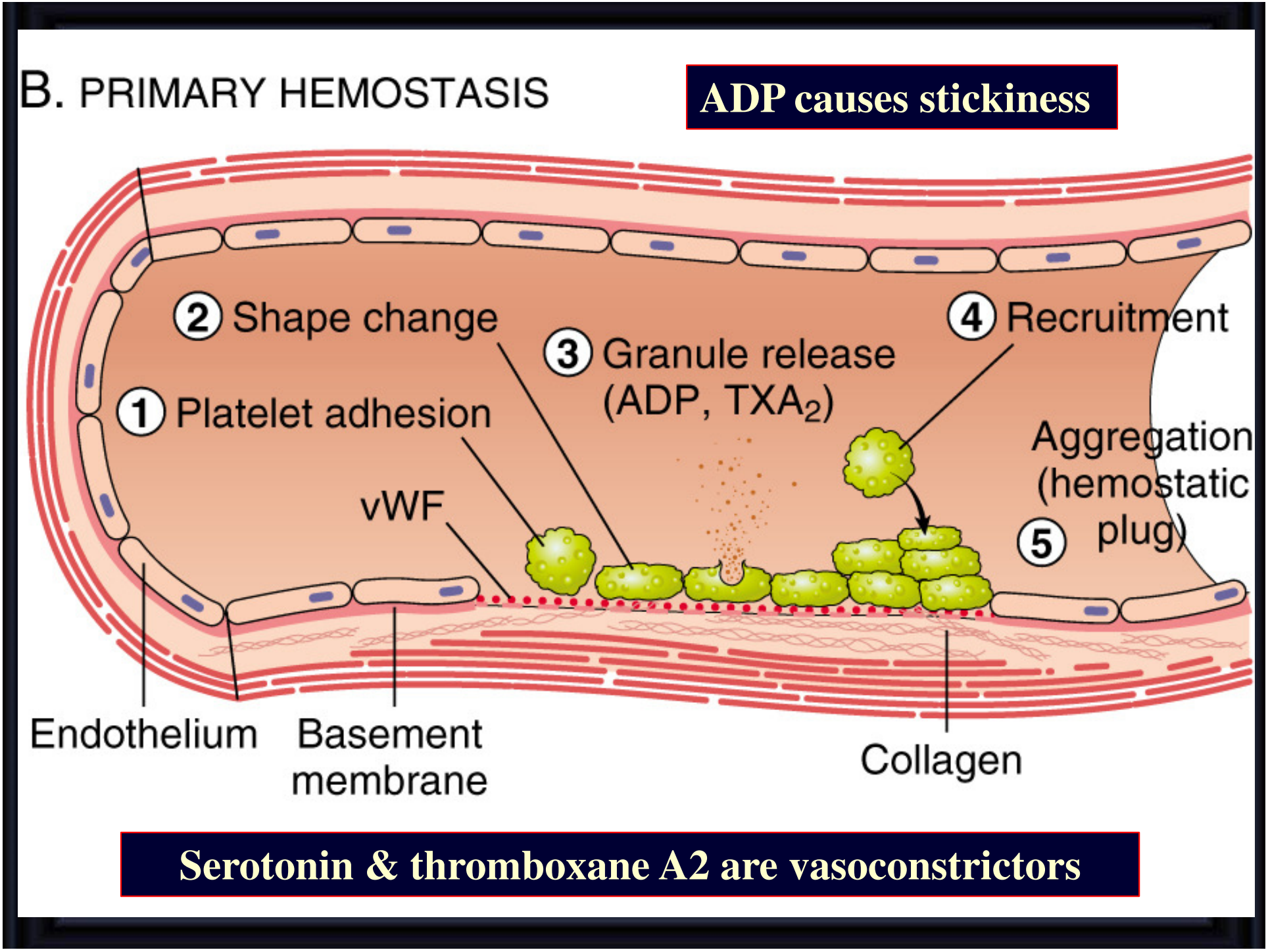
Importance ►enough to stop bleeding from small vascular damage



Secrete

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


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

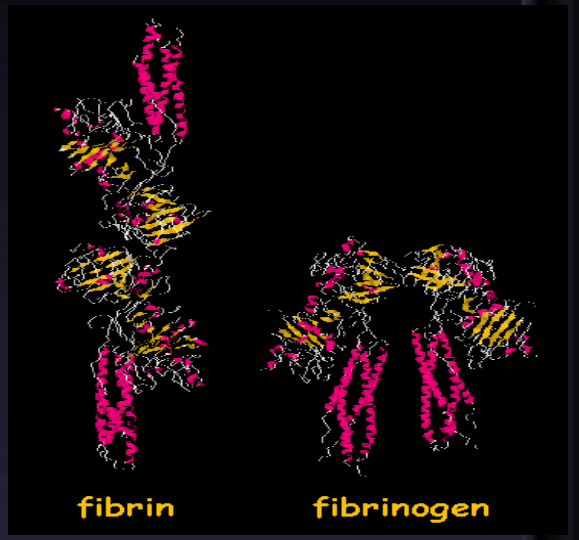

B. PRIMARY HEMOSTASIS
ADP causes stickiness
2 Shape change
3 Granule release (ADP, TXA2)
4 Recruitment
1 Platelet adhesion
vWF
Aggregation (hemostatic plug)
5
Endothelium
Basement membrane
Collagen
Serotonin & thromboxane A2 are vasoconstrictors

