# Coagulation: the bare essentials

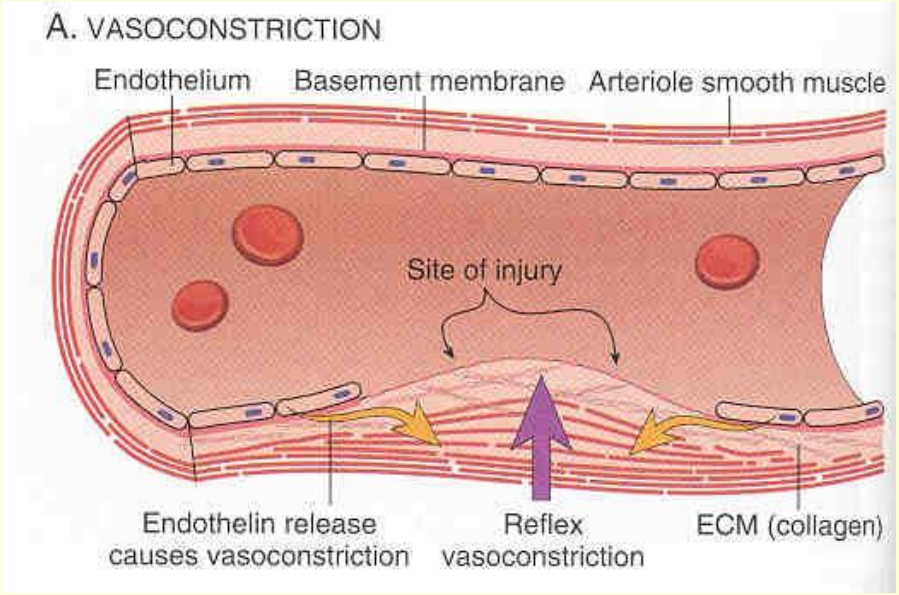
Md.Amer Wahed

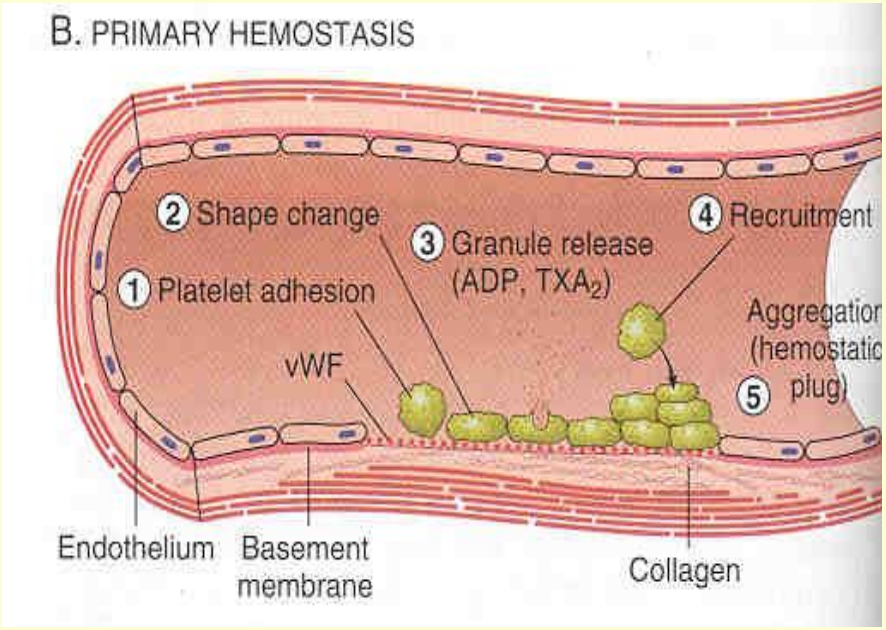
# Normal hemostasis

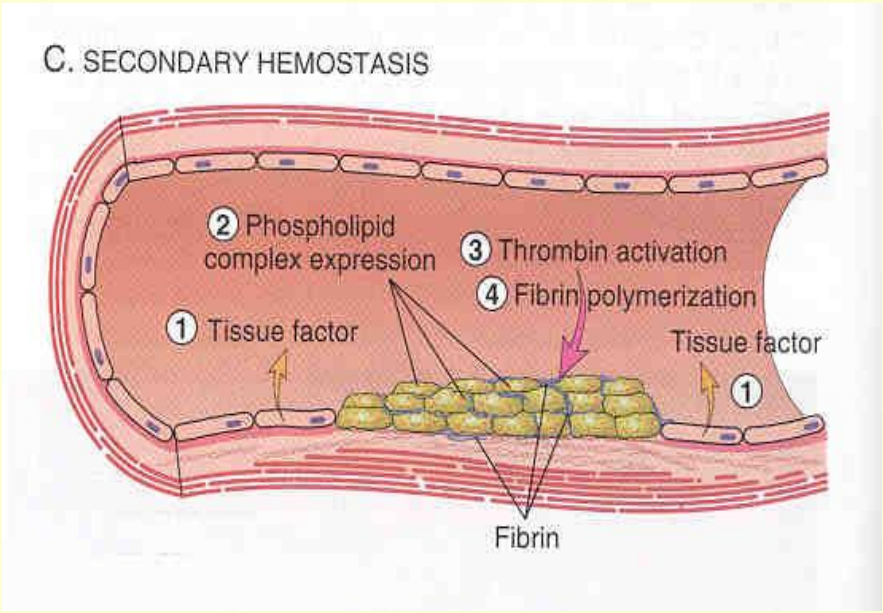
- Vasoconstriction:

  Reflex neurogenic mechanisms

  Augmented by endothelin
- Platelet plug (primary hemostasis)
- Activation of the coagulation cascade (secondary hemostasis)


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


B. PRIMARY HEMOSTASIS
2 Shape change
3 Granule release (ADP, TXA2)
4 Recruitment
1 Platelet adhesion
vWF
Aggregation (hemostatic plug)
5
Endothelium
Basement membrane
Collagen

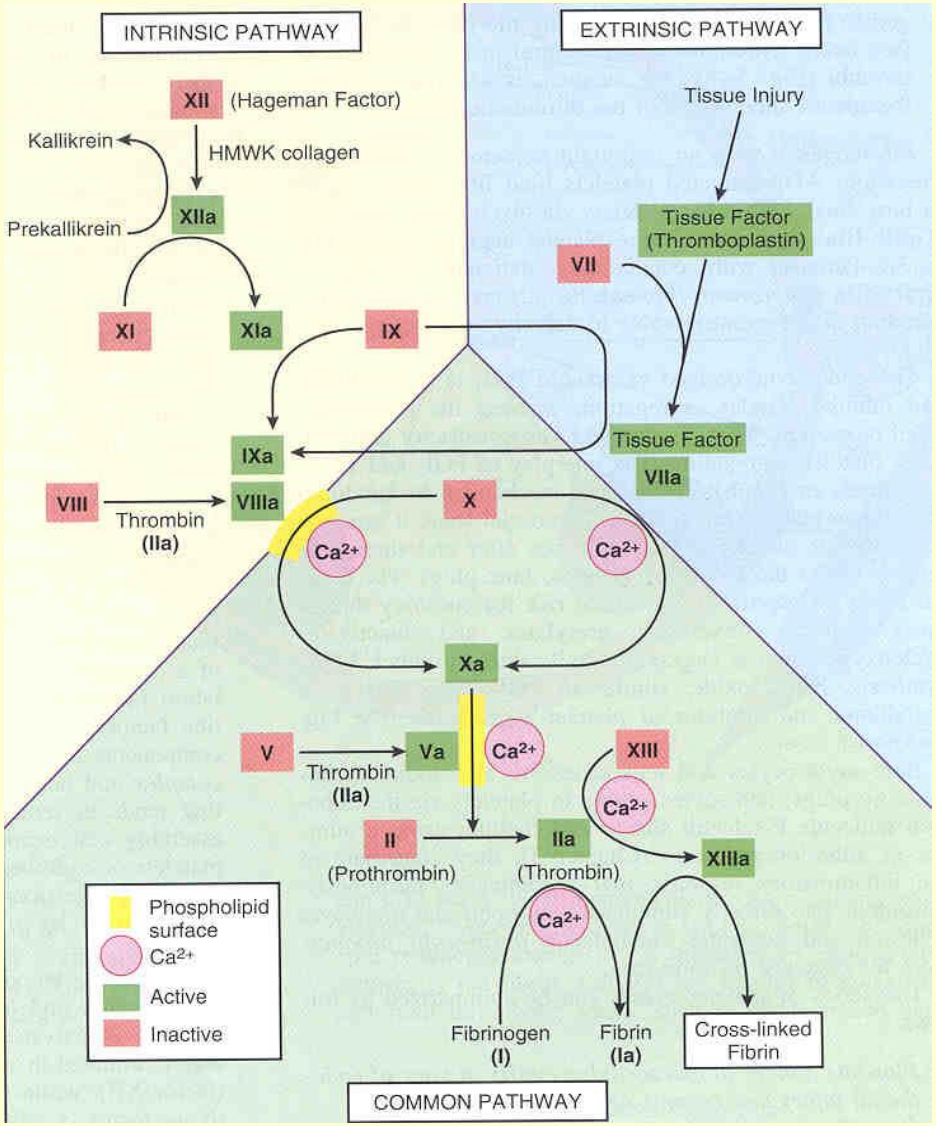

C. SECONDARY HEMOSTASIS
2 Phospholipid complex expression
3 Thrombin activation
4 Fibrin polymerization
1 Tissue factor
Tissue factor
1
Fibrin

