PLATELETS STRUCTURE & FUNCTIONS

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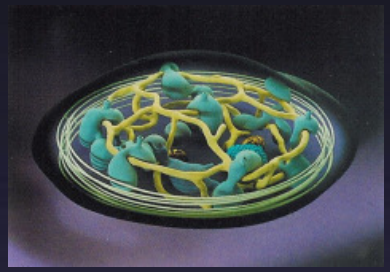
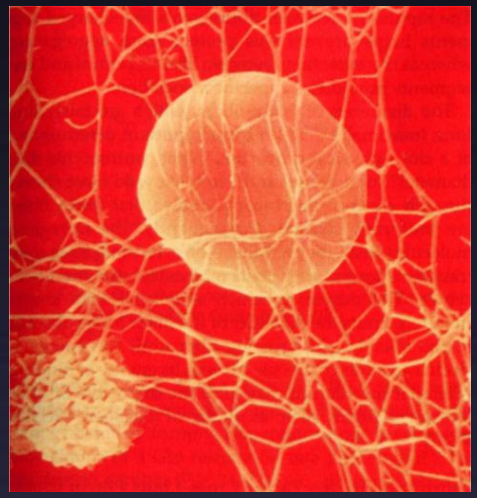




HANDOUTS…1/19/2020

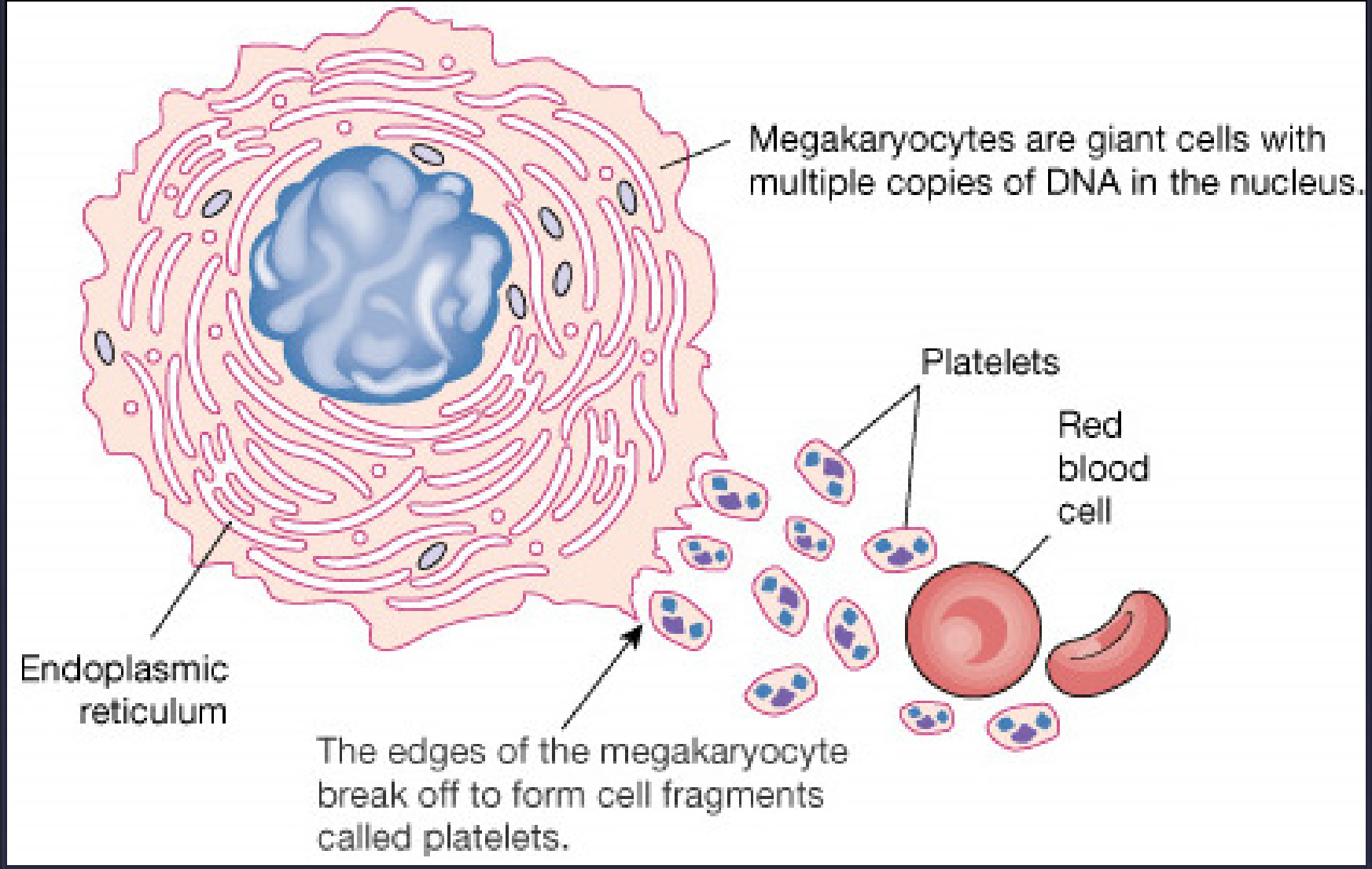
OBJECTIVES

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

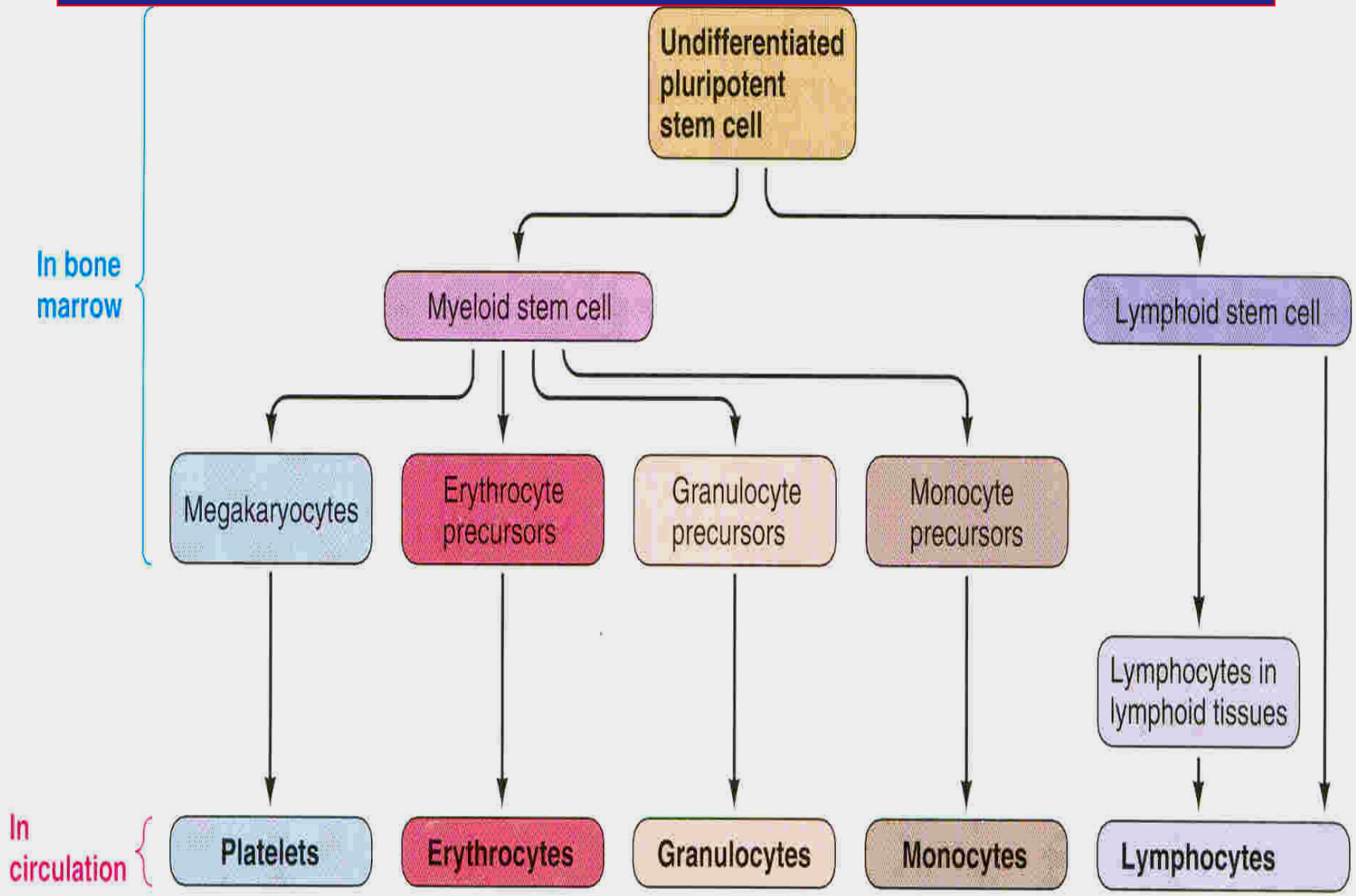
- **Describe formation and development of platelets**
- **Understand platelet normal ultrastructure**
- **Describe the functions of different platelets organelles and surface receptors**
- **Describe the mechanisms of platelet functions**
- **Relate membrane receptors and granule content to normal function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

