COAGULATION MECHANISMS

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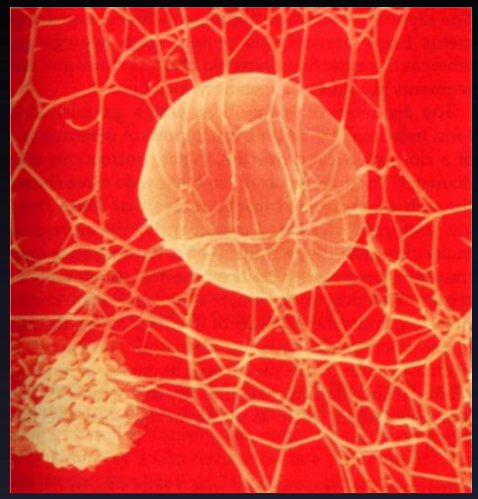


Vessel injury

Antithrombogenic
(Favors fluid blood)

Thrombogenic
(Favors clotting)

HANDOUTS…12/19/2020

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

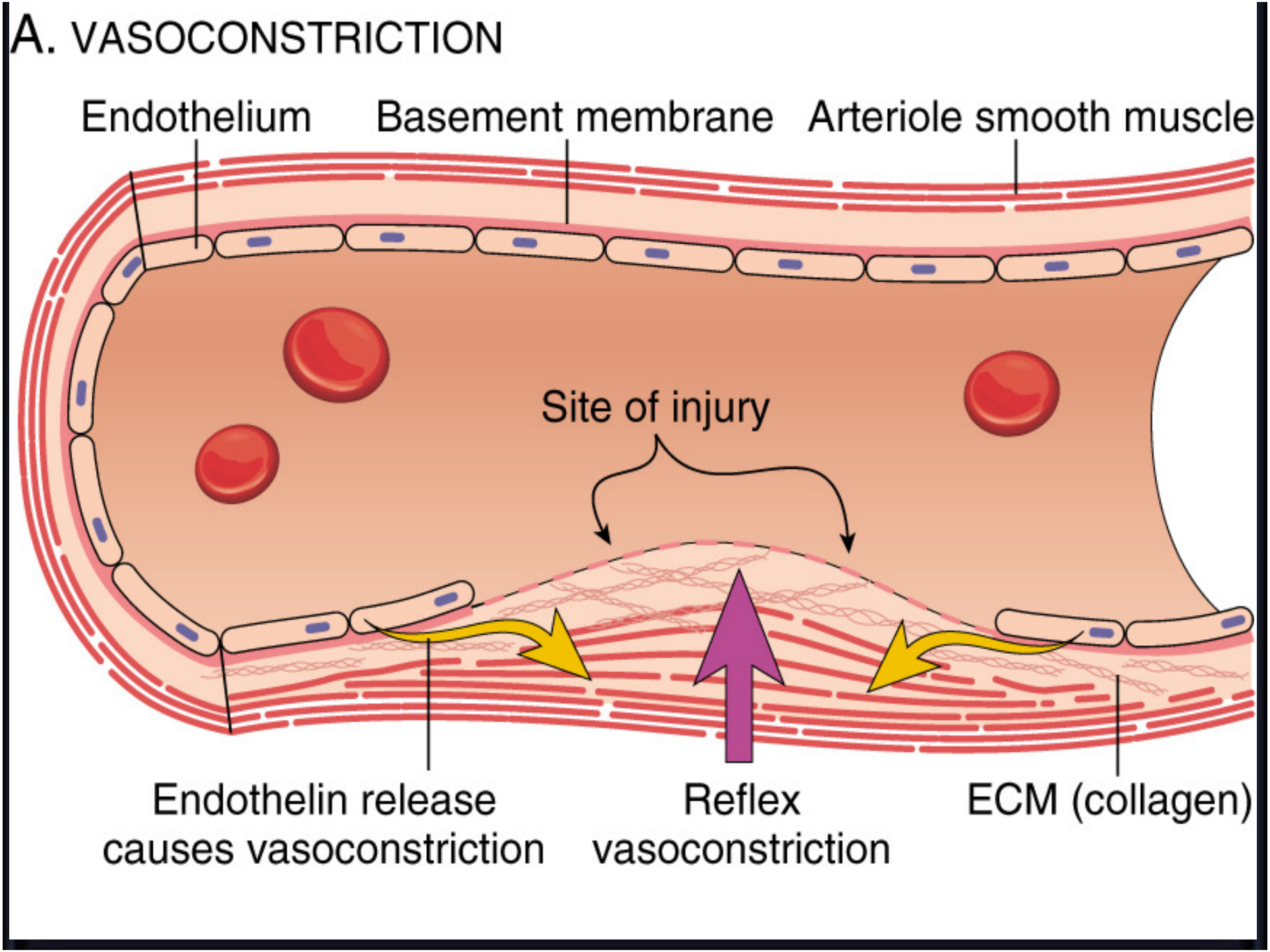
1. **VASCULAR PHASE ► Vascular Spasm**
2. **PLATELET PHASE ► Formation of platelet plug**
3. **COAGULATION PHASE ► Blood Coagulation & Clot Retraction**
4. **FIBRINOLYTIC PHASE ► Fibrinolysis**

1-VASCULAR SPASM (Vascular Constriction)

Immediately After injury there is localized Vasoconstriction.

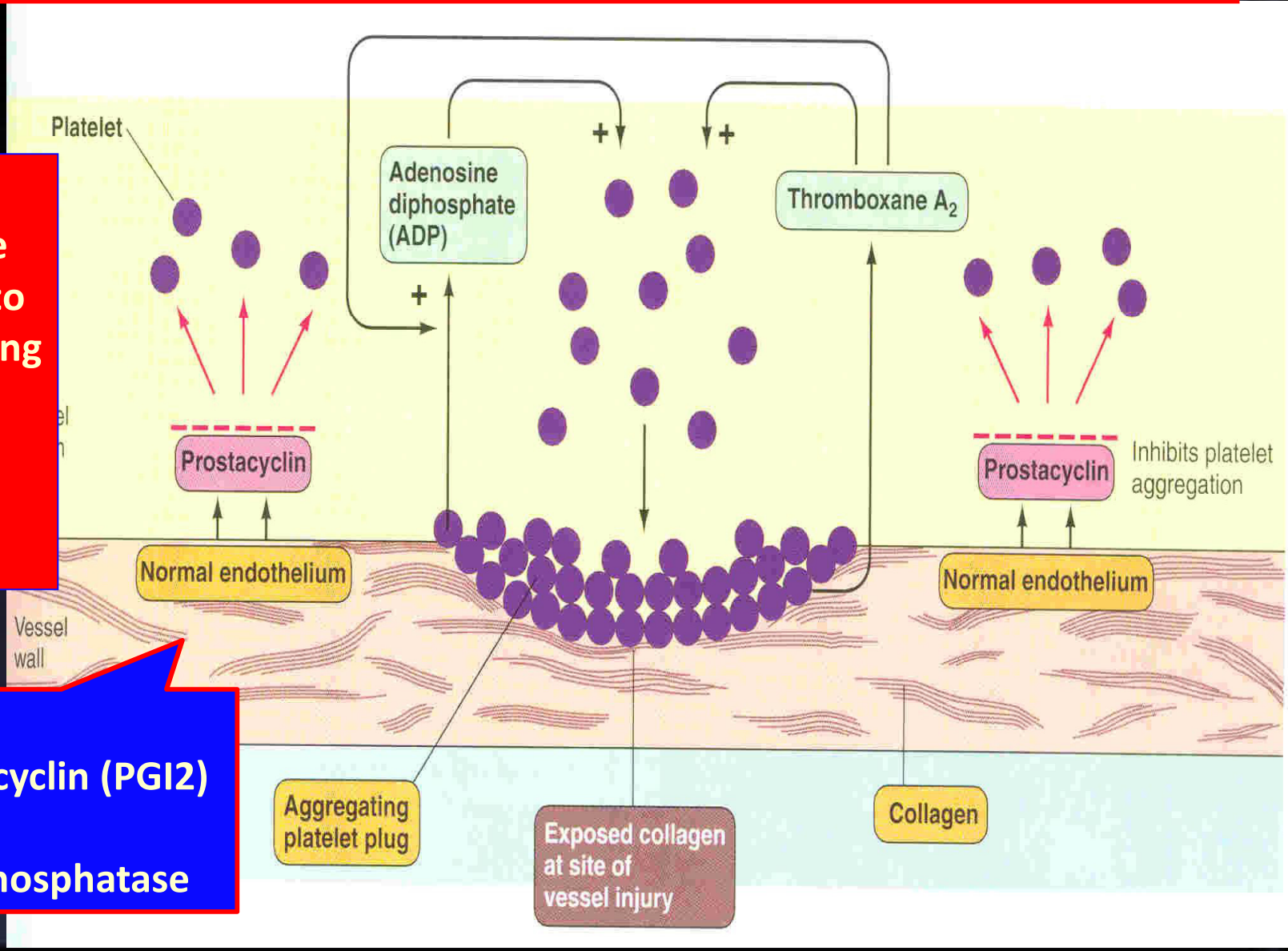
- **Causative Factors are three (3)**
  1. Nervous reflexes
  2. Local myogenic spasm
  3. Local humoral factors….Platelets → Thromboxane A_2 [TXA2] (Vasoconstrictor)
- **Importance**
  - Crushing injuries → Intense spasm → No lethal loss of blood

TXA2 is inhibited by aspirin…How?

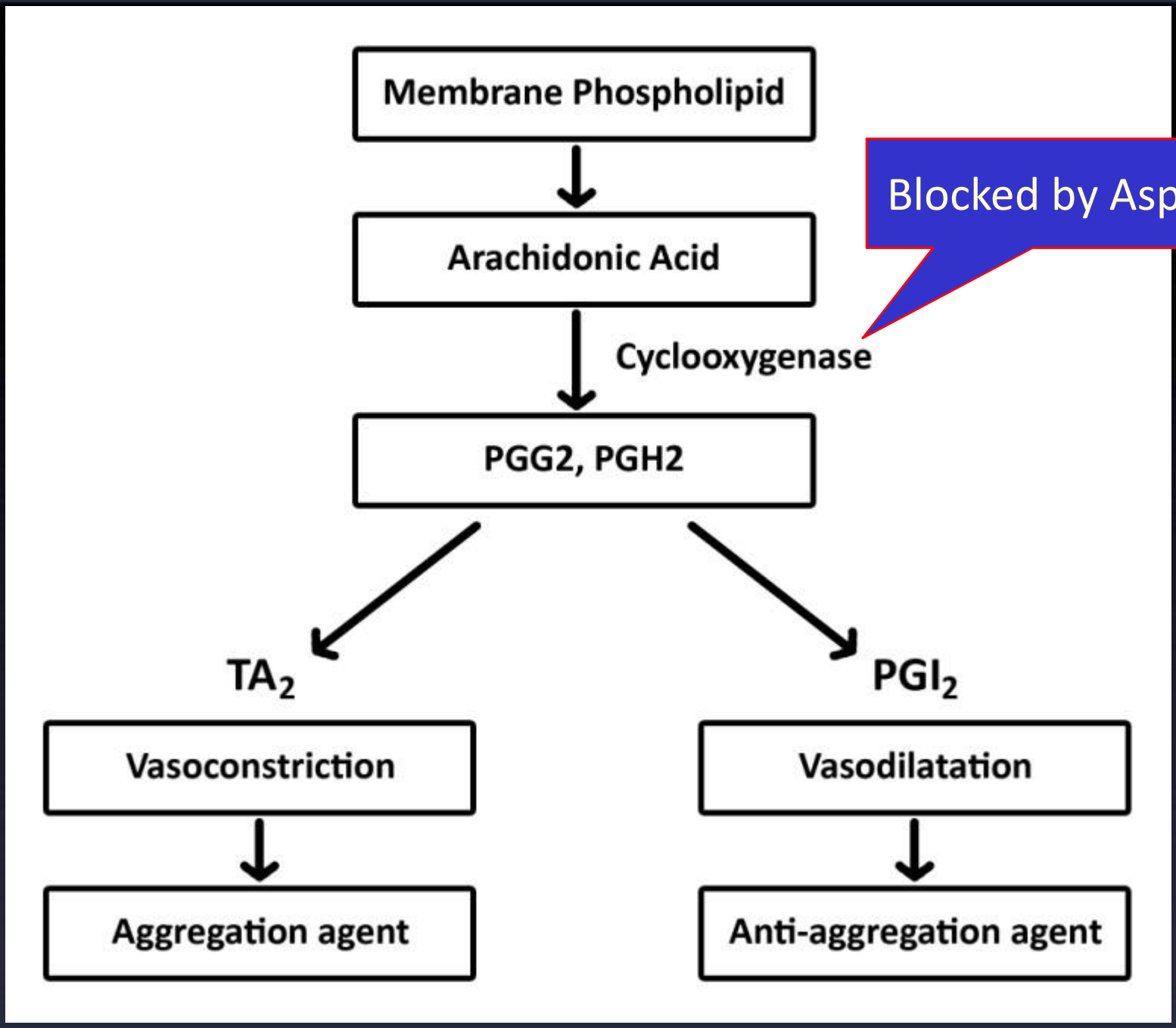

A. VASOCONSTRICTION
Endothelium
Basement membrane
Arteriole smooth muscle
Site of injury
Endothelin release causes vasoconstriction
Reflex vasoconstriction
ECM (collagen)

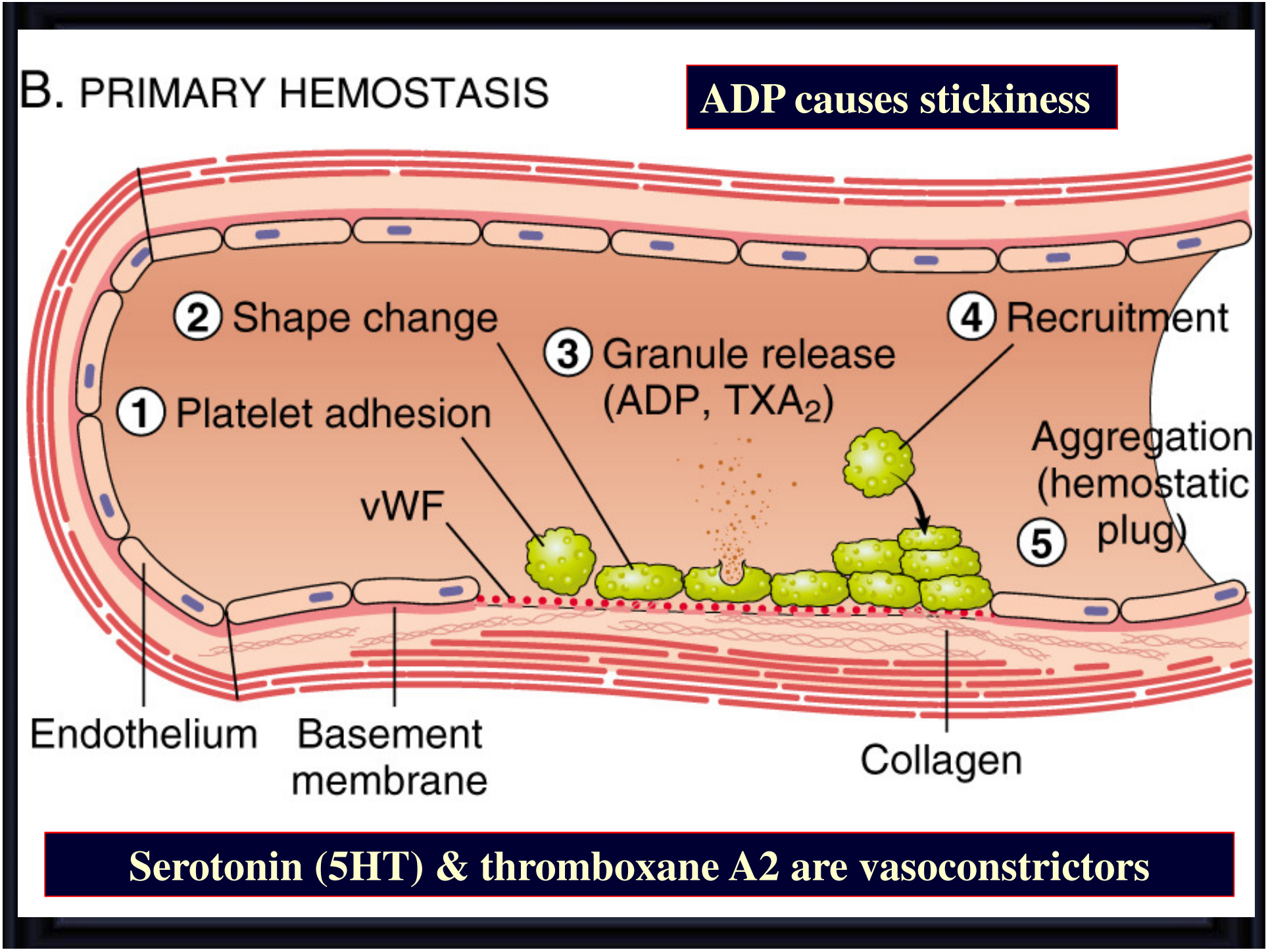
2-FORMATION OF PLATELET PLUG [PRIMARY HEMOSTASIS]

