Lecture 1: Introduction to Hemostasis
MEDT4238

Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Course Syllabus

1. Introduction to Haemostasis
2. Primary Haemostasis
3. Coagulation Factors
4. Coagulation Cascade
5. Fibrinolysis System
6. Vitamin K Cycle and Coagulation
7. Bleeding disorders part 1
8. Bleeding disorders part 2
9. Inherited and acquired thrombotic disorders
10. Drugs that affect Haemostasis

Grades

- Midterm exam ..... 40 marks
- Final exam ..... 60 marks

Introduction

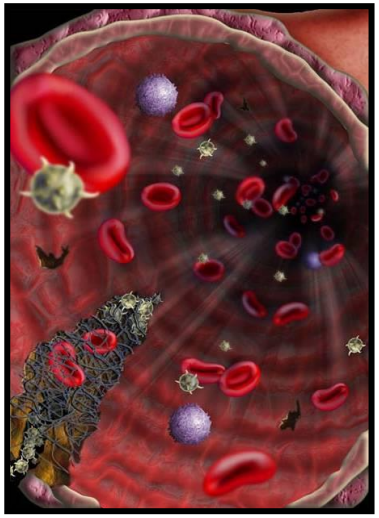
- Blood is always in a fluid state, normally circulates within the blood vessels in a closed system.

- When you are exposed to a traumatic injury, such as a cut in your finger, bleeding will result. If you lose too much blood you will be exposed to a hypovolemic shock, and then you will simply die.

- To minimize blood loss, platelets and dissolved proteins within your blood mobilize to form insoluble mass or a barrier that closes the injured vessel.

- This barrier is limited to the injury site so that normal circulation is maintained in your blood vessels.

Hemostasis



Definition:

Haemostasis or Hemostasis:

Drives from the Greek : aimóstasis, from aíma "blood" + stásis "stagnation, to halt or to stop") meaning

"The stoppage of blood flow"

- Hemostasis is a complex physiologic process that keeps circulating blood in a fluid state and then, when an injury occurs, produces a clot to stop the bleeding, confines the clot to the site of injury, and finally dissolves the clot as the wound heals.

- Most times this includes the changing of blood from a fluid to a solid state and then to fluid state.

If bleeding occurs

Functions of Hemostasis

The Haemostatic mechanisms have several important functions:

1. To arrest bleeding at the site of injury or blood loss by formation of a haemostatic plug;

2. To maintain blood in a fluid state while it remains circulating within the vascular system;

3. Repair and reestablish the blood flow through the injured vessels; and

4. To ensure the eventual removal of the haemostatic plug when healing is complete.

Hemostatic Mechanism

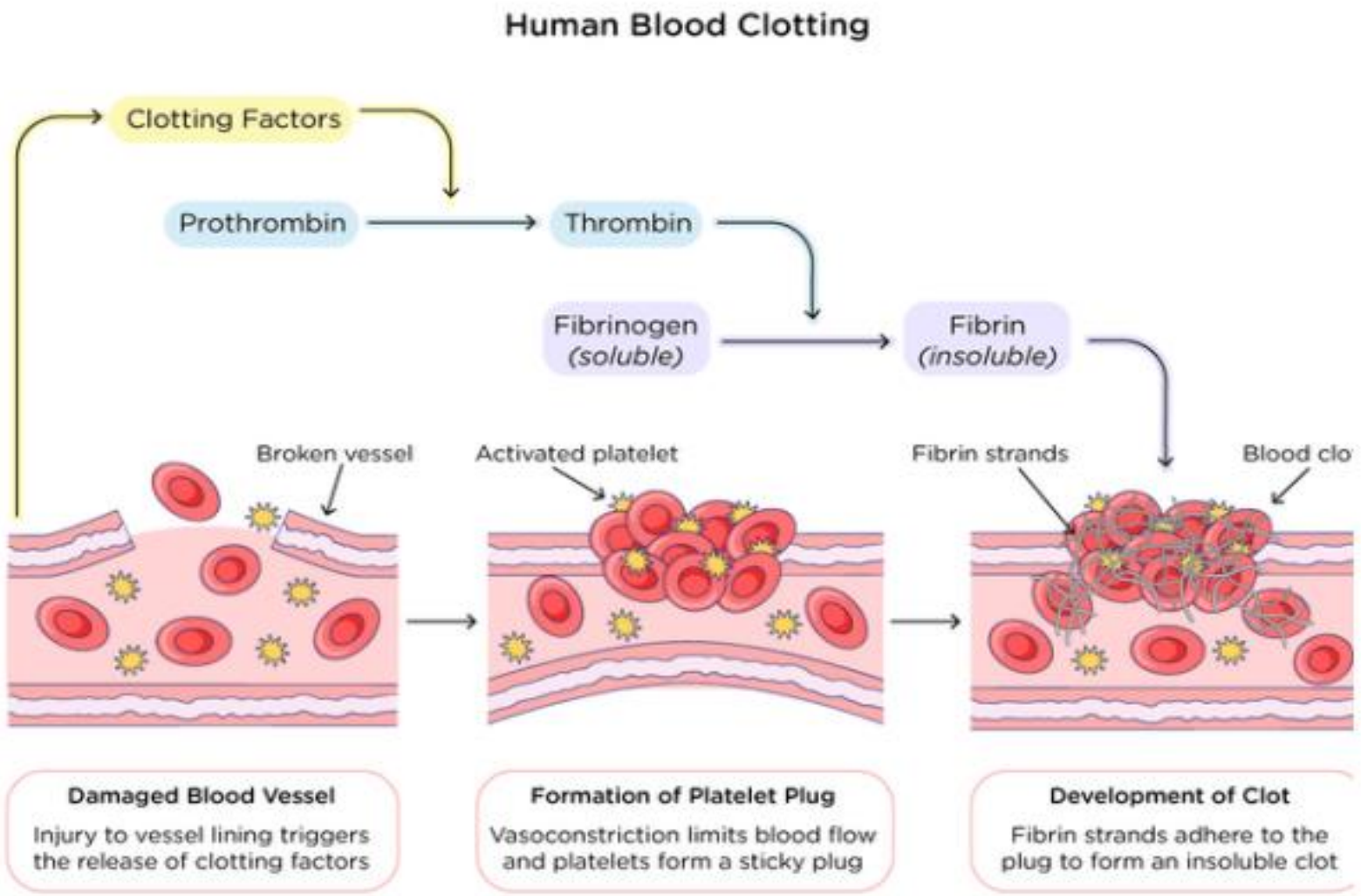
- This mechanism is only activated normally when it is needed, and where it is needed.

- Haemostasis involves a series of delicately balanced physical and biochemical changes following an injury to a blood vessel. Hemostasis has three steps:

  - Step 1: Vasoconstriction, As the most immediate response, the blood vessel constrict

  - Step 2: Platelet Plug formation, platelets adhere and aggregate at the site of the injury and form a plug.

  - Step 3: Formation of a fibrin blood clot.


Human Blood Clotting
Clotting Factors
Prothrombin
Thrombin
Fibrinogen
(soluble)
Fibrin
(insoluble)
Broken vessel
Activated platelet
Fibrin strands
Blood clo
Damaged Blood Vessel
Injury to vessel lining triggers the release of clotting factors
Formation of Platelet Plug
Vasoconstriction limits blood flow and platelets form a sticky plug
Development of Clot
Fibrin strands adhere to the plug to form an insoluble clot

Hemostasis Phases

Three stages process: Stages of normal hemostasis are recognized as:

I. Primary hemostasis

II. Coagulation (Secondary hemostasis)

III. Fibrinolysis (Tertiary hemostasis)

Components of Normal Hemostasis

- The hemostatic components remain inert in the presence of intact vascular tissue or endothelium.
- Following injury, each component must function optimally.
- Coagulation factors are normally circulate in the plasma as (inert form) inactive proteins except Tissue factors not circulate in plasma.
- On activation some factors form enzymatic proteins known as Serine Proteases that activate other specific factors in the coagulation sequence.

Components of Normal Hemostasis

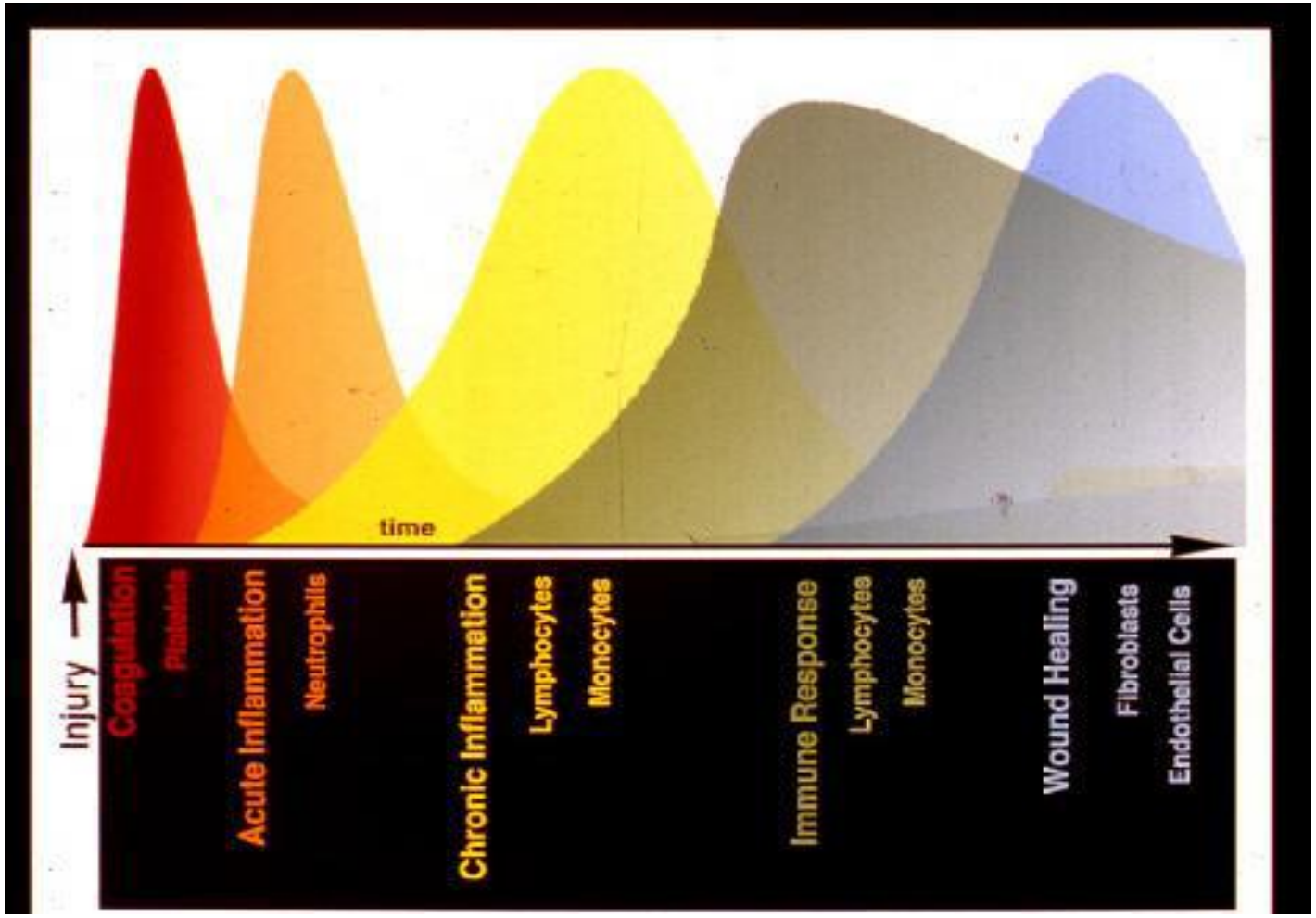
1. Blood vessels
2. Platelets in quantity and quality
3. Coagulation System
4. Fibrinolytic system
5. Regulators for all these systems (activators and inhibitors)

Hemostasis is also a body defense mechanism??