PLATELETS

Formed by fragmentation from megakaryoctyes



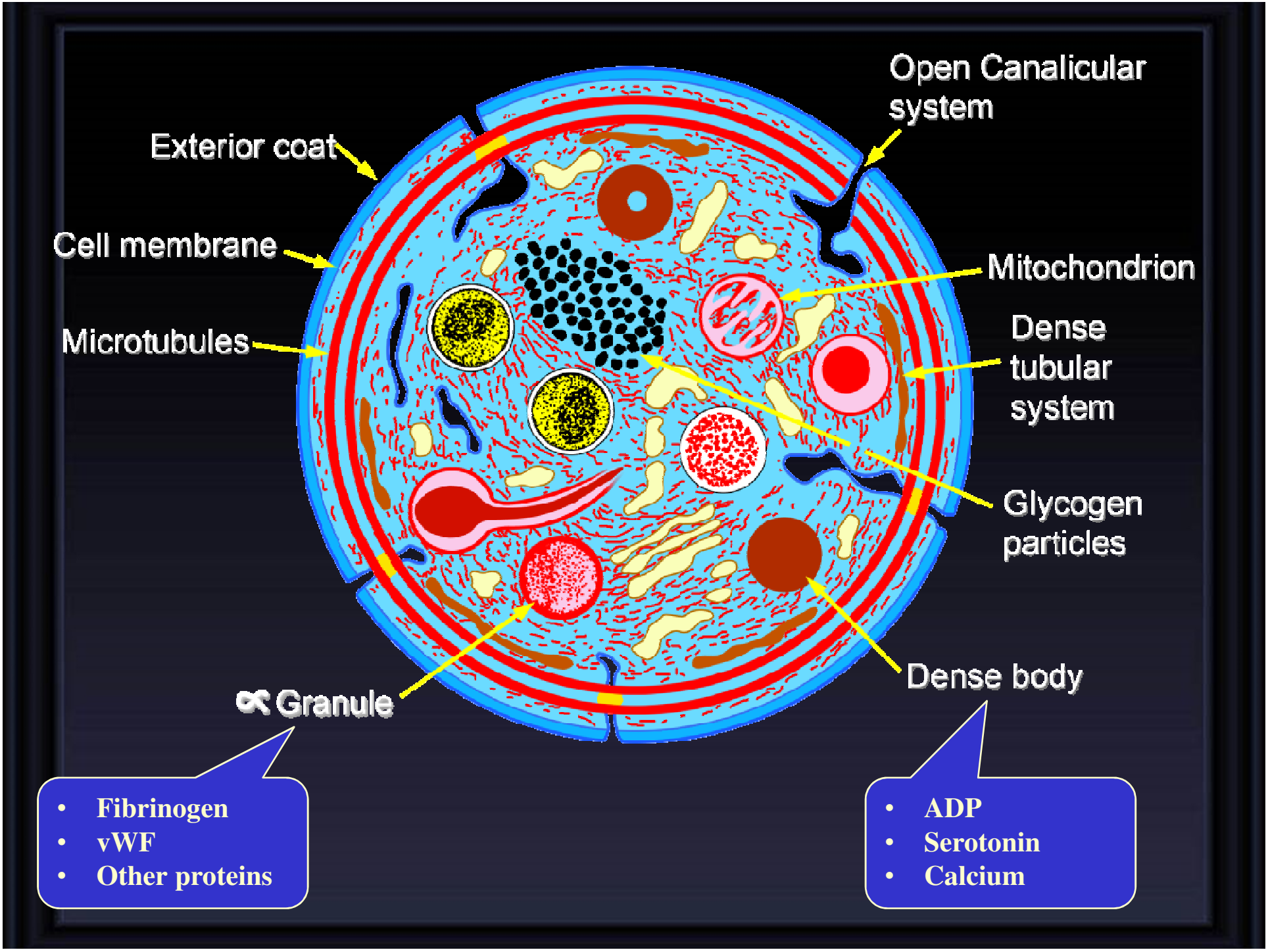
SITE OF FORMATION
Bone-marrow



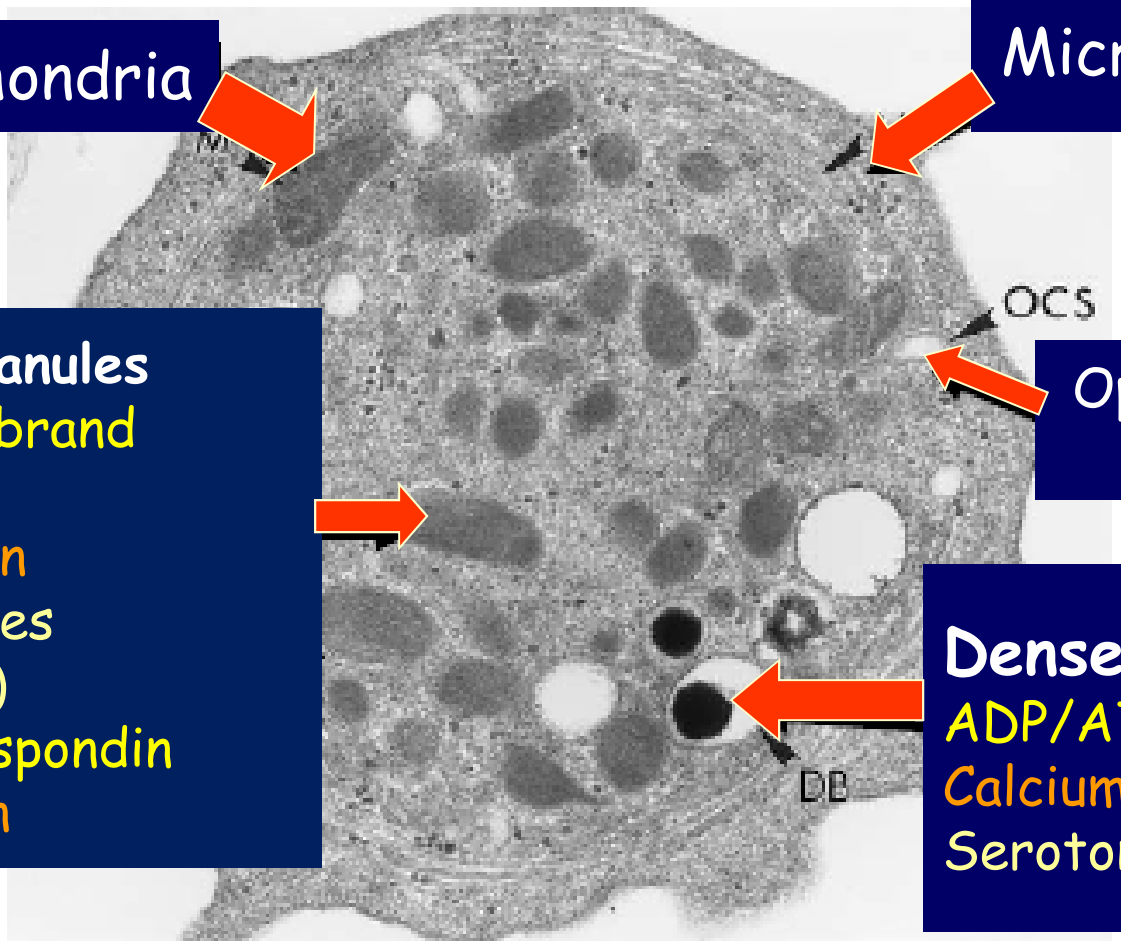
PLATELETS (Characteristics)

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
α Granule
Dense body
• Fibrinogen
• vWF
• Other proteins
• ADP
• Serotonin
• Calcium