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

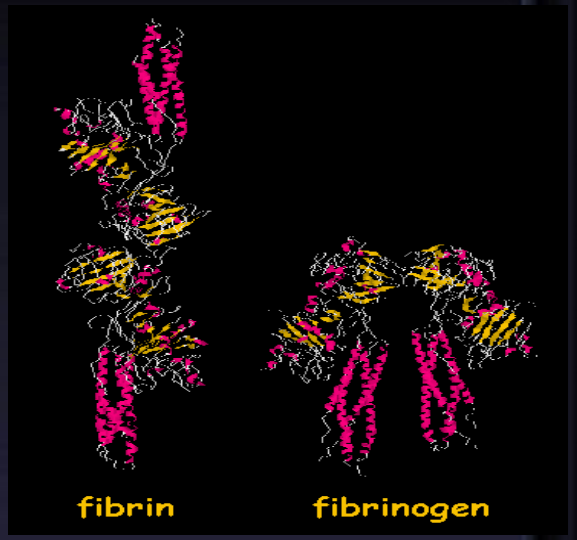

B. PRIMARY HEMOSTASIS
ADP causes stickiness
2 Shape change
3 Granule release (ADP, TXA2)
4 Recruitment
1 Platelet adhesion
vWF
Aggregation (hemostatic plug)
5
Endothelium
Basement membrane
Collagen
Serotonin (5HT) & thromboxane A2 are vasoconstrictors

3-BLOOD COAGULATION

Formation of Clot or Thrombus

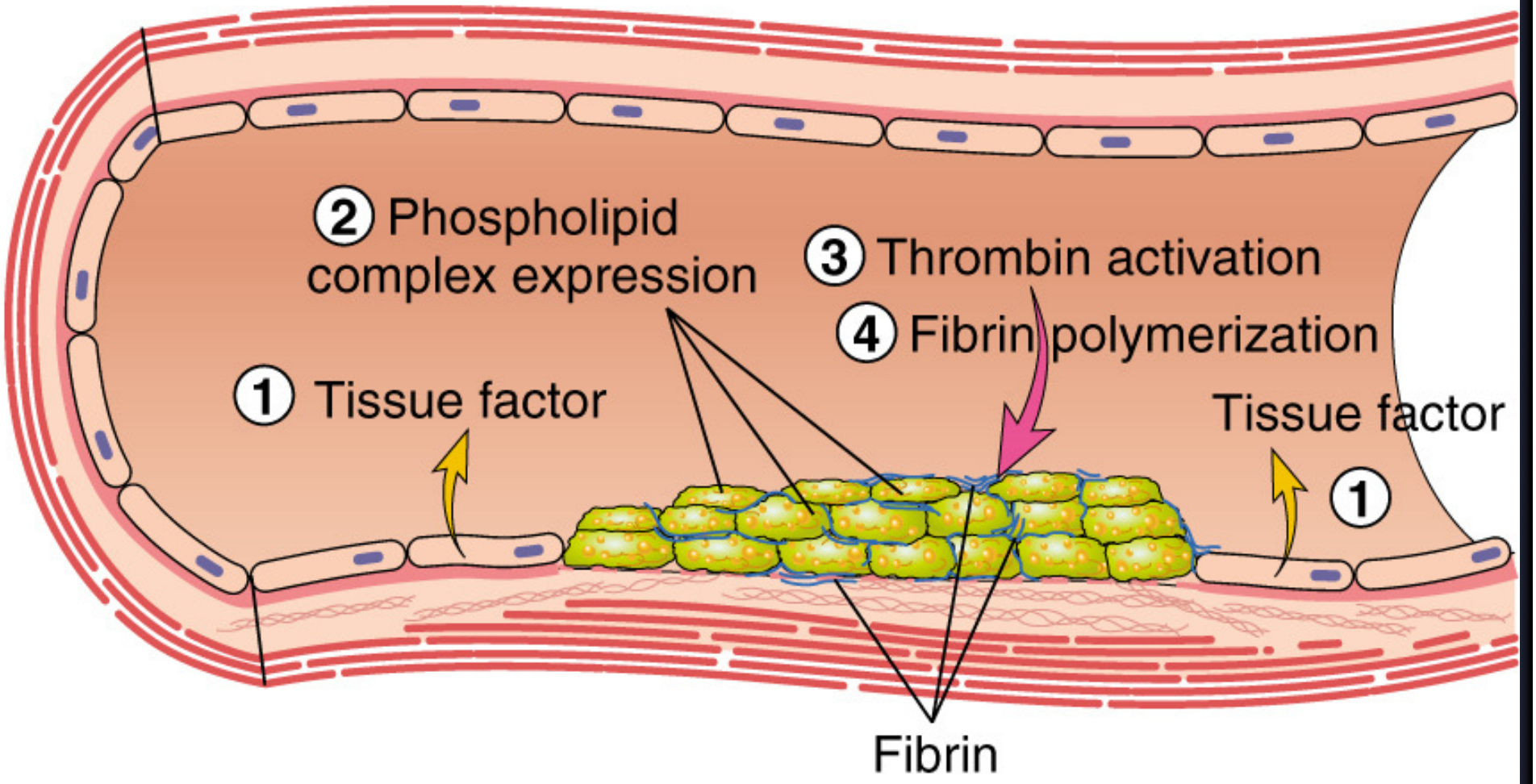
[SECONDARY HEMOSTASIS]

- 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

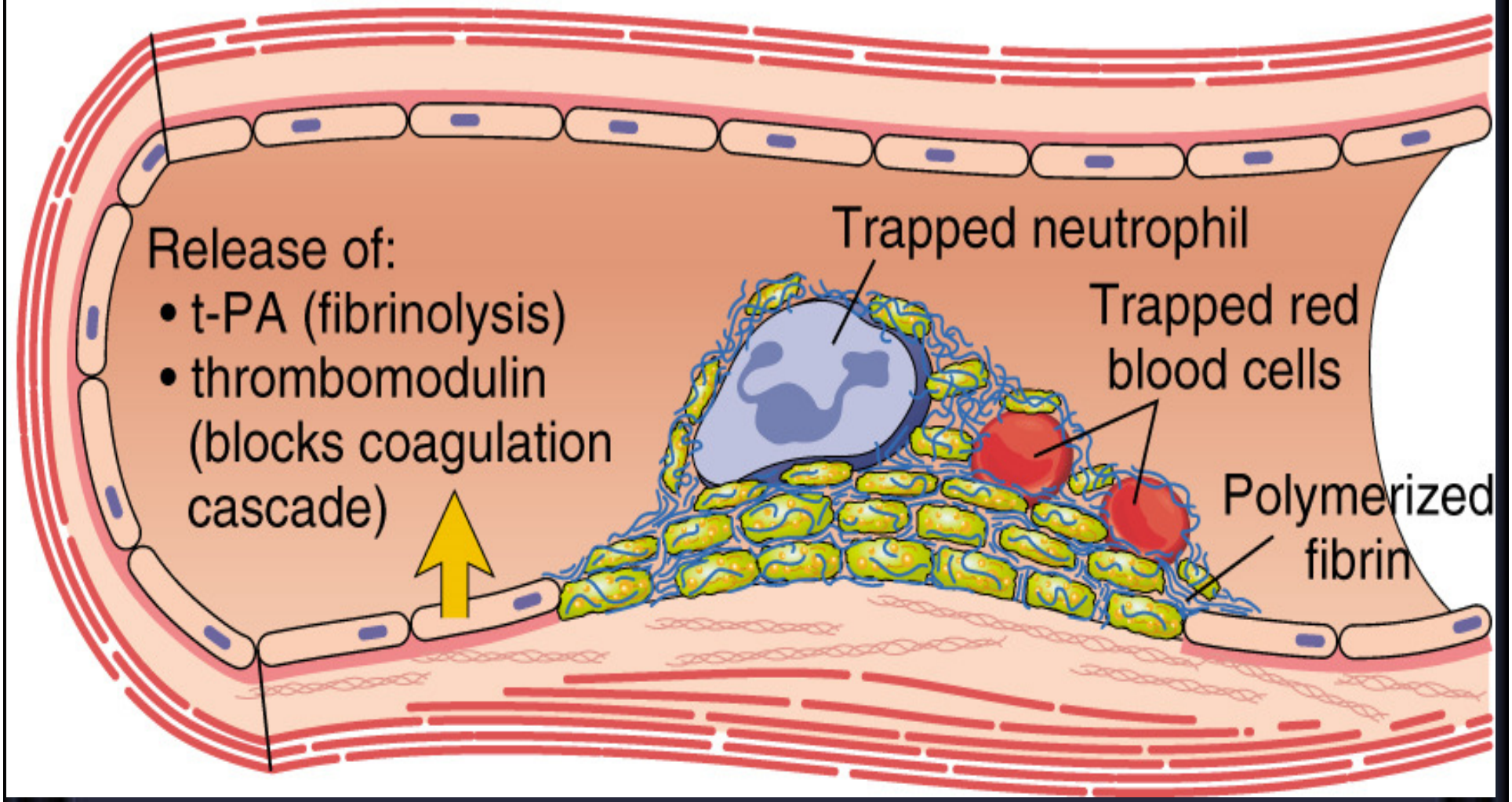


CLOT is a meshwork of fibrin fibers running in all directions entrapping blood cells, platelets and plasma.

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	

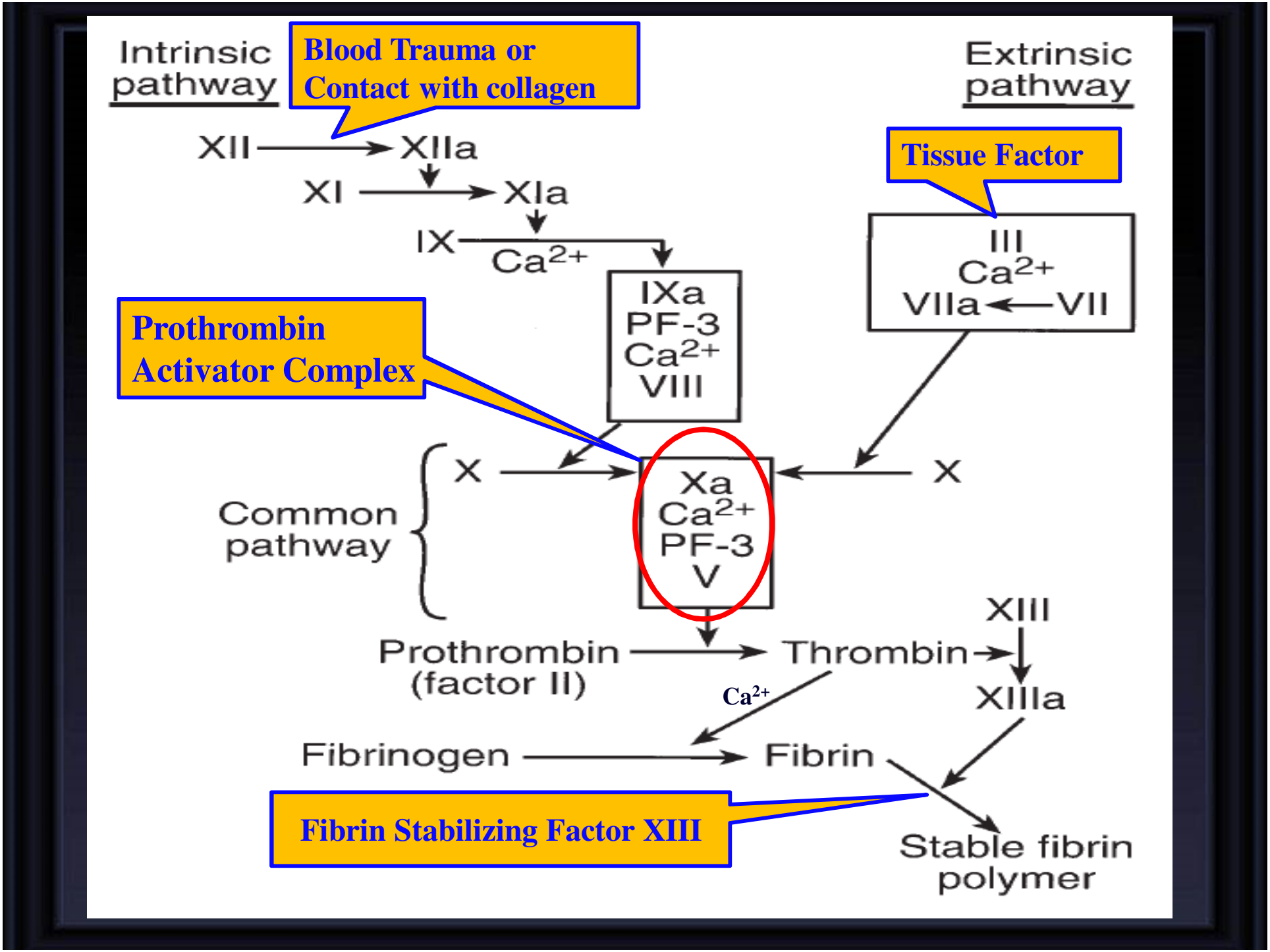
Clotting Factors

Ganong

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

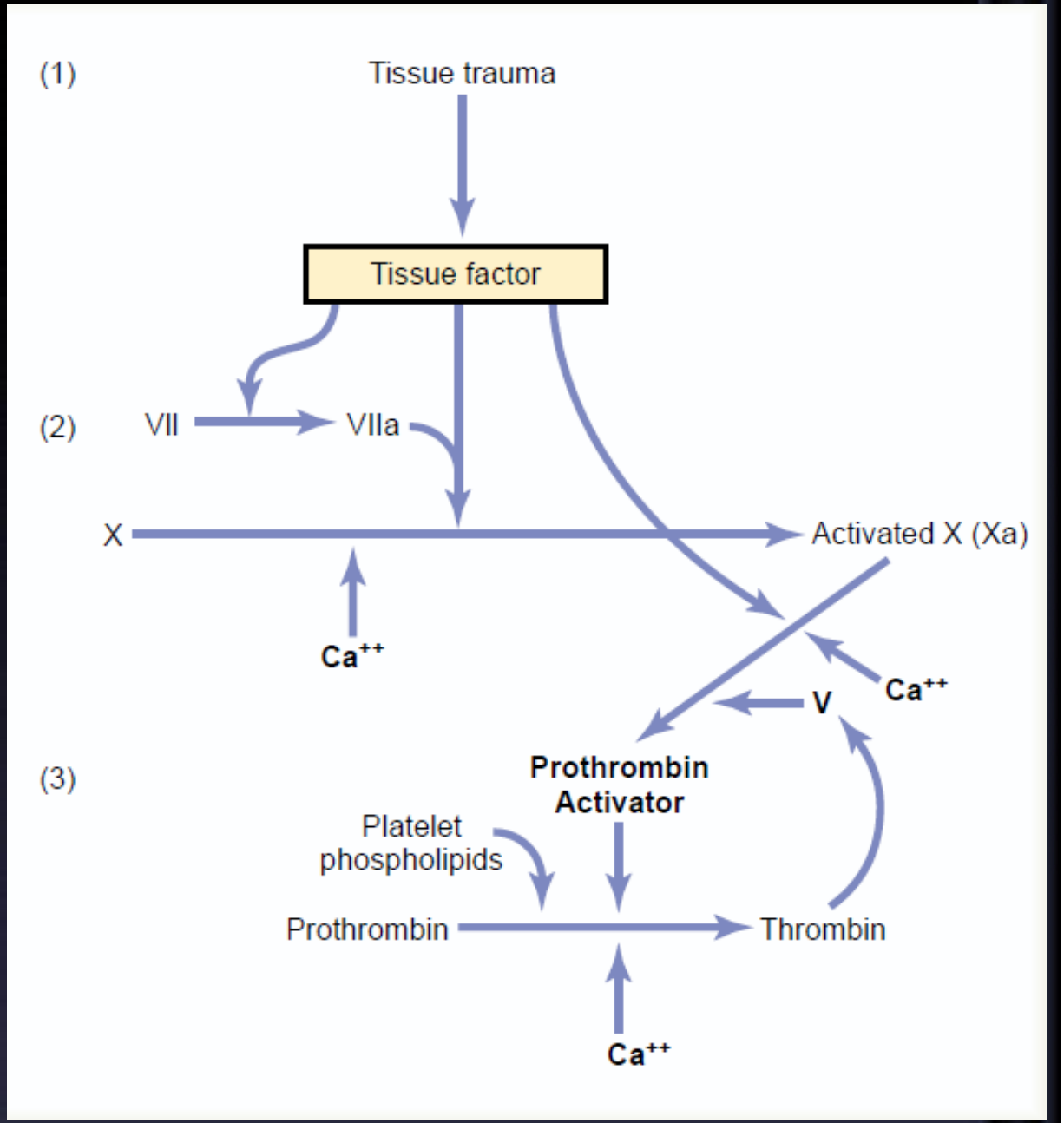
Factor[a]	Names
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Proaccelerin, labile factor, accelerator globulin
VII	Proconvertin, SPCA, stable factor
VIII	Antihemophilic factor (AHF), antihemophilic factor A, antihemophilic globulin (AHG)
IX	Plasma thromboplastic component (PTC), Christmas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