- Yes, hemostasis is linked with other elements of body defense response, such as complement and kinin generating processes and phagocytosis.

Coagulation: Response to Injury

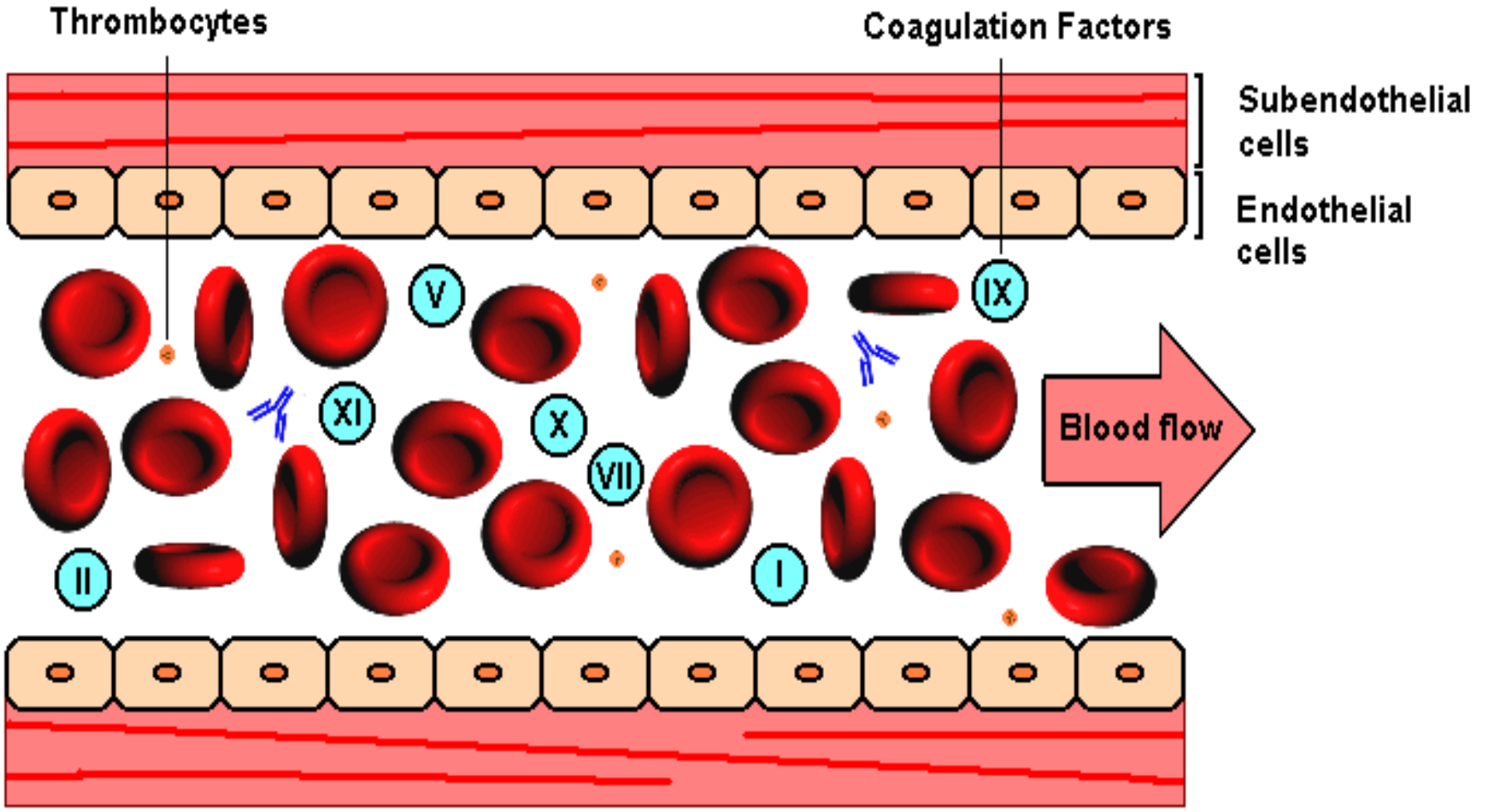
when there is an injury, the body must mount a response to halt immediate damage, deal with an infection, and heal the wound and restore tissue fxn. the first step in this process is coagulation which not only stops bleeding, but produces mediators such as growth factors and cytokines which help condition and direct the rest of this process...



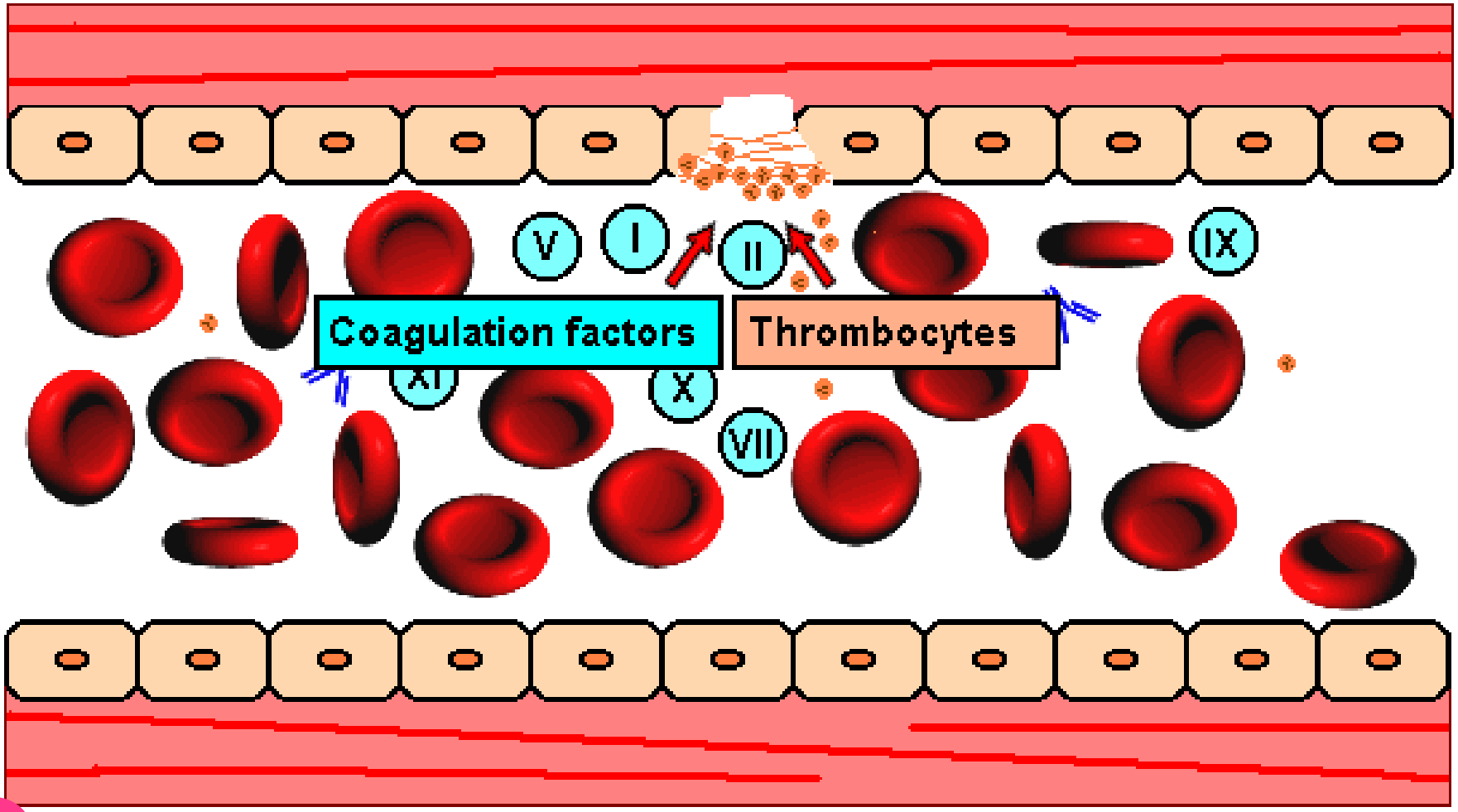
The effectiveness or failure of haemostatic mechanism to control bleeding depends on :

- The type & degree of injury.
- The size & ability of injured vessel to contract.
- The pressure within the vessel & surrounding tissues.
- The availability & activity of the platelets.
- The quantity & functional ability of blood clotting factors.
- The absence of inhibitors.