# Clinical manifestations in a patient with bleeding disorder

| Findings | Coagulation Disorders | Platelet or Vessel Disorders |
|---|---|---|
| Petechiae | Rare | Characteristic |
| Deep hematomas | Characteristic | Rare |
| Hemarthroses | Characteristic | Rare |
| Delayed bleeding | Common | Rare |
| Bleeding from superficial cuts | Minimal | Persistent |
| Patient gender | Most inherited disorders in men | Most inherited disorders in women |
| Mucosal bleeding | Minimal | Typical |

| Vascular bleeding disorders |
| --- |
| **Hereditary** |
| Hereditary haemorrhagic telangiectasia |
| Ehlers–Danlos syndrome |
| Marfan's syndrome |
| Osteogenesis imperfecta |
| Fabry's syndrome |
| **Infections** |
| Bacterial |
| Viral |
| Rickettsial |
| **Allergic** |
| Henoch–Schönlein syndrome |
| Systemic lupus erythematosus |
| Drugs |
| Food |
| **Atrophic** |
| Senile purpura |
| Cushing's syndrome and corticosteroid therapy |
| Scurvy purpura |
| Dysproteinaemia |
| Amyloid |
| **Miscellaneous** |
| Simple easy bruising |
| Factitious |
| Autoerythrocyte sensitization |
| Fat embolism |

# Secondary hemostasis




FVIII produced by endothelium, not liver

Individuals with FXII, Prekallikrein and HMWK def do not bleed

FVII has the shortest $T^{1/2}$

# PT

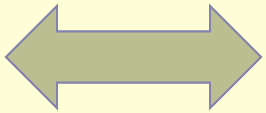
- Patient's platelet-poor plasma, tissue thromboplastin, and calcium are mixed; and clotting time is determined.
- Assessment of extrinsic pathway and common pathway
- Reported as INR (international normalized ratio)
- Thromboplastin used may vary from lab to lab and country to country; giving variable PT
- INR is used for standardization
- INR=[Patient PT/Mean of normal PT range]$^{ISI}$

(ISI: international sensitivity index)

# Prolonged PT

- Coumadin
- Vit K def
- Failure of absorption of Vit K (cholestasis, short-bowel syndrome, etc.)
- Liver disease
- Factor def in extrinsic and common pathways

# Coumarin

- Vit K  Vit K epoxide

  epoxidase

  reductase

- Coumadin blocks reductase and non-functional epoxide accumulates

# Vit K

- The Vit K dependent factors (II, VII, IX, X) have 9-12 glutamic acid residues near the amino terminal end, which needs to be carboxylated (Vit K dependent).
- Vit K dependent proteins:
  II, VII, IX, X, proteins C and S

# aPTT

- Patient's platelet-poor plasma, surface activating agent (silica) and platelet substitute (crude phospholipid or partial thromboplastin) are mixed, and clotting time is determined.
- Assessment of intrinsic and common pathways.

# An isolated prolonged PTT

- Heparin
- Factor deficiency: VIII, IX, XI, XII
- Inhibitors: VIII and IX inhibitors, lupus anticoagulant
- vWD
- HMWK (Fitzerald) def
- Pre-kallikrein (Fletcher) def

# R/O Heparin

- History
- Prolonged Thrombin Time
- Normal reptilase time

# Thrombin Time (TT)

- Patient's plasma and thrombin is mixed, and clotting time is determined.
- Heparin produces prolonged TT but normal reptilase time
- Functional fibrinogen (Clauss method) is based on TT, using diluted plasma sample

# Prolonged TT

- Heparin
- Hypofibrinogenemia
- Dysfibrinogenemia
- Thrombolytic therapy

# Approach to a prolonged PTT

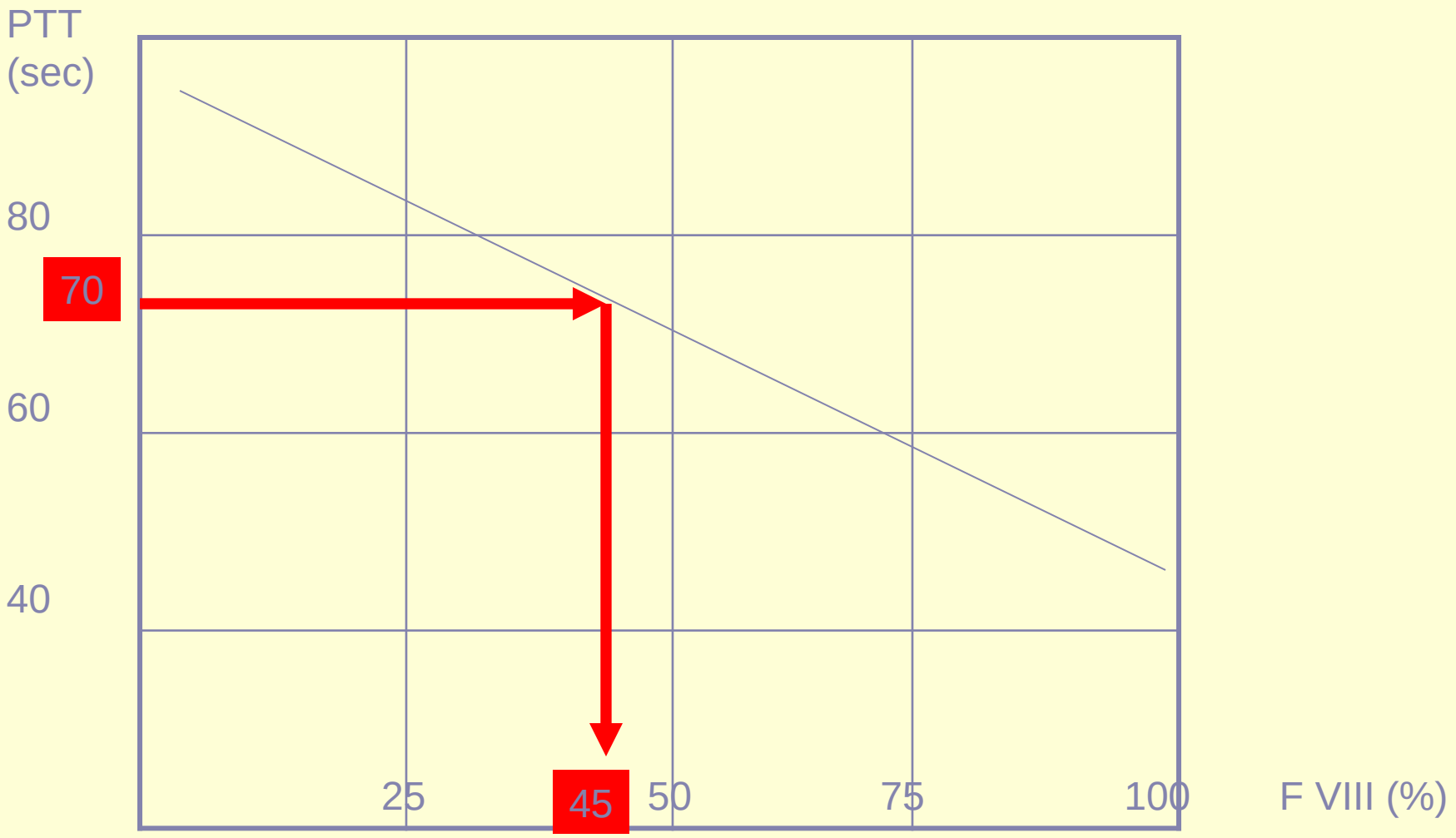
# Mixing study

- Patient's plasma is mixed with an equal volume of normal plasma (1:1 mix)
- PTT measured at 0 hour (immediate) and 1-2 hours after incubation.
- Failure of correction of prolonged aPTT means inhibitors
- If results at 0 hour and 1-2 hours are similarly prolonged-> lupus anticoagulant, heparin
- If results show time-dependent prolongation -> coag factor antibody (esp. F VIII inhibitor)

# Factor VIII assay

- Factor VIII level is inversely proportional to PTT
- A standard curve (PTT vs F VIII) is first set up using commercial assayed samples
- Mutiple dilutations of patient's sample (using F VIII-deficient substrate) are tested for PTT.
- These PTT's are plotted on the standard curve to intrapolate for F VIII
- Each F VIII is multiplied by the dilutation factor to obtain the actual F VIII before dilution
- F VIII level is the mean of F VIII's from multiple dilutions

# Factor VIII Standard Curve



# Factor assay

- For IX, XI, XII, prekallikrein and HMWK: the same principle as for FVIII
- For factors V, VII and X : the same principle except that PT is used instead.
- For fibrinogen: Clauss method based on Thrombin Time with diluted plasma (report level as mg/dl)

# Hereditary clotting factor deficiencies

- Hemophilia A (VIII def), B (IX def), C (XI def)
- I, II, V, VII, X, XIII deficiency
- Dysfibrinogenemia

- Hemophilia A, B are X-linked recessive
- Dysfibrinogenemia : autosomal dominant
- All others: autosomal recessive

# Acquired Clotting factor deficiency

- Anticoagulants (coumadin)
- Fibrinolytic therapy
- DIC
- Liver disease
- CP bypass

# Hemophilia A

- Mild: >10% activity
- Moderate: 2-10% activity
- Severe:<1% activity
- Treatment:
- DDAVP for mild cases (2-10 fold increase in Factor VIII level)
- Factor VIII replacement (1 unit/kg raises FVIII level by 2% ($T^{1/2}$:8hrs)

# Target FVIII activity

- 100%: surgery; CNS bleeding; GI and genitourinary bleeds.
- 40-80%: bleeding into joints and muscle

# Calculation

- Blood volume= weight (in kg) x 70 ml
- Plasma volume (PV) = blood volume x (1-Hct)
- Dosage of FVIII= PV x change in level

- Example: 80 kg_patient, Hct = 30%, needs to increase FVIII from 50% to 100%

  PV=80x70 (1-0.30)=3920 ml
  Dosage=3920x(100-50)/100=
  1960 IU of FVIII concentrates

# Factor Inhibitors

- Mixing study: no correction
- Spontaneous inhibitors (typically in autoimmune diseases) can go away
- Acquired inhibitors (in hemophilia A patients with chronic FVIII infusions) are persistent

# Inhibitors

- Lupus anticoagulant: dilute Russell Viper Venom Time (DRVVT), confirm with Platelet Neutralization Procedure (PNP)
- Factor VIII or IX inhibitor:

  Mixing study does not show correction

  Factor VIII/IX levels very low (functional activity)

# DRVVT and PNP

- DRVVT: is based on the principle that LA exacerbates the prolongation of phospholipid-dependent clotting time when phospholipid is diluted.
- PNP: LA prolongs aPTT due to its phospholipid inhibiting property. Addition of lysed platelets would correct the aPTT by adsorbing the antibody.