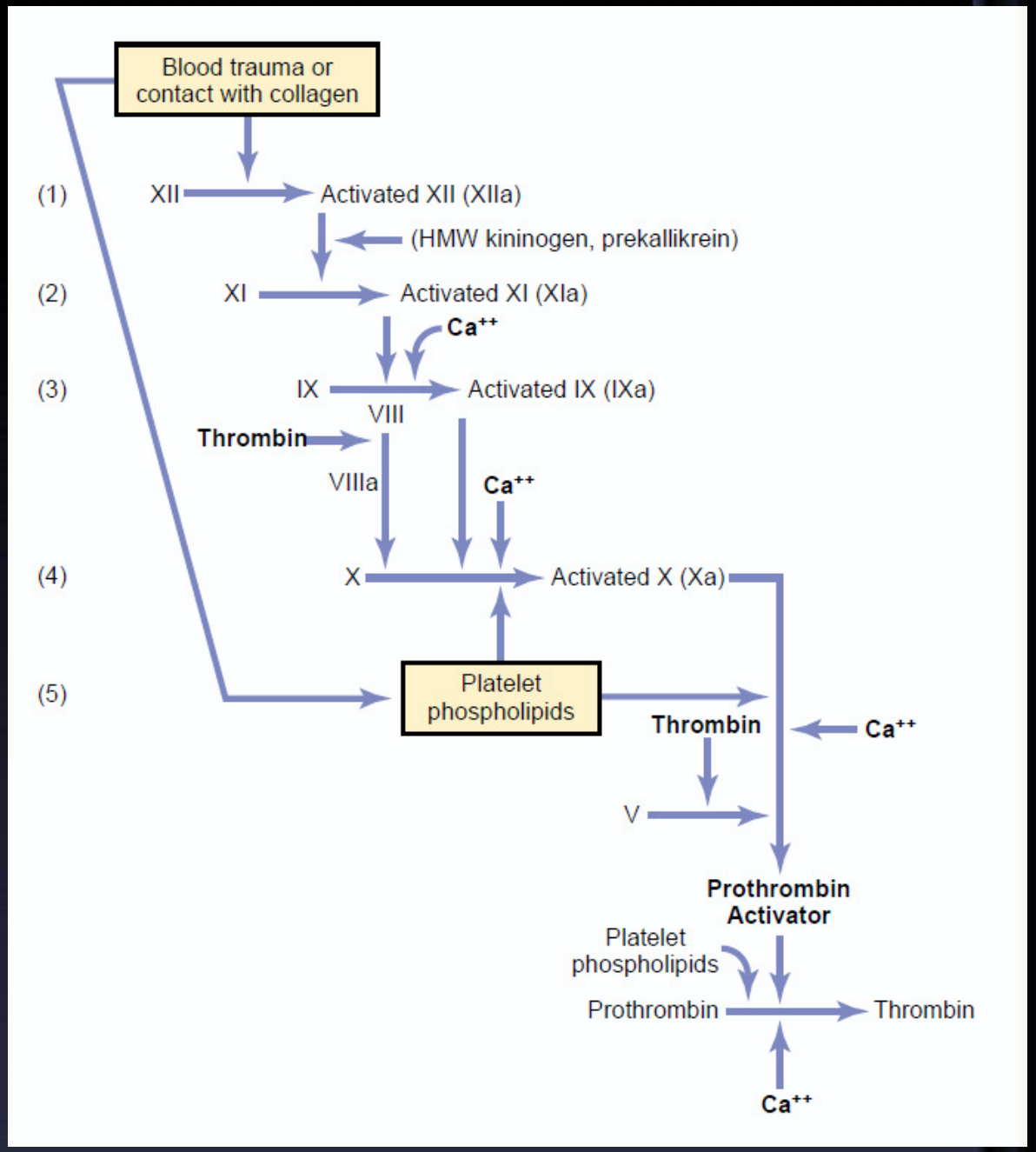
EXTRINSIC MECHNANISM FOR INITIATING CLOTTING

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

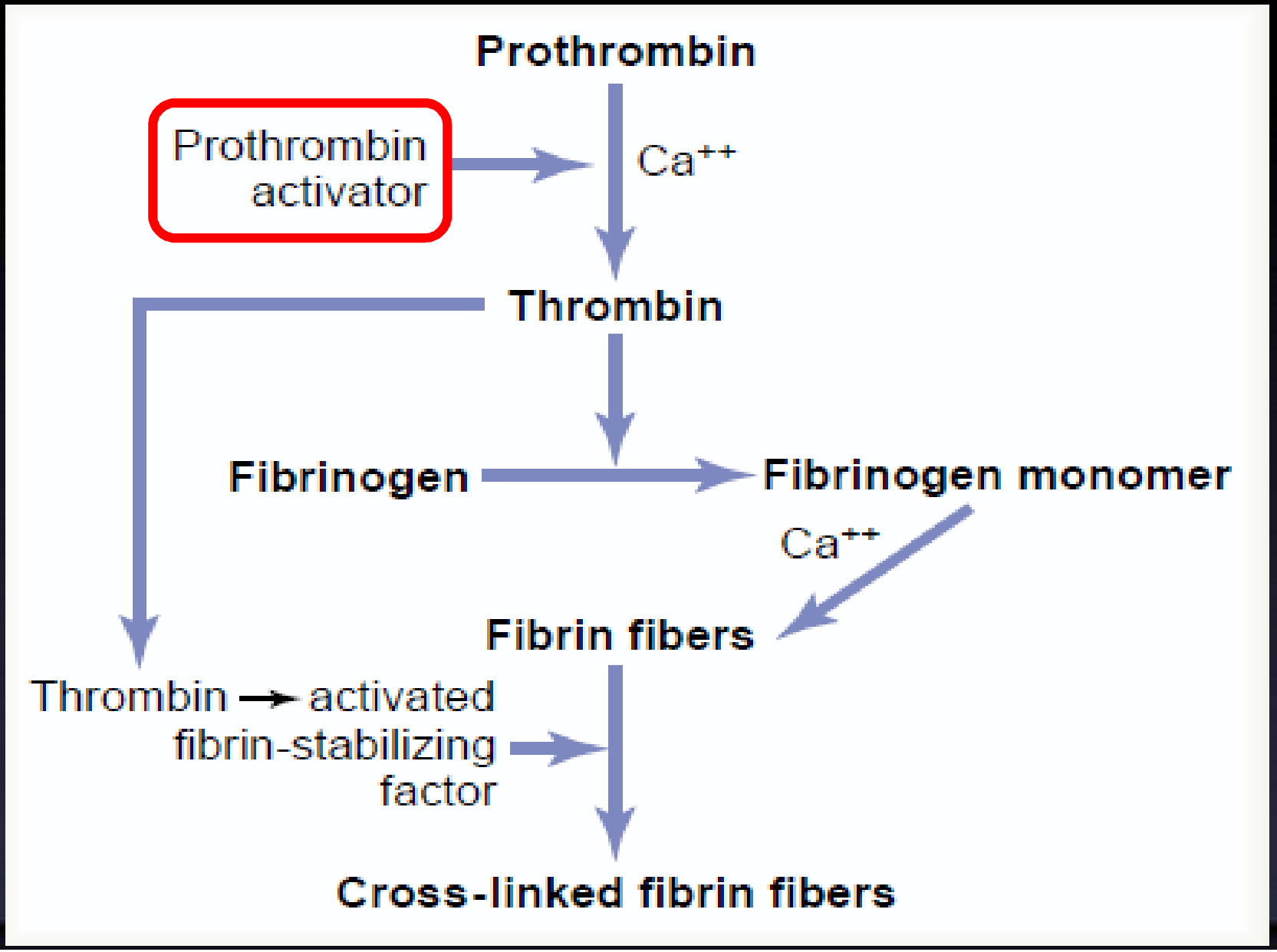


INTRINSIC MECHNANISM FOR INITIATING CLOTTING

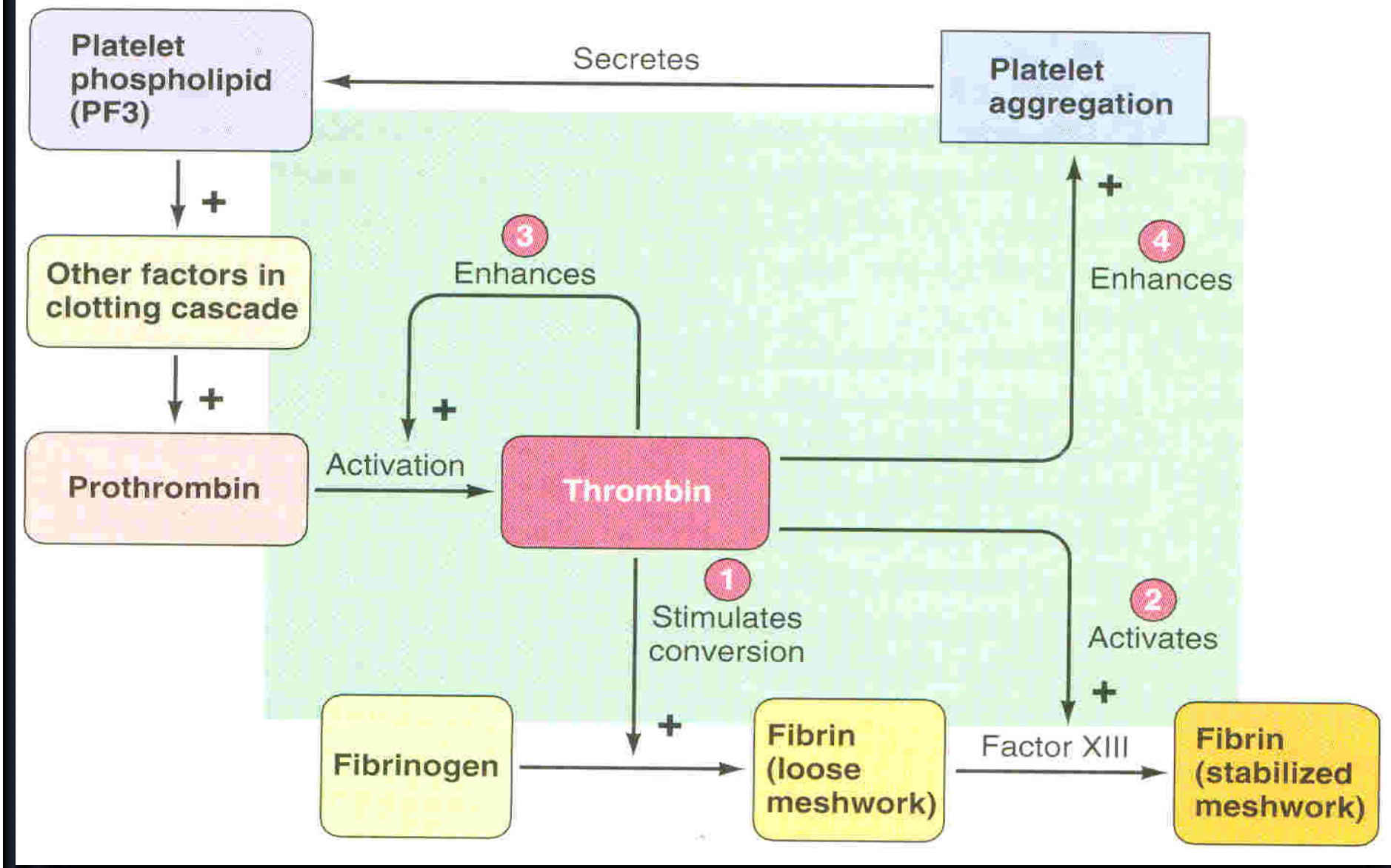
Trauma to the blood itself or exposure of the blood to collagen (from a traumatized blood vessel wall), foreign surface/glass



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:

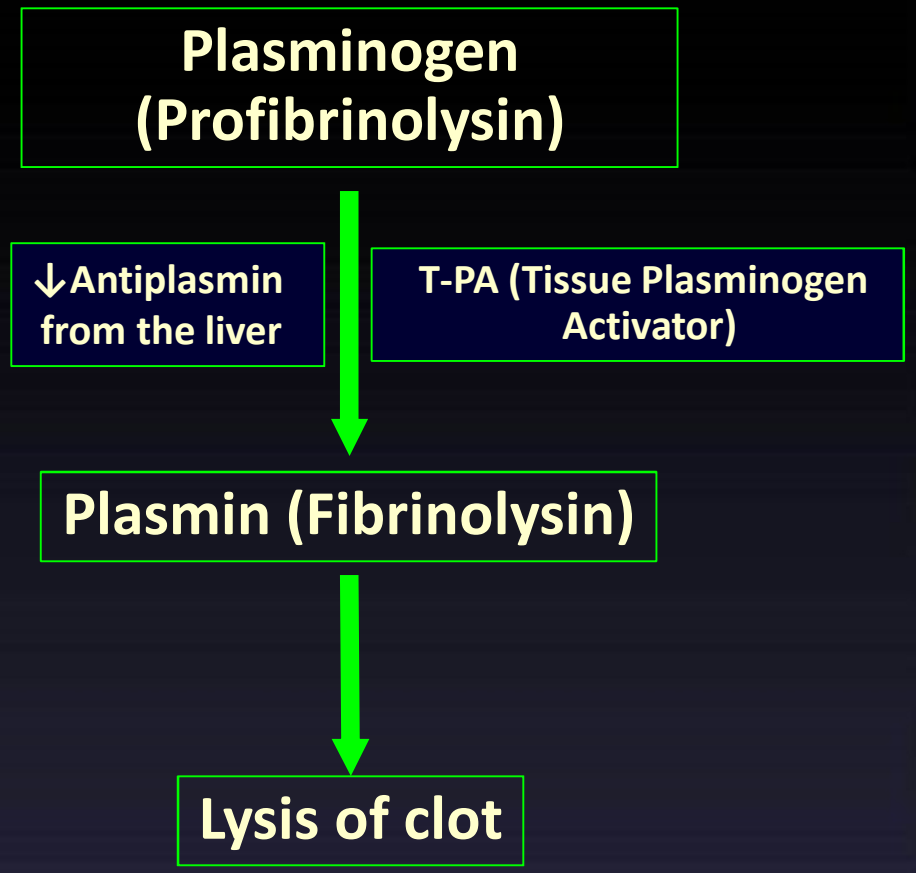
Lysis or Fibrous tissue Formation

(platelet derived growth factor)