3-BLOOD COAGULATION

Formation of Clot or Thrombus

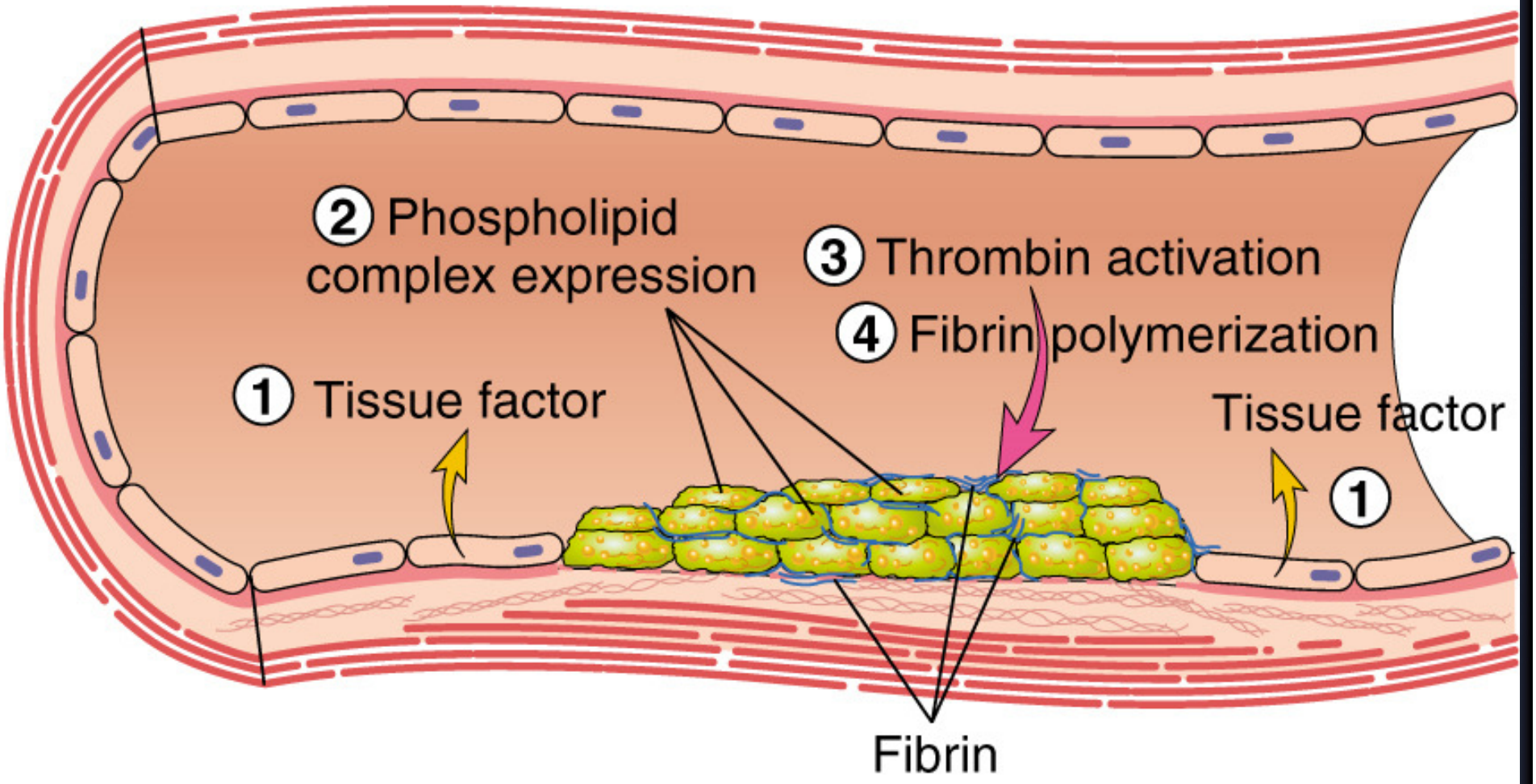
[SECONDARY HEMOSTASIS]