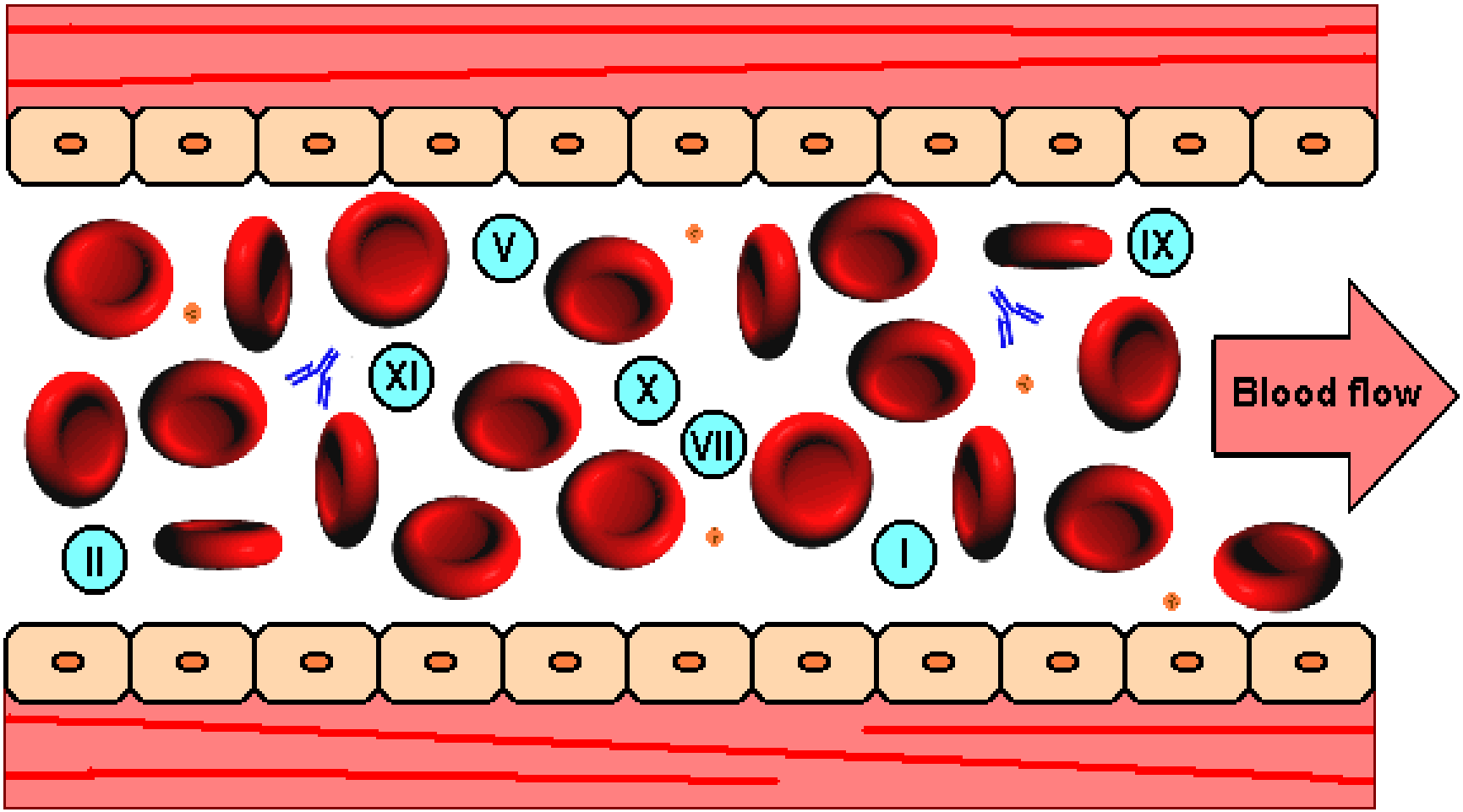
Vessel wall, Blood flow, platelets & Coagulation Substances



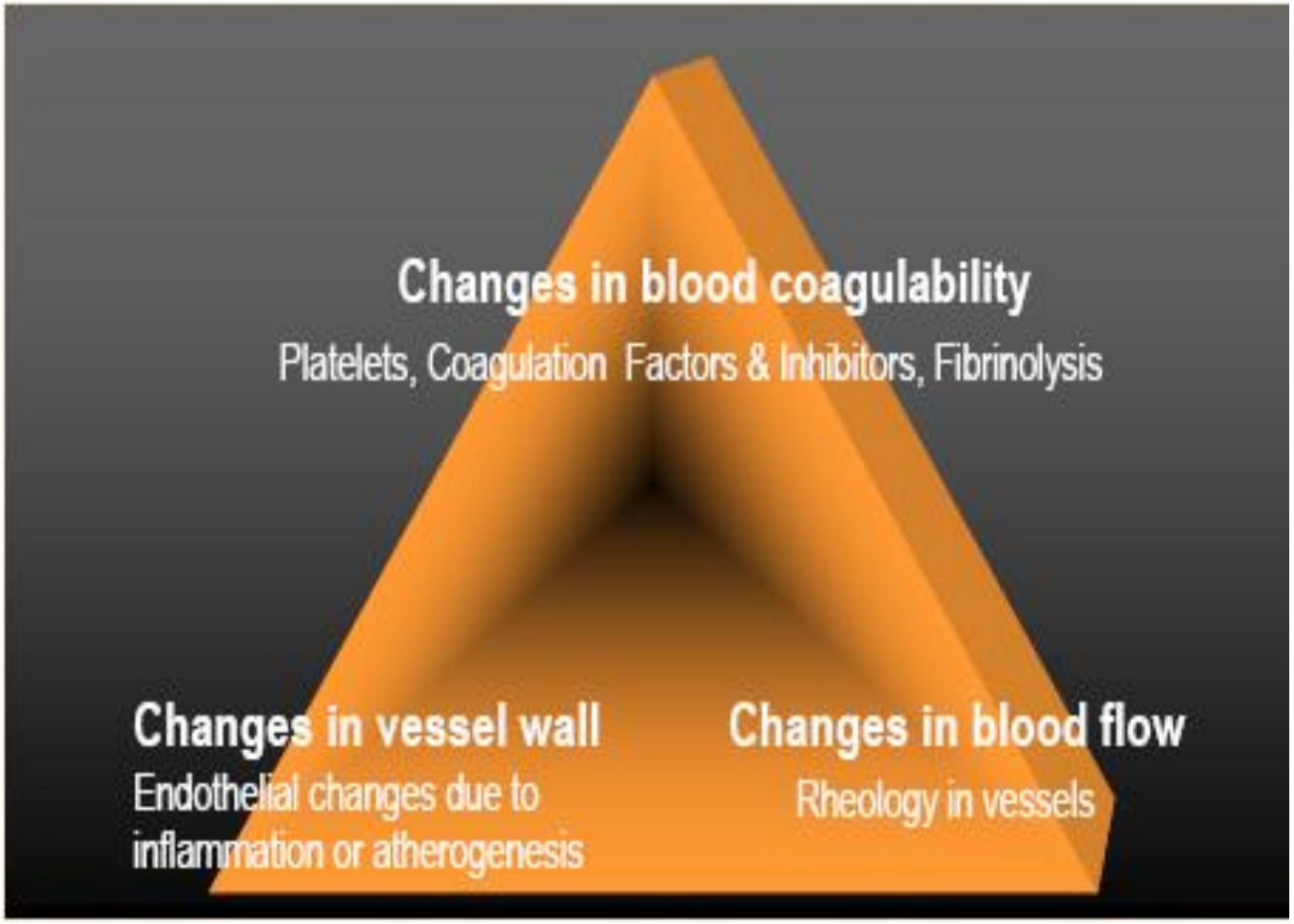
In Case if there is an Endothelial Injury
(Bleeding must be prevented at site of injury)

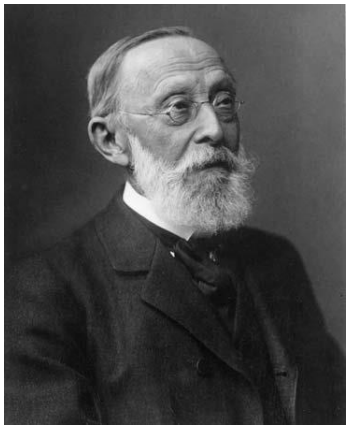


Flow must be Maintained



Virchow's Triad





Rudolf Virchow - German pathologist(1821-1902)

Virchow Law

There are three haemostatic components:

1. The extra-vascular (The tissues surrounding blood vessels) involved in Hemostasis when local vessel is injured.
   - It plays a part in Hemostasis by providing back-pressure on the injured vessel through swelling and trapping of escaped blood.

2. The vascular (The blood vessels through which blood flow) it depends on the size, amount, of smooth muscle within their walls and integrity of the endothelial cell lining.

3. The intra-vascular (The platelets and plasma proteins that circulate within the blood vessels).
   - These components are involved in Coagulation (clot or thrombus formation) or Fibrinolysis (clot or thrombus dissolution).

Hemostasis Phases

Three –phase process

I. Primary hemostasis

II. Coagulation (Secondary hemostasis)

III. Fibrinolysis (Tertiary hemostasis)

Primary hemostasis

- Involves the vascular and the platelet response to vessel injury.
  - Blood vessels (vascular vasoconstriction phase and release of tissue or exogenous factors)
  - Thrombocytes (platelet or endothelial-thrombocyte phase, platelet aggregation and release of platelet factors).
- Initial, rapid, short-lived response to vessel damage (seconds to occur).
- This interaction results in a temporary platelet plug or primary hemostatic plug. But this plug is not strong enough and it can be removed or washed away easily as a result of blood flow pressure.
- The plug must be reinforced by fibrin.

Secondary Hemostasis (Coagulation):

Involves :

- ✓ The coagulation factors response to such injury.
- ✓ The catastrophic reaction in secondary hemostasis is the generation of thrombin.
- ✓ Its final goal is the formation of fibrin.
- ✓ Consequently a thrombus is formed.
- ✓ Takes 5 to 10 minutes by formation of fibrin
- ✓ Reinforces the platelet plug.

NOTE:

Together blood vessels, platelets, and coagulation factors combine to stop bleeding and allow for vessel repair through formation of a stable fibrin-platelet plug at the site of injury.

Tertiary Hemostasis (Fibrinolysis):

- ✓ Essential final step in any hemostasis mechanism,
- ✓ Occur in 48 to 72 hours.
- ✓ Break, digest and fragment this fibrin plug to remove this clot and healing the injured vessel. These components collectively are called the fibrinolytic system.
- ✓ Fibrinolysis will ensure that excessive coagulation (no propagation of the clot formation) does not occur, at site of injury or expand elsewhere within the circulation.