# Factor VIII inhibitor

- Seen in approximately 1% of all hemophiliacs (typically severe type)
- 1 Bethesda unit (BU) of inhibitor is the amount of inhibitor that inhibits half the factor VIII activity in an equal mixture of patient and normal plasma.
- Treatment:
  FEIBA (factor VIII inhibitor bypass activity)
  Novo Seven
  Porcine F VIII

# Bleeding with normal PT/PTT

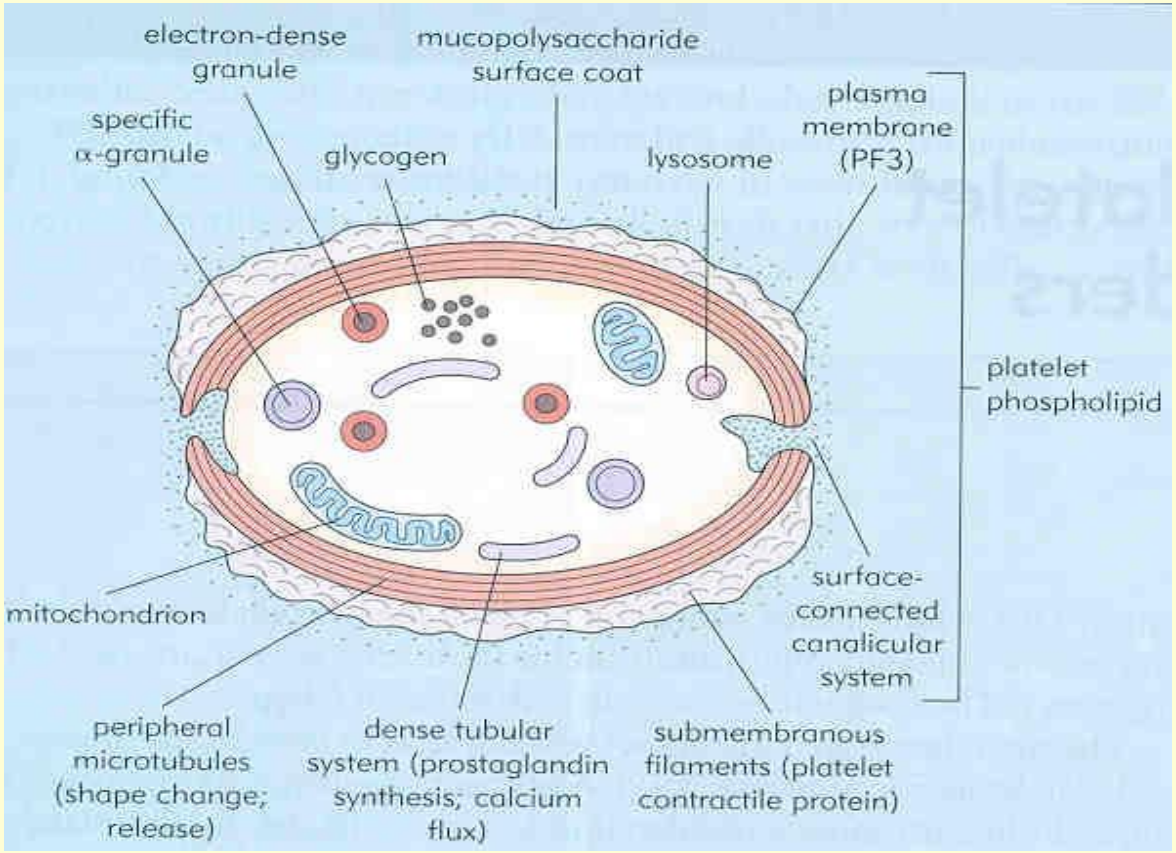
- Factor XIII deficiency (clot is soluble in 5M urea solution in 24 hours); Tx: cryo
- Alpha2-antiplasmin def, Tx: amino caproic acid (EACA)

# Fibrinogen correction using cryoprecipitates

## Number of Units (bags) of Cryo:

- $\frac{\text{Plasma volume ml} \times (\text{desired level} - \text{initial level}) \text{ mg/dl}}{100} = X$
- Number of units of cryo = X/150

# Platelets




Circulate for 10 days; 1/3 sequestered in spleen

# Platelets

- Alpha granules:

  Fibrinogen, fibronectin

  Factor V, vWF, PF-4, PDGF, TGF-beta

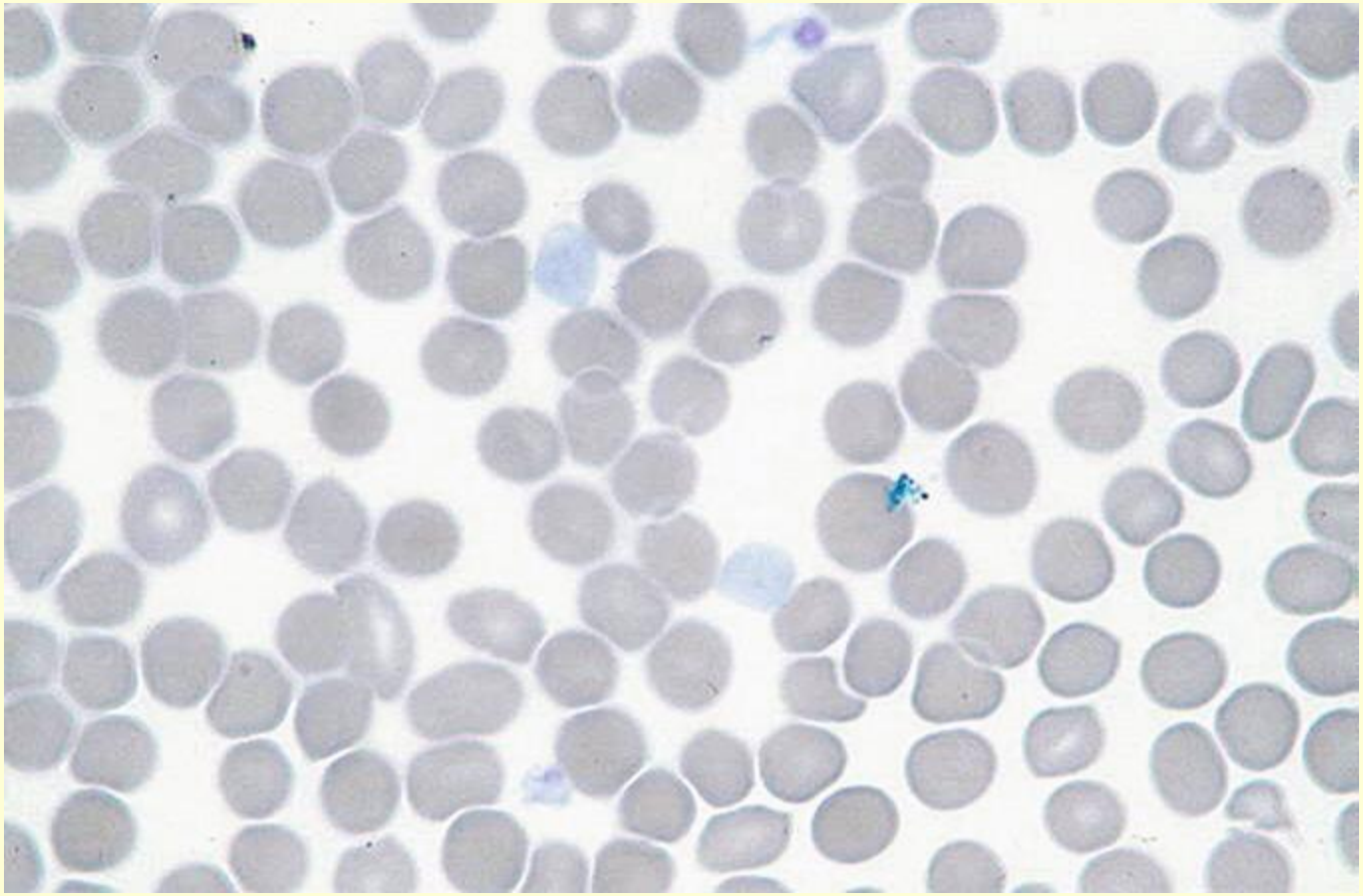
  Thrombospondin
- Dense bodies (delta granules):

  ATP, ADP, ionized calcium, histamine,

  5-HT, epinephrine
- Lysosomes containing acid hydrolases

- Alpha granules: stained by Wright-Giemsa stain
- Delta granules: electron dense due to calcium