- **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**

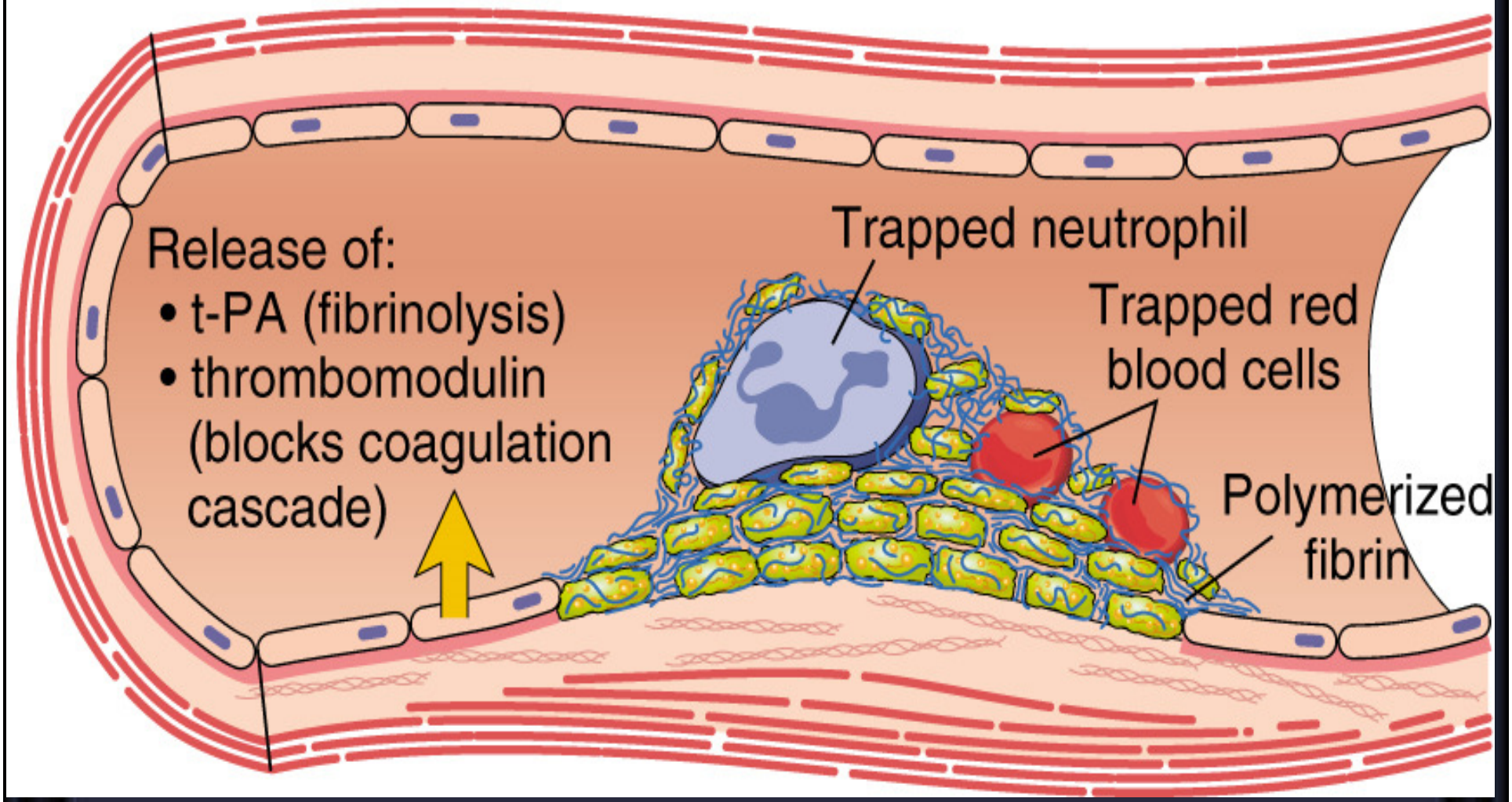


CLOT is a meshwork of fibrin fibres running in all directions entrapping blood cells, platelets and plasma.