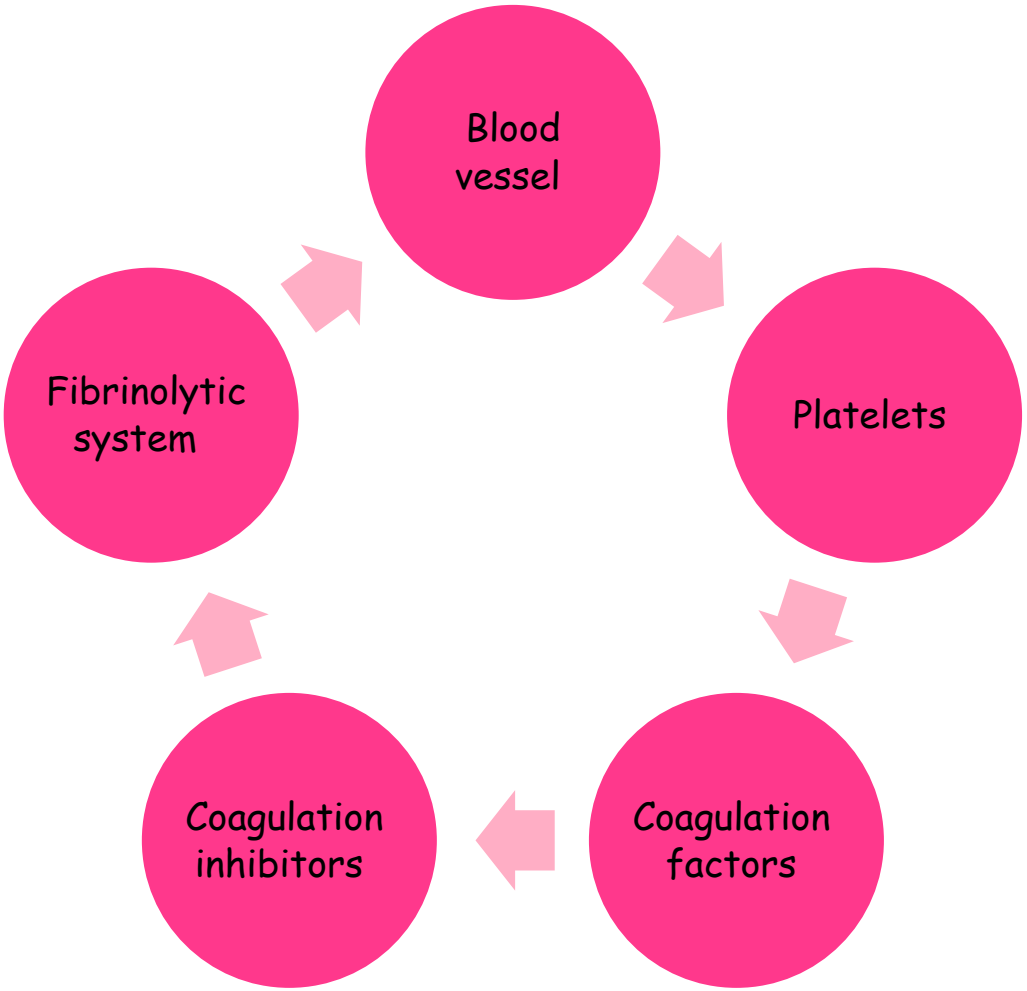
Fibrinolysis

- Fibrinolysis is concerned with the breakdown of fibrin clots.
- Fibrin is degraded by plasmin, that is derived from its precursor plasminogen.
- This plasminogen proteolysis is mediated by tissue-plasminogen activator (tPA), primarily secreted by endothelial cells.
- The activity of tPA is regulated by plasminogen activator inhibitor (PAI-1).

Coagulation inhibitors

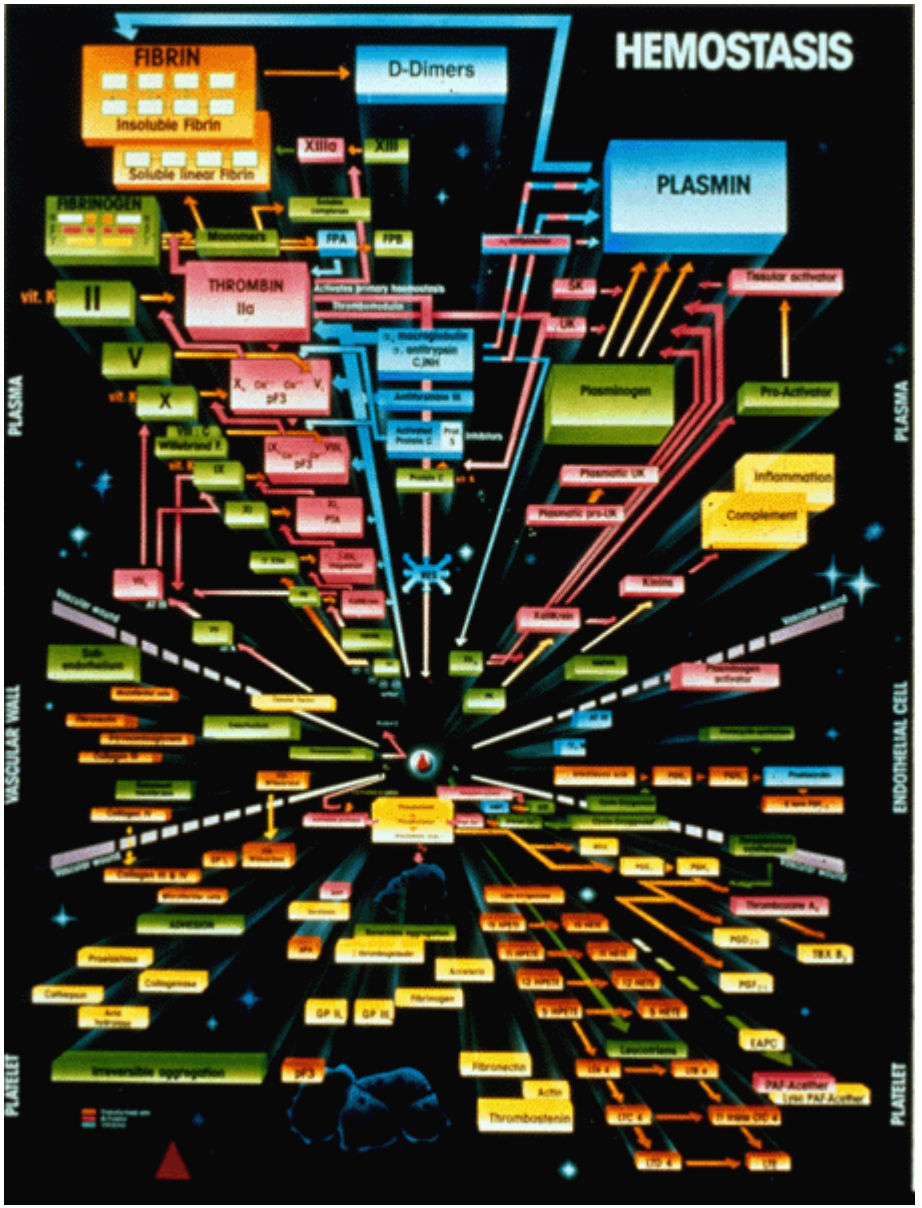
- Not only fibrinolytic system inhibit propagation of clot formation but also Coagulation inhibitors play a role in inhibiting clot formation in other sites also prevent propagation of clot that cause occlusion of blood vessel.

We see how the five components of hemostasis collectively work together in complete work



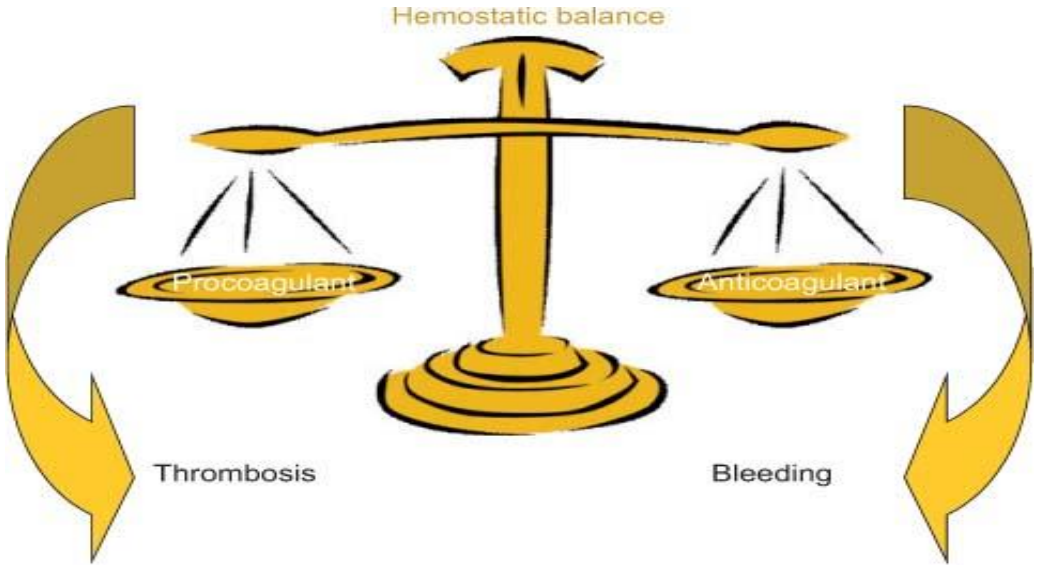
How can we make sense of hemostasis?

its complicated, but we will try to highlight key features that will help us make sense of things that happen in our pts



When hemostasis systems are out of balance:

- Failure or deficiencies in any of the above-mentioned five systems can lead to a varying degrees of uncontrolled hemorrhage (bleeding) or thrombosis (pathological clotting) can be life-threatening.

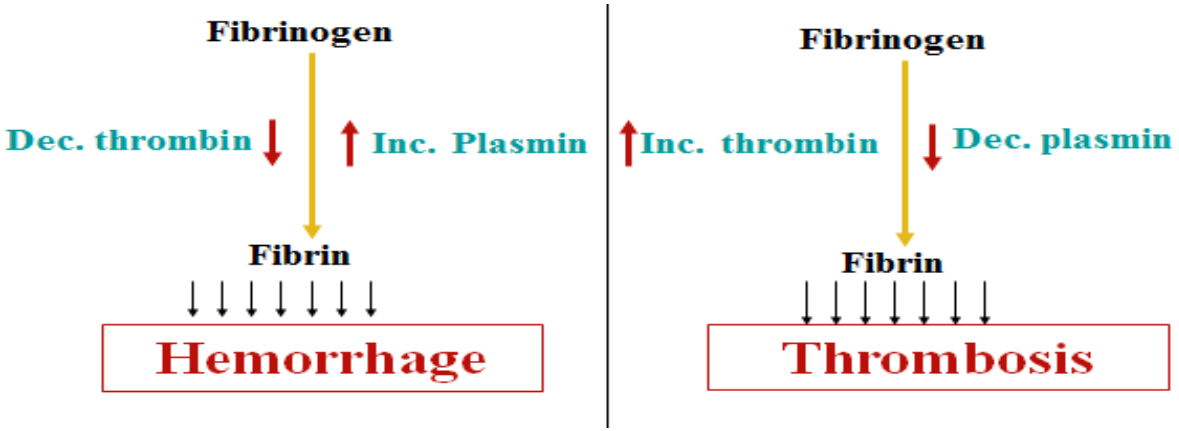


So abnormalities in hemostasis can be classified into:

- Vessel wall disorders
- Abnormalities of blood platelets
  - Quantitative platelets defects and/or
  - Qualitative platelets defects
- Disturbance of coagulation in circulation
  - Deficiency of coagulation factors
- Increase or deficiency of anticoagulation substances
- Fibrinolysis Hyper-function

Importance of Balance in Hemostasis

- *Any disruption* in the balance between clot formation and clot dissolution results in:



- Without this balance, the individual may experience either excessive bleeding (hemorrhaging due to hypocoagulation).
- (poor clot formation or excessive Fibrinolysis).