Platelet Ultrastructure



Mitochondria

Microtubules

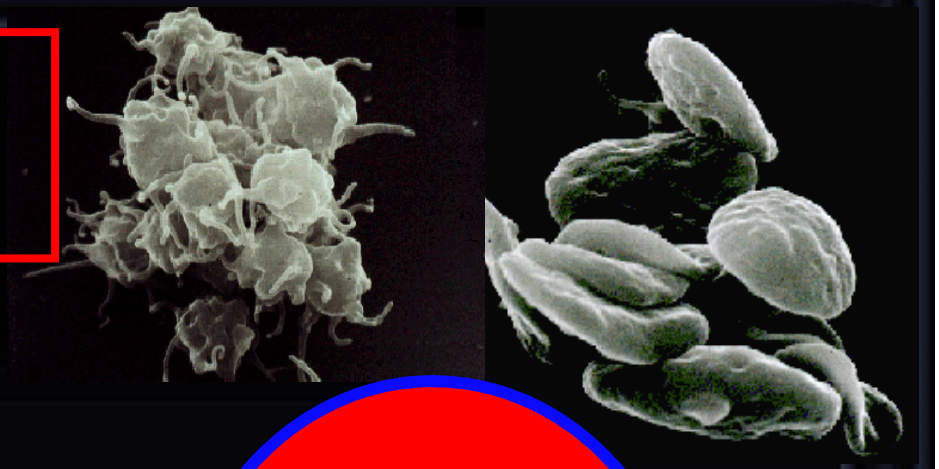
Open canalicular system

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)**

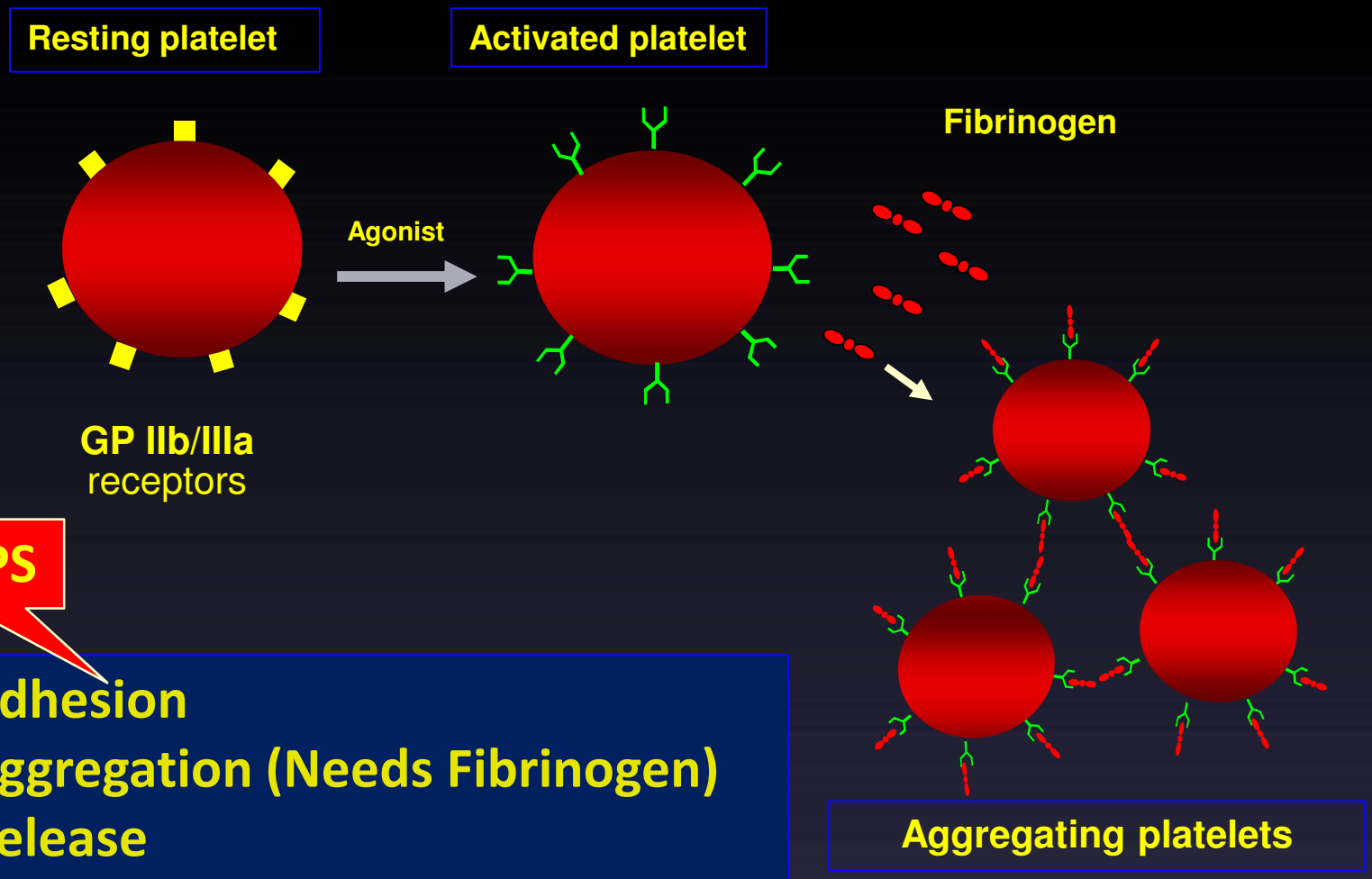
Dense or δ granules

- Serotonin
- ADP
- Ca^{++}

Alpha α granules

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

Platelets Activation



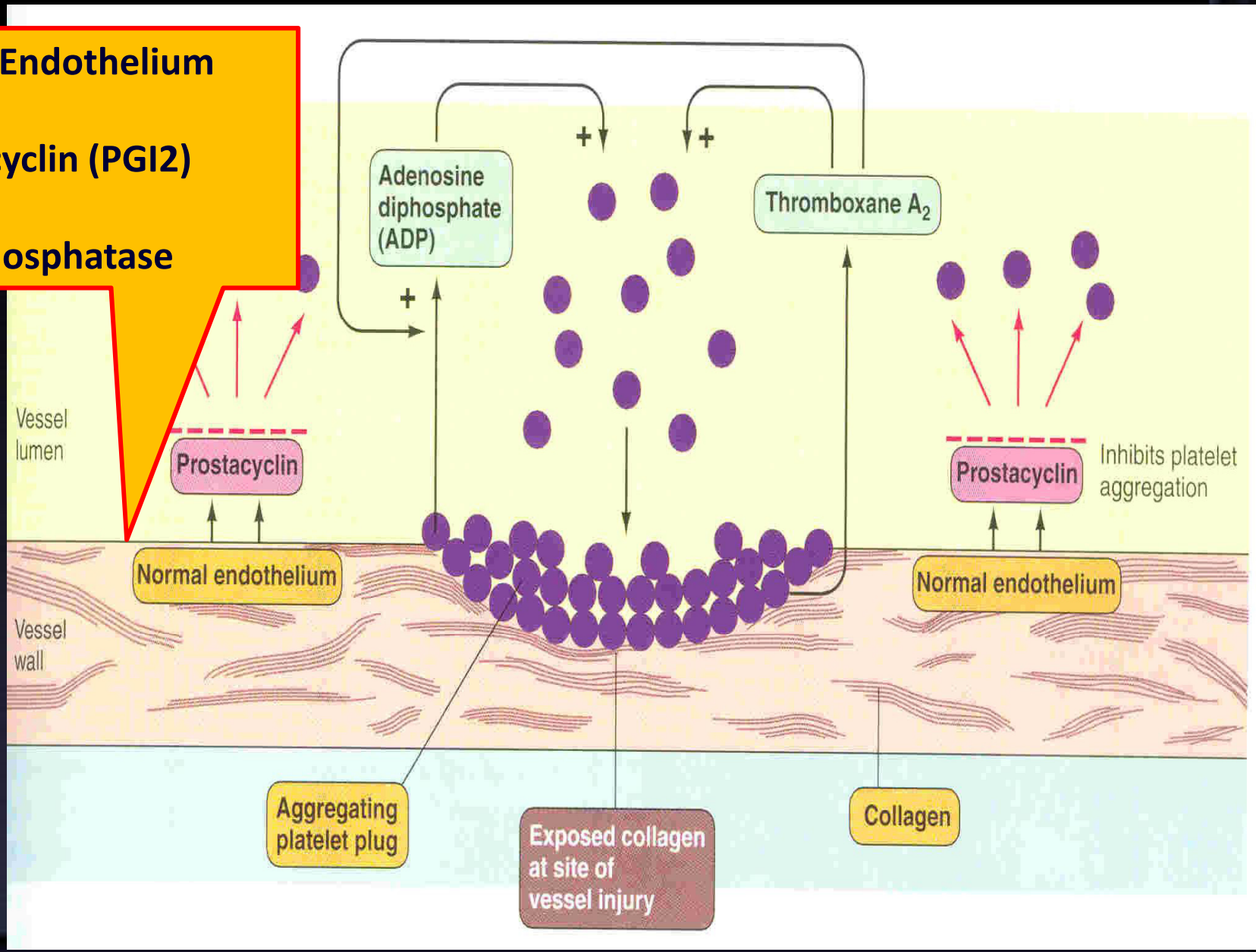
4 STEPS

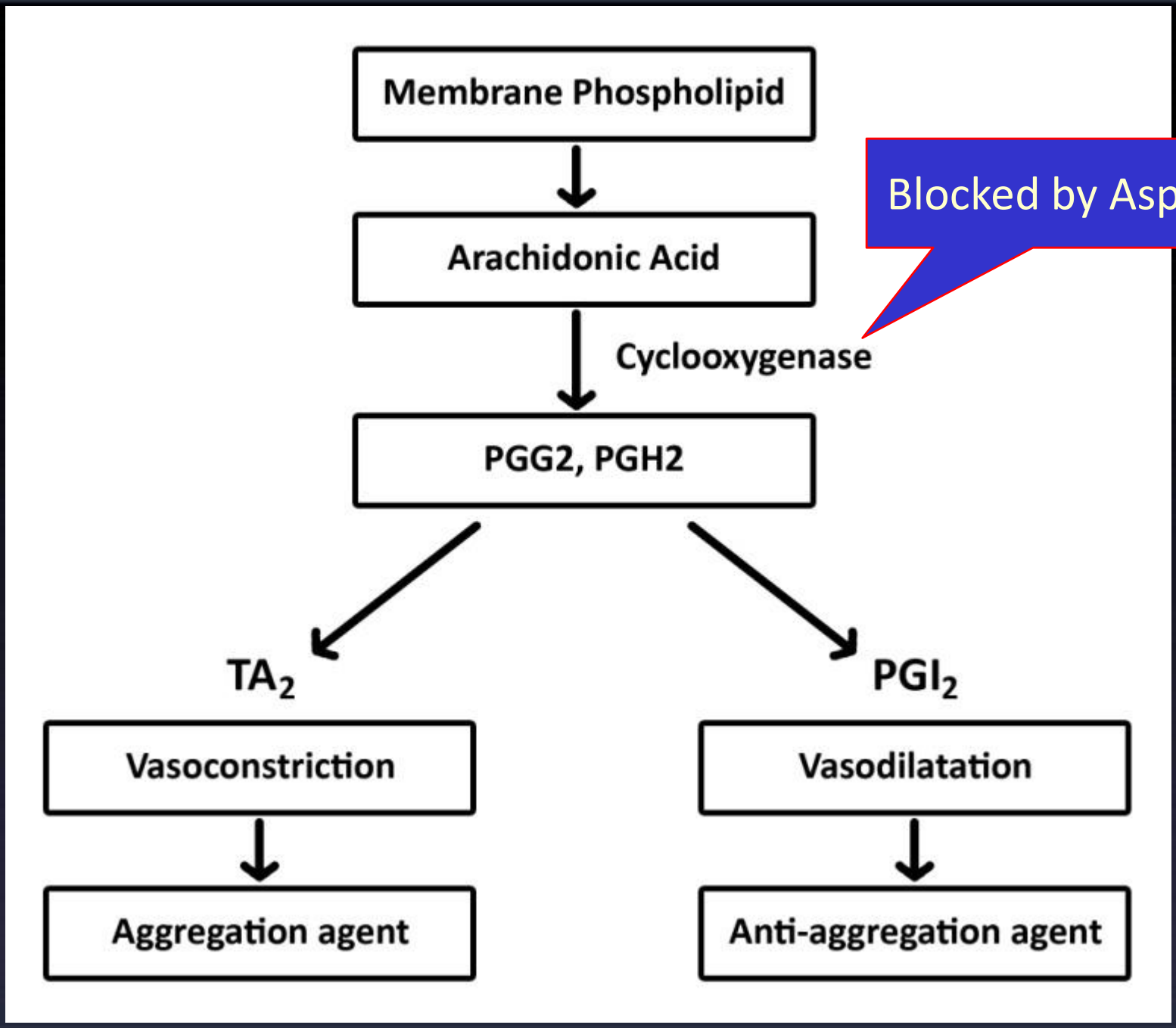
1. Adhesion
2. Aggregation (Needs Fibrinogen)
3. Release
4. Clot Retraction