# Platelet granule deficiency: blood smear

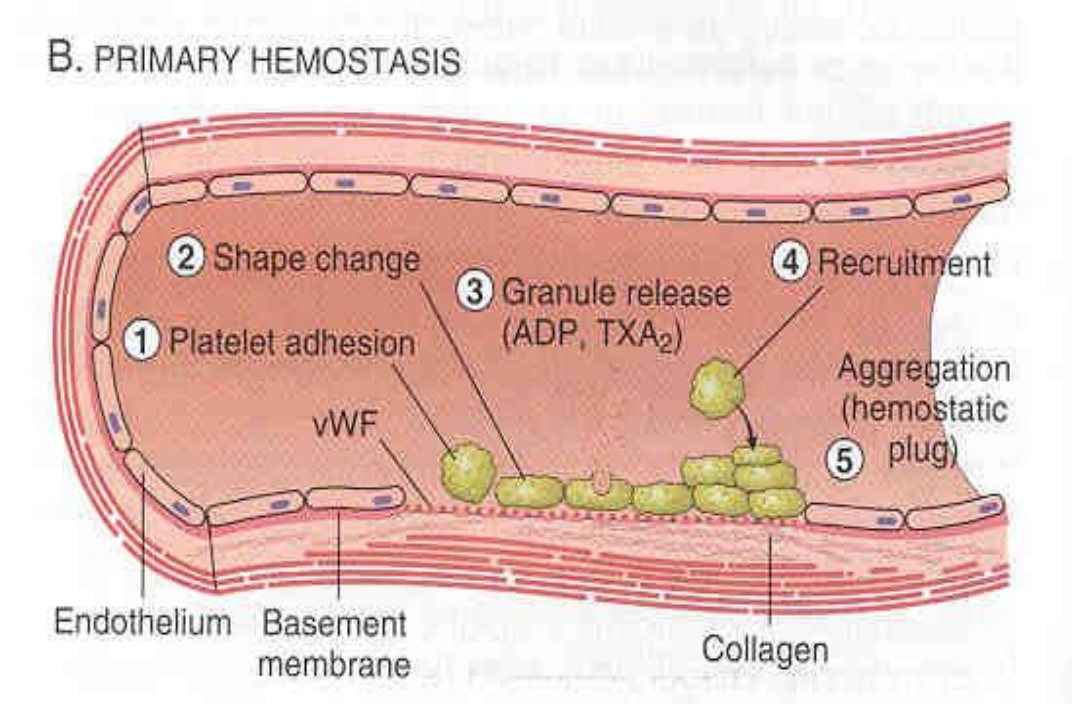


# Platelet events

- Adhesion and shape change
- Platelet release reaction
- Aggregation

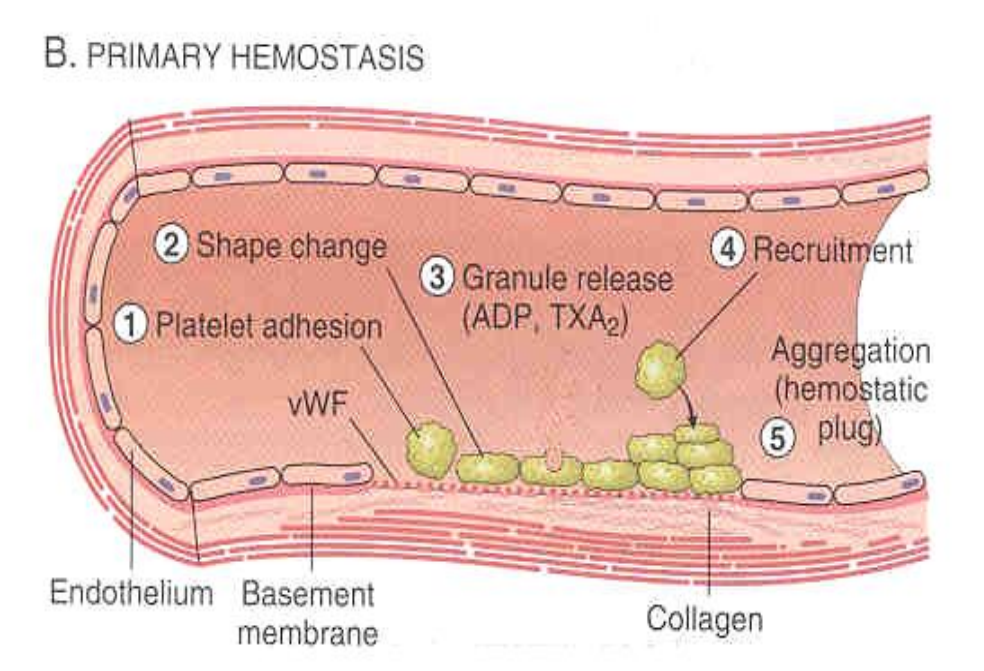
# Platelet Adhesion

- Interaction between vWF and GP Ib/IX/V receptors
- Conformational change in HMW multimers of vWF upon exposure to subendothelial collagen



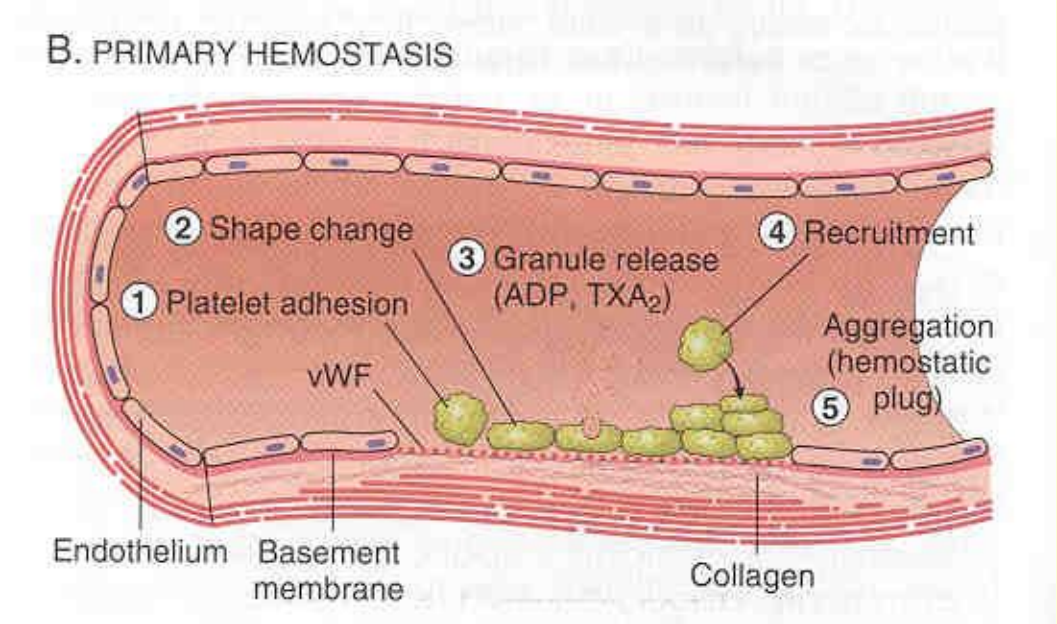
# Platelet Activation

- Agonists: ADP, Thrombin, Tx A2, Collagen, vWF
- Rapid rise in cytoplasmic calcium
- Shape change; extension of pseudopodia
- Release reaction
- Activation of ligand binding site on GP IIb/IIIa
- Translocation of phosphatidylserine to external surface



# Platelet Aggregation

- Fibrinogen mediates binding of activated GP IIb/IIIa receptors on adjacent platelets
- Augmented by Thrombospondin; a component of $\alpha$-granules



# Platelet bleeding disorders

- Thrombocytopenia
- Dysfunctional platelets

# Clinical presentation

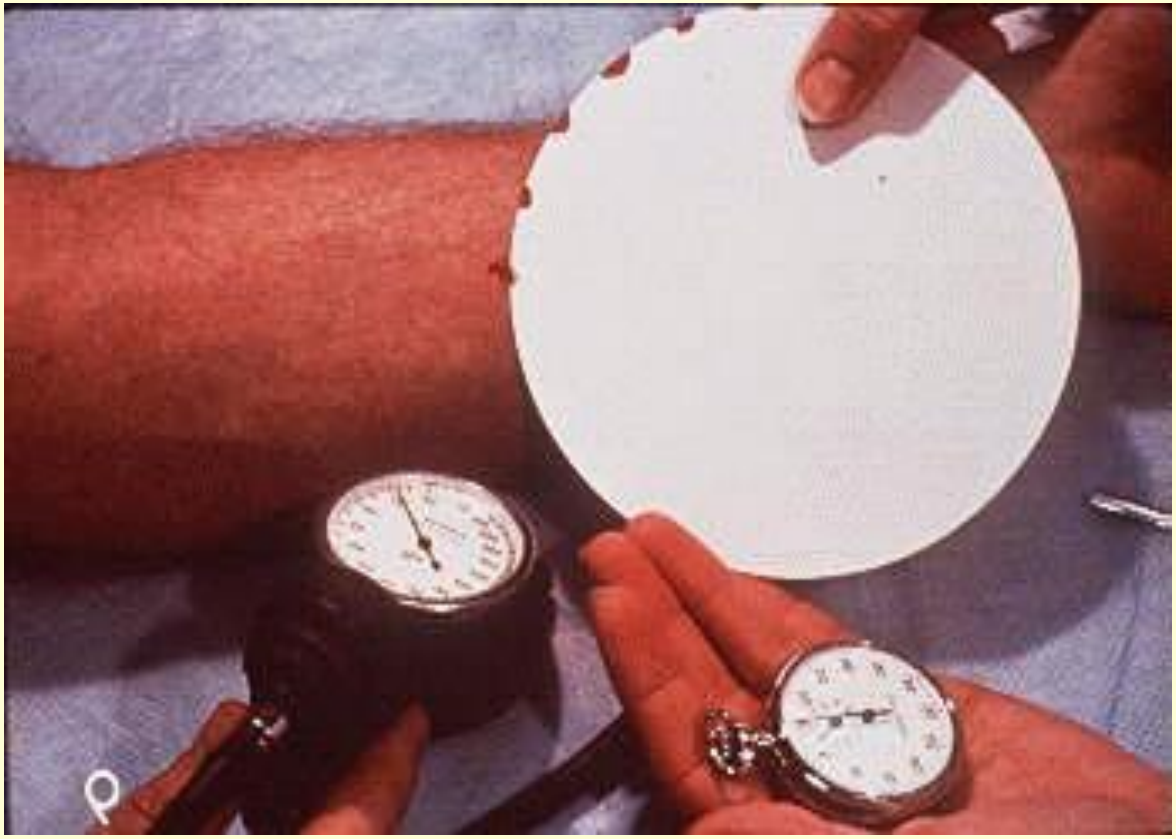
- Purpura
- Mucosal  bleeding
- Prolonged bleeding from superficial cuts and abrasions
- Menorrhagia

# Investigations for platelet disorders

- Bleeding time (poor predictive value)
- CBC, peripheral smear
- BM examination
- Platelet aggregation studies

# Bleeding time

- BP cuff at 40mmHg
- Two small punctures on flexor aspect of forearm
- Drops of blood are absorbed with filter paper disks every 30s.
- Poor clinical correlation; can be useful for patients with bleeding manifestation