- Vaso-occlusion (thrombosis due to hypercoagulation) (uncontrolled formation of thrombin in vascular system, occluding vessels and depriving organs of blood).

Bleeding

- There are certain conditions associated with excessive bleeding are referred to as: Hypo-coagulable states.
  - Such as, Hemophilia or deficiency in one of the plasma coagulation proteins such as factors VIII.
  - Or deficiency in one component of the fibrinolytic system as seen in alpha2 anti-plasmin that inhibits plasmin.
  - Acquired conditions such as DIC, Liver and Kidney diseases.

Thrombosis

- Other conditions are related to uncontrolled thrombosis are called Hyper-coagulable state and thrombophilia.
- This is related to an appropriate formation of thrombi in the vascular vessels that occlude normal blood flow.
- These conditions may be inherited or acquired.

Thank you

Next Lecture : Primary Hemostasis

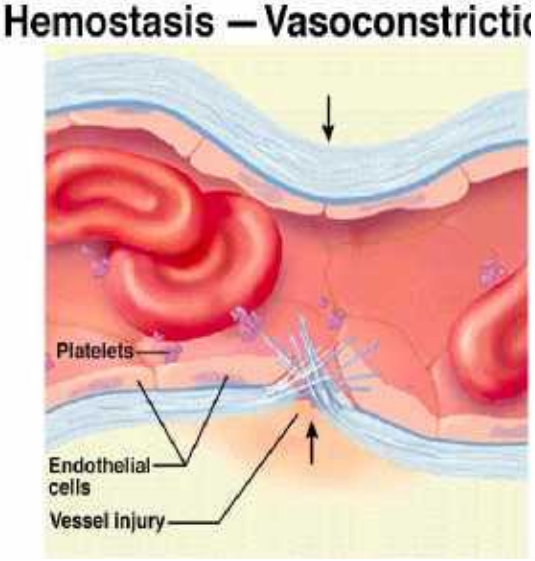
Lecture 2: Primary Hemostasis Part-1

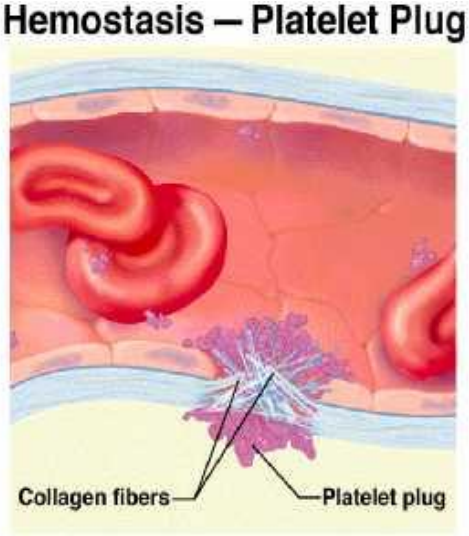
Miss: Amany S. AlHindi

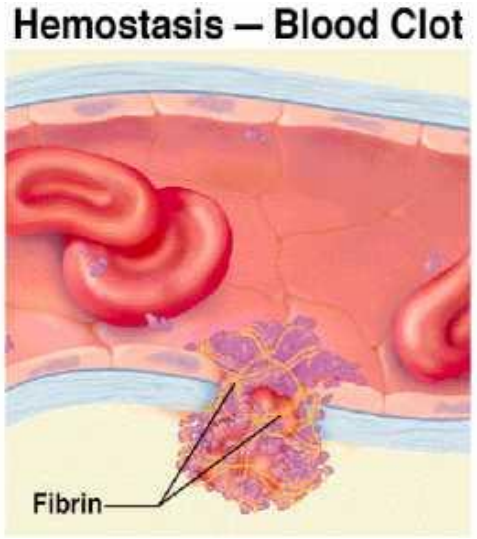
M.Sc. in Biological sciences from Islamic University of Gaza

Hemostasis

- ✓Vasoconstriction
- ✓Platelet activation
- ✓Platelet plug
- ✓Coagulation: Stable clot formation
- ✓Clot dissolution





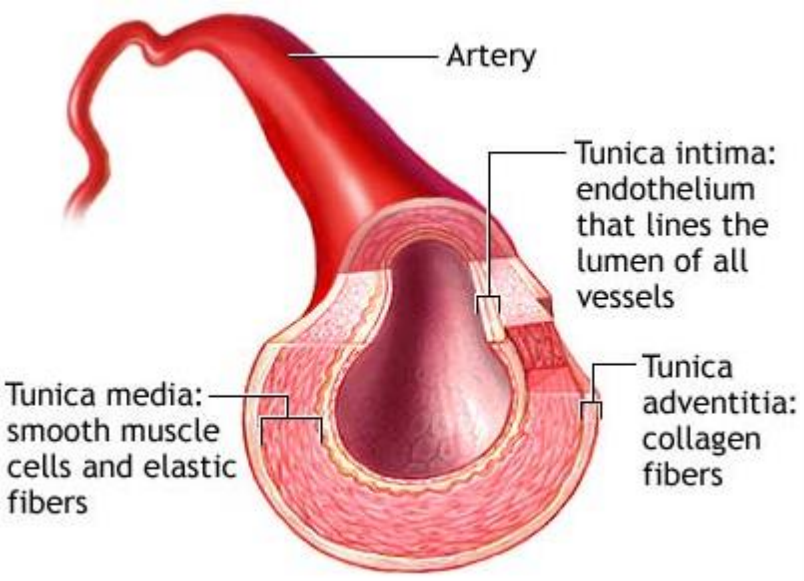


Now we will talk about primary hemostasis in more details

Primary Hemostasis

- Involves:
  - Vasoconstriction
  - Platelet adhesion
  - Platelet change in shape
  - Platelet aggregation
  - Platelet secretion

Blood Vessels



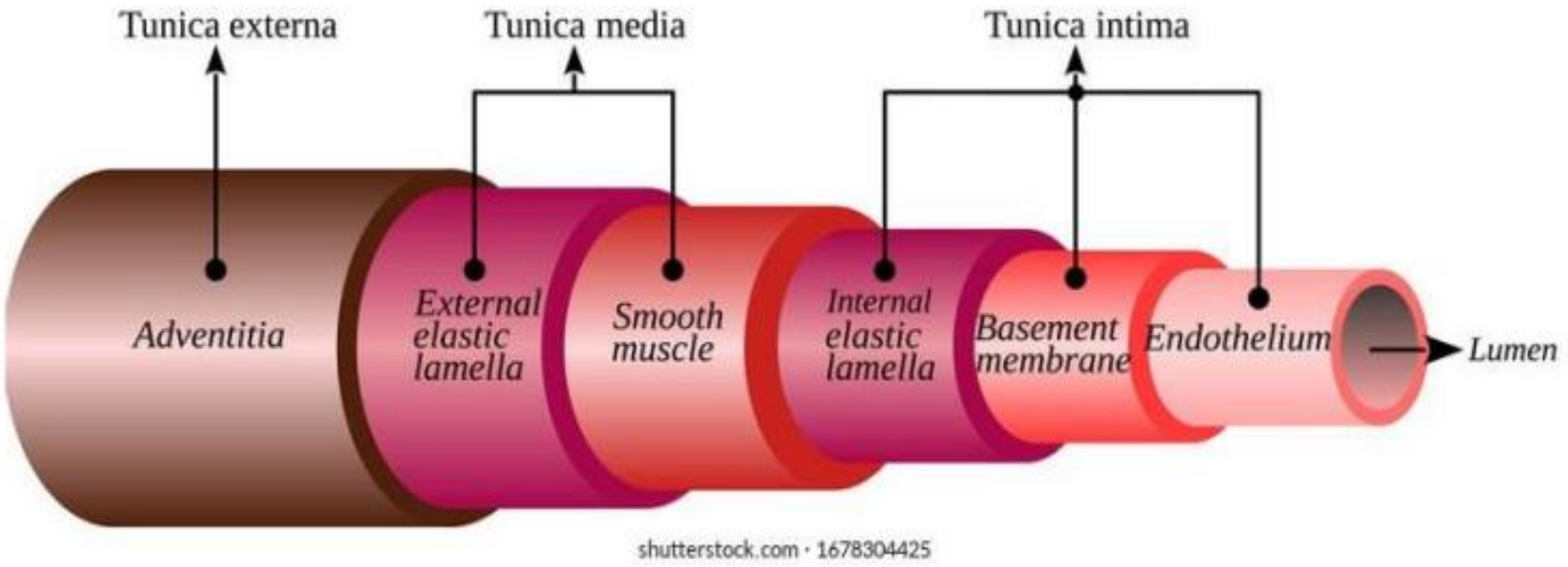
Vascular System

- Blood Vessels
  - Arteries
    - Carry blood from the heart to capillaries
    - Thickest walls of the vasculature
  - Veins
    - Return blood from capillaries to the heart
    - Thinnest walls of vasculature
  - Capillaries
    - No vessel wall
    - Do not contribute to hemostasis

Where does hemostasis works?