PLATELETS ACTIVATION

Normal Endothelium Secrete:

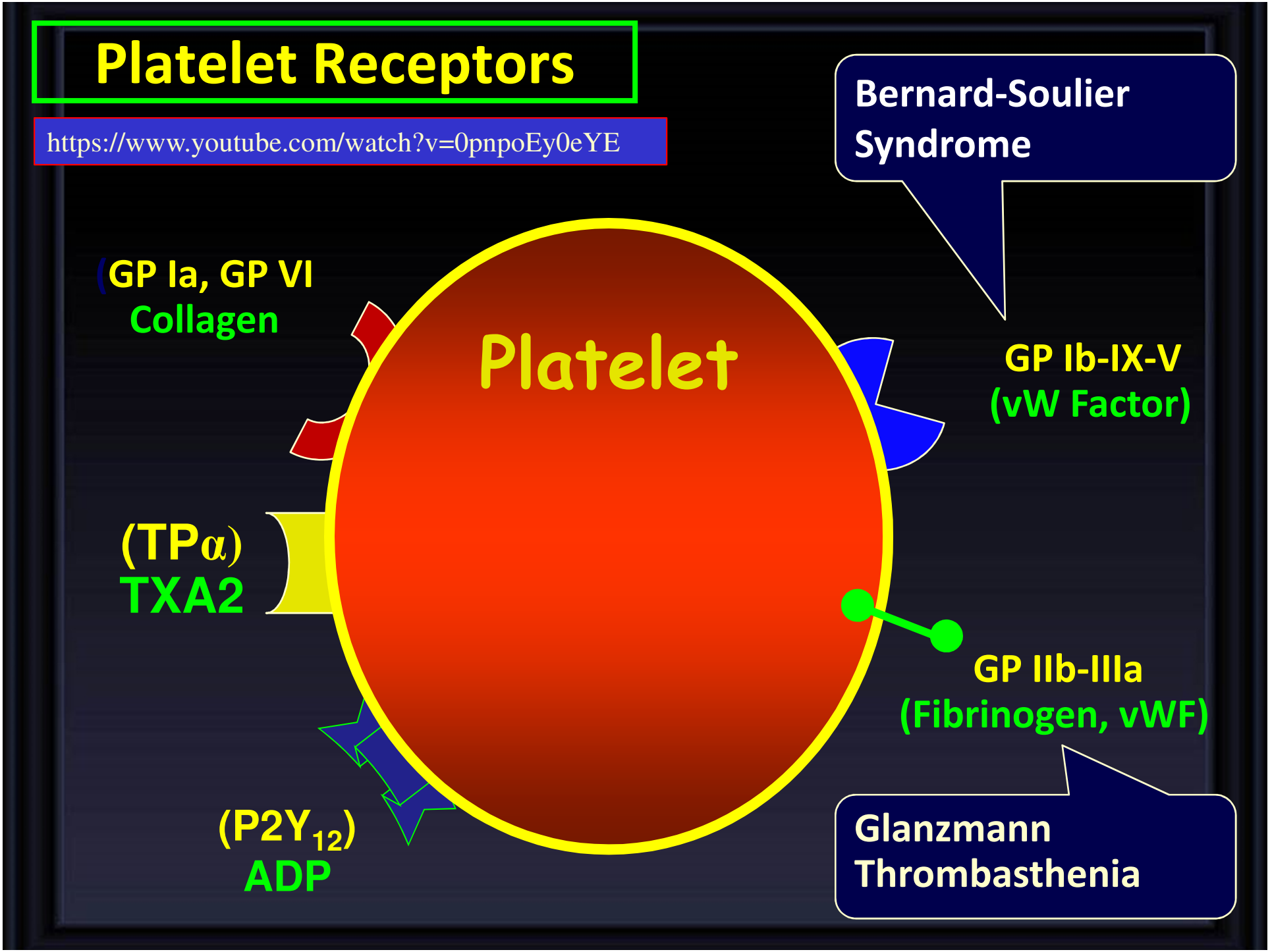
- **prostacyclin (PGI2)**
- **NO**
- **ADP phosphatase**

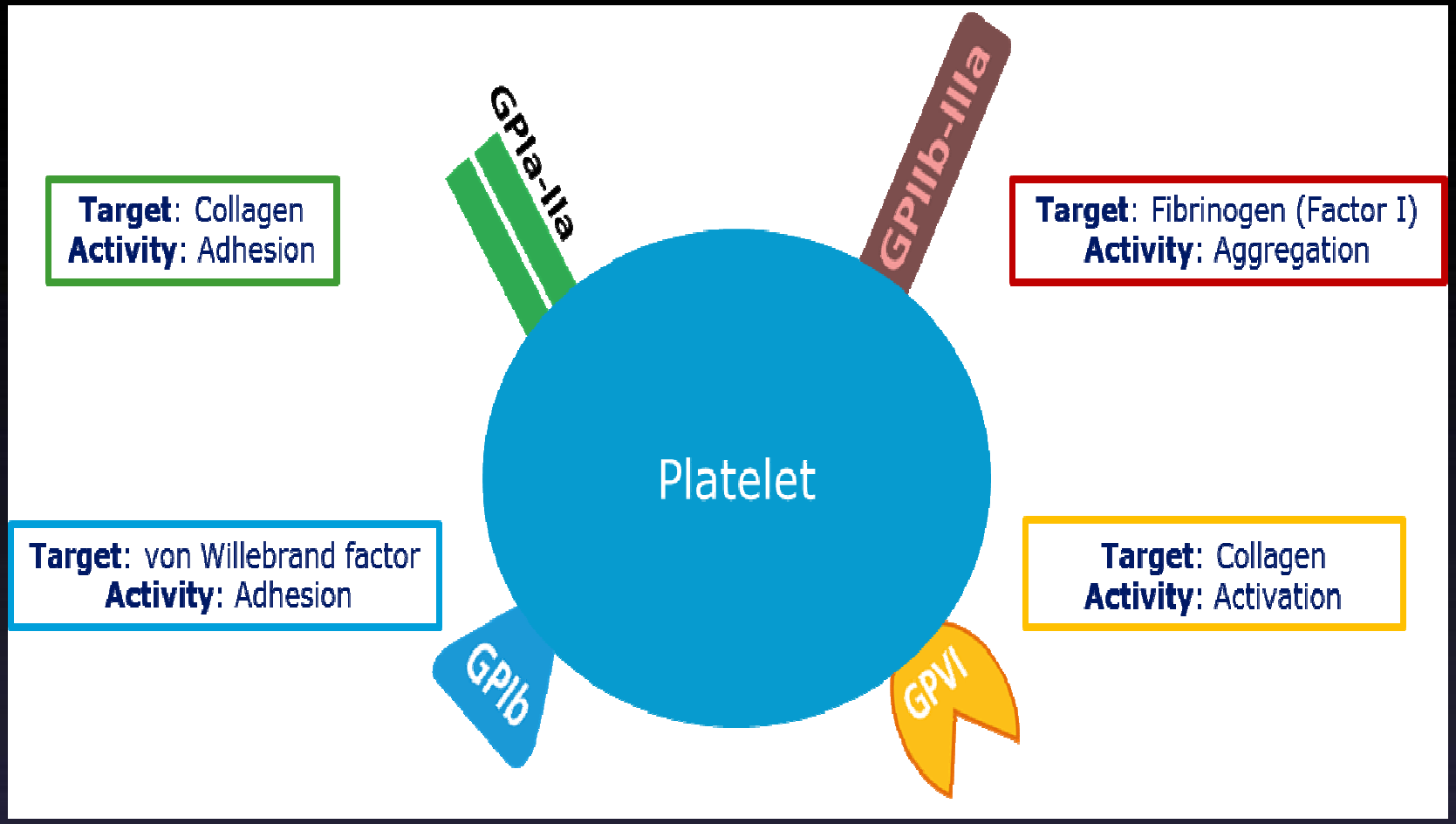



Membrane Phospholipid
Arachidonic Acid
Blocked by Aspirin
Cyclooxygenase
PGG2, PGH2
TA_2
PGI_2
Vasoconstriction
Vasodilatation
Aggregation agent
Anti-aggregation agent

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
GPIb
GPVI
Target: Collagen
Activity: Activation

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:

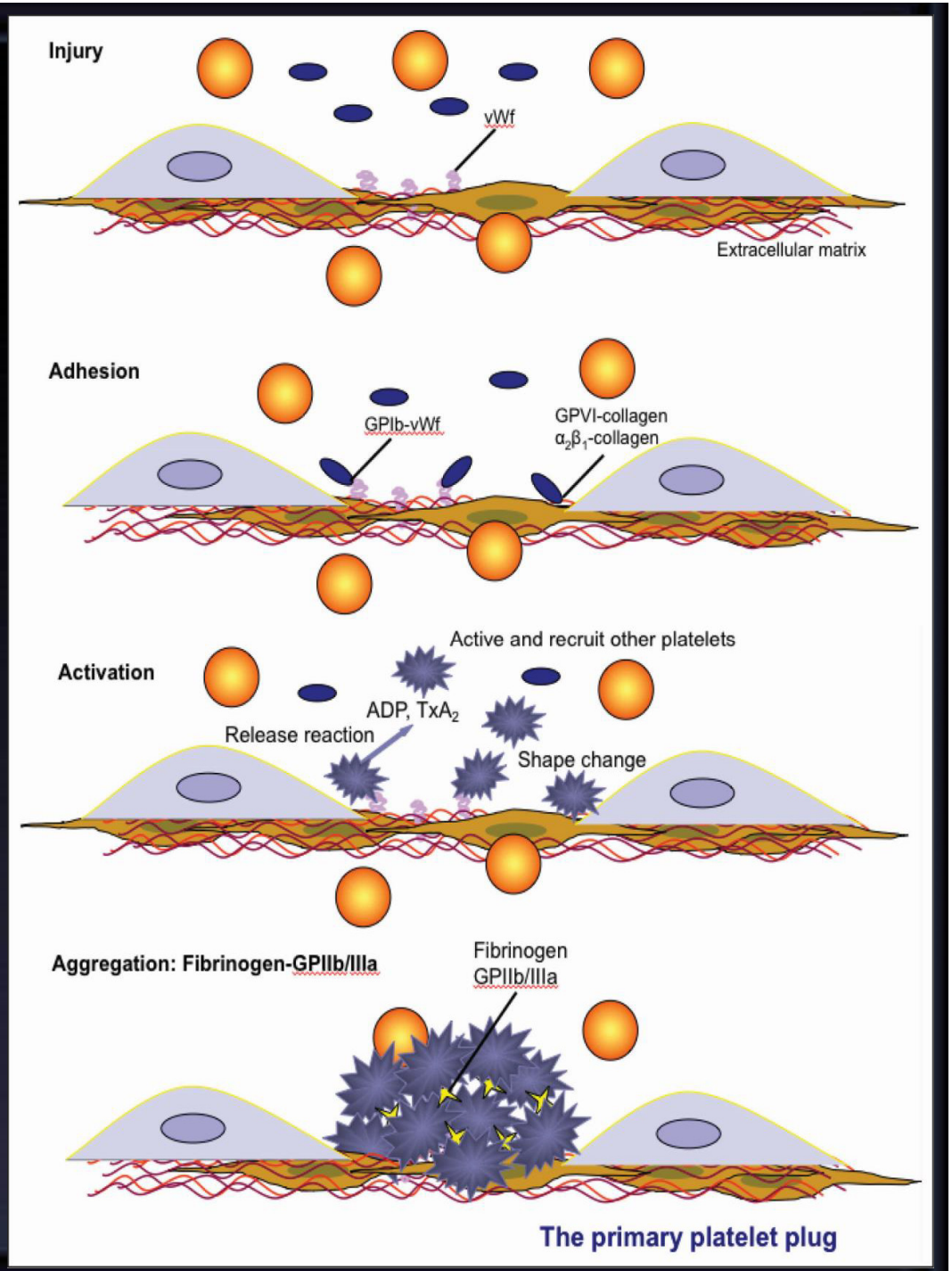
–**vasoconstriction**

–**Platelet aggregation**

(TXA2 is inhibited by aspirin)

Aggregation:

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

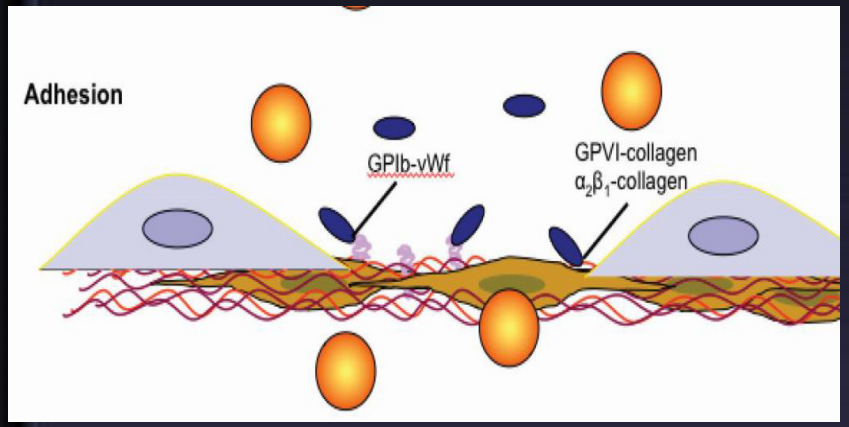


Congenital Platelet Disorders

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

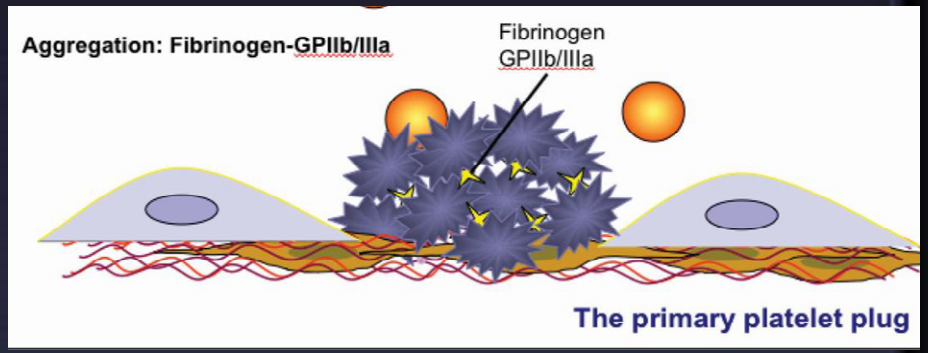


Adapted from Balduini et al, Haematologica 88[5]: 582-592, May 2003

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

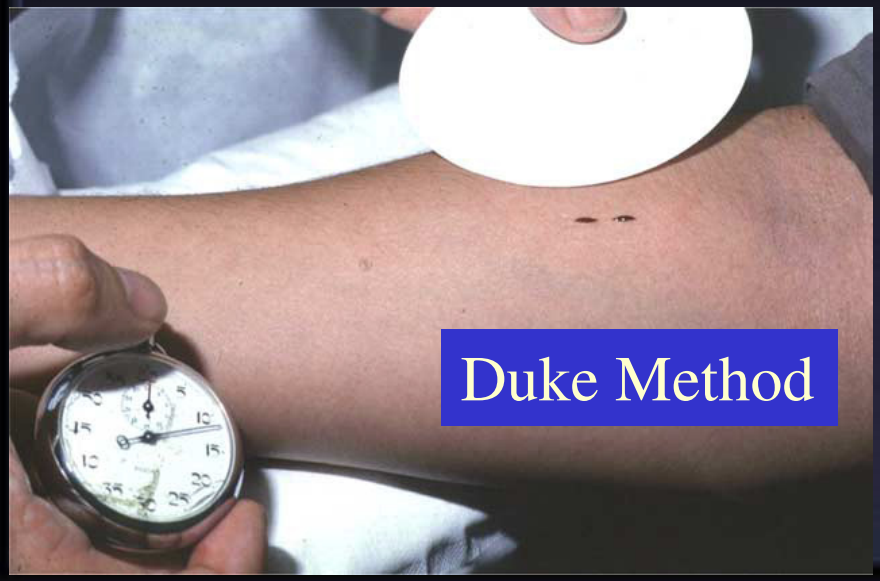


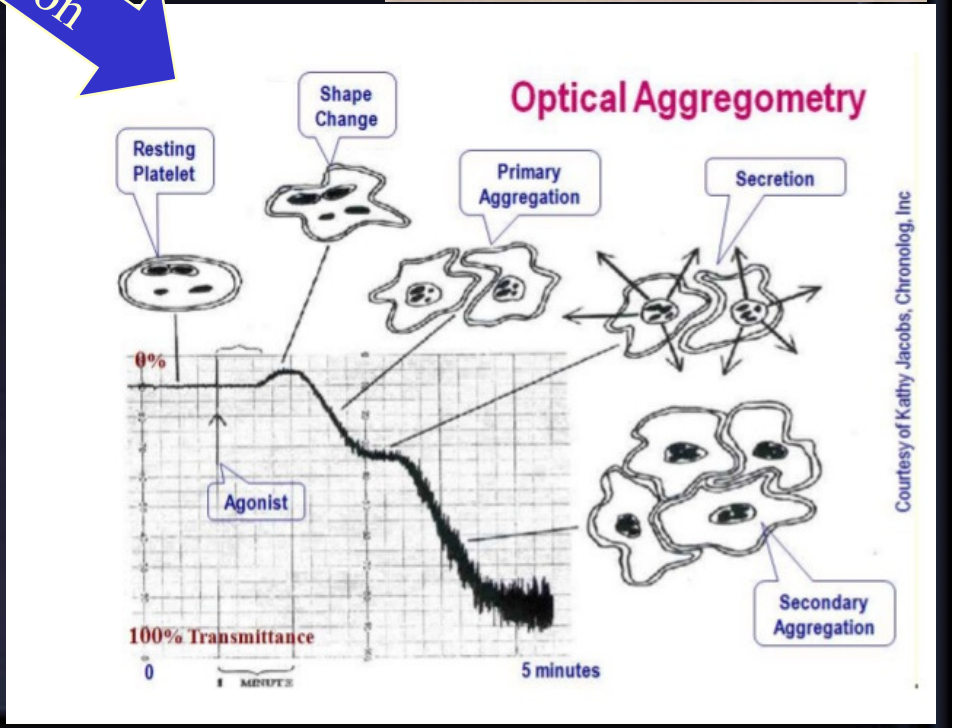
Testing Platelet Functions

- 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

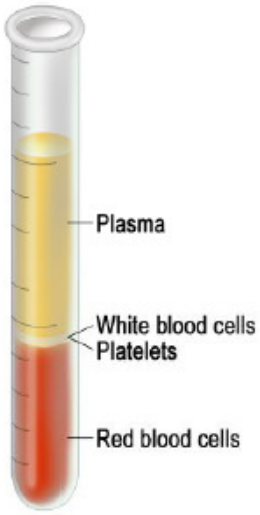




Laboratory Testing of Platelet Functions

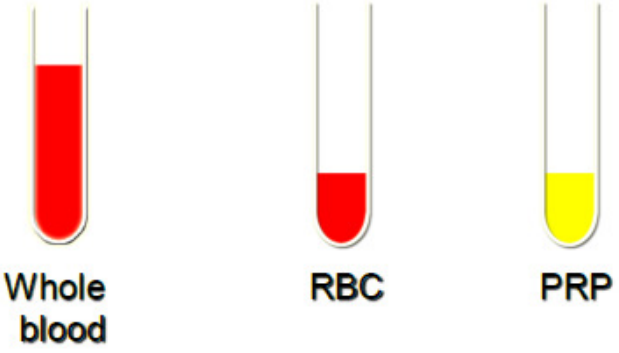
- **By Platelet Aggregation Method:** Provides information on time course of plat. activation.
- **Agonists:**
  - ADP
  - Adrenaline
  - Collagen
  - Arachidonic acid
  - Ristocetin
  - Thrombin
- Reference ranges need to be determined for each agonist