LYSIS OF BLOOD CLOTS BY PLASMIN

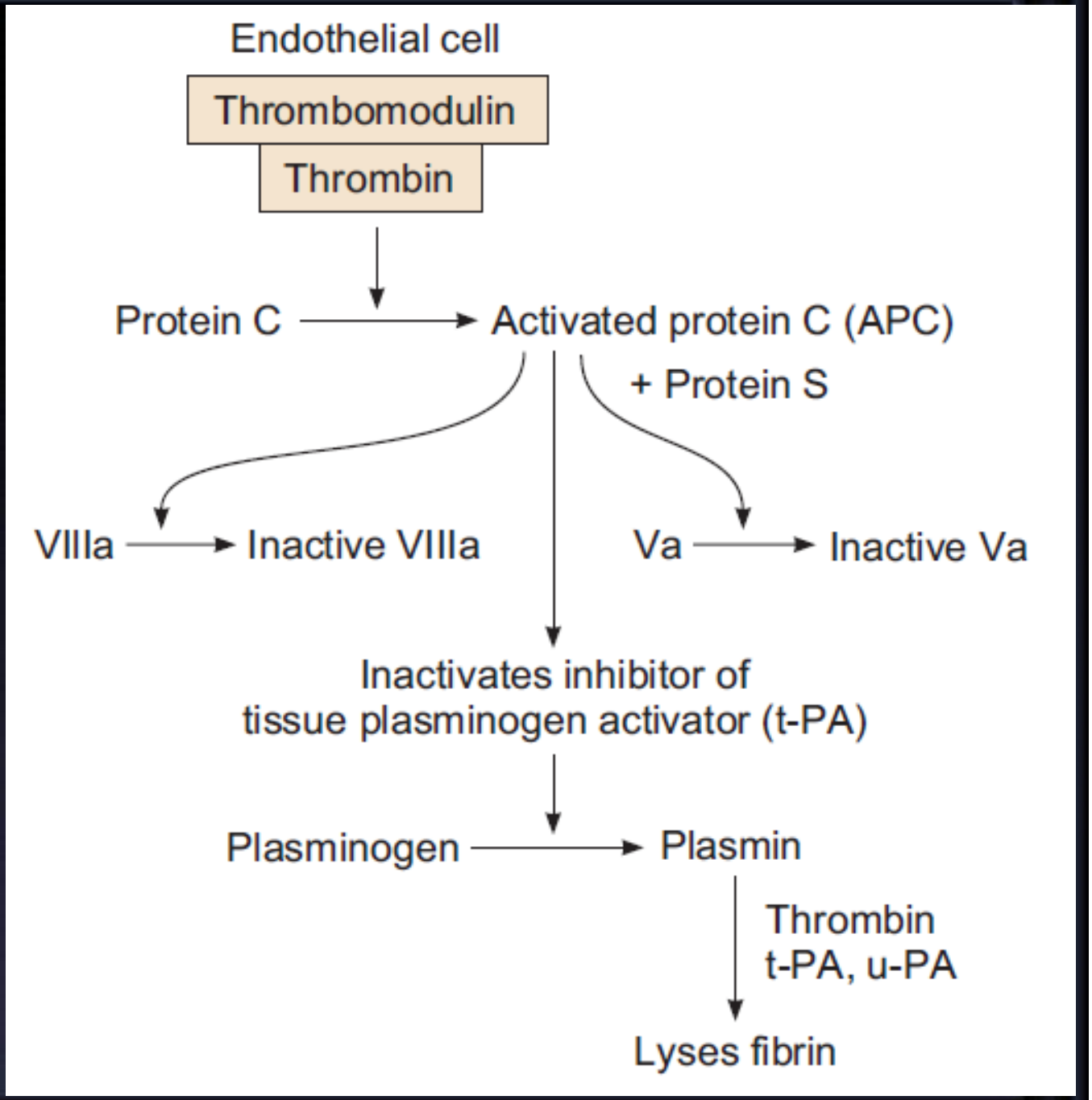
Formed blood clot can either become fibrous or dissolve.

- **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

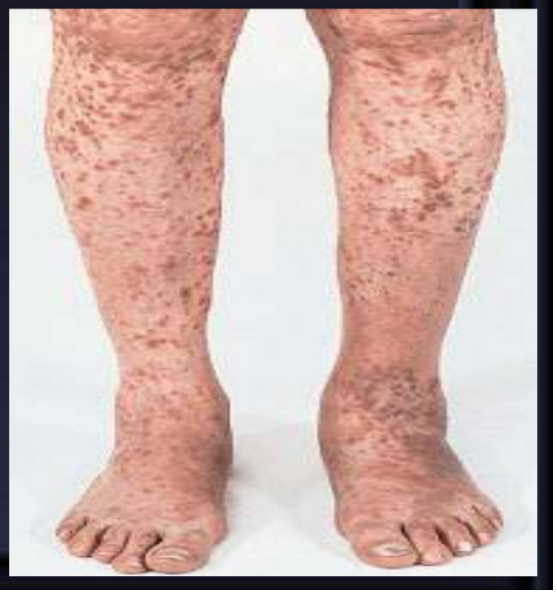
- Aplastic anemia
- Leukemia
- Drugs
- Infections (HIV, Measles)

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

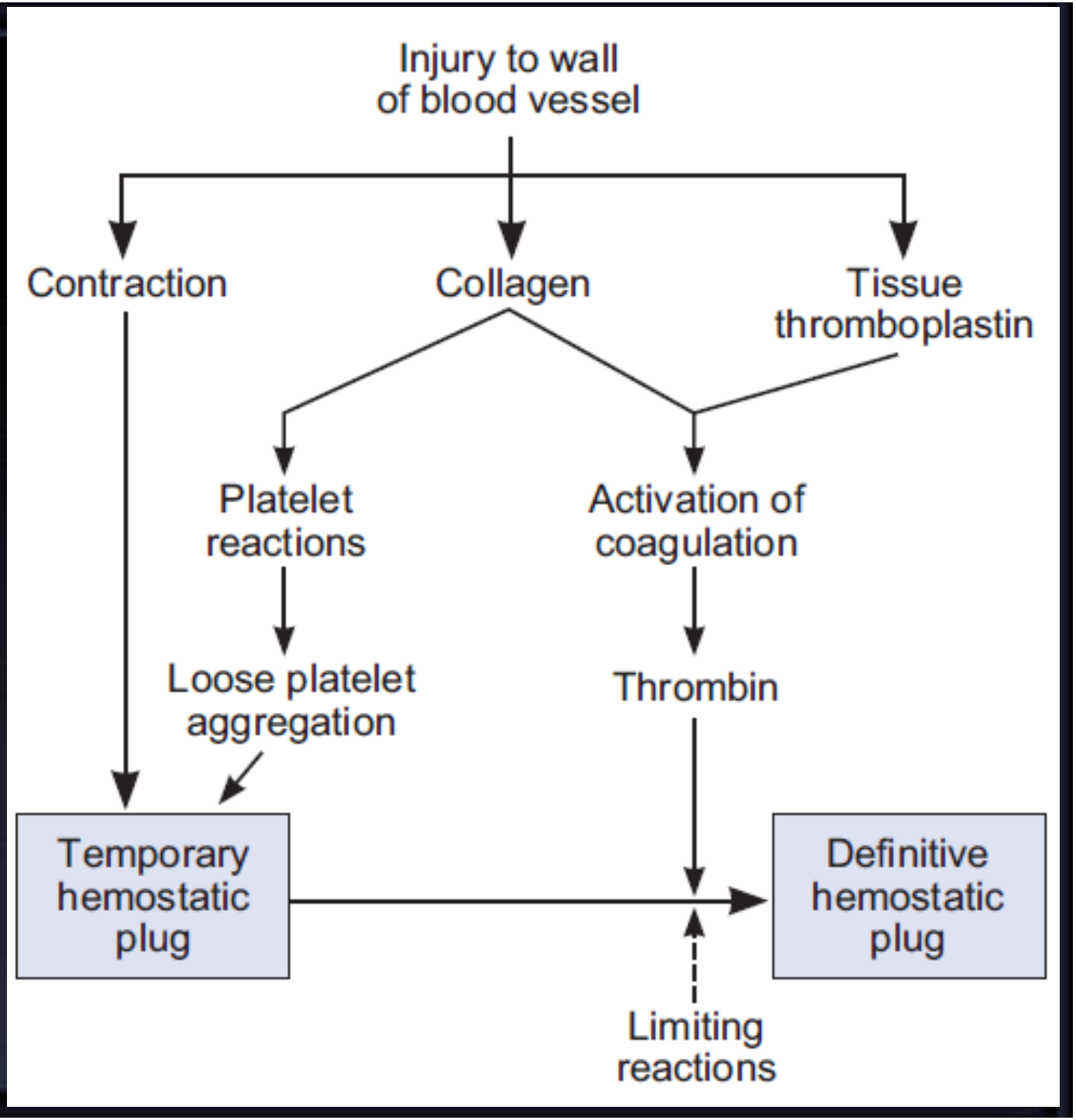
Test	Mechanism Tested	Normal Value	Disorder
Bleeding time (BT)	Hemostasis, capillary & platelet function	3-7 min beyond neonate	Thrombocytopenia , von Willebrand disease
Platelet count	Platelet number	150 000 – 450 000 / mm^3	Thrombocytopenia
Prothrombin time (PT)	Extrinsic & common pathway	< 12 sec 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

Summary of reactions involved in hemostasis.




THANKS

PLATELETS STRUCTURE & FUNCTIONS

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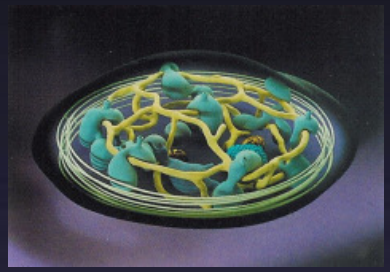
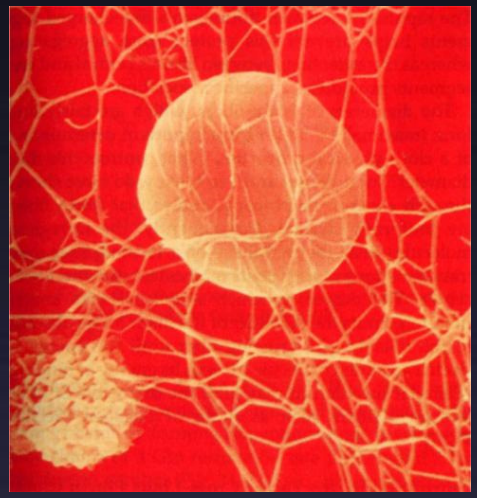




HANDOUTS…12/19/2020

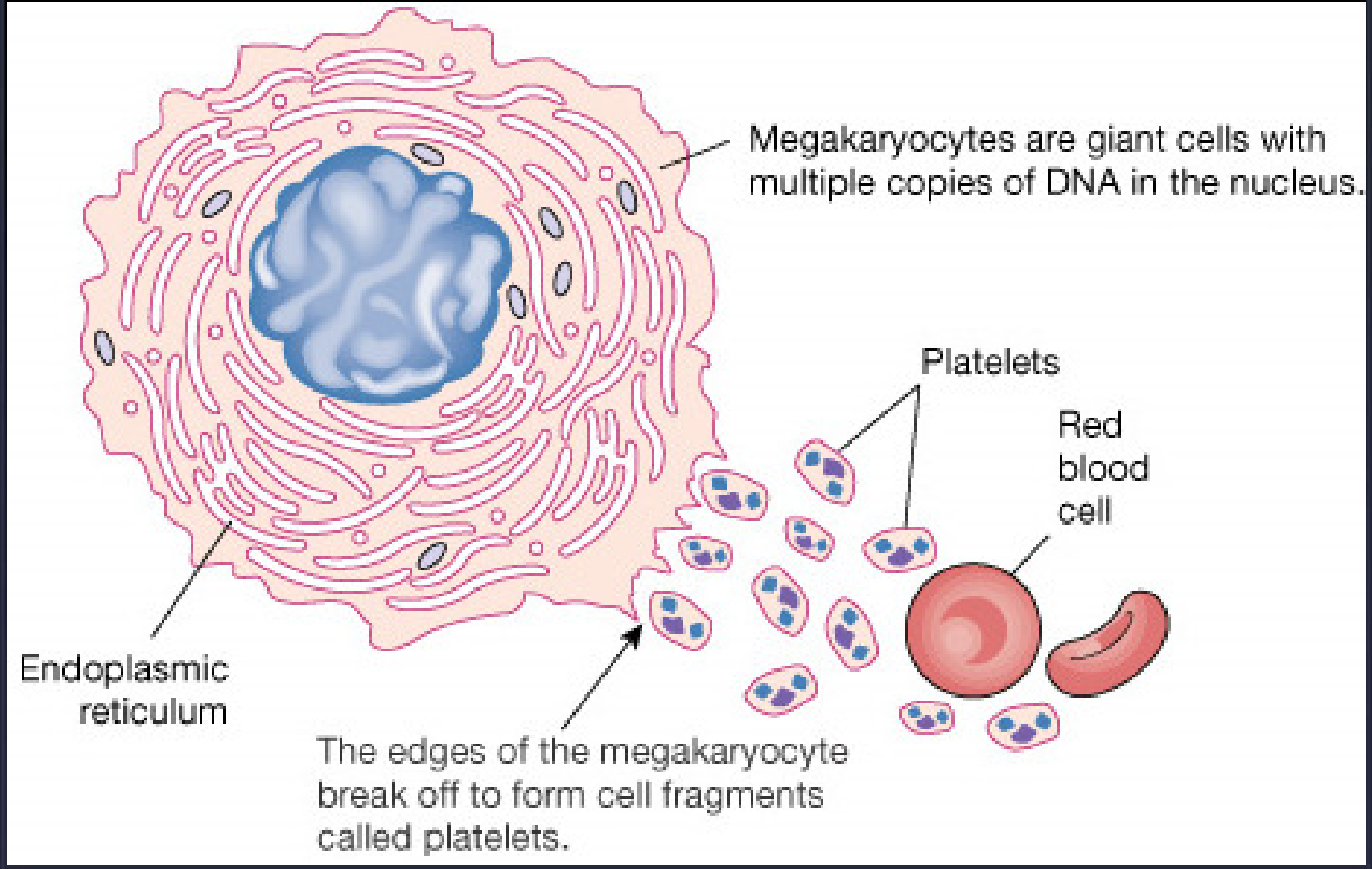
OBJECTIVES

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

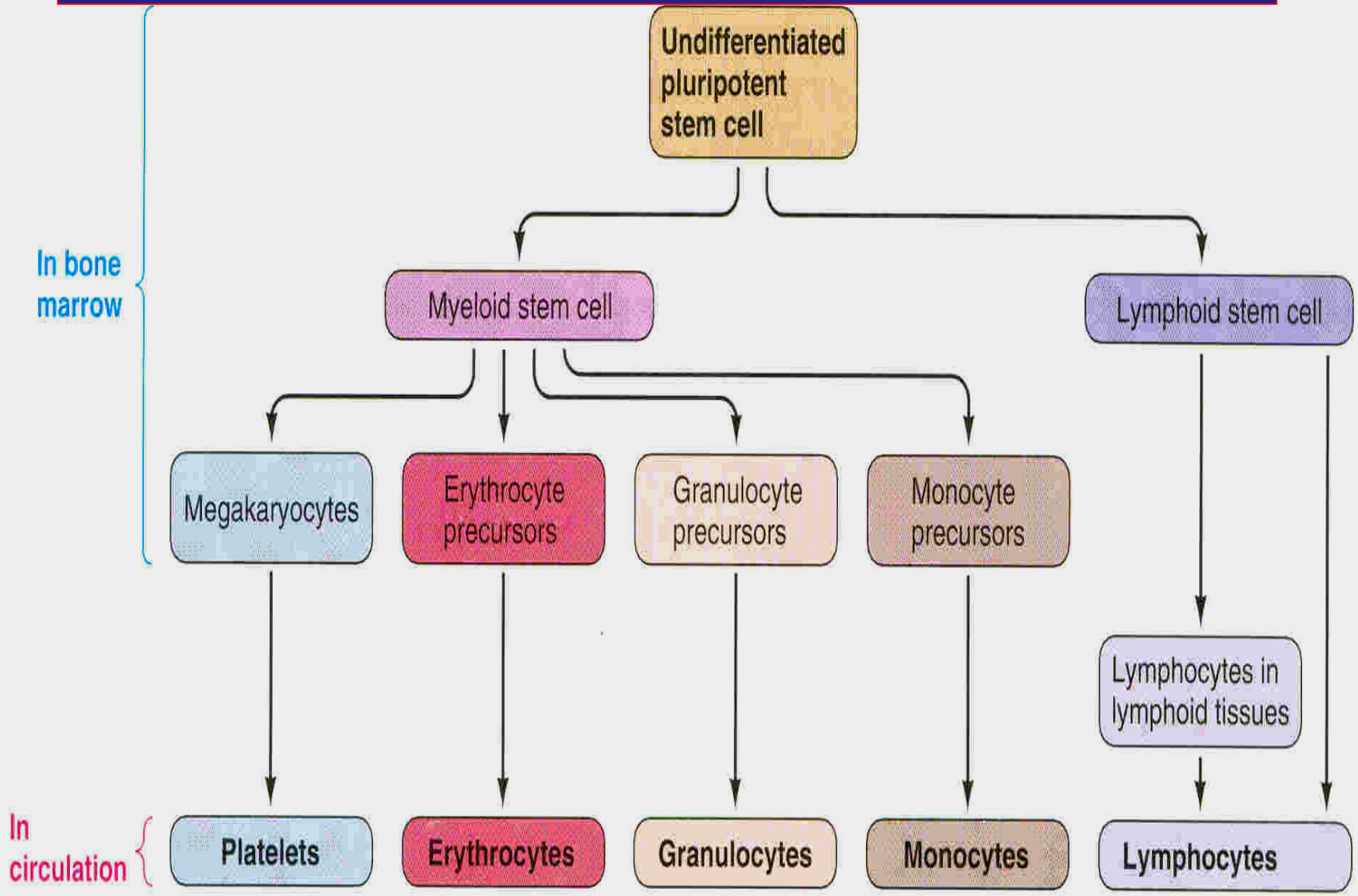
- **Describe formation and development of platelets**
- **Understand platelet normal ultrastructure**
- **Describe the functions of different platelets organelles and surface receptors**
- **Describe the mechanisms of platelet functions**
- **Relate membrane receptors and granule content to normal function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