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	

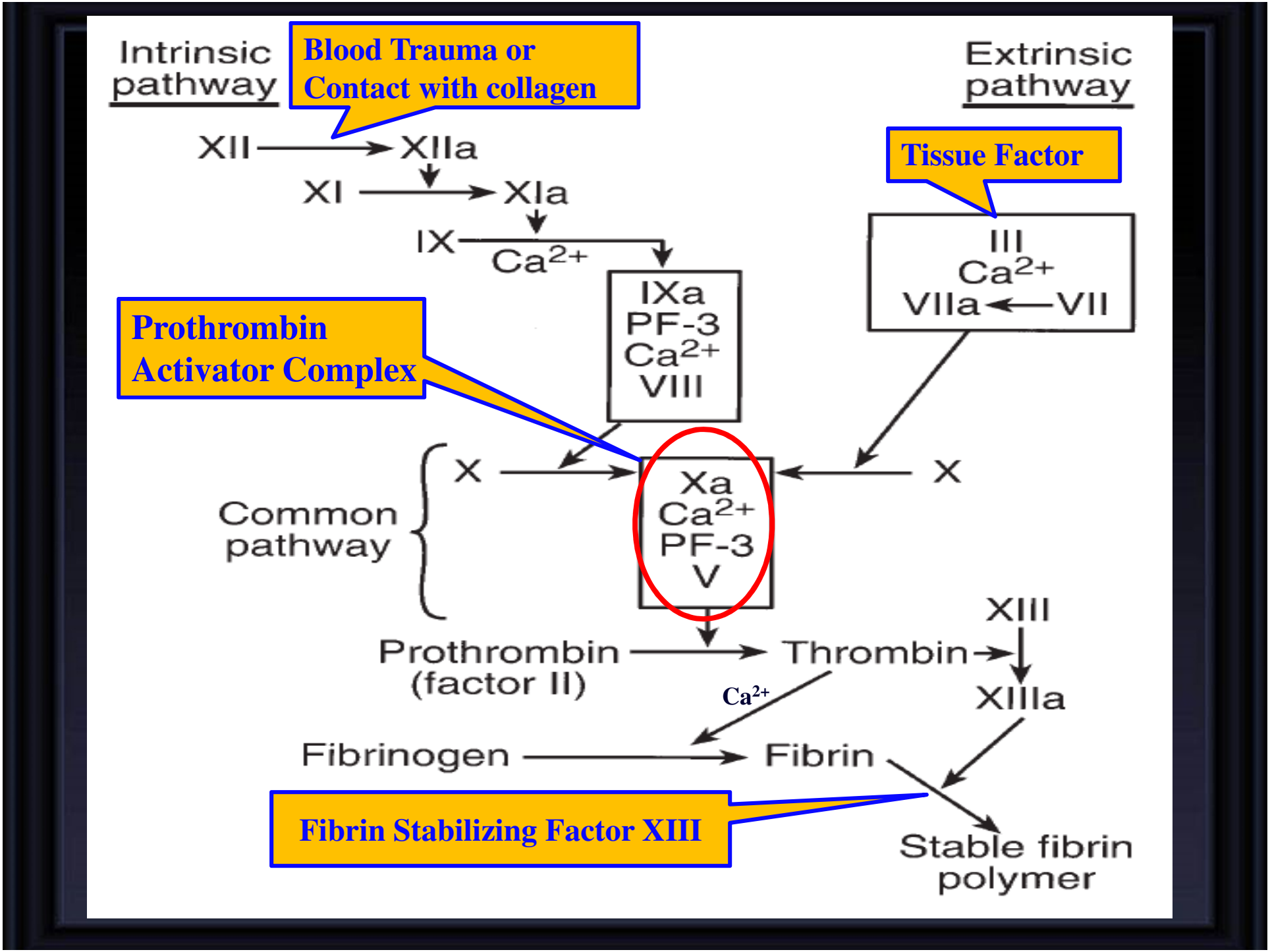
Clotting Factors

Ganong

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

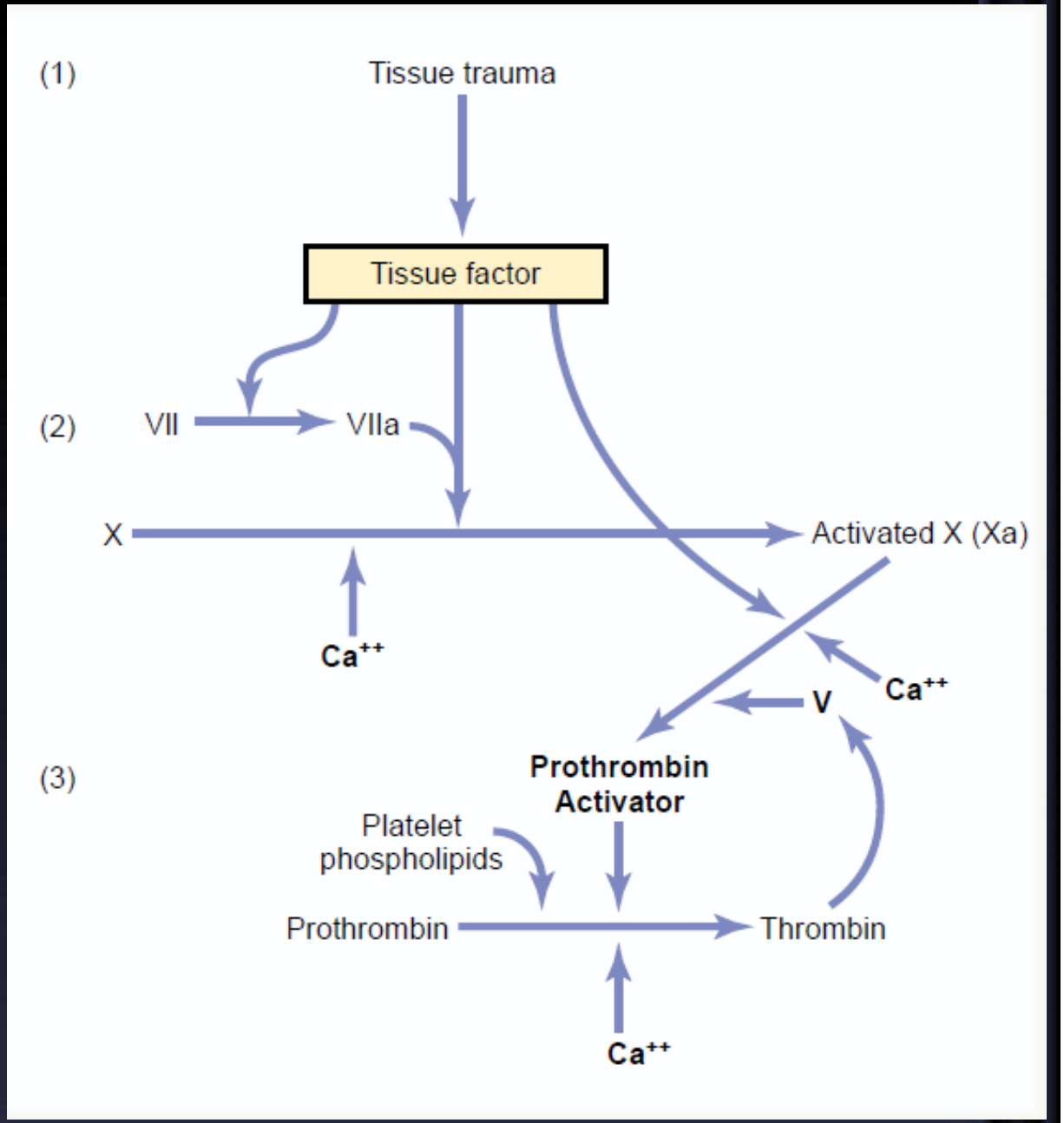
Factor[a]	Names
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Proaccelerin, labile factor, accelerator globulin
VII	Proconvertin, SPCA, stable factor
VIII	Antihemophilic factor (AHF), antihemophilic factor A, antihemophilic globulin (AHG)
IX	Plasma thromboplastic component (PTC), Christmas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