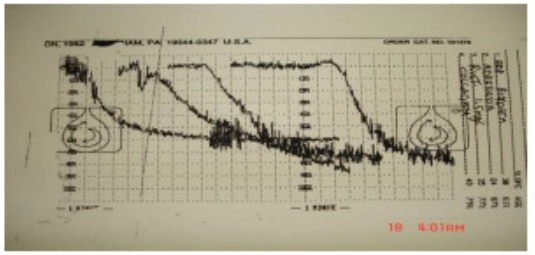
You need in Platelet rich plasma (PRP)



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

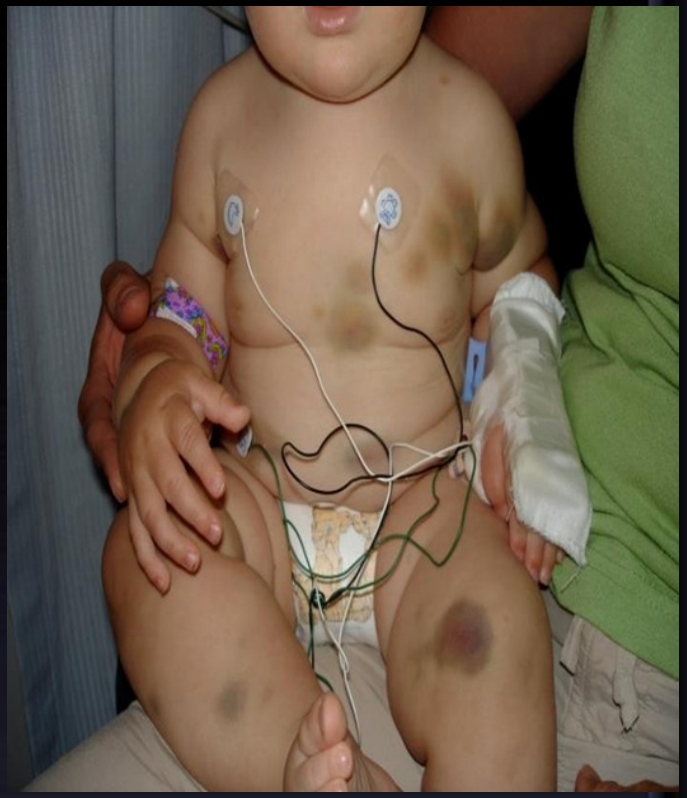
Test	Normal Value	Importance
PLATELET COUNT	100,000 - 400,000 CELLS/MM^3	Thrombocytopenia
PLATELET FUNCTIONS	Normal Aggregation	Thrombocytopathy (normal count) [Congenital or Acquired…Aspirin]
BLEEDING TIME (BT)	2-8 MINUTES	Bleeding disorders
PROTHROMBIN TIME (PT)	10-15 SECS	Measures Effectiveness of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

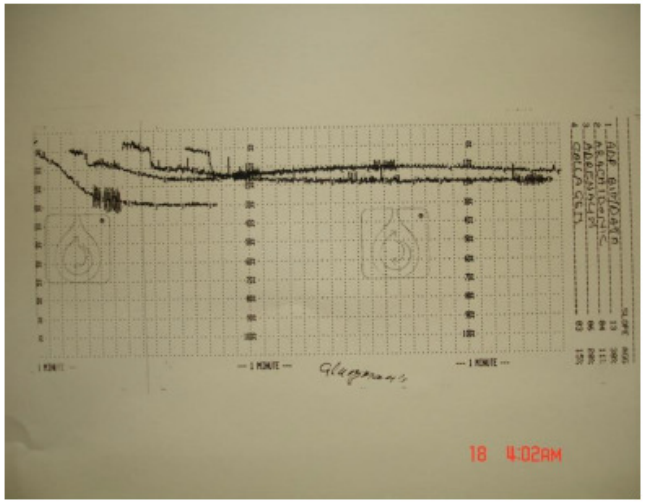
  Absent platelet aggregation in response to ADP,collagen ,thrombin and epinephrine.



Diagnosis:
Glanzmann's Thrombasthenia

Aggregometry:

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



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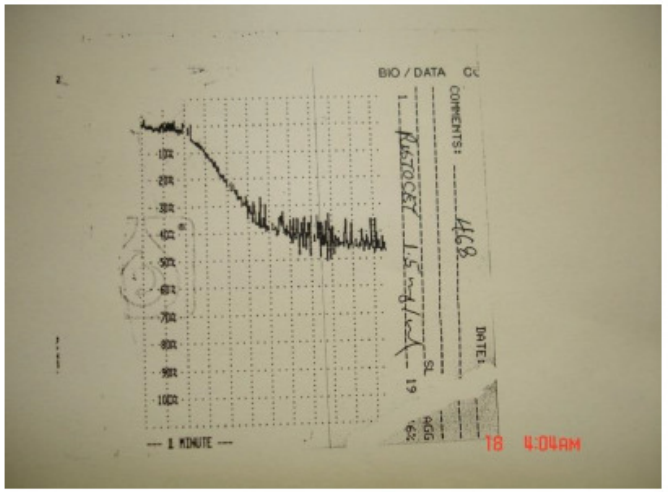
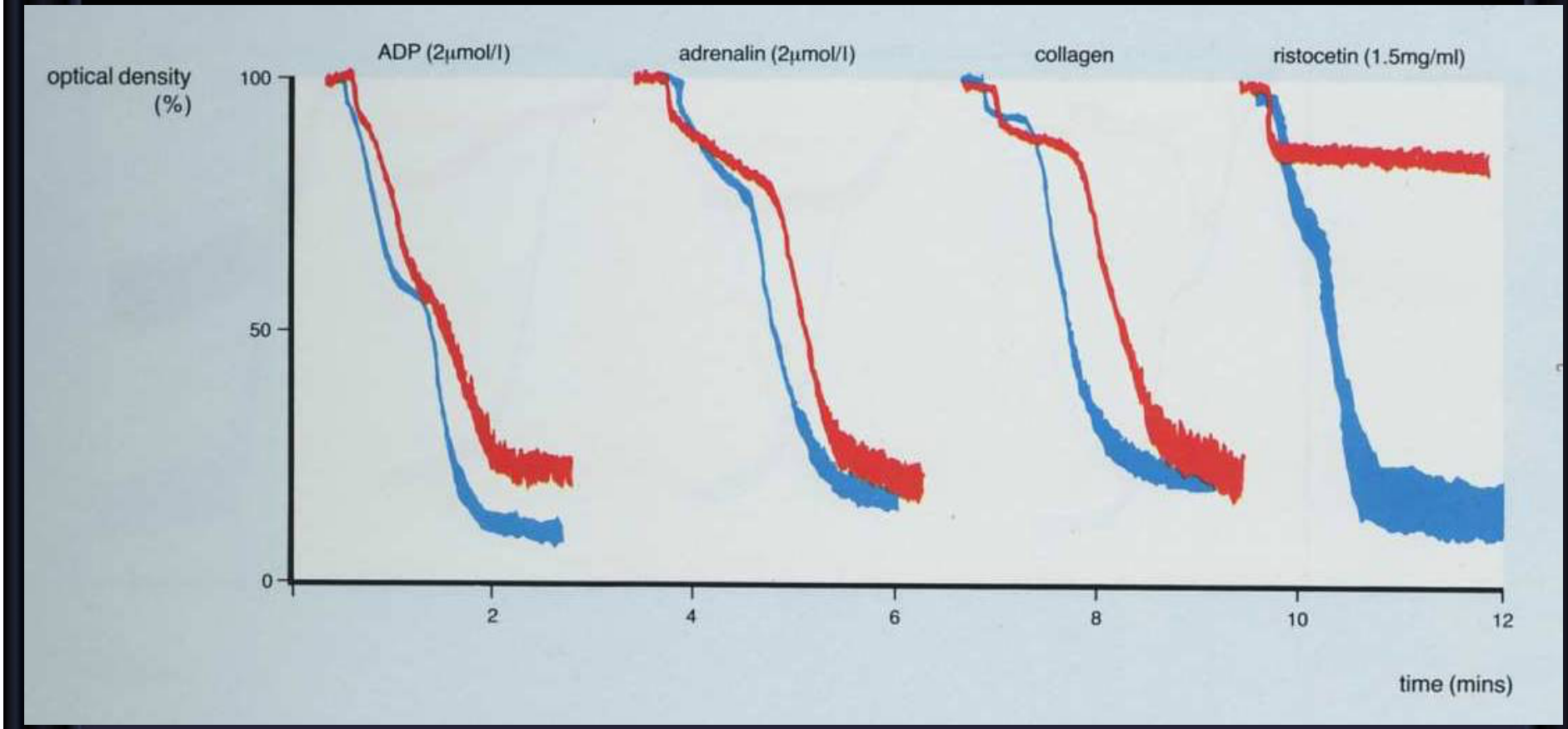
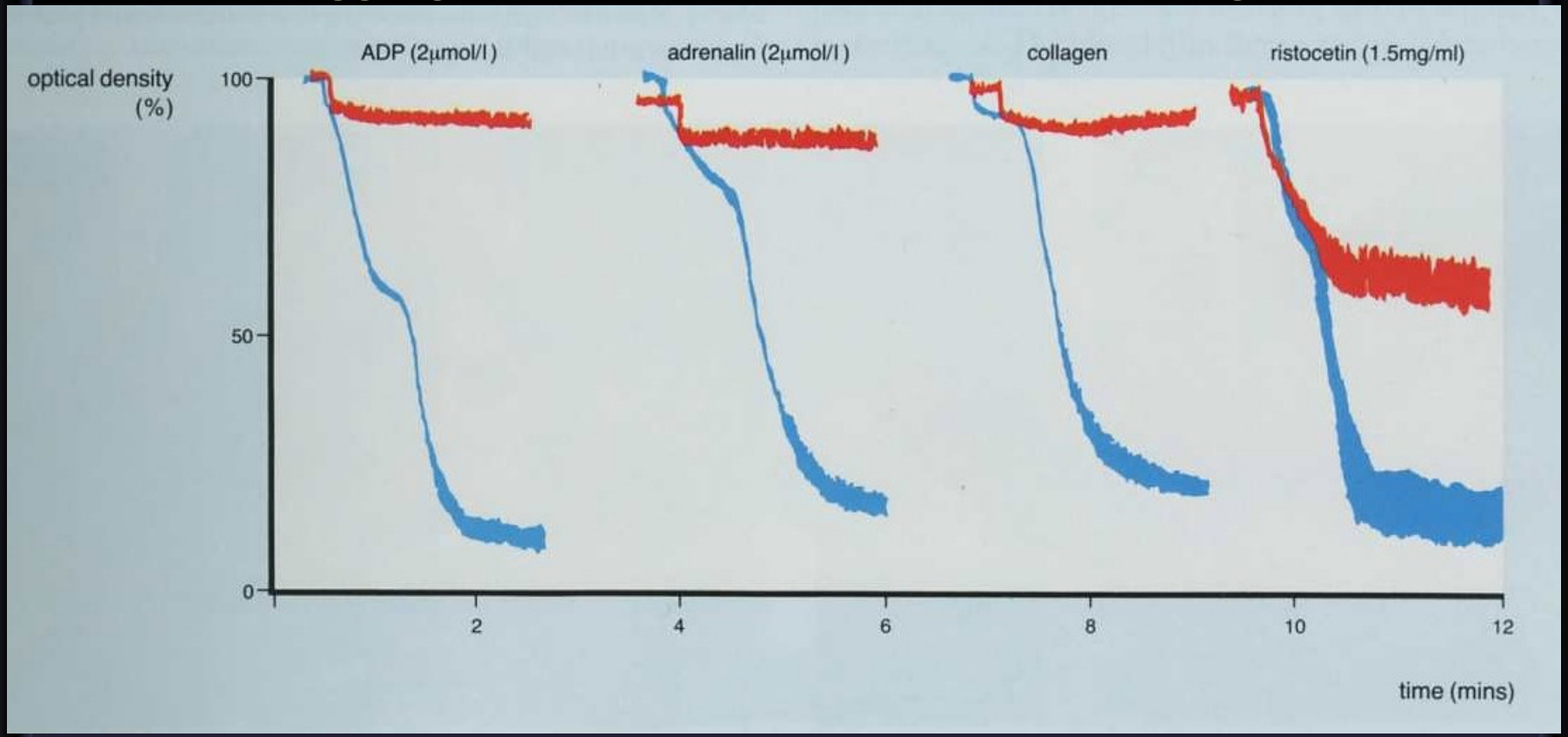