PLATELETS

Formed by fragmentation from megakaryoctyes



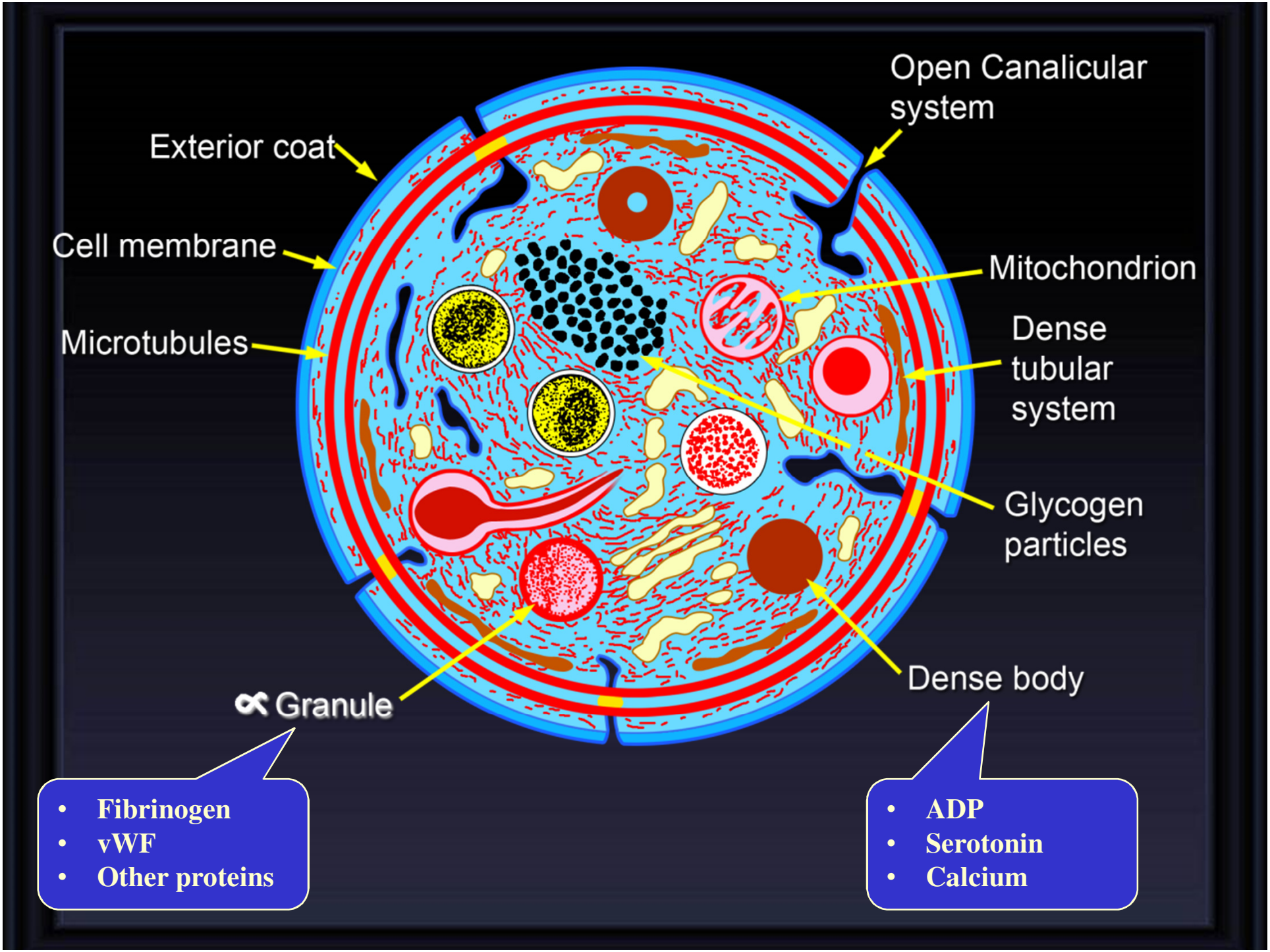
SITE OF FORMATION
Bone-marrow



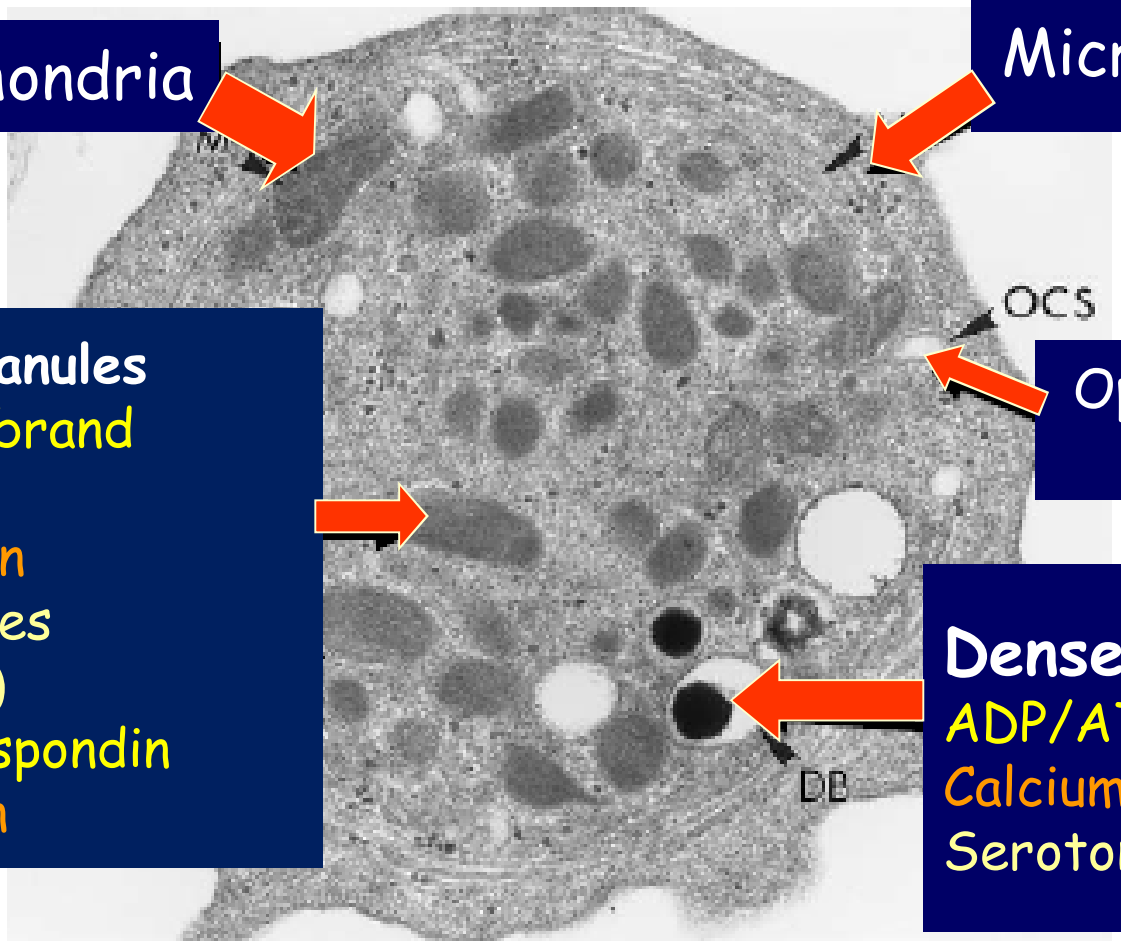
PLATELETS (Characteristics)

SHAPE: MINUTE ROUND OR OVAL DISCS
SIZE: 1.5-3.0 um IN DIAMETER
LIFE SPAN: 7-10 DAYS
COUNT: 150,000 – 300,000/ microlitrer
LOCATION: 80% in blood & 20% in spleen (hypersplenism may lead to low platelet counts)

- Anuclear and discoid cell
- Contractile, adhesive, cell fragments.
- Store coagulation factors & enzymes
- Surface Binding Antigens Glycoproteins


Open Canalicular system
Exterior coat
Cell membrane
Mitochondrion
Microtubules
Dense tubular system
Glycogen particles
Granule
Dense body
• Fibrinogen
• vWF
• Other proteins
• ADP
• Serotonin
• Calcium

Platelet Ultrastructure



Mitochondria

Microtubules

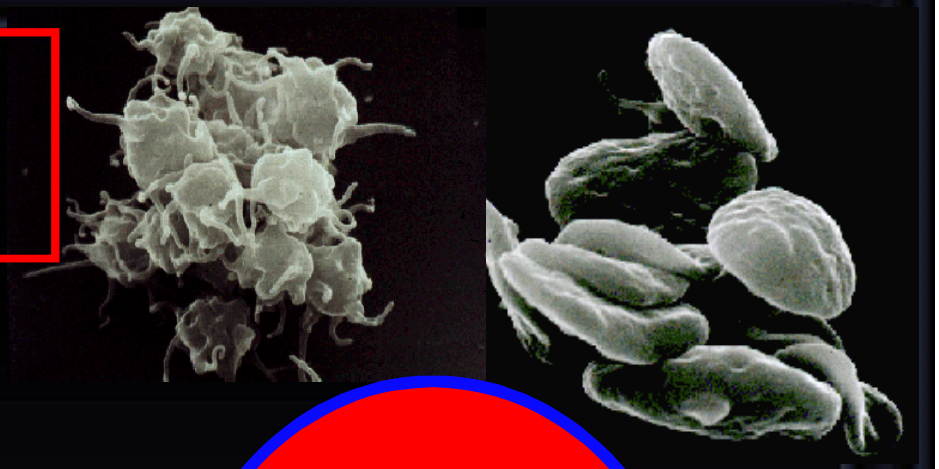
Open canalicular system

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 such as for Synthesis Of Prostaglandins
- **Garnules (α & δ)**