- It works in the small vessels.
- It works largely in venules and to a lesser extent in the arterioles.
- Both of them have a diameter of 20-200 µm

Structure of blood vessels: The arteries, arterioles, veins and venules



Three layers in blood vessels

Tunica intima:

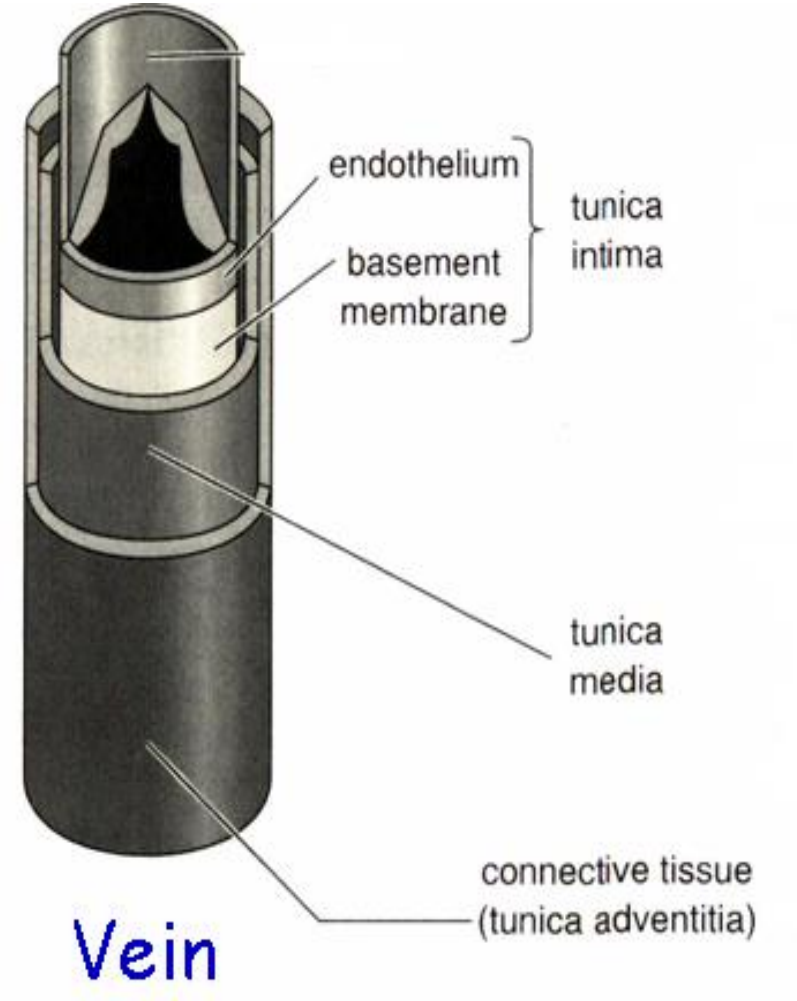
- It is the monolayer (single) endothelial, the basement membrane, and the sub-endothelial CT that holds them together.

Tunica Media:

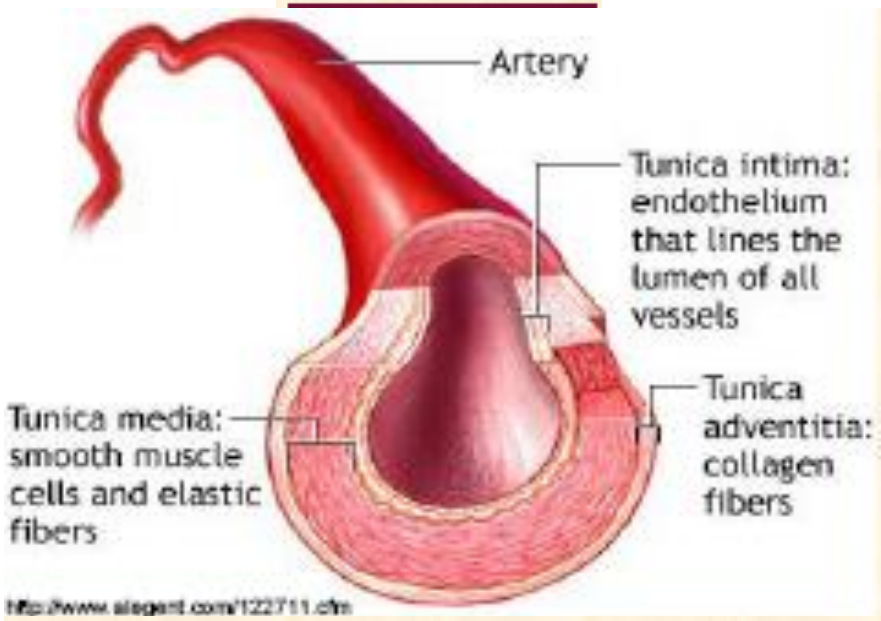
- It is the middle layer that contains smooth muscle cells and elastin fibers.
- This layer that can contract or relax and thus is responsible for vasoconstriction or vasodilatation of blood vessels.

Tunica Adventitia

- It is the outer coat, which consists of few fibroblasts embedded in the collagen and other CT tissue.
- Fibroblasts synthesizes and secretes the fibers.
- Mast cells may be present in this layer.



Vessel Wall



Tunica intima

- Endothelium - inner most layer of cells that separate the remainder of the vessel from the lumen
- Basement membrane – thin layer of spongy connective tissue that secretes elastic collagen

Tunica intermedia

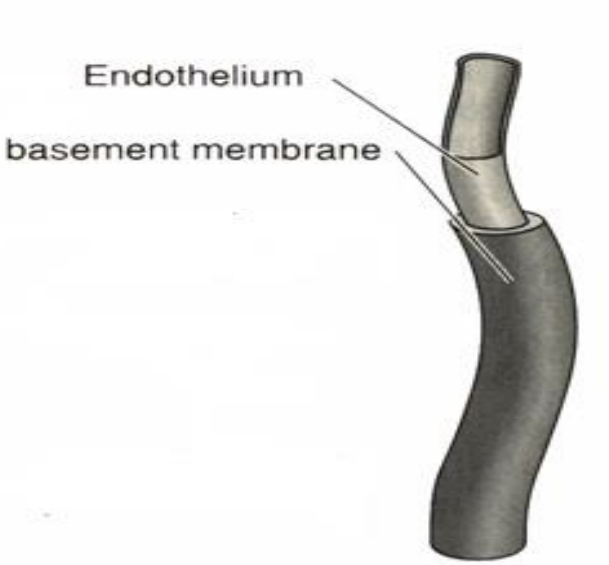
- Surrounds the tunica intima
- Smooth muscle – layer of smooth muscle cells that are under involuntary control and can dilate or constrict
- Connective tissue – produces collagen fibers whose elasticity is reduced by hypertension

Tunica adventitia

- Surrounds the tunica media
- Connective tissues – produce elastic and non-elastic collagen fibers
- Prevents ballooning of vessel with high systolic blood pressure
- Aneurysm – weaknesses in the tunica adventitia

Blood Capillaries

- Do not contribute to hemostasis
- Only a single layer of endothelial cells and a basement membrane.



Role of Blood Vessels in Hemostasis

- Blood normally carried within vessels whose physical capabilities include Contraction (narrowing) and Dilation, which are controlled by the smooth muscle of the vessel media.

- Vasoconstriction and Vasodilatation provide the means for control blood flow rate and blood pressure.

- Substances released from the endothelial cells and sub- endothelial smooth muscles also contribute to normal blood flow and prevent abnormal formation of clot.

Vascular System

- Initiate hemostasis
  - Vasoconstriction of the arterioles
    - Minimizes blood flow to injured area
    - Prevents blood loss
    - Immediate
    - Short-lived

- The first response of the blood vessels to the injury is constriction or narrowing of the vessel lumen thus minimizing blood flow at injury site.

- Vasoconstriction occurs immediately and lasts only for a short period of time.

Vasoconstriction

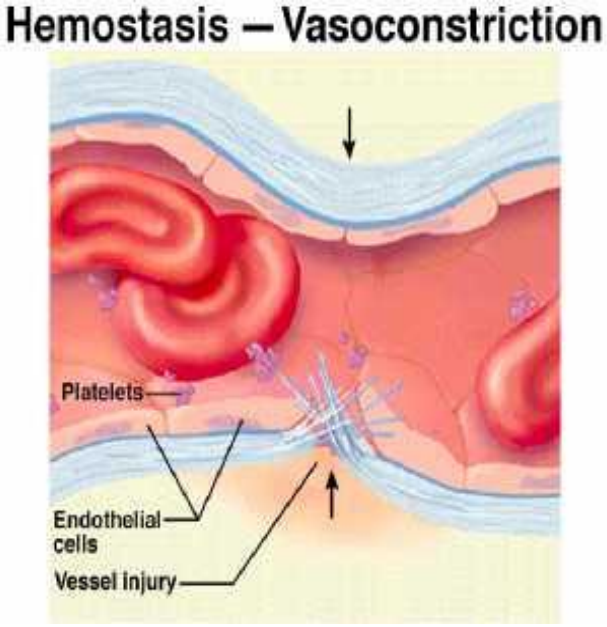
- It is really a complex process:
  - ✓ in part it is caused by neurological factors and
  - ✓ by several regulatory substances that interact with receptors on the surface of cells of the blood vessel wall.

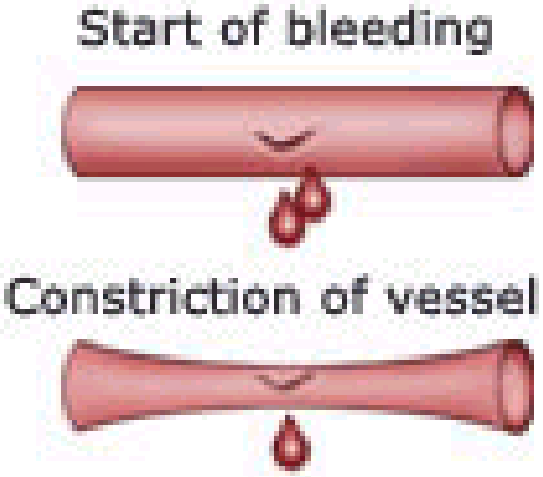
- The regulatory substances include:
  1. Serotonin (also called 5` hydroxy tryptamine),
  2. Thromboxane A2 (TXA2) (both of these substances are secreted by platelets upon their activation from their granules).
  3. Endothelin-1 (which is produced by damaged endothelial cells).

- These regulatory substances may aid in prolonging vasoconstriction.

Vasoconstriction

- Vasodilation Counteracts Vasoconstriction.
- When hemostasis begins to work we need the vessels to be vasodilated, so that more blood is coming carrying more platelets and more coagulation factors to act on the injured site.
- This is why vasoconstriction lasts for a short period!