Bleeding time: reference range < 9 min

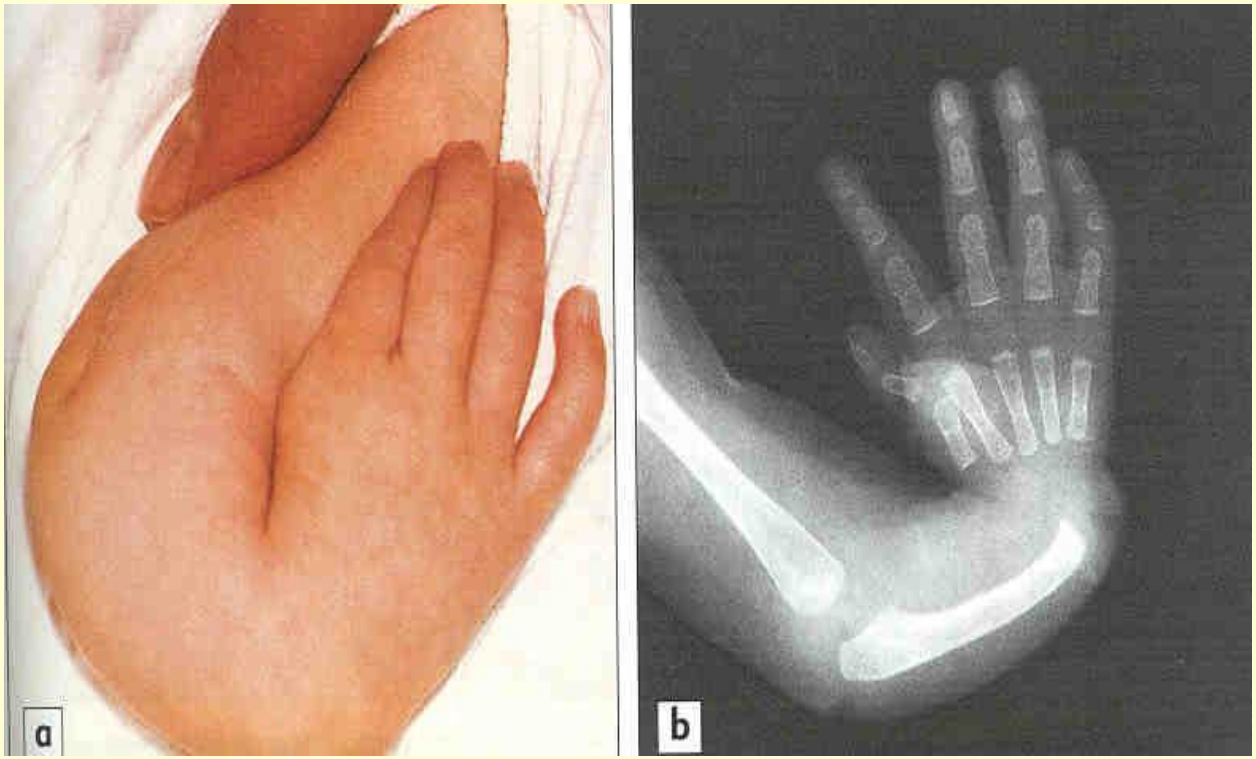
# Thrombocytopenia

- Decreased production
  Generalized BM failure
  Selective megakaryocyte depression
- Increased breakdown: ITP, HIT, Neonatal and post-transfusion purpura
- Increased utilization: DIC, TTP, HUS
- Increased sequestration: Kasabach-Merritt syndrome (hemangioma)

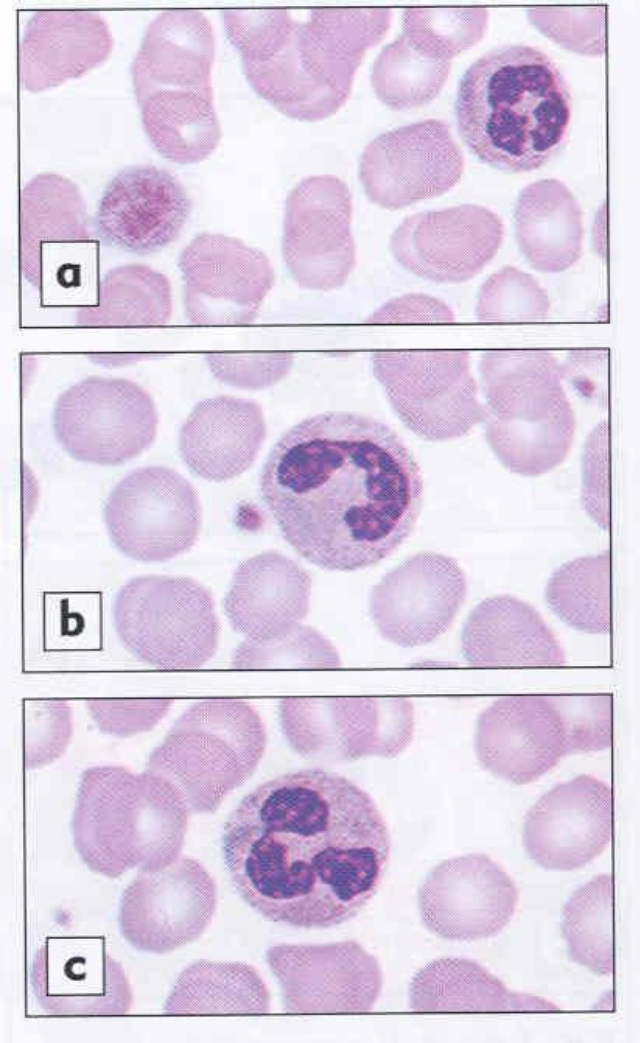
# Congenital diseases associated with reduced platelet production

- TAR syndrome (autosomal recessive)
- Fanconi's anemia (autosomal recessive)
- Wiskott-Aldrich syndrome (X-linked recessive)
- May-Hegglin anomaly (autosomal dominant)

# TAR syndrome
# (thrombocytopenia with absent radius)



# May-Hegglin anomaly

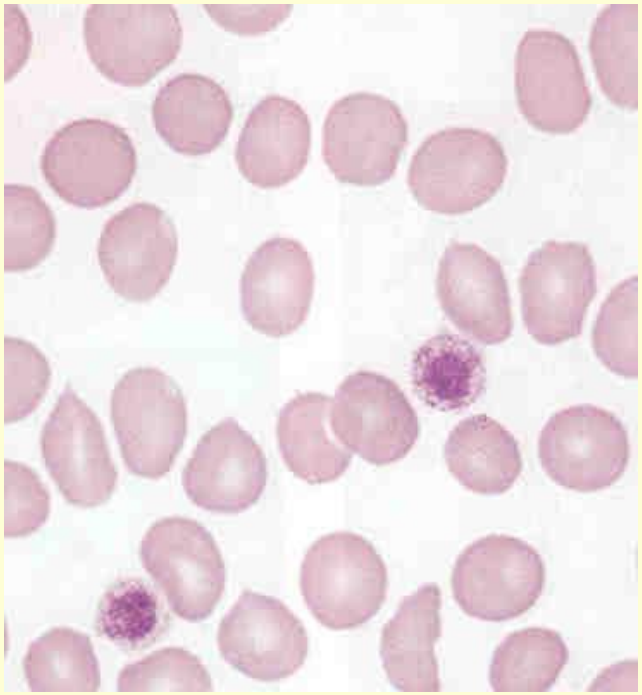



Thrombocytopenia, giant platelets
Dohle-like bodies
Autosomal dominant

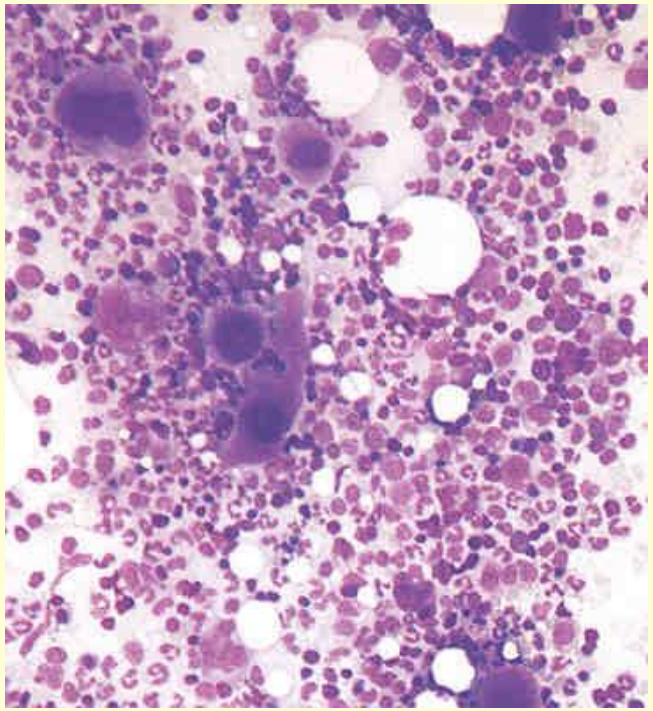
# ITP

- Immune destruction of platelets
- Increased megs in BM; large and giant platelets in peripheral smear
- Acute: self limiting
- Chronic: >1 year; 10% with splenomegaly
- Antibody against pathogen which cross reacts with GPIb/IX, GPIIb/IIIa

# ITP



Giant platelets



Increased megakaryocytes in BM

# ITP

- ITP may present as part of Evan's syndrome (with autoimmune hemolytic anemia)
- ITP may occur in patients with SLE, HIV, CLL and following stem cell transplantation
- Treatment:

  Steroids

  IVIg

  Rituximab

  Oncovin

  Splenectomy

# HIT/HAT

- Antibody binds to PF-4/heparin and results in platelet aggregation and subsequent thrombosis
- 7-10 days post heparin

# Investigation and management of HIT

- Heparin induced platelet aggregation studies: patient's serum and normal platelets with heparin, look for positive response (aggregation > 25%)
- 14C-Serotonin release: serum samples from patients with heparin-induced thrombocytopenia initiate 14C-serotonin release from labeled platelets at therapeutic but not high concentrations of heparin.