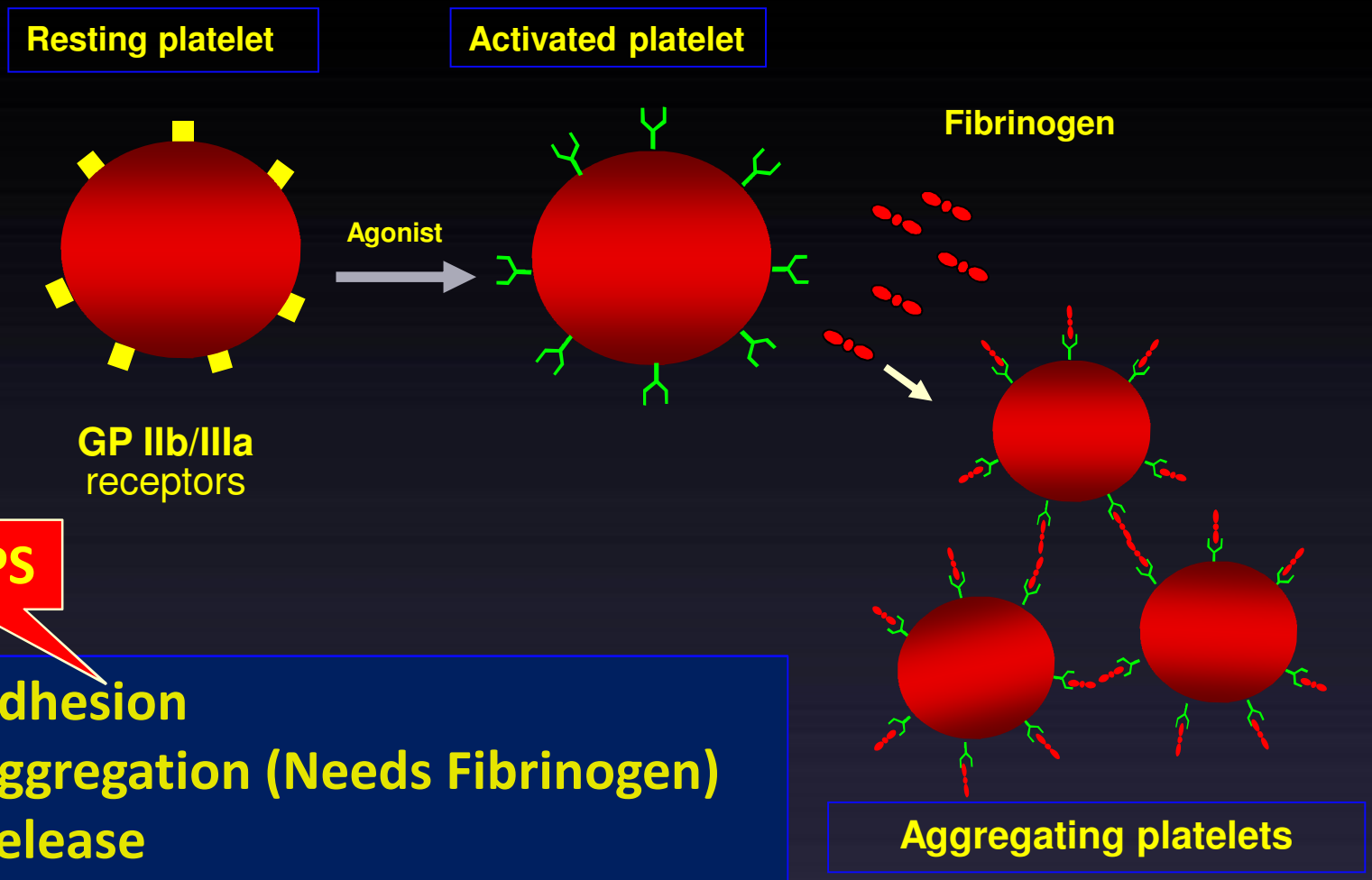
Dense or δ granules

- Serotonin
- ADP
- Ca^{++}

Alpha α granules

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

Platelets Activation



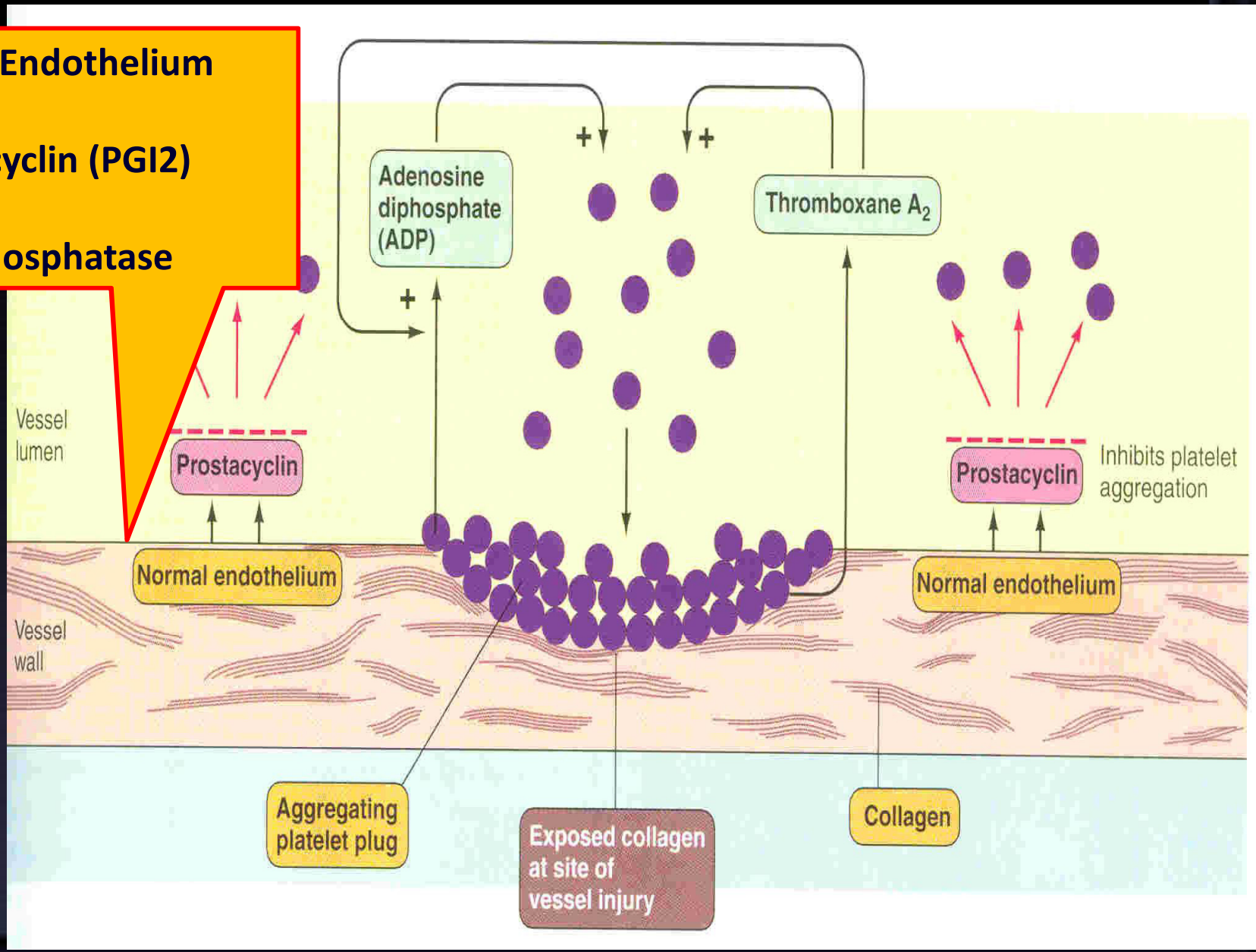
4 STEPS

1. Adhesion
2. Aggregation (Needs Fibrinogen)
3. Release
4. Clot Retraction

PLATELETS ACTIVATION

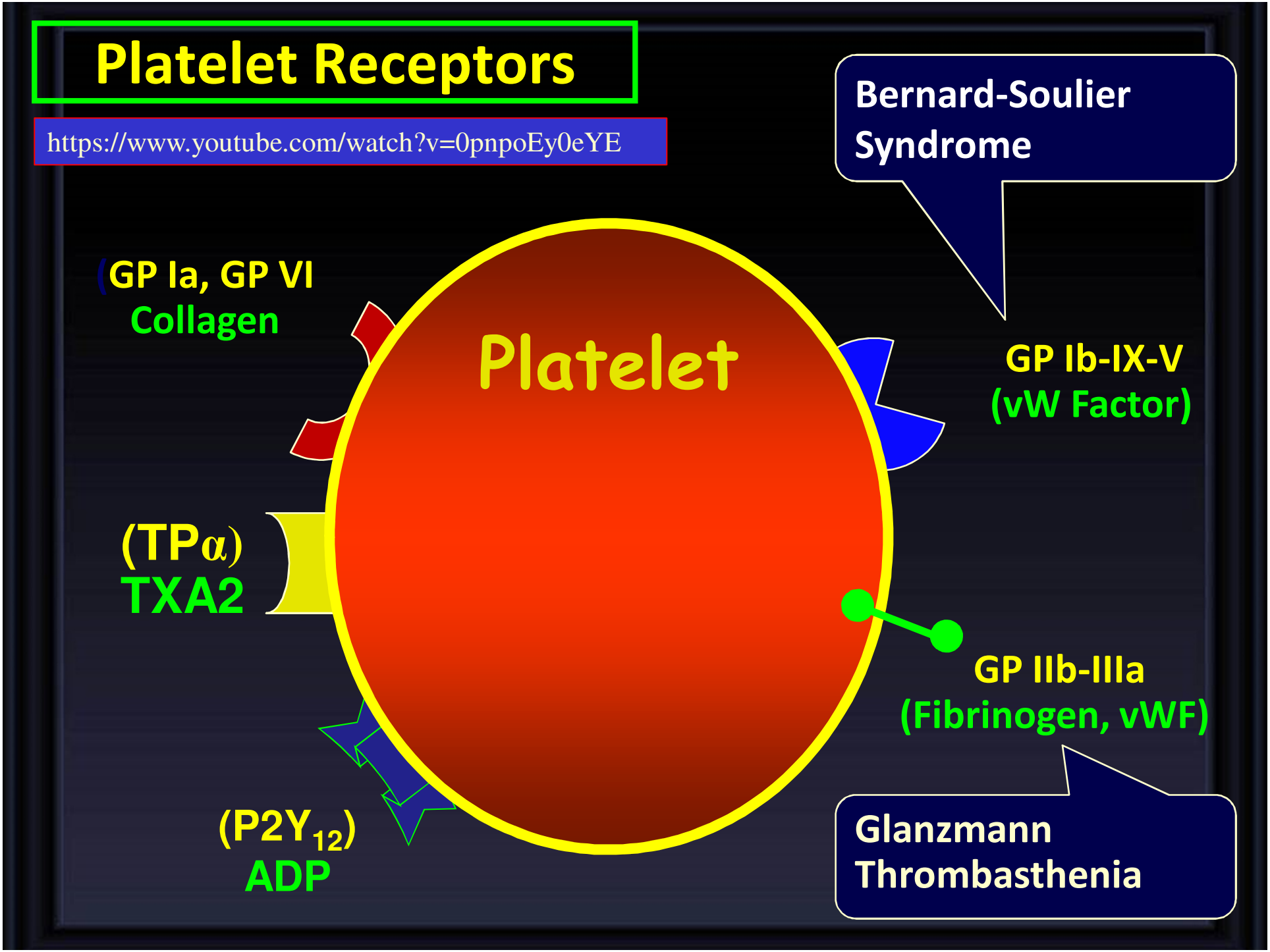
Normal Endothelium Secrete:

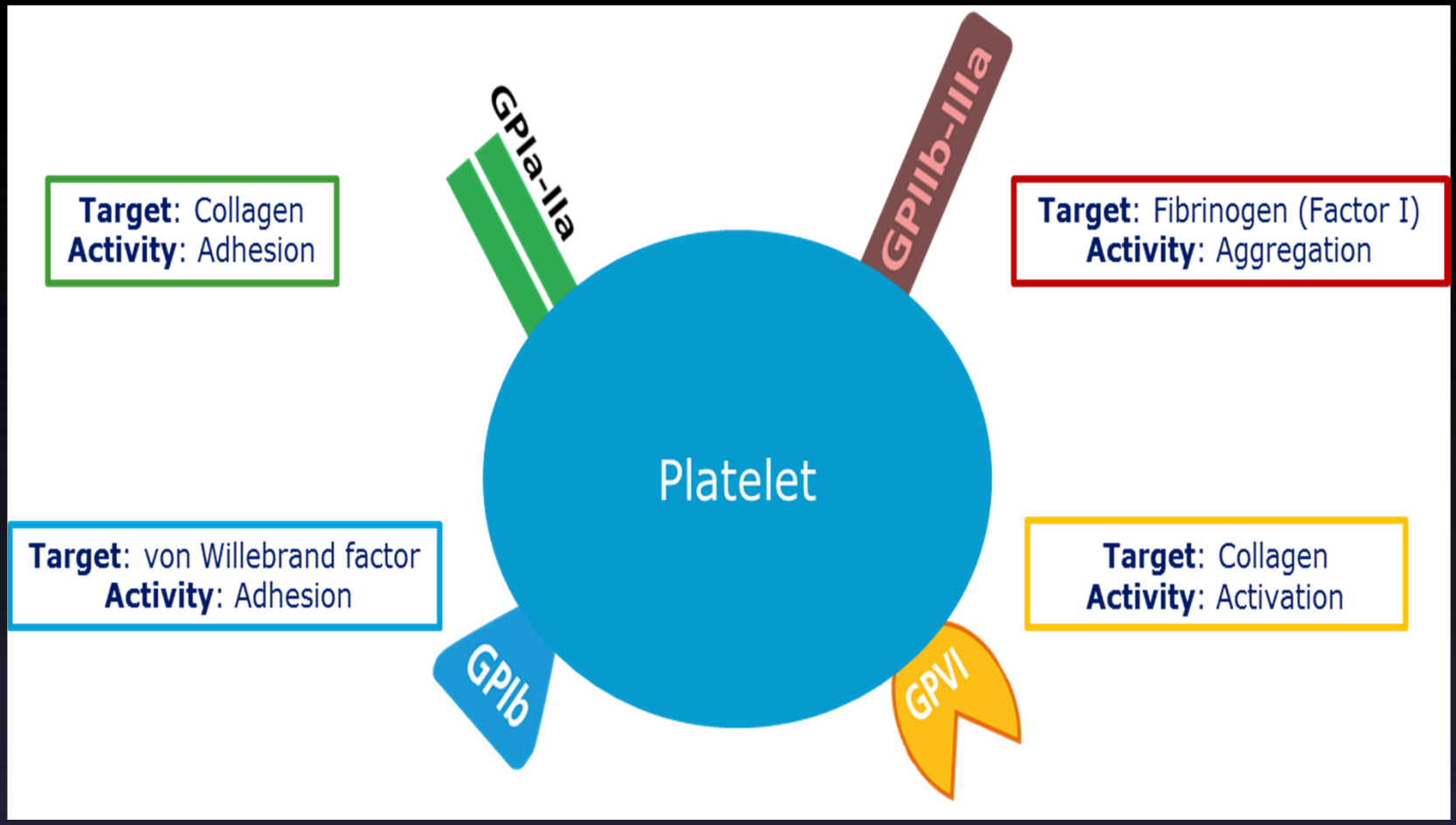
- **prostacyclin (PGI2)**
- **NO**
- **ADP phosphatase**



Platelet Receptors

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




GPIa-IIa
GPIIb-IIIa
Target: Collagen
Activity: Adhesion
Target: Fibrinogen (Factor I)
Activity: Aggregation
Platelet
Target: von Willebrand factor
Activity: Adhesion
Target: Collagen
Activity: Activation
GPIb
GPVI

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)

Activated Platelets Secrete:

1. **ADP** → Adhesion
2. **5HT→vasoconstriction**
3. **Platelet phospholipid (PF3)→ clot formation**
4. **TXA2 is a PG formed from arachidonic acid →**

Functions:

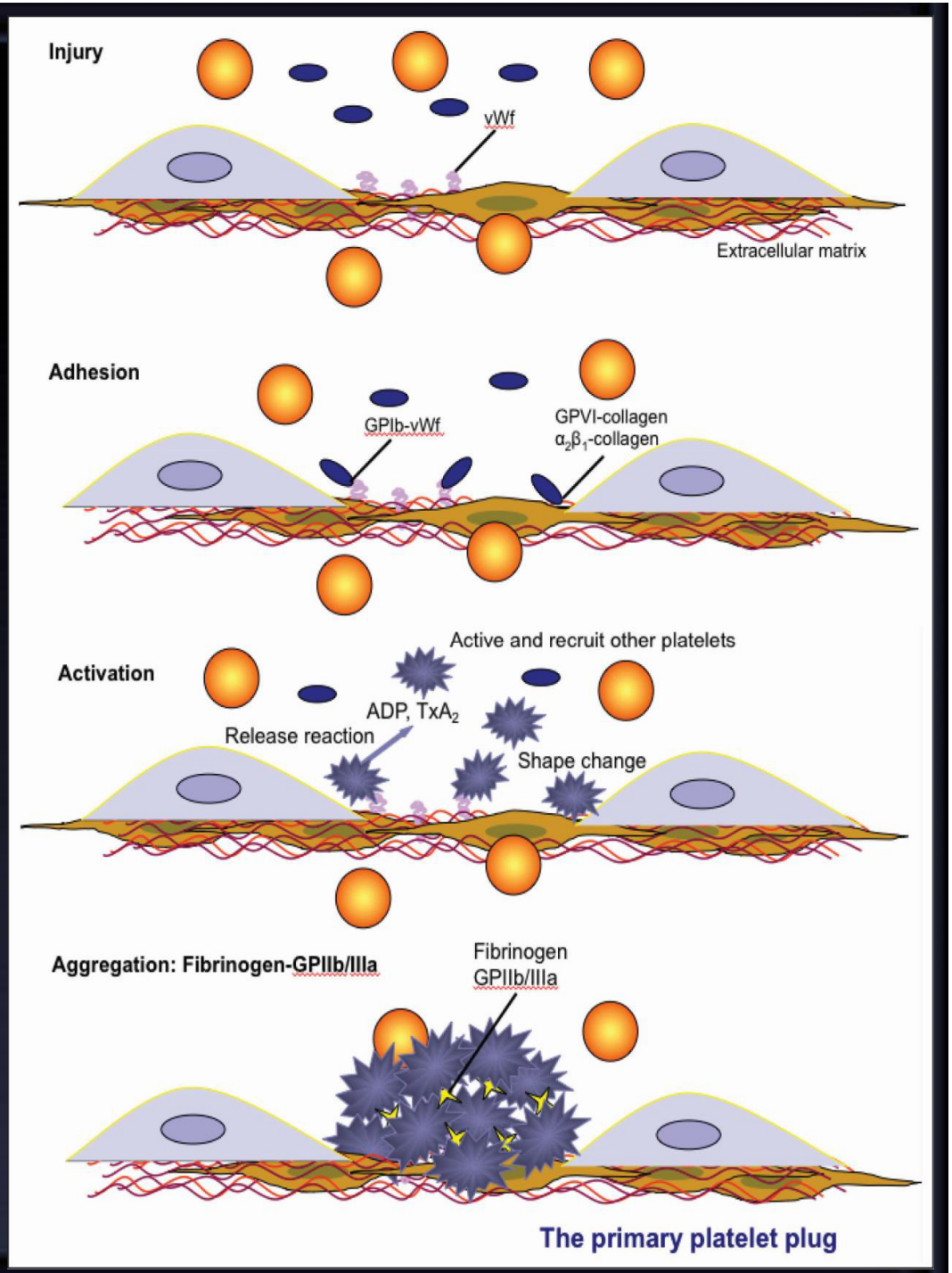
–**vasoconstriction**

–**Platelet aggregation**

(TXA2 is inhibited by aspirin)

Aggregation:

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

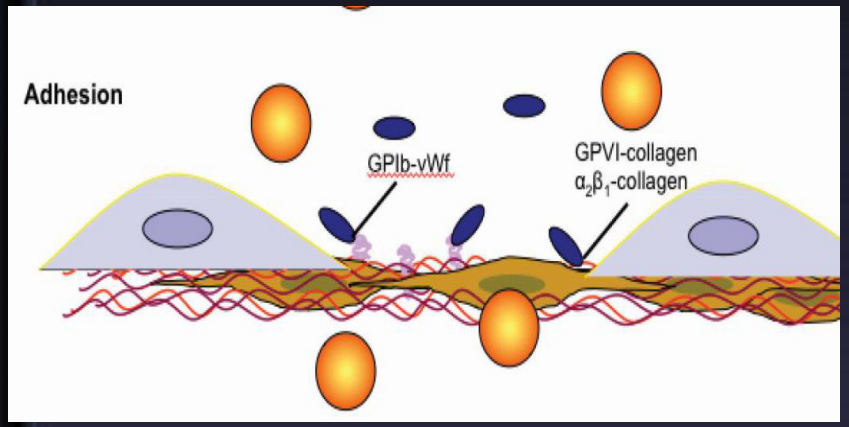


Congenital Platelet Disorders

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

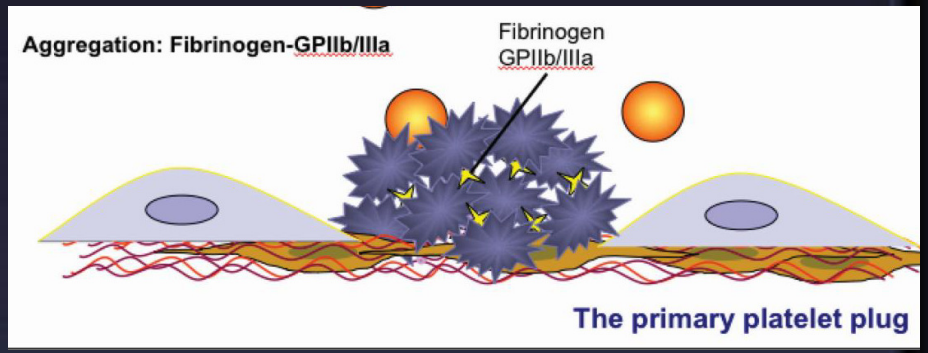


Adapted from Balduini et al, Haematologica 88[5]: 582-592, May 2003

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

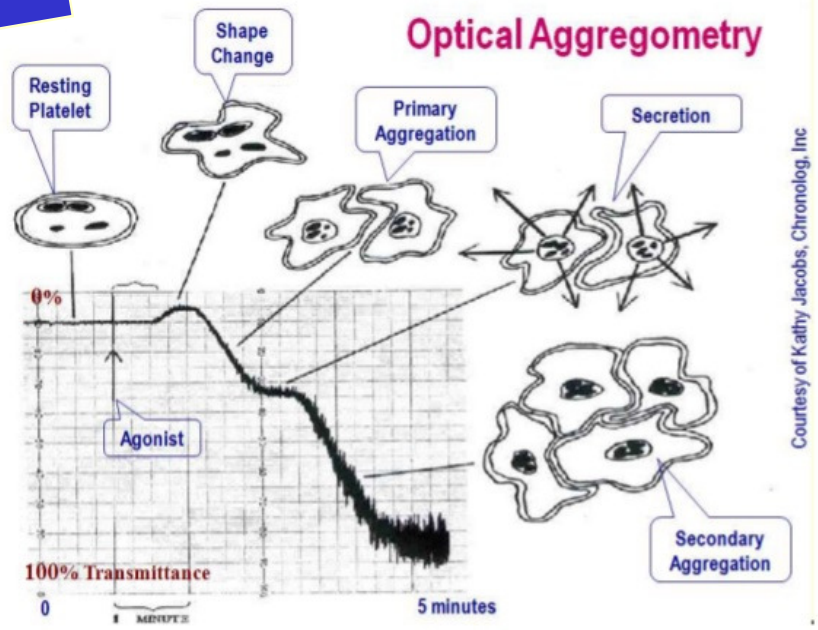


Testing Platelet Functions

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

Automated

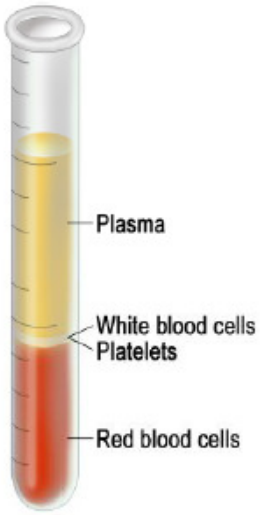
Aggregation



Laboratory Testing of Platelet Functions

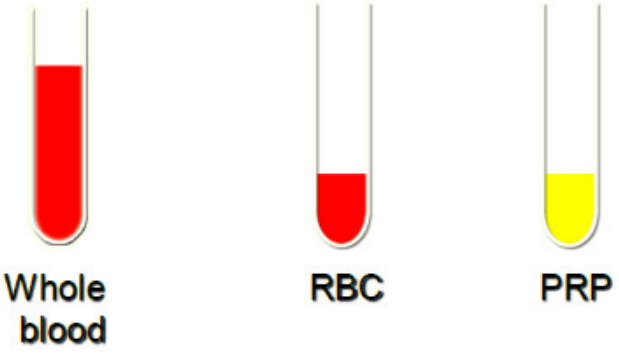
- **By Platelet Aggregation Method:** Provides information on time course of plat. activation.
- **Agonists:**
  - ADP
  - Adrenaline
  - Collagen
  - Arachidonic acid
  - Ristocetin
  - Thrombin
- Reference ranges need to be determined for each agonist