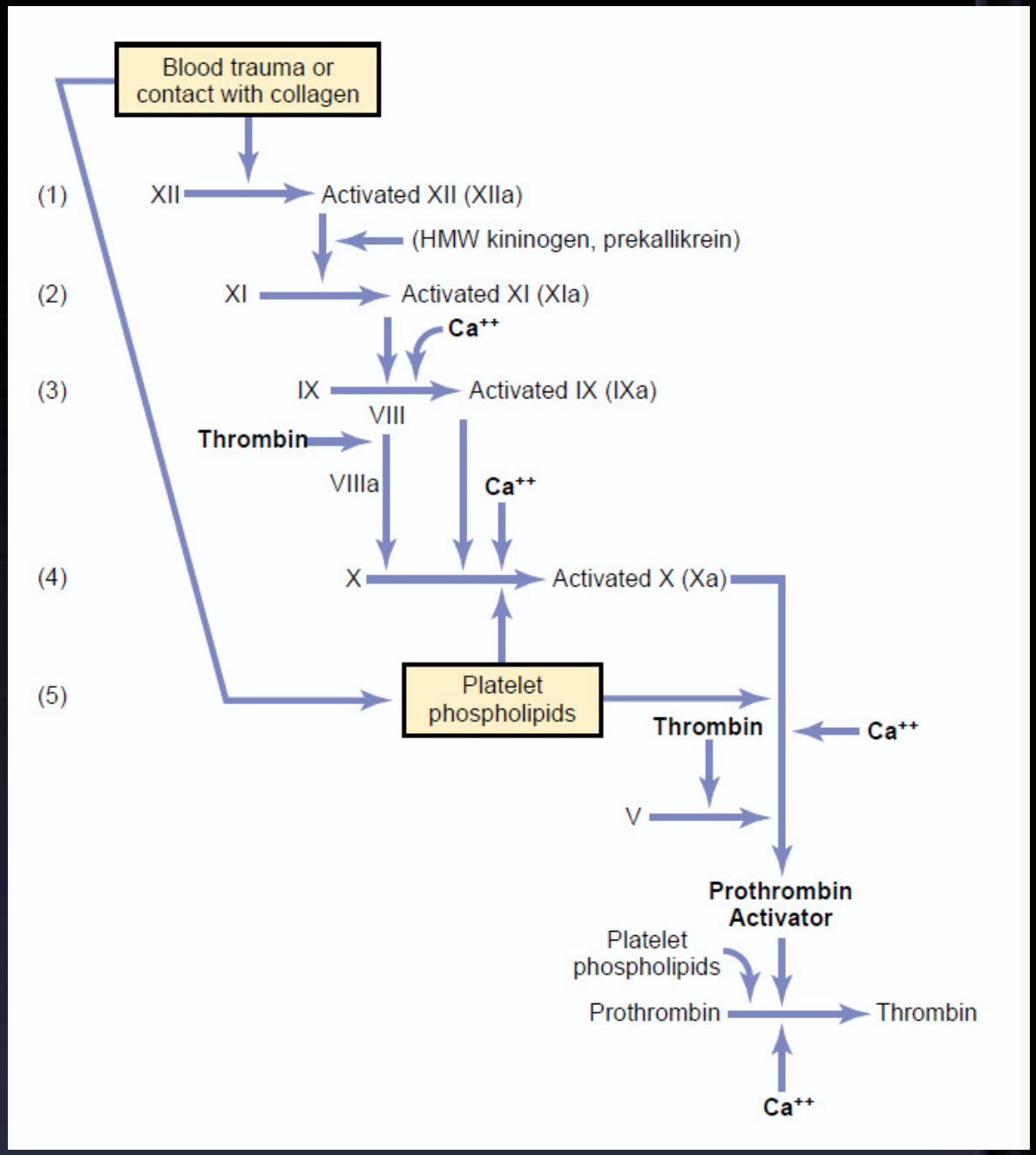
EXTRINSIC MECHNANISM FOR INITIATING CLOTTING

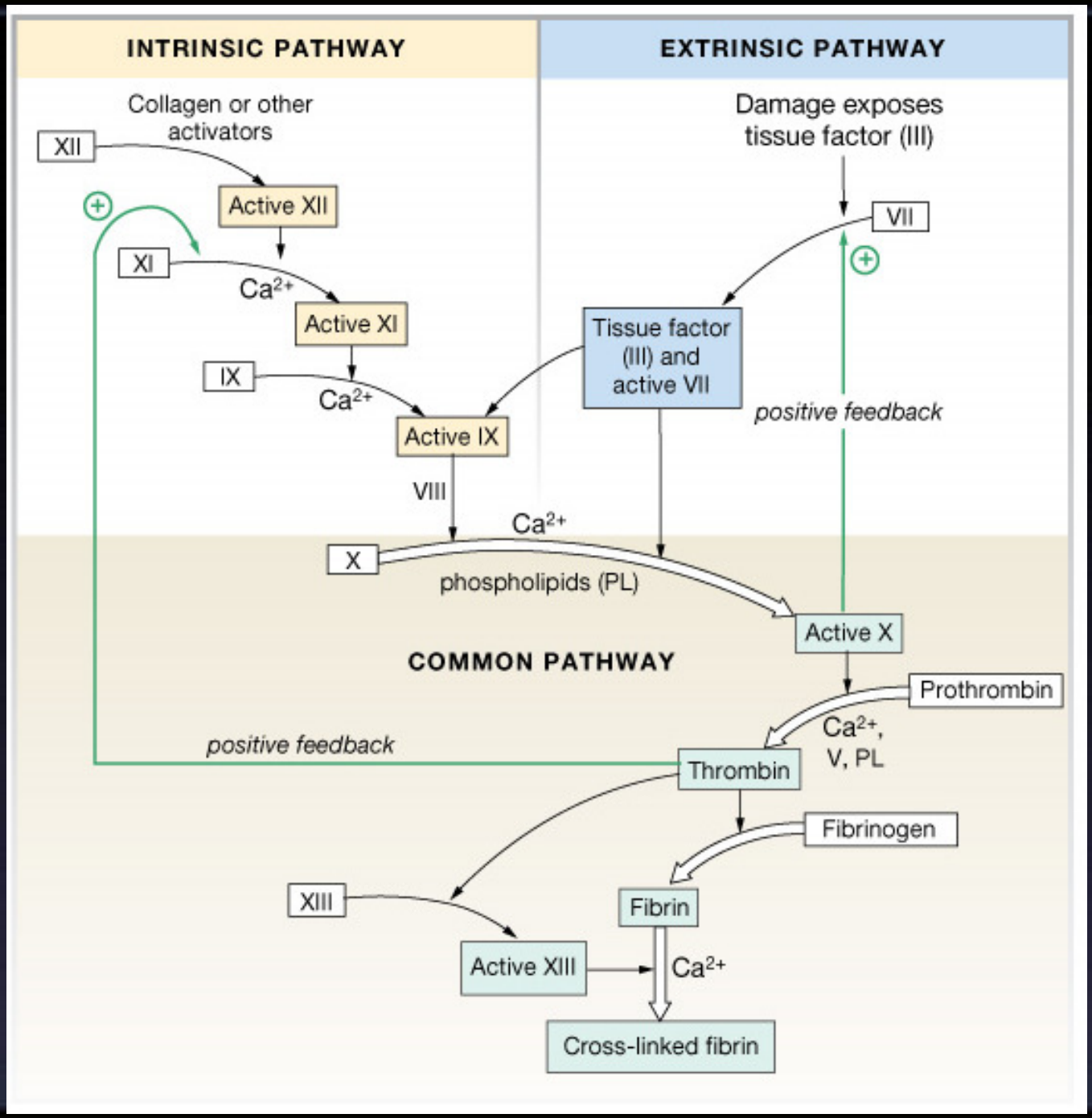
TF or tissue thromboplastin; includes phospholipids from the membranes of the tissue plus a lipoprotein complex that functions mainly as a proteolytic enzyme.