# Treatment of HIT

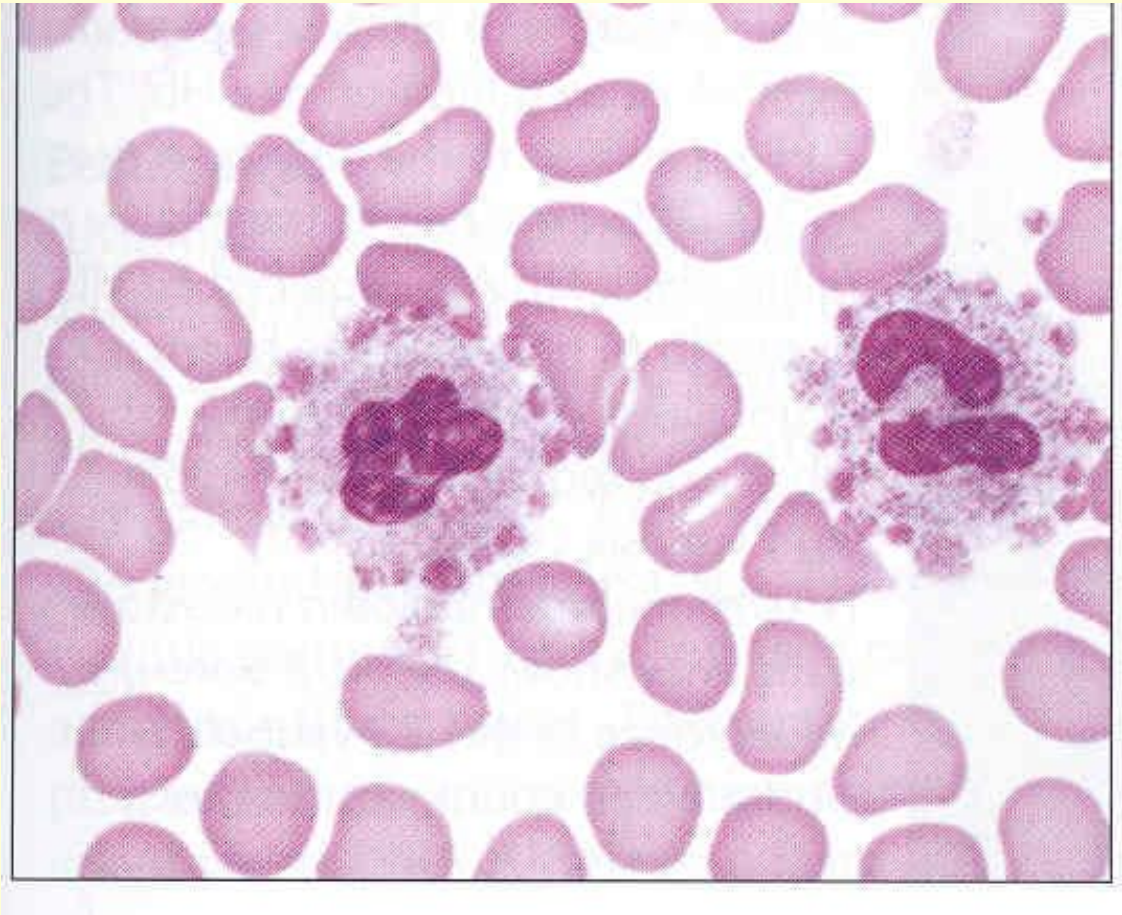
- Direct anti-thrombin agents:

  Lepirudin (Hirudin analog)

  Danaparoid (10% cross reaction)

  Argatroban
- LMW Heparin: not recommended due to cross-reaction

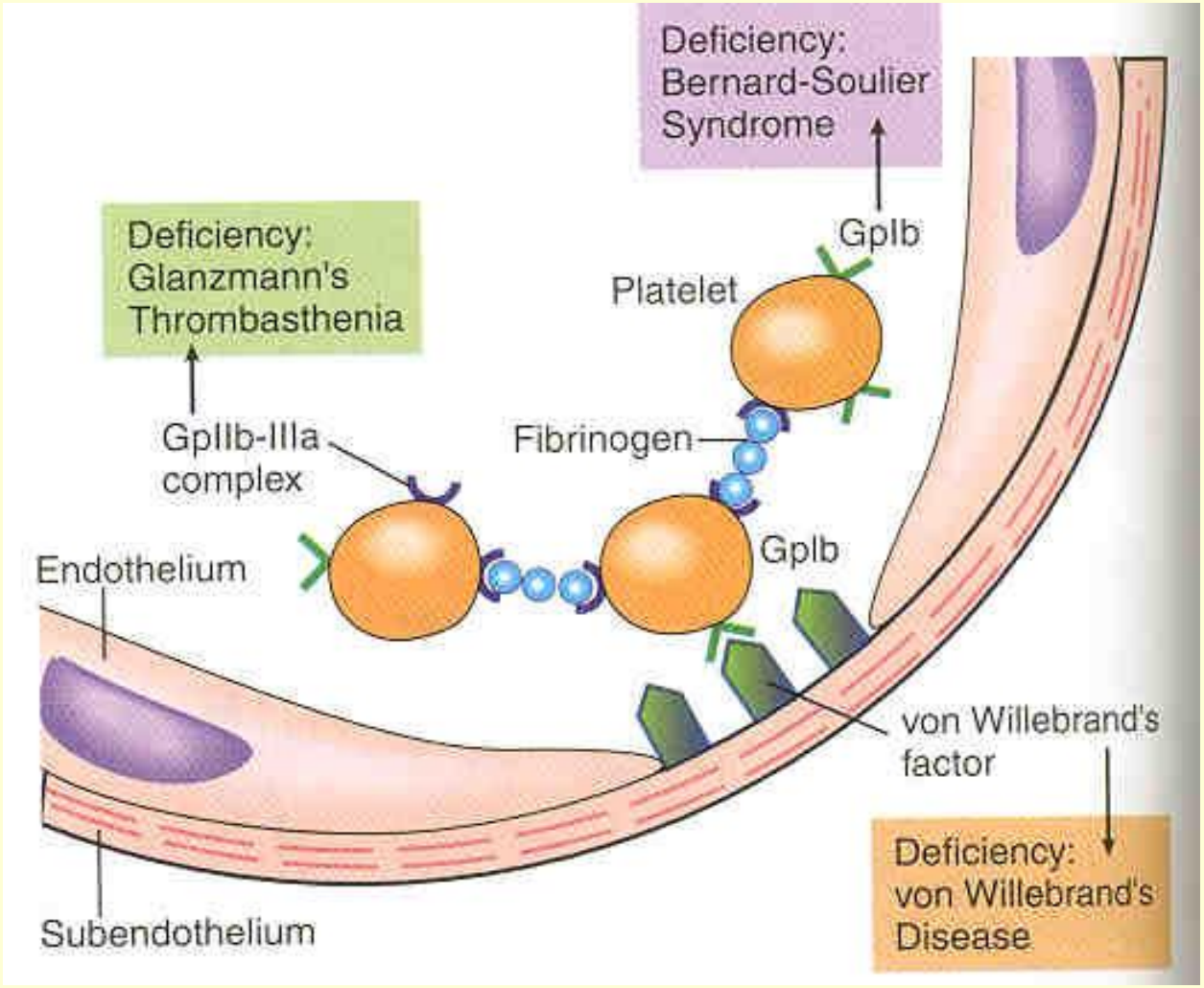
# Pseudothrombocytopenia due to EDTA antibody



# Congenital Platelet Disorders

- Disorders of platelet adhesion:
  - von Willebrand's disease
  - Bernard-Soulier syndrome
- Disorders of platelet activation
- Disorders of platelet aggregation:
  - Glanzmann's syndrome

# Defects in platelet adhesion and aggregation



# Dysfunctional Platelets

| Membrane Receptor Disorders | Storage Pool Disorders | Biochemical Signaling Disorders |
|---|---|---|
| Collagen receptor deficiency | Hermansky-Pudlak syndrome | Cyclooxygenase deficiency |
| $\alpha_2$-Adrenergic receptor deficiency | Wiscott-Aldrich syndrome | Thromboxane synthetase deficiency |
| ADP receptor deficiency | Chediak-Higashi syndrome | Decreased arachidonic-acid release |
| $TxA_2$ receptor abnormality | Idiopathic dense granule deficiency | Phospholipase C disorders |
| | Gray platelet syndrome | G-protein disorders |
| | Quebec platelet disorder | Abnormal phosphatidylinositol metabolism Idiopathic |

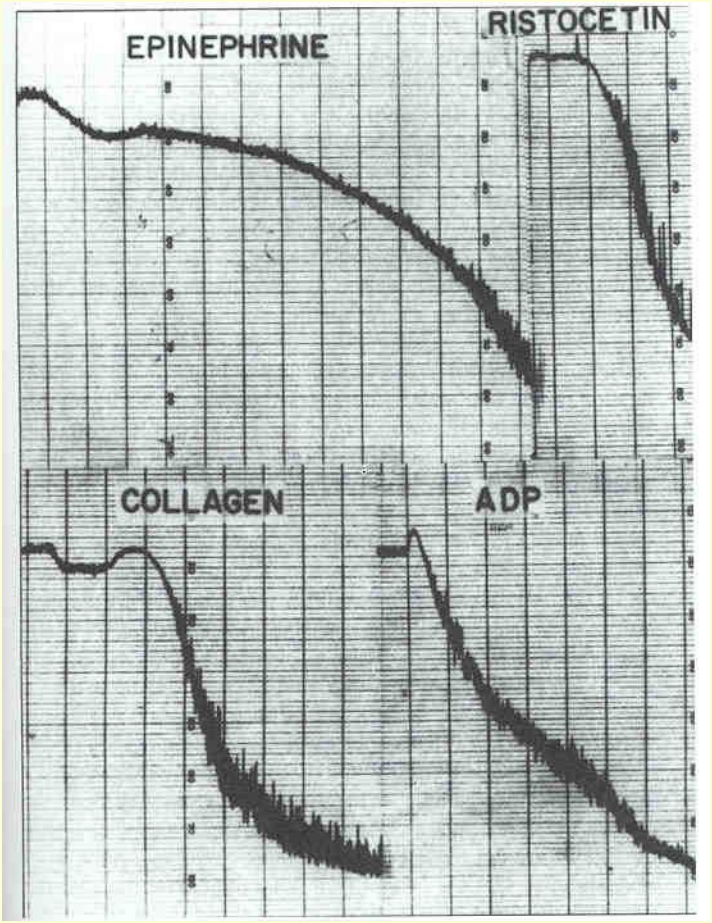
# Acquired Dysfunctional Platelets

- Drugs: Aspirin, other NSAIDs
- Uremia
- Acquired VWD
- Myeloproliferative diseases
- Anti-platelet antibodies