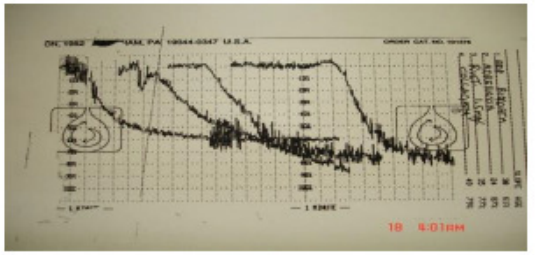
You need in Platelet rich plasma (PRP)



Agonists:

- ADP
- Adrenaline
- Collagen
- Arachidonic acid
- Ristocetin
- Thrombin





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

LAB TESTS IN BLEEDING AND CLOTTING

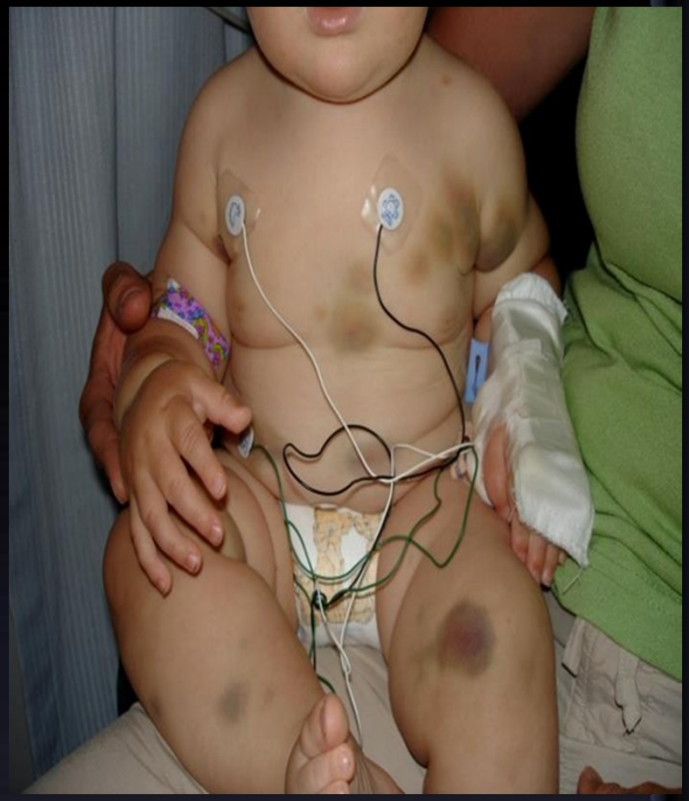
Test	Normal Value	Importance
PLATELET COUNT	100,000 - 400,000 CELLS/MM^3	Thrombocytopenia
PLATELET FUNCTIONS	Normal Aggregation	Thrombocytopathy (normal count) [Congenital or Acquired…Aspirin]
BLEEDING TIME (BT)	2-8 MINUTES	Bleeding disorders
PROTHROMBIN TIME (PT)	10-15 SECS	Measures Effectiveness of 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

Case study

- A 7 years old girl complaining of severe bruising since birth and if she had injury she would bleed for days. She had epistaxis which lasted for days ,her mother said :"she just bruise more easily than her older sister."
- **Investigation:**
  - CBC
  - RBC
  - WBC
  - platelet
- **Platelet morphology:**

  Normal
- **Aggregometry :**

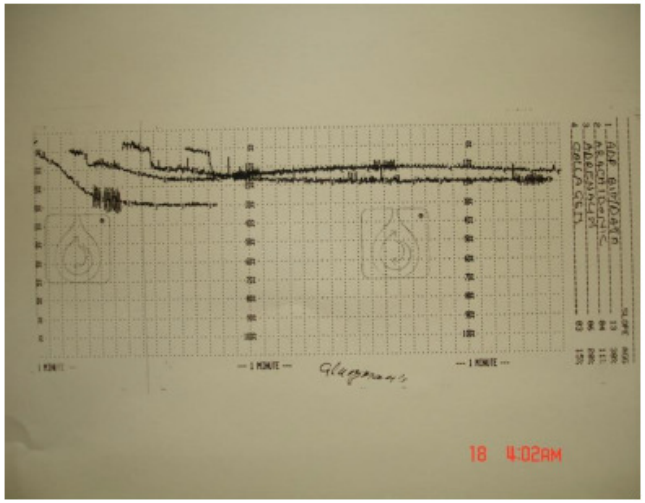
  Absent platelet aggregation in response to ADP,collagen ,thrombin and epinephrine.



Diagnosis:
Glanzmann's Thrombasthenia

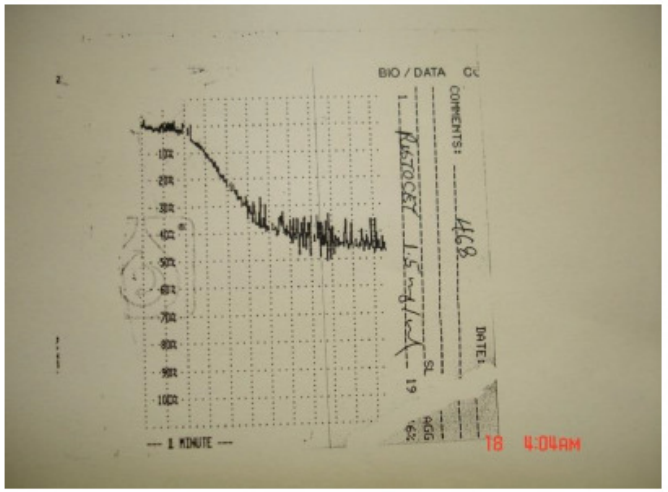
Aggregometry:

Absent platelet aggregation in response to ADP, collagen, thrombin, & epinephrine.

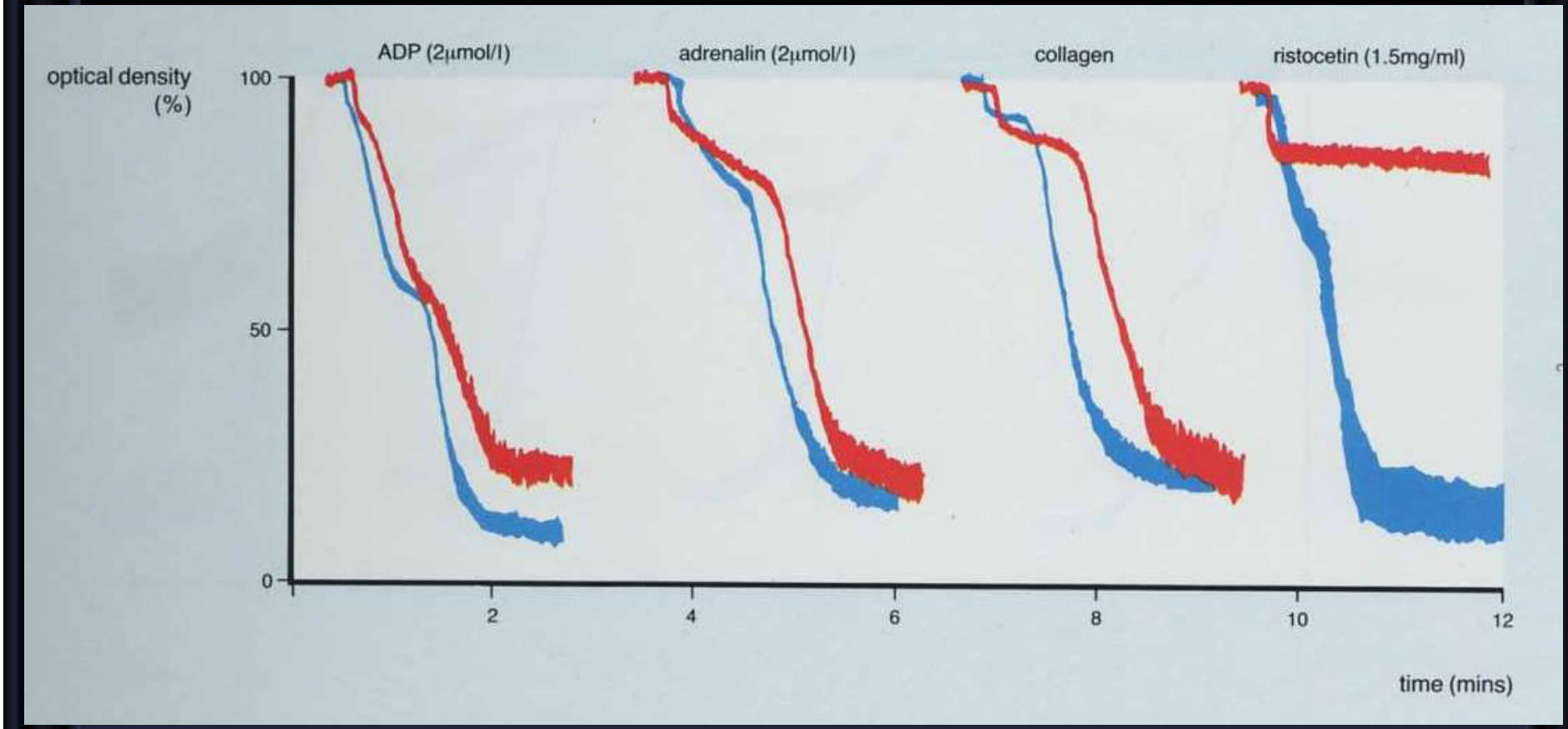


Diagnosis:

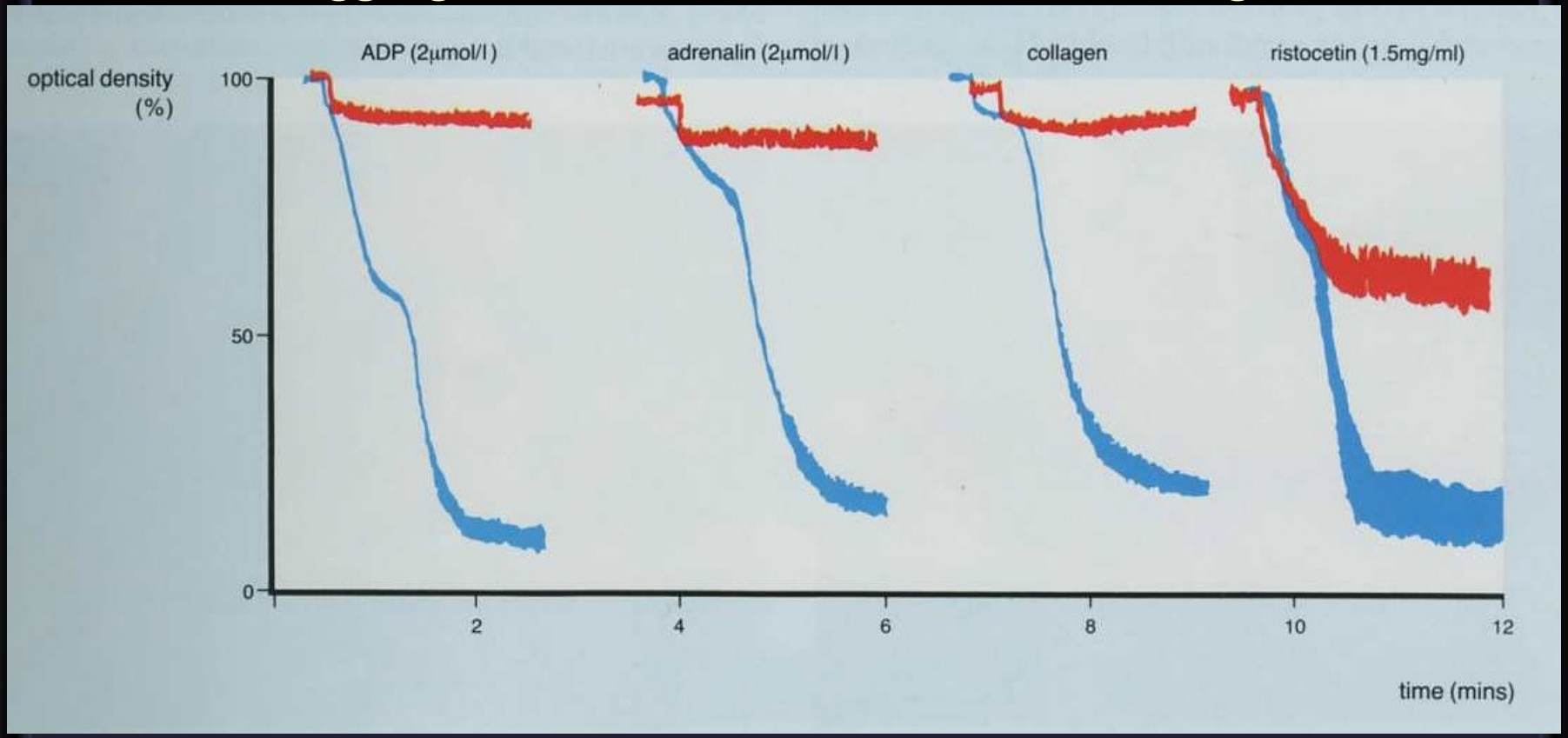
Glanzmann's Thrombasthenia



1. In the patient shown below, the only abnormality is a lack of agglutination with Ristocetin. Possible diagnoses are therefore, Von Willebrand Disease or Bernard Soulier Syndrome.