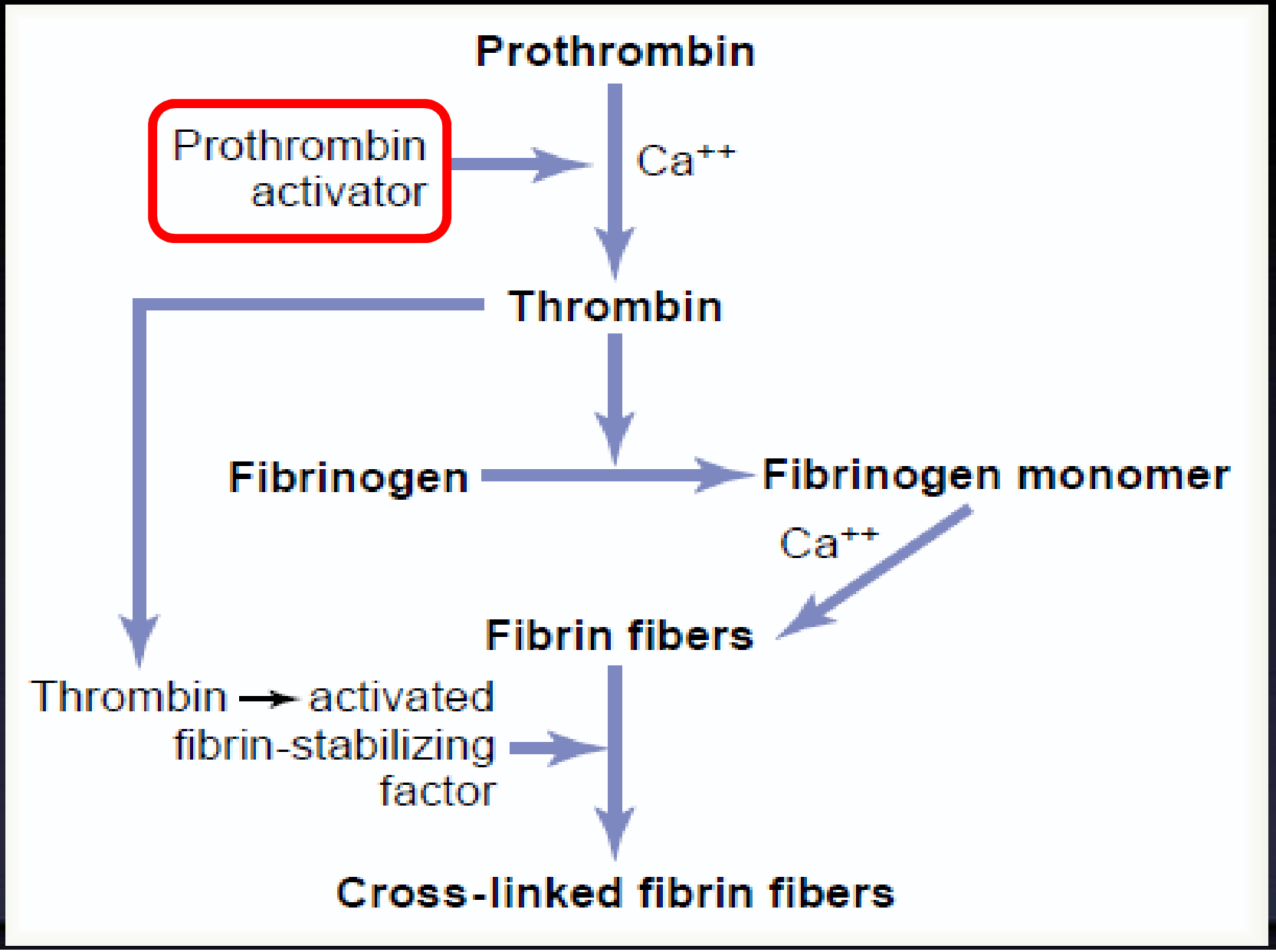
INTRINSIC MECHNANISM FOR INITIATING CLOTTING

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

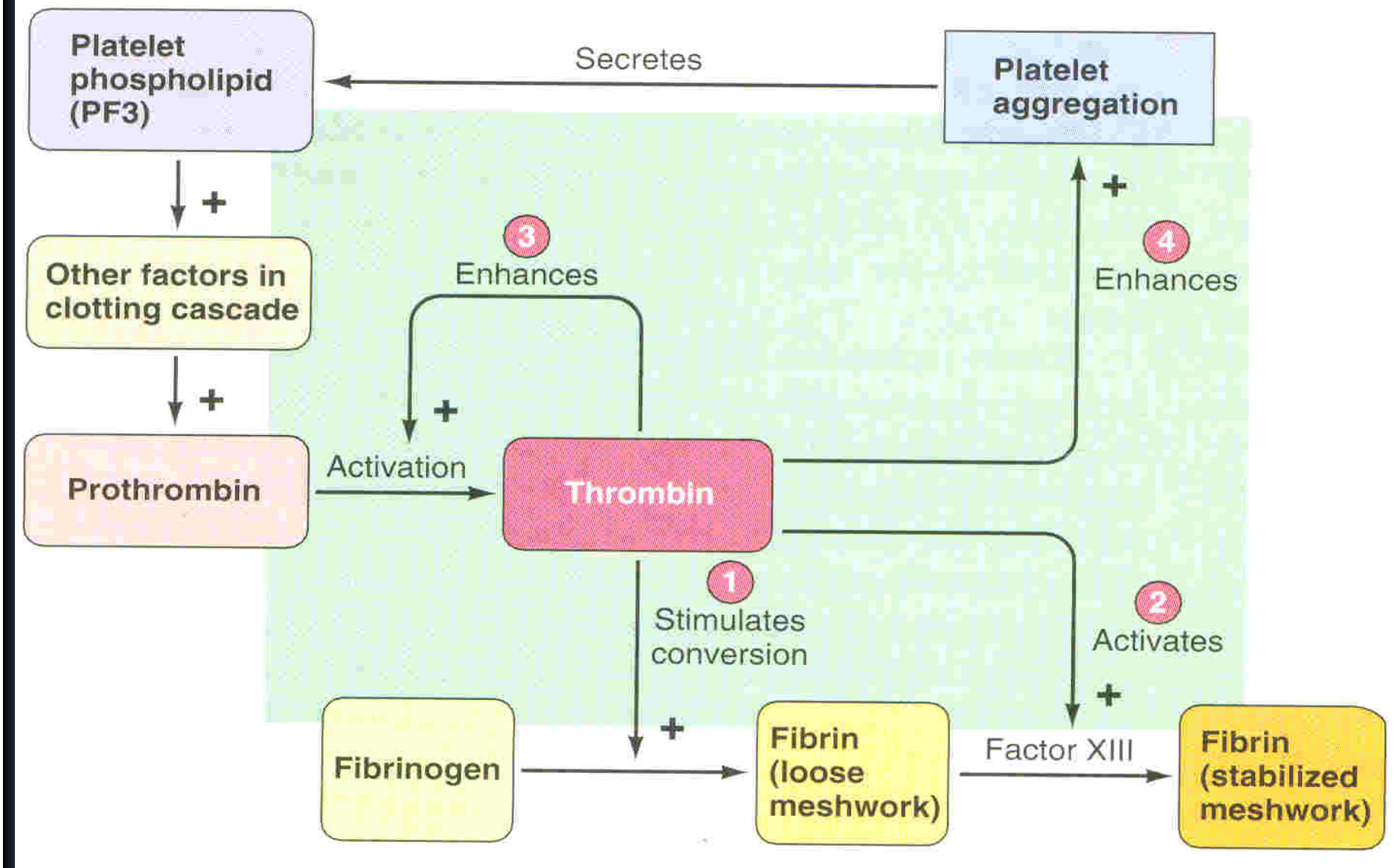



INTRINSIC PATHWAY
EXTRINSIC PATHWAY
Collagen or other activators
XII
Active XII
XI
Ca2+
Active XI
IX
Ca2+
Active IX
VIII
Damage exposes tissue factor (III)
VII
Tissue factor (III) and active VII
positive feedback
Ca2+
X
phospholipids (PL)
Active X
COMMON PATHWAY
Prothrombin
Ca2+, V, PL