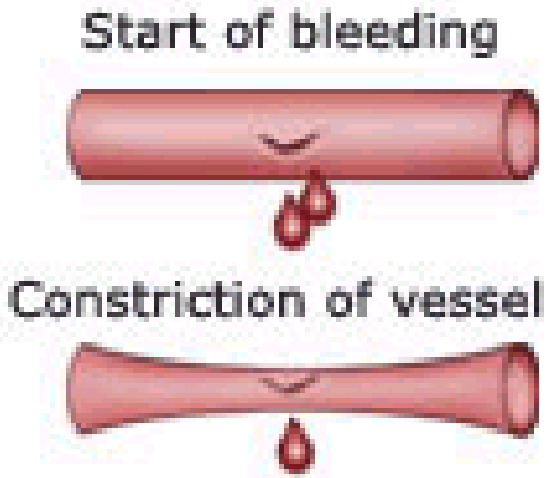


Thought question…

- Think about the last time you cut your finger with a piece of paper. Did your finger bleed immediately?
- If not, what might have prevented the bleeding?

Answer..

- No, the finger probably did not bleed immediately, due to vasoconstriction of the blood vessels



Discussion

- What actions of the endothelial cells prevent clotting from occurring within the blood vessels?

Functions of endothelial cells

1. **Endothelium is a critical blood vessel component influencing vascular hemostasis.**
   - They either modulate functions that aid in blood clot formation or appose formation of blood clots.
   - They express receptors or synthesize or secrete substances that aids their function and their role.
2. ECs form a physical barrier separating procoagulant proteins and platelets in blood from collagen in the internal elastic lamina that promotes platelet adhesion, and tissue factor in fibroblasts and smooth muscle cells that activates coagulation.
3. **Regulation of blood flow and vascular tone.**
   - Normal endothelium is anti-thrombotic.
   - Activated/injured endothelium is pro-thrombotic.
4. **Fluid distribution**
5. **Inflammation**
6. **Healing/ repair**
7. **Hemostasis**

Causes of endothelial injury

- Inflammation
  - Infectious (bacteria, viruses)
  - Non- infectious (immune mediated)
- Necrosis
  - Toxins
  - viruses
- Trauma
  - Invasion
  - Inflammatory or neoplastic masses

Endothelium in Hemostasis

- Normal endothelium generally induces vascular relaxation and anti-clotting properties.

Mediators:

1. Nitric oxide (NO): induce vascular relaxation and inhibits platelets activation. Participates with protein C and anti-thrombin to suppress thrombin production.
2. prostacyclin (PGI2)
3. Endothelium derived hyperpolarizing factor: induce vascular relaxation.

Anticoagulant Properties of Intact Endothelium

Composed of rhomboid cells presenting a smooth, contiguous surface.

- Secretes the eicosanoid platelet inhibitor *prostacyclin*
- Secretes vascular "relaxing" factor *nitric oxide*
- Secretes the anticoagulant glycosaminoglycan *heparan sulfate*
- Secretes coagulation extrinsic pathway regulator *tissue factor pathway inhibitor*
- Expresses endothelial protein C receptor
- Expresses cell membrane *thrombomodulin,* a protein C coagulation control system activator.
- Secretes TPA, thereby activating *fibrinolysis*

Endothelium in Hemostasis

- Damaged endothelium has predominately pro-clotting properties.
  - Release of tissue factor from activated endothelium and sub endothelial tissues. Inflammatory cytokines (IL-1, TNF) induce TF release.
  - Exposure of underlying collagen and other sub-endothelial components provide sites for platelet adhesion.

Endothelial mediators: Pro-coagulant

- Smooth muscle cells in arterioles and arteries: Induce vasoconstriction
- Exposed subendothelial rich in collagen: Binds VWF and platelets
- Damaged or activated Ecs: Secrete von Willebrand factor VWF, Secrete adhesion molecules: P-selectin, ICAMs, PECAMs induce leukocyte adhesion.
- Exposed smooth muscle cells and fibroblasts: Tissue factor exposed on cell membranes
- ECs in inflammation: Tissue factor is induced by inflammation.
- Plasminogen activator inhibitor-1: inhibits fibrinolysis

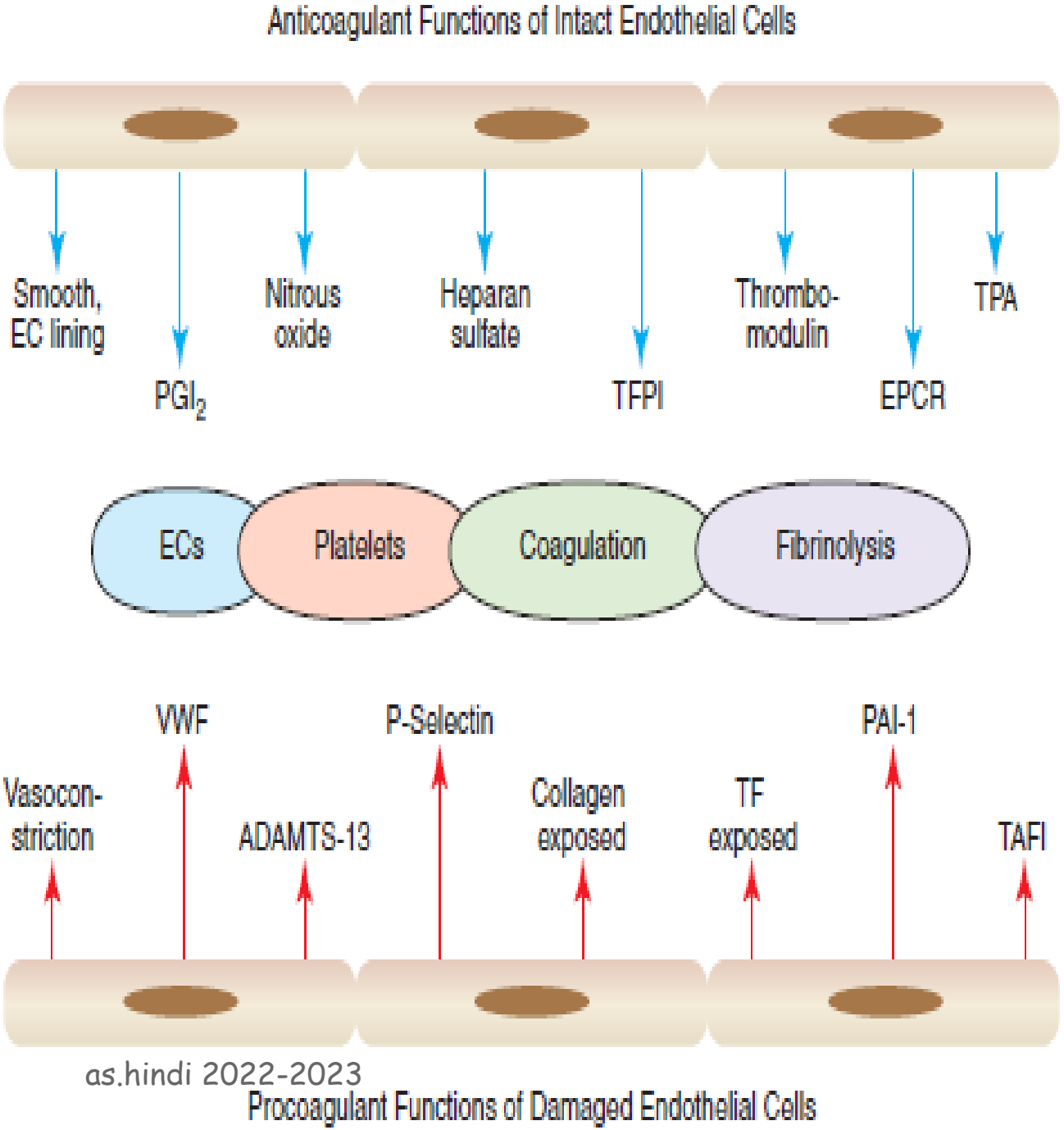


Figure 37-2 Anticoagulant functions of normal intact endothelial cells and procoagulant properties of endothelial cells when damaged. *EC*, Endothelial cells; *PGI_2*, prostacyclin or prostaglandin I_2; *TFPI*, tissue factor pathway inhibitor; *EPCR*, endothelial cell protein C receptor; *TPA*, tissue plasminogen activator; *VWF*, von Willebrand factor; *ADAMTS-13*, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; *TF*, tissue factor; *PAI-1*, plasminogen activator inhibitor-1; TAFI, thrombin-activatable fibrinolysis inhibitor.

Nitric oxide

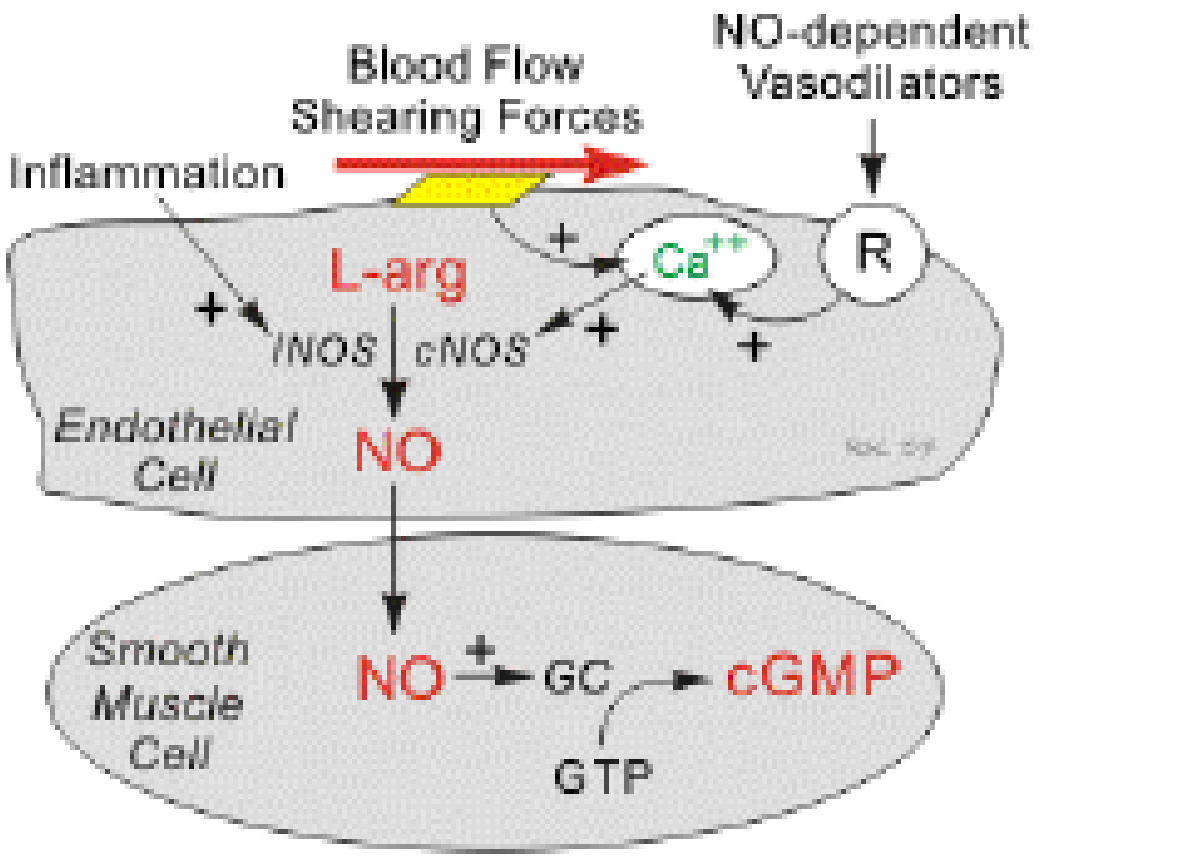
- Nitric oxide is synthesized in ECs, vascular smooth muscle cells, neutrophils, and macrophages.