1. In the patient shown below, the only abnormality is a lack of agglutination with Ristocetin. Possible diagnoses are therefore, Von Willebrand Disease or Bernard Soulier Syndrome.

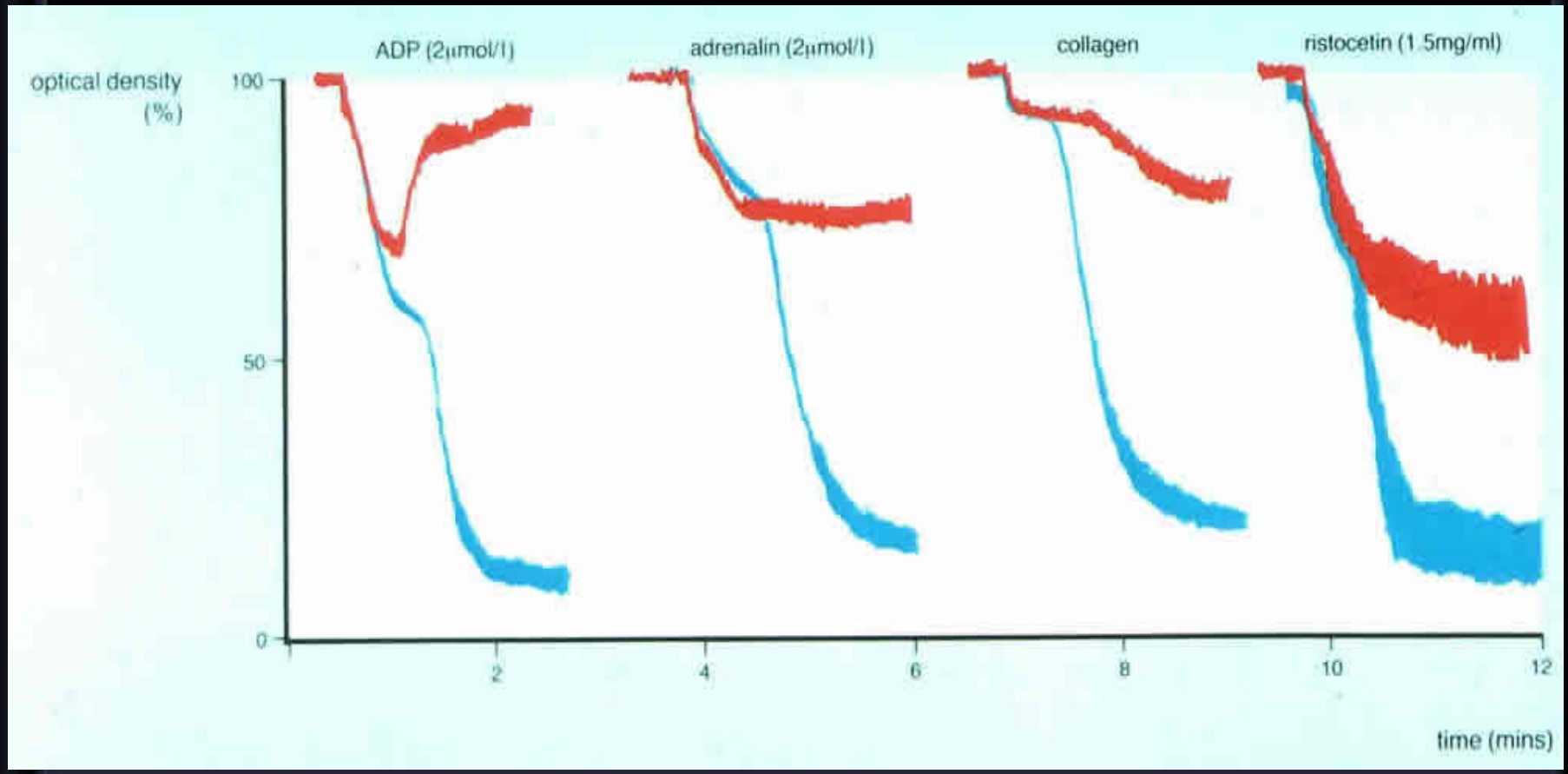


2. This is the converse of the first patient and the only agglutination [and this is not complete] is seen with Ristocetin. There is no aggregation with ADP, adrenaline or collagen.



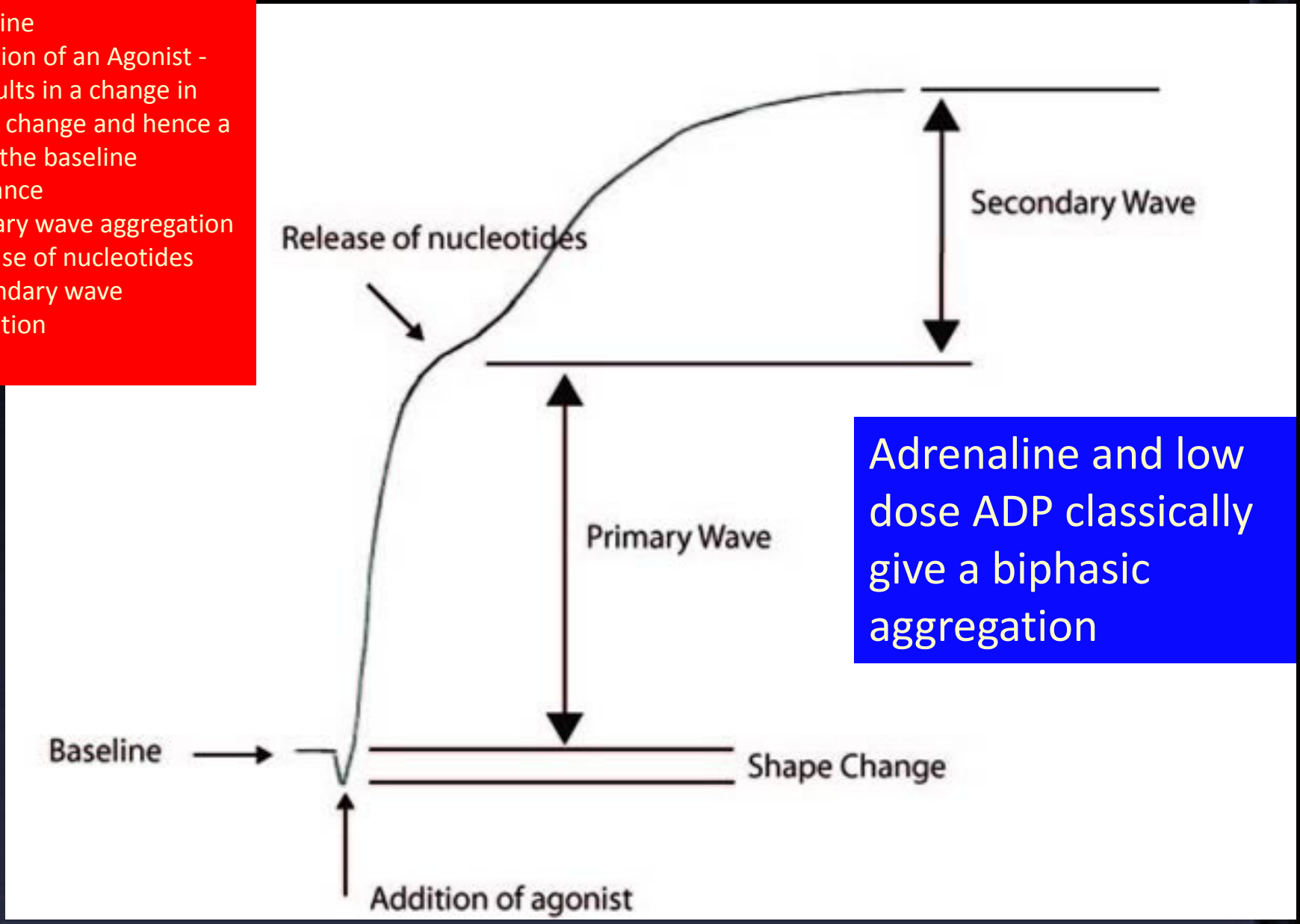
Possible diagnoses include Glanzmann's Thrombasthenia or Afibrinogenaemia.
[Remember, platelet agglutination with Ristocetin occurs independently of Fibrinogen.]