positive feedback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

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.

ROLE OF CALCIUM IONS IN CLOTTING

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

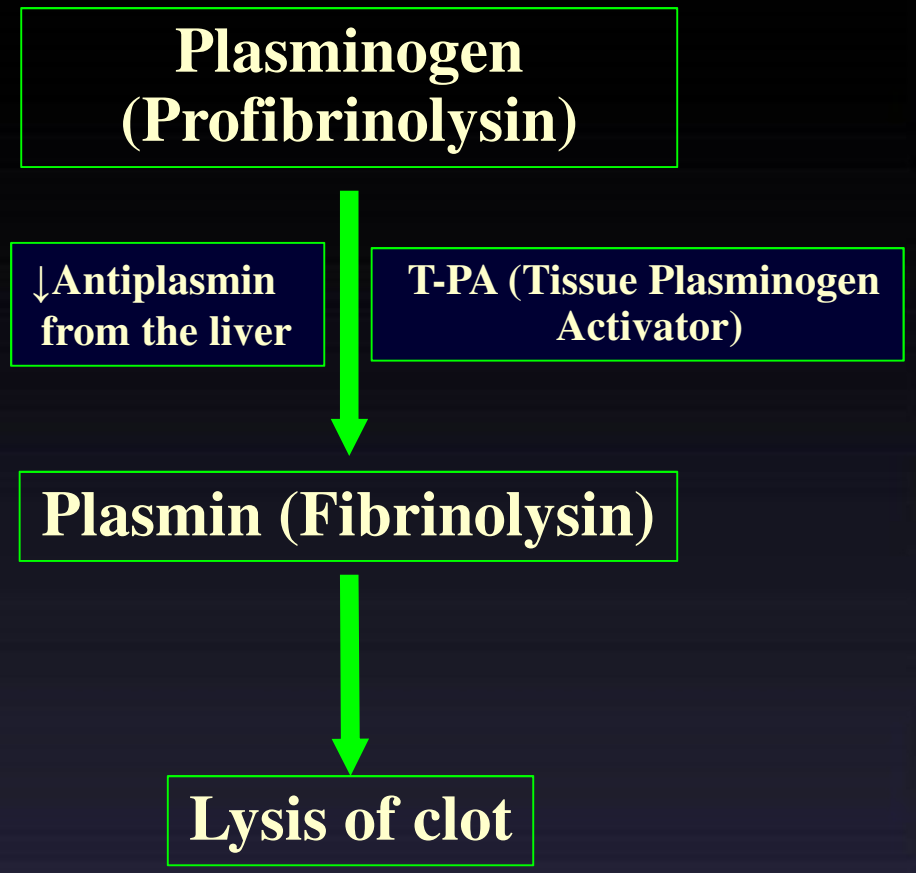
Blood samples are prevented from clotting by:

- **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 (Monitored by PTT time)
- **Warfarin**: ↓ production of Factors VII, IX and X by liver (Monitored by PT time)
- **EDTA** → chelates (binds) calcium ions