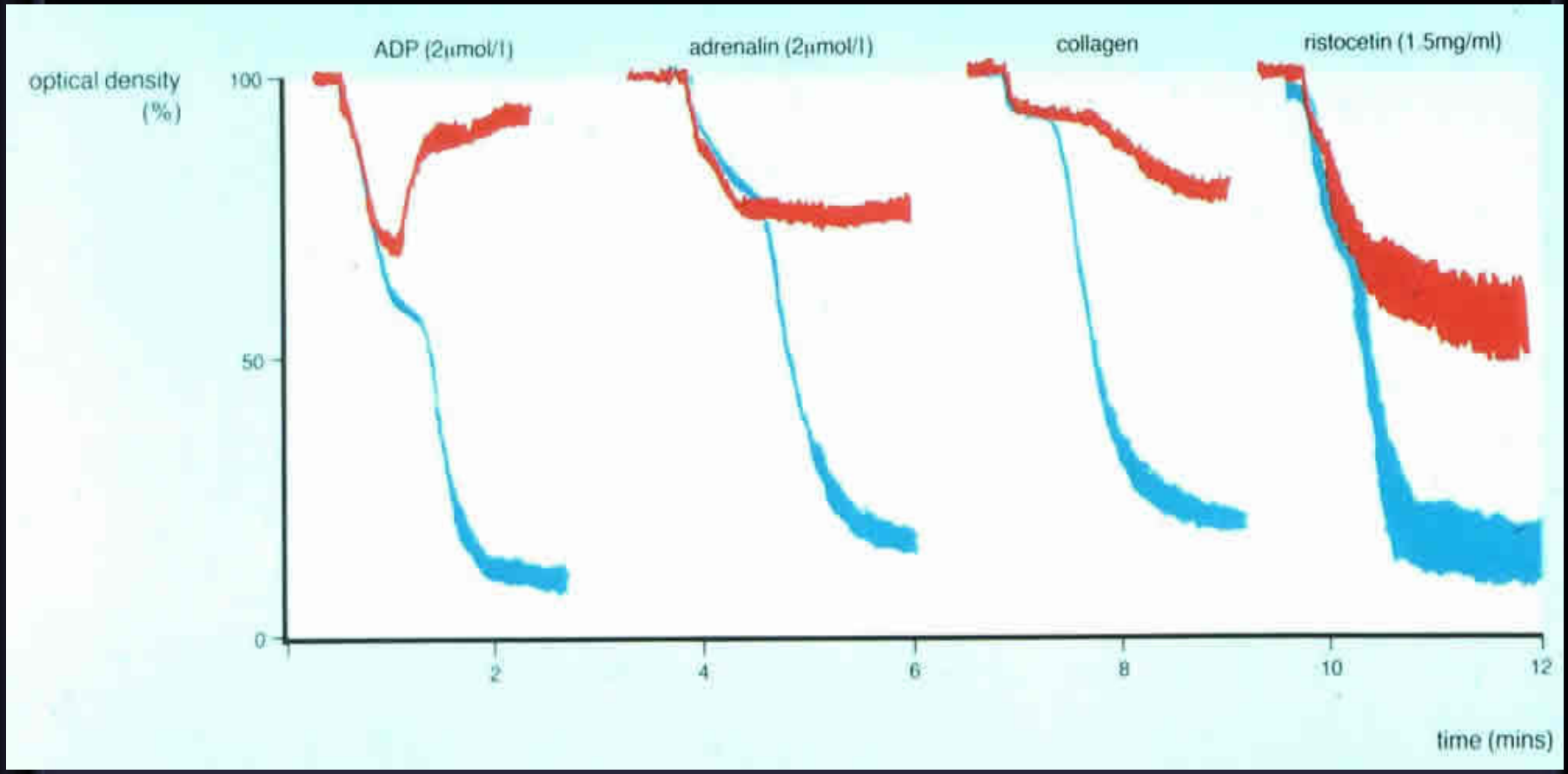


2. This is the converse of the first patient and the only agglutination [and this is not complete] is seen with Ristocetin. There is no aggregation with ADP, adrenaline or collagen.



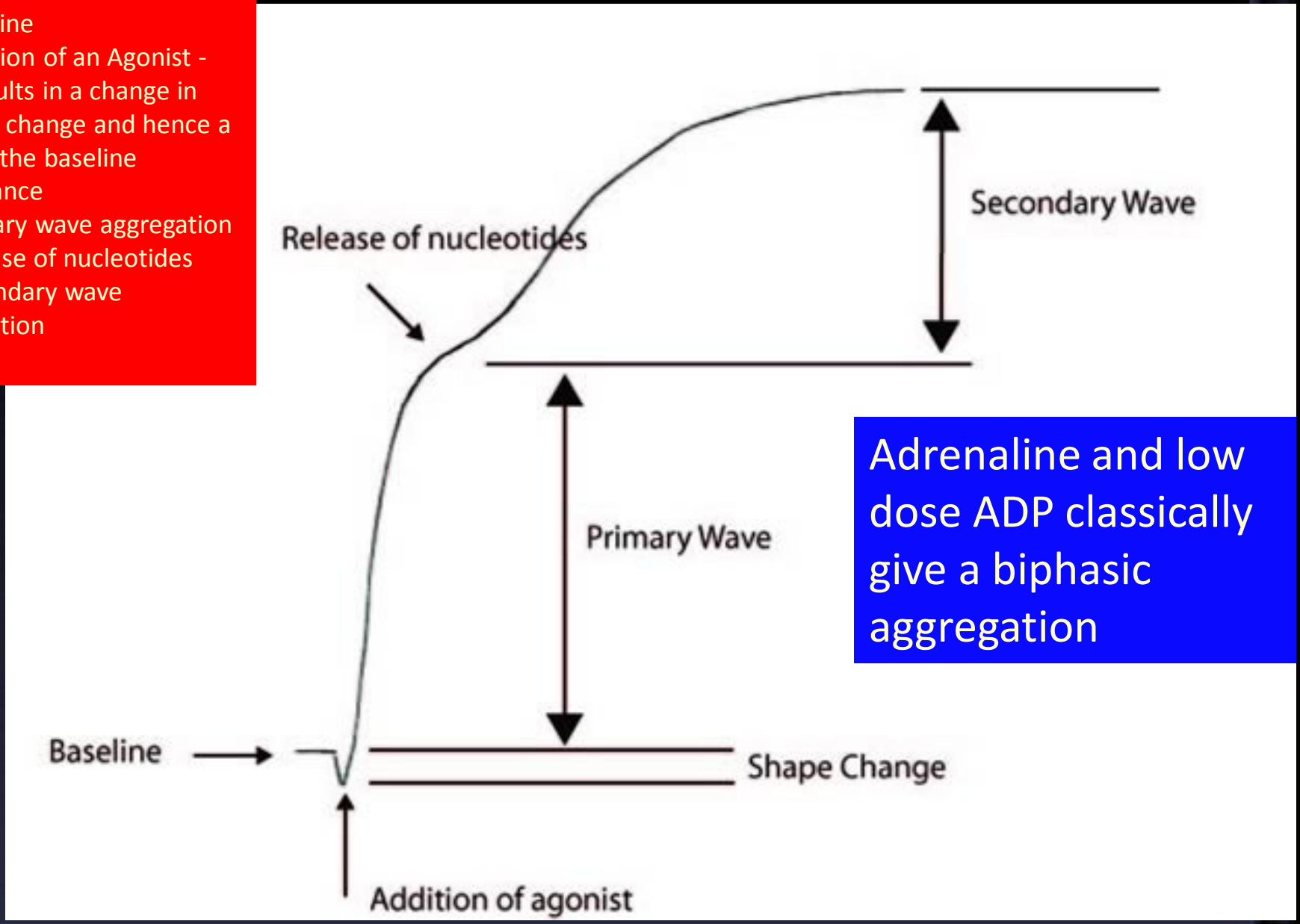
Possible diagnoses include Glanzmann's Thrombasthenia or Afibrinogenaemia.
[Remember, platelet agglutination with Ristocetin occurs independently of Fibrinogen.]

3. In this patient reversible, first wave aggregation is seen with ADP, adrenaline and collagen and only partial agglutination with Ristocetin. The picture is clearly different from the two traces above 1) or 2): the results suggest a failure of granule release and is consistent with either platelet Storage Pool Disorder or a defect in nucleotide release.

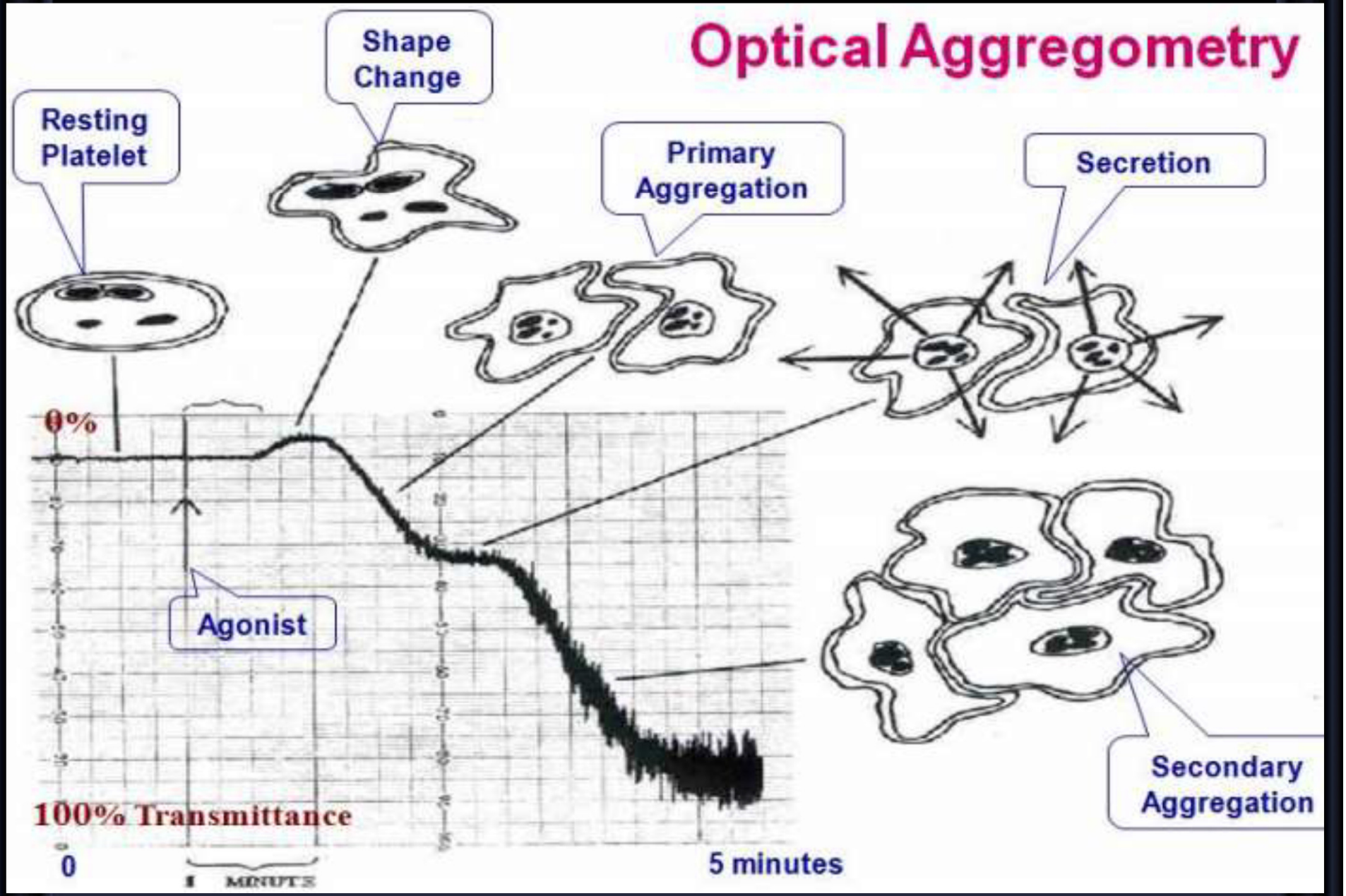


Classic biphasic aggregation:

1. Baseline
2. Addition of an Agonist - this results in a change in platelet change and hence a drop in the baseline absorbance
3. Primary wave aggregation
4. Release of nucleotides
5. Secondary wave aggregation

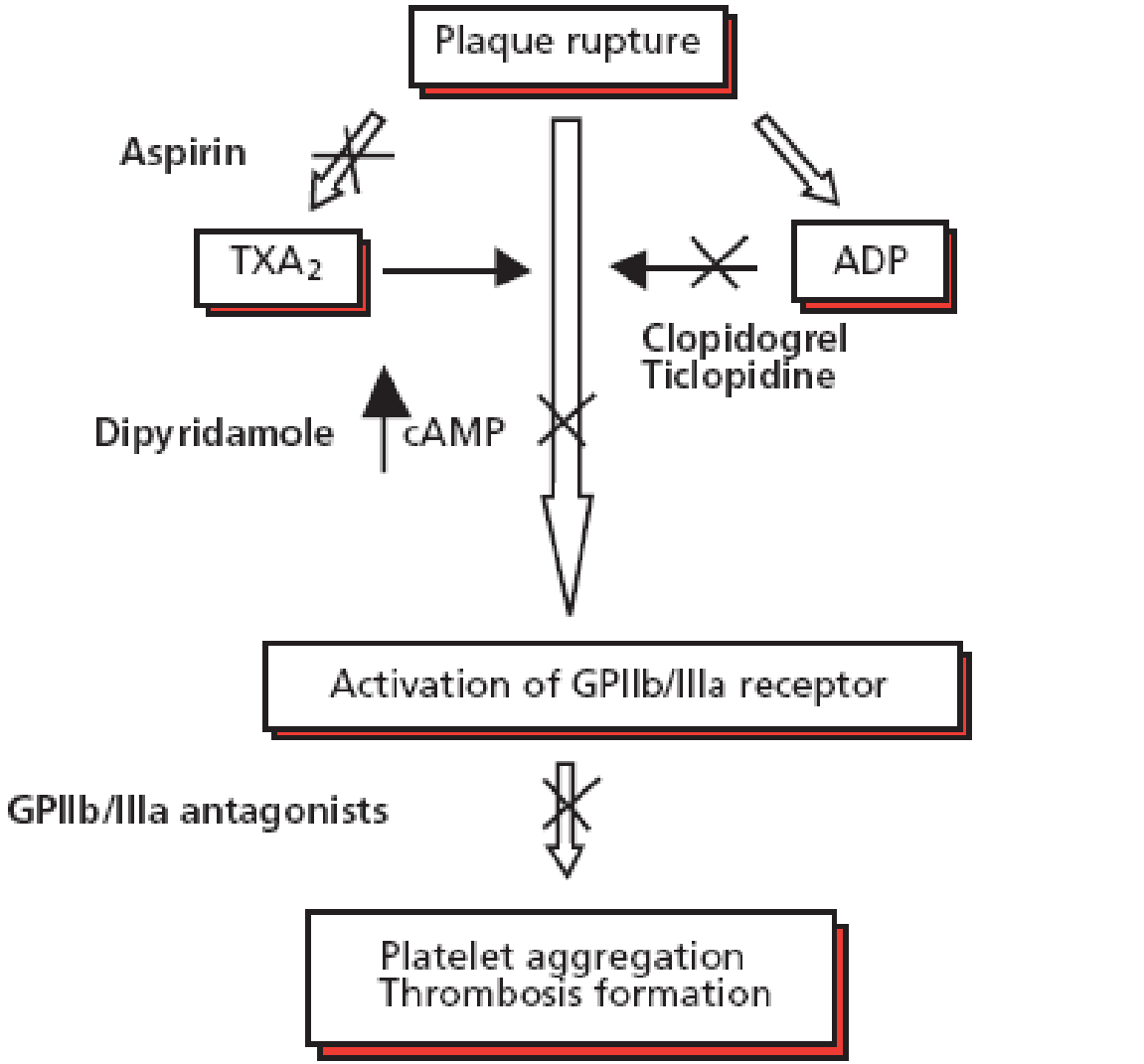


Adrenaline and low dose ADP classically give a biphasic aggregation


Optical Aggregometry
Shape Change
Resting Platelet
Primary Aggregation
Secretion
0%
Agonist
100% Transmittance
0
1 MINUTE
5 minutes
Secondary Aggregation

Disorder	Characteristic Findings on LTA
Glanzmann's Thrombasthenia OR afibrinogenaemia	Absent or markedly impaired aggregation to all agonists except Ristocetin. Ristocetin-induced agglutination shows only primary wave - aggregation cannot occur because fibrinogen cannot bind. Afibrinogenaemia gives similar results.
Bernard Soulier Syndrome OR Von Willebrand Disease	Absent or markedly reduced platelet agglutination with Ristocetin.
Storage Pool Disorder OR Platelet Release Defect	Primary aggregation only with ADP, adrenaline and collagen and only partial agglutination with Ristocetin suggesting a failure of granule release or a deficiency of platelet granules.
Aspirin [or defects in the COX pathway]	Absent aggregation to Arachidonic acid. Primary wave aggregation only with ADP. Decreased or absent aggregation with collagen.
Clopidogrel	Absent aggregation with ADP

Aspirin inhibits platelet cyclooxygenase by irreversible acetylation, thereby preventing the formation of thromboxane A2 which is a powerful stimulant of platelet aggregation . Clopidogrel, a thienopyridine, acts by inhibiting adenosine receptors, which inhibits the early step of platelet activation



Key: TXA_2 = thromboxane; GP = glycoprotein; ADP = adenosine diphosphate; CAMP = cyclic adenosine monophosphate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K-EDTA
Clot Activator
Plain
EDTA.K
Heparin Li
Gel Clot Activ
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4ml