# Platelet aggregation study

- Principle: aggregation in response to an added chemical stimulus can be monitored by change in transmittance
- Stimulating agent: arachidonic acid, ADP, collagen, epinephrine, and ristocetin
- Platelet functional disorders have typical aggregation patterns

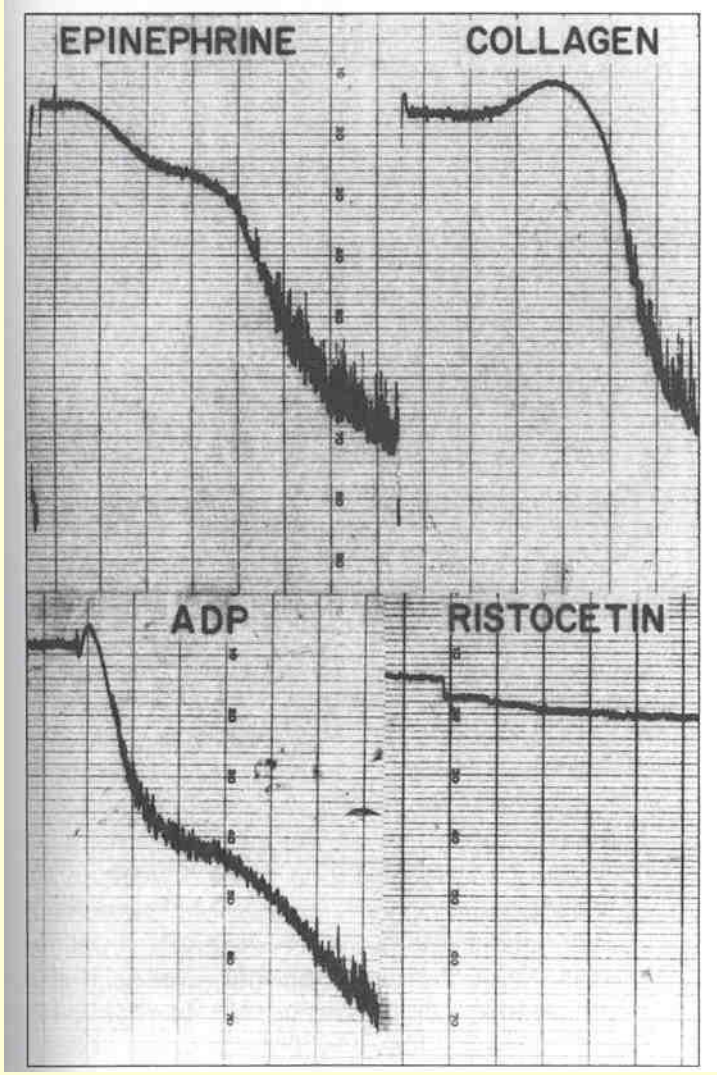
# Normal pattern




ADP and epinephrine exhibit two waves of aggregation, primary and secondary

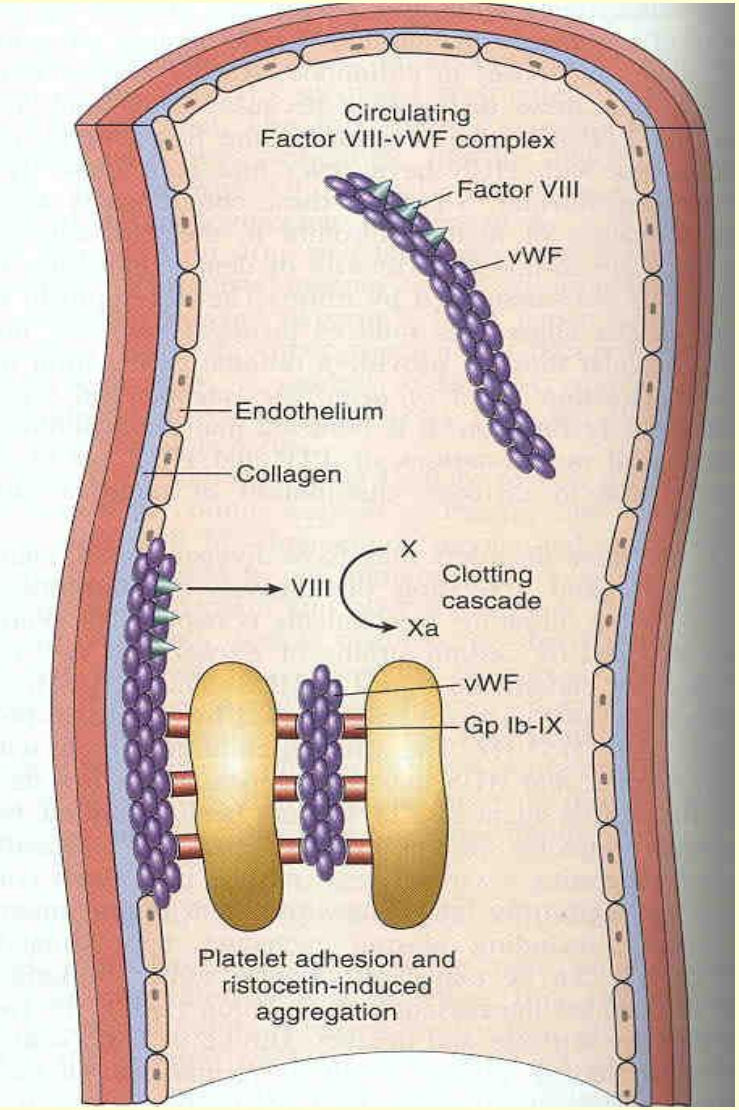
The secondary wave is due to release of endogenous ADP

# Abnormal platelet aggregation studies




VWD and Bernard Soulier have similar aggregation pattern: Aggregation in response to ristocetin is abnormal

# Von Willebrand factor




HMW glycoprotein, synthesized by endothelium and megakaryocytes

VWD is the most common congenital bleeding disorder

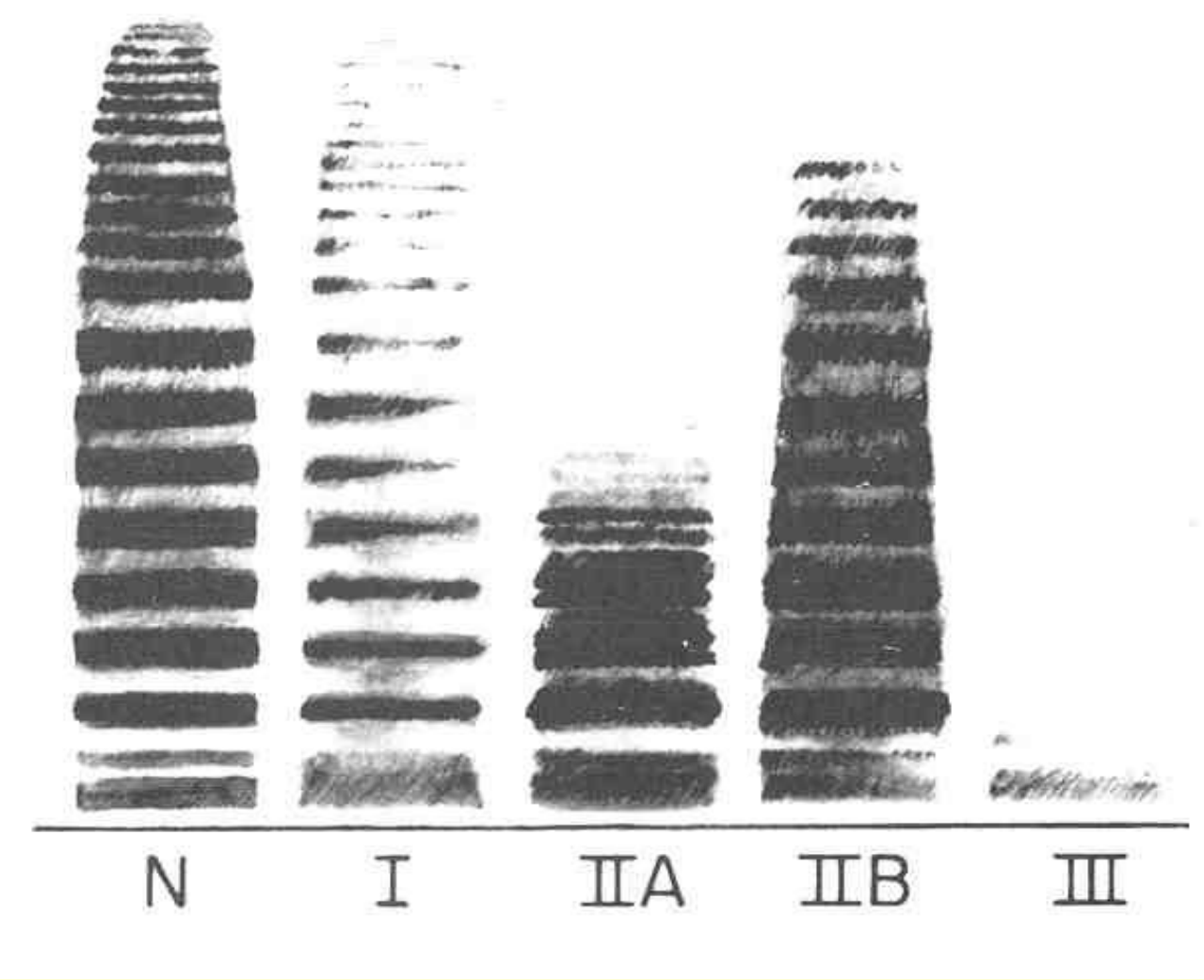
# Classification of von Willebrand disease