LYSIS OF BLOOD CLOTS BY PLASMIN

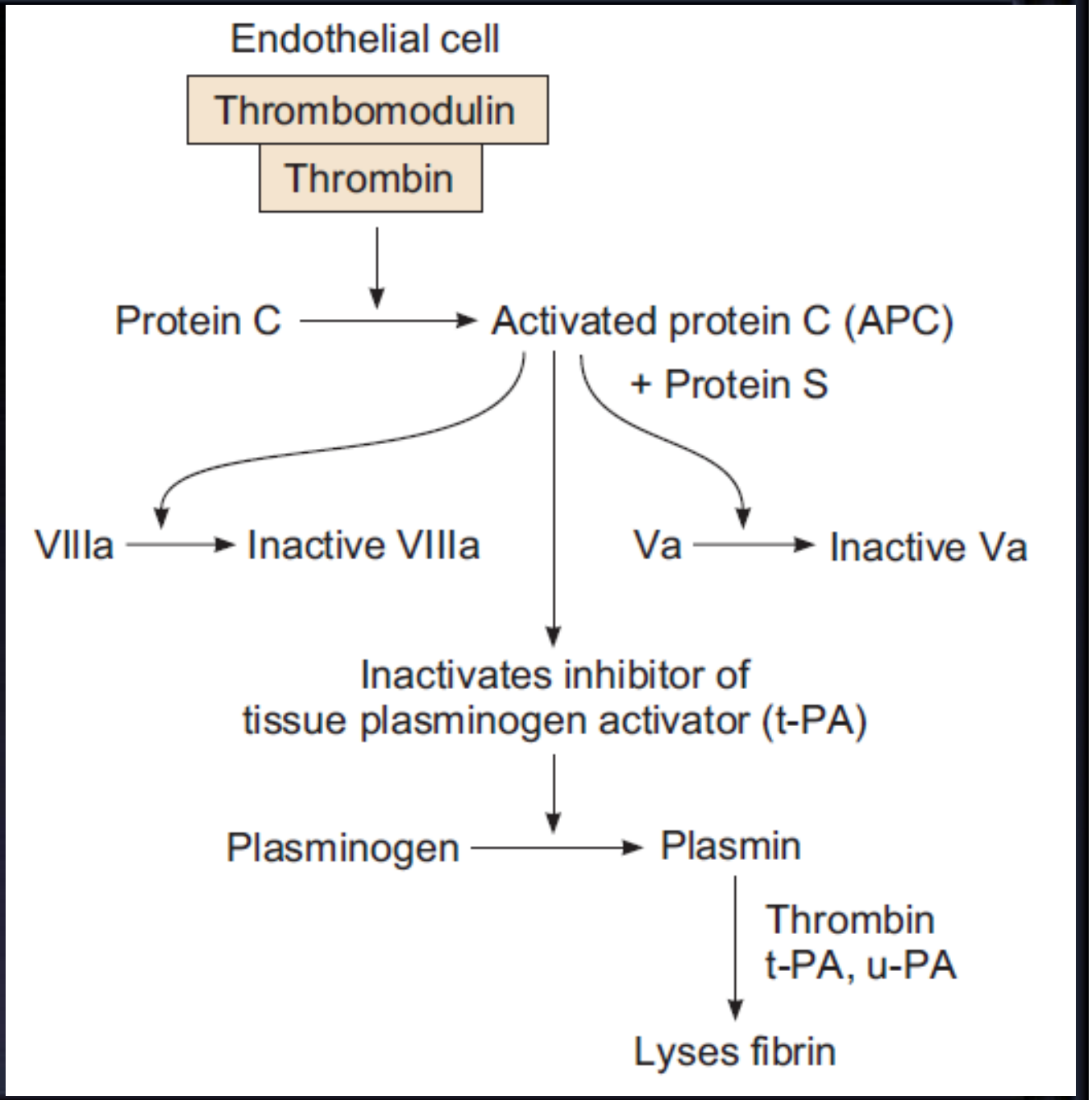
Formed blood clot can either become fibrous or dissolve.

- **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.

The fibrinolytic system and its regulation by Protein C



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**
   - Acts as a binding agent for several coagulation factors

BLEEDING & CLOTTING DISORDERS

A. Hemophilia

B. Thrombocytopenia

C. Liver diseases & Vitamin-K deficiency

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

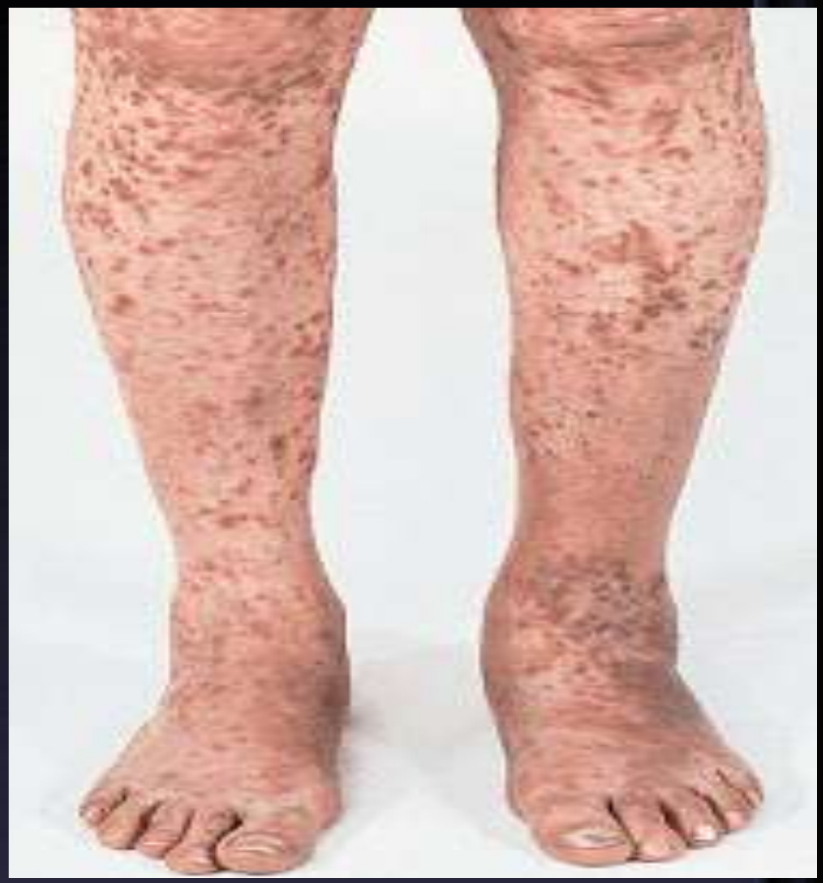
- ITP
- Drugs
- Infections (HIV)

Clinical Features

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

THROMBOCYTOPENIA (cont.)

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



HEMOPHILIA

- Genetic disorders
- Transmitted by female chromosome as recessive trait
- Transmitted by female chromosome as recessive trait. Occurs exclusively in males Females are carriers

❖ **HEMOPHILIA A**

- ❖ Classic Hemophilia
- ❖ 85 % cases
- ❖ Def. Of factor VIII

❖ **HEMOPHILIA B**

- ❖ 15 % cases
- ❖ Def. Of factor IX

- 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

BLEEDING DISORDERS

Liver diseases & Vitamin-K deficiency

- e.g. Hepatitis, Cirrhosis
  - Decreased formation of clotting factors
  - Icnreased 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
- 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

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

Summary of reactions involved in hemostasis.