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.

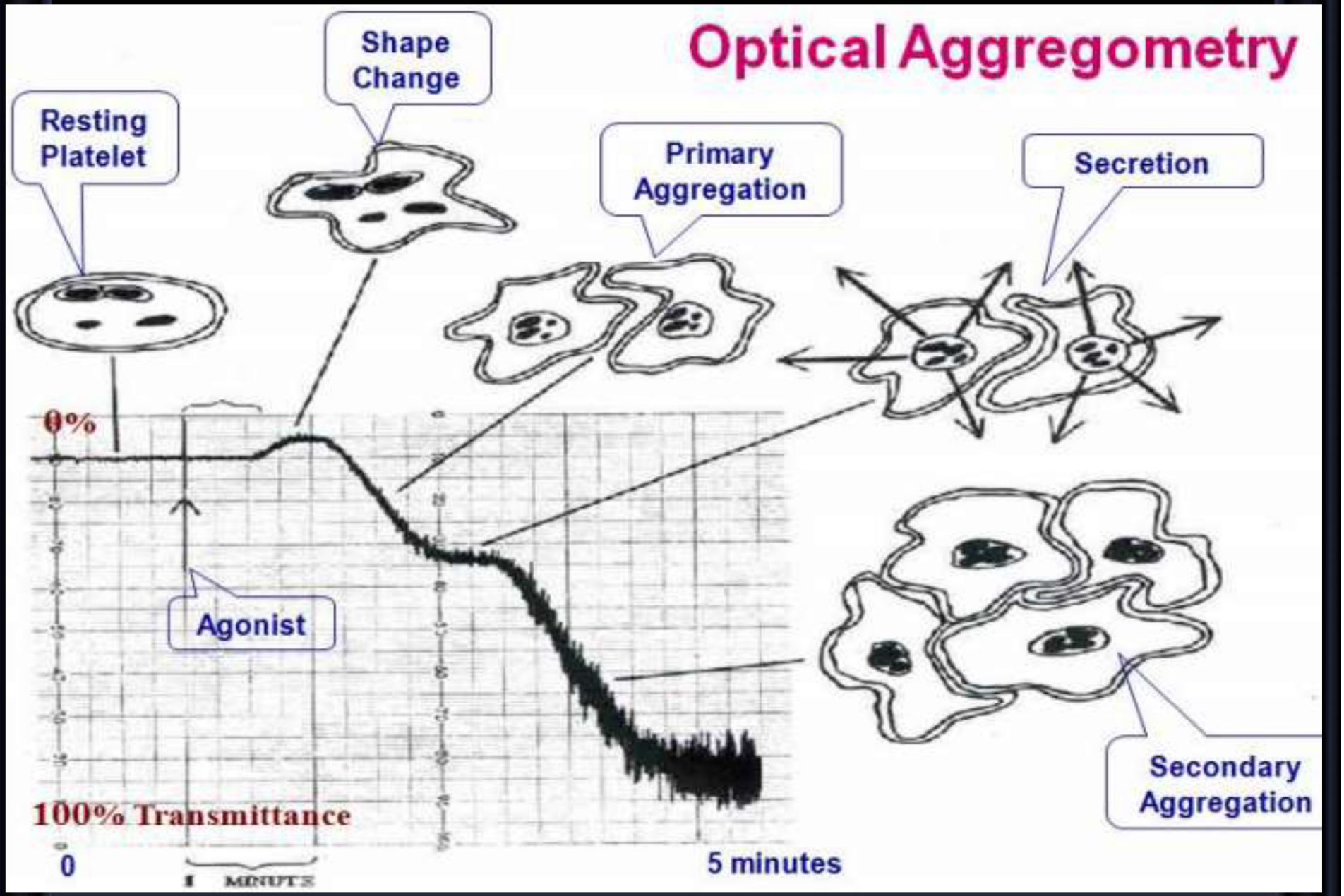


Classic biphasic aggregation:

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

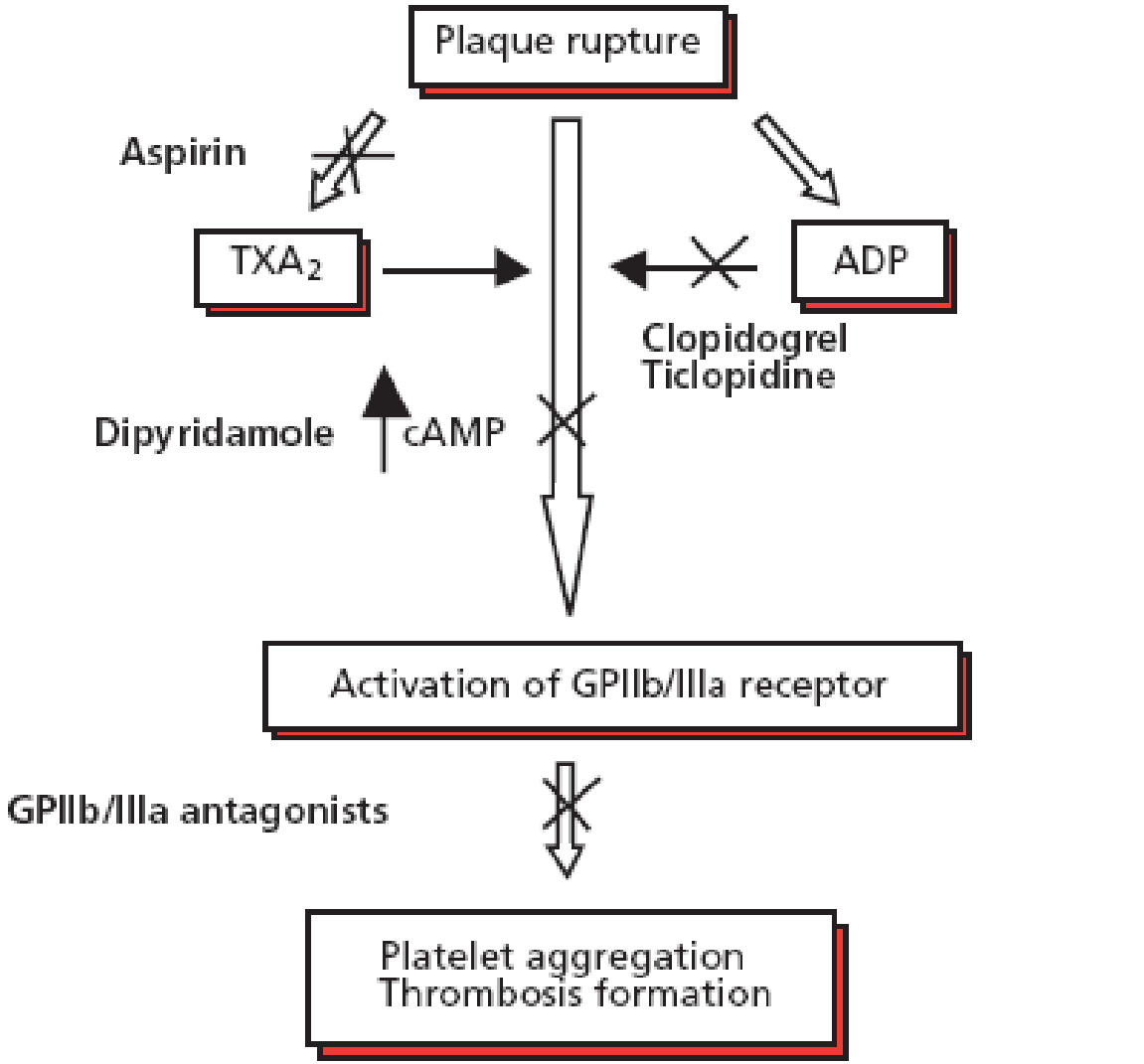


Adrenaline and low dose ADP classically give a biphasic aggregation


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

THROMBOCYTOPENIA

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

Decreased production

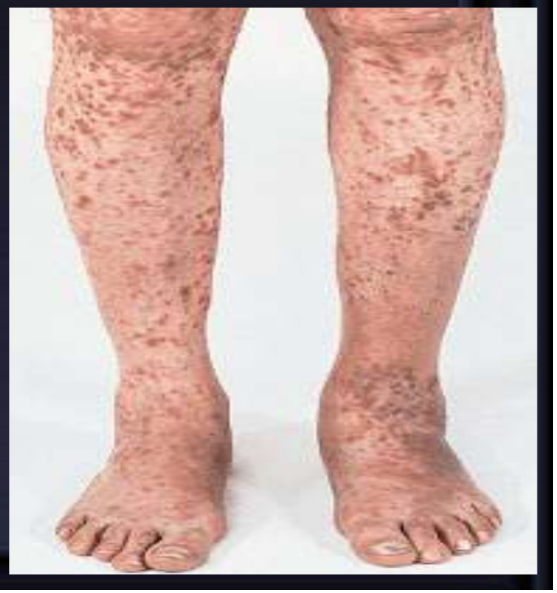
- Aplastic anemia
- Leukemia
- Drugs
- Infections (HIV, Measles)

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