| VWD Sub-Type | Defect | Inheritance |
|---|---|---|
| Type 1 | Decreased quantity of all vWF multimers<br>High-molecular-weight multimers present<br>Concordance between vWF:ag and vWF:Rcof | Autosomal dominant |
| Type 2A | High molecular weight multimers absent<br>Abnormal platelet dependent vWF function<br>Discordance between vWF:ag and vWF:Rcof | Autosomal dominant |
| Type 2B | Loss of some high-molecular-weight multimers<br>Increased affinity of vWF for GP Ib/IX/V<br>Association with thrombocytopenia | Autosomal dominant |
| Type 2M | Abnormal platelet-dependent vWF function<br>Discordance between vWF:ag and vWF:Rcof<br>High-molecular-weight multimers present | Autosomal dominant |
| Type 2N | Decreased affinity for factor VIII<br>Normal platelet-dependent vWF function<br>Decreased factor VIII, prolonged APTT | Autosomal recessive |
| Type 3 | Severe deficiency of vWF<br>Multimeric analysis cannot be performed<br>Factor VIII also markedly decreased | Autosomal recessive |
| Platelet-Type | Abnormal GPIb/V/IX (Ibα)<br>Increased affinity for normal vWF<br>Association with thrombocytopenia | Autosomal dominant |

# Investigation of VWD

- Prolonged PTT
- Abnormal platelet aggregation studies
- VWD panel:
- Factor VIII:C activity
- vWF- Antigen
- vWF- Ristocetin co-factor activity
- (ability to aggregate normal platelets
- in the presence of ristocetin)
- Multimeric analysis

# Multimeric Analysis of vWF



# VWD

- Type I: most common
- Type IIB and platelet type: large multimers absent; these two types show aggregation with low-dose ristocetin

# Treatment of vWD

- **Desmopressin:** twofold to tenfold increase in plasma vWF level; contraindicated in vWD type 2B and platelet-type vWD as release of abnormal vWF may induce thrombocytopenia
- **vWF/FVIII concentrates**

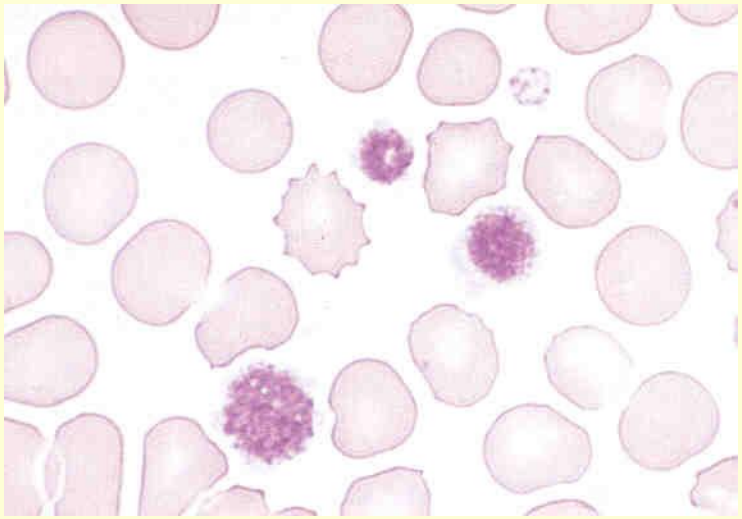
# Acquired vWD

- Presentation: sudden onset of mucocutaneous bleeding in a previously asymptomatic patient
- Mechanisms:

  - Antibody to vWF: increased clearance of vWF from circulation or inhibition of vWF function

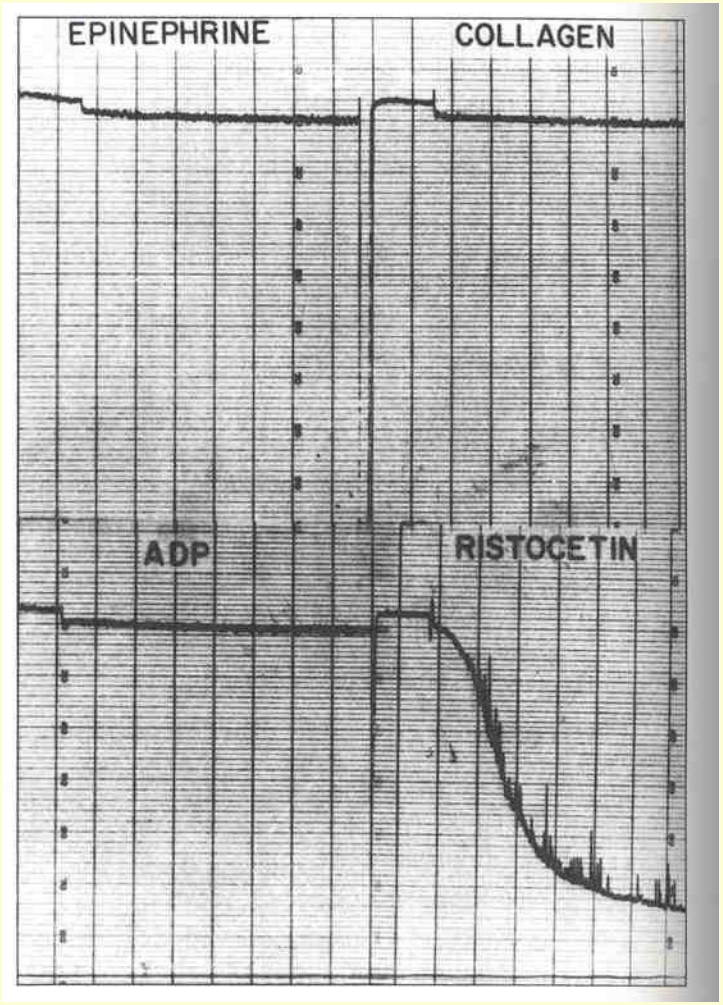
  - Adsorption by tumor cells: tumor cells may have aberrent GP Ib$\alpha$ receptor expression

# Bernard-Soulier disease
# Autosomal recessive



Thrombocytopenia with giant platelets
Decreased expression of GP Ib/IX or
decreased affinity of GP Ib/IX for VWF

# Abnormal platelet aggregation studies: Glanzmann's thrombasthenia

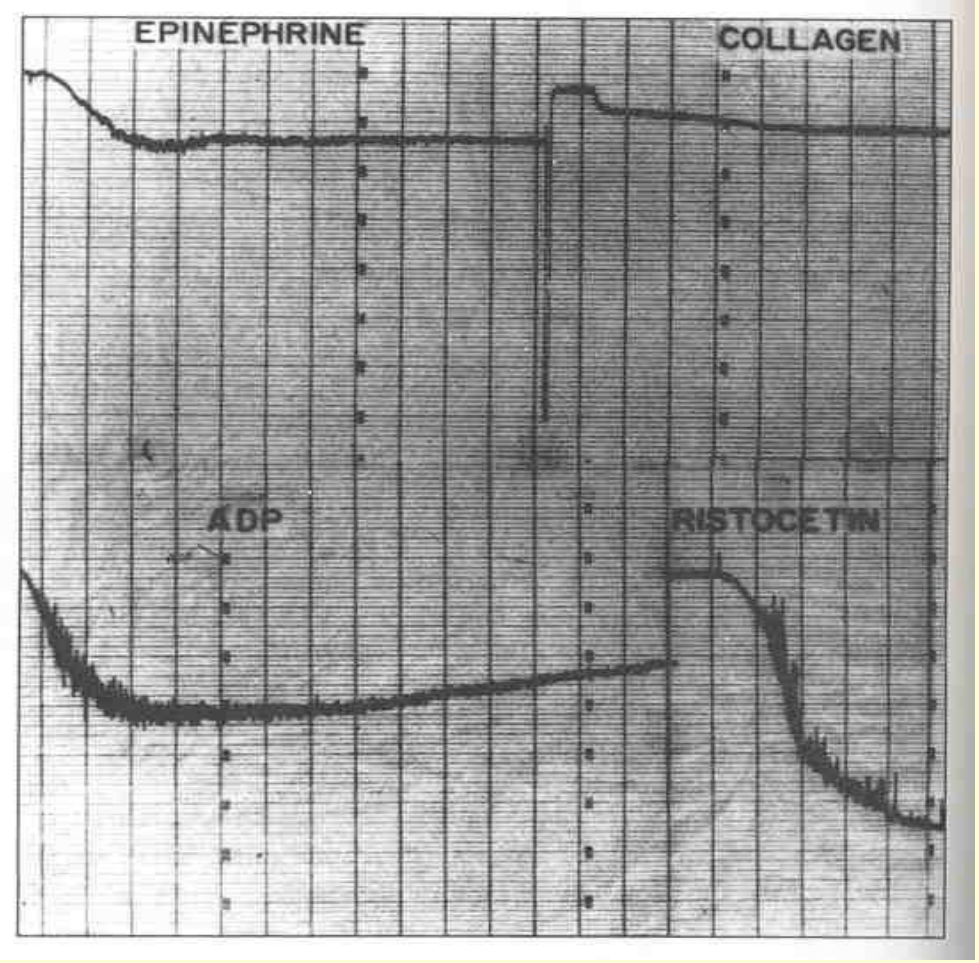



Primary wave defect for all reagents except Ristocetin

Autosomal recessive

# Abnormal platelet aggregation studies:

## Storage pool disease or defective release of storage pool contents (aspirin like defect)




Secondary waves to ADP and epinephrine absent