- Nitric oxide induces smooth muscle relaxation and subsequent vasodilation, inhibits platelet activation, and promotes angiogenesis and healthy arterioles.

Vascular NO functions

- NO inhibits platelets' and leucocytes' adhesion to endothelial cells.
- It inhibits platelet aggregation
- It facilitates the dissolution of small platelet aggregates.
- NO affect the fibrinolytic activity by regulating the release of t-PA & PAI-1.
- The crucial role of vascular NO in the control of blood fluidity has been demonstrated by the regulation of the bleeding time in humans.

Nitric Oxide



Prostacyclin (called prostaglandin I2 or PGI2)

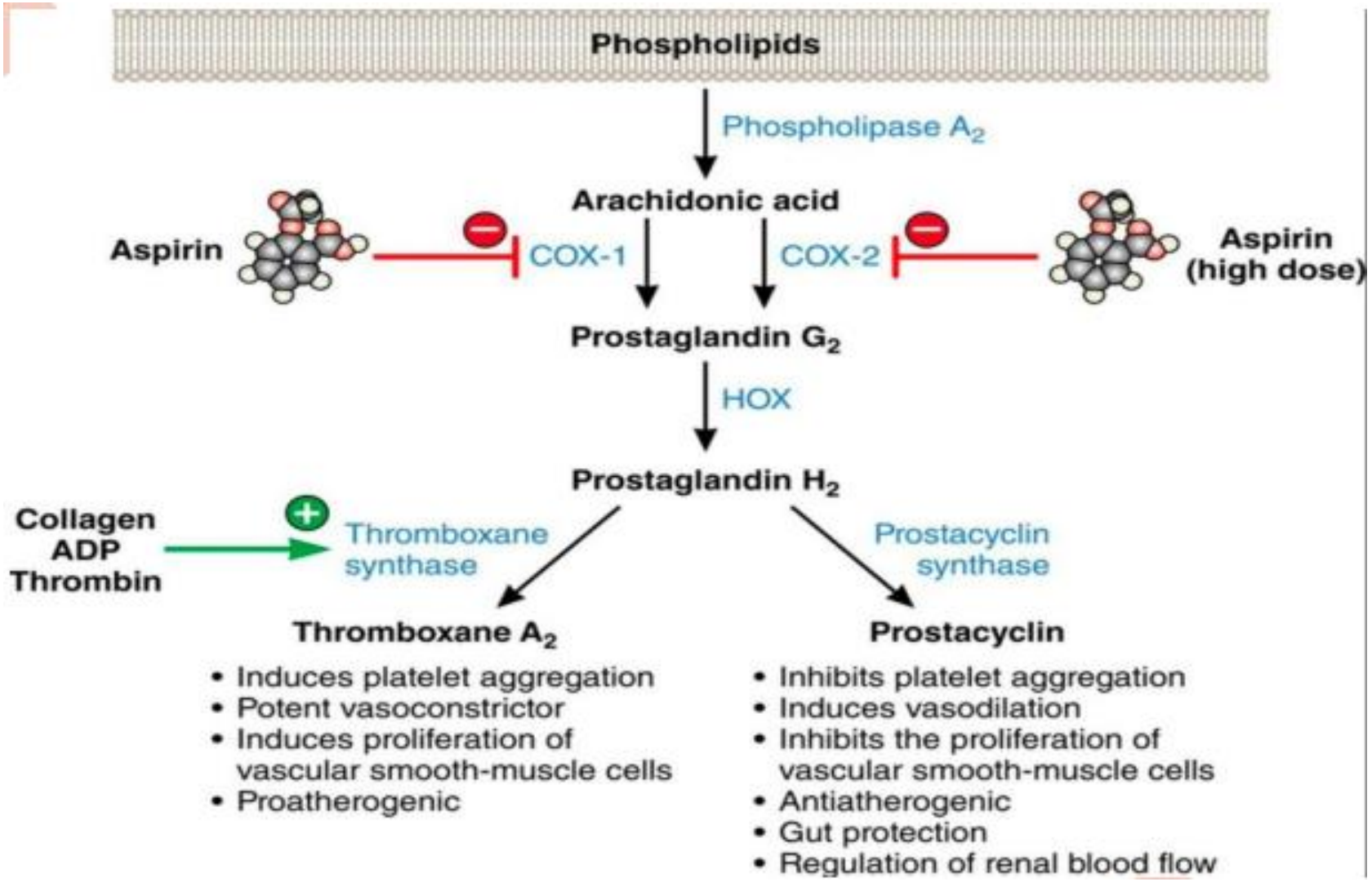
- Prostacyclin is a prostaglandin member of the eicosanoid family of lipid molecules.

- Prostacyclin is produced in endothelial cells, which line the walls of arteries and veins, from prostaglandin H2 (PGH2) by the action of the enzyme prostacyclin synthase. Although prostacyclin is considered an independent mediator, it is called PGI2 (prostaglandin I2) in eicosanoid nomenclature, and is a member of the prostanoids (together with the prostaglandins and thromboxane).

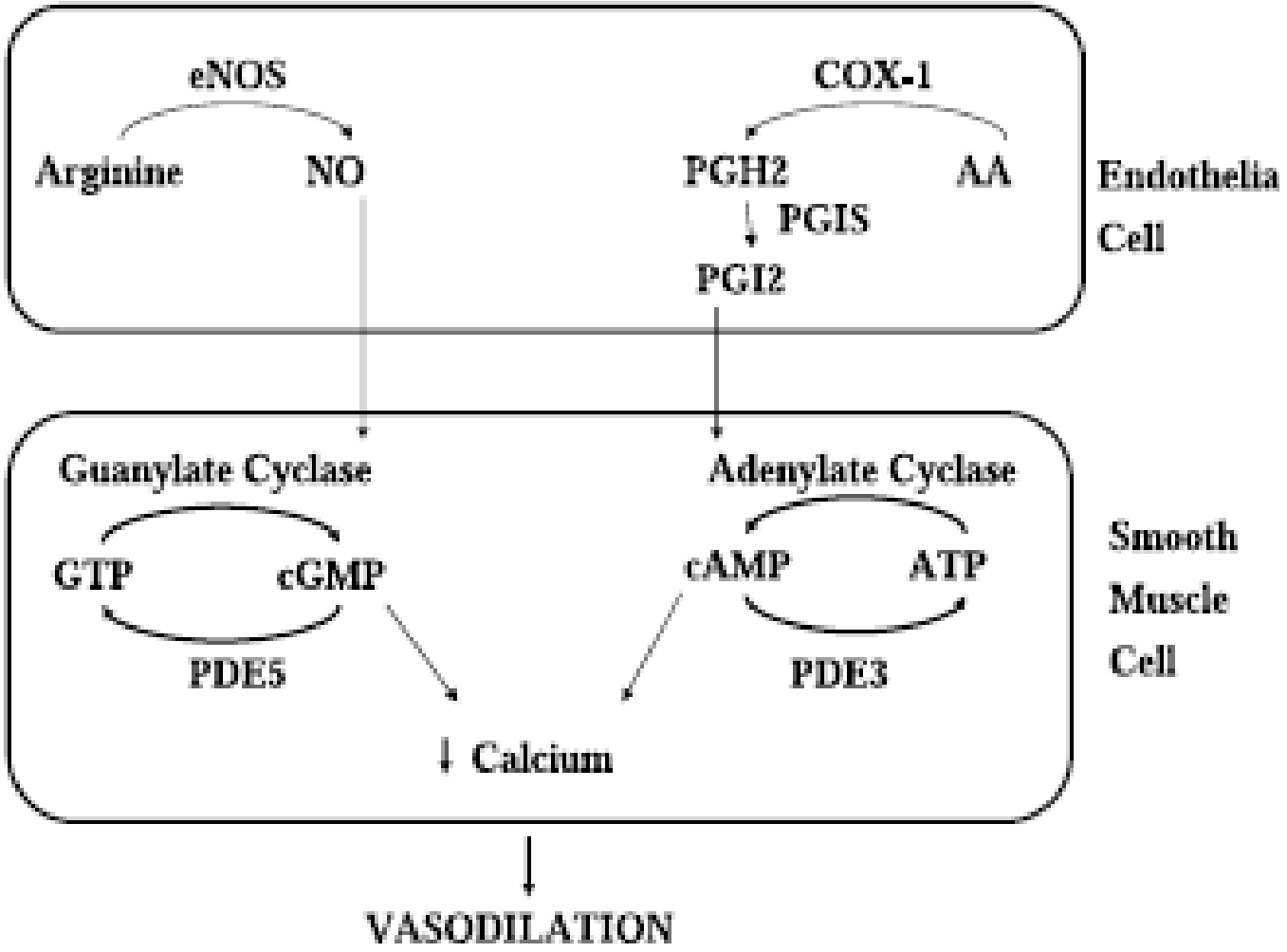
- It inhibits platelet activation and is also an effective vasodilator.

Prostacyclin

- A product of arachidonic acid metabolism produced mainly by endothelium
- Increases the cAMP level in vascular smooth muscle.
- A potent vasodilator
- A potential synergistic effect on the vascular tone when used combined with NO.
- Intravenous and aerosol administration
- Short half-life


Phospholipids
Phospholipase A2
Arachidonic acid
Aspirin
COX-1
COX-2
Aspirin (high dose)
Prostaglandin G2
HOX
Prostaglandin H2
Collagen
ADP
Thrombin
Thromboxane synthase
Prostacyclin synthase
Thromboxane A2
• Induces platelet aggregation
• Potent vasoconstrictor
• Induces proliferation of vascular smooth-muscle cells
• Proatherogenic
Prostacyclin
• Inhibits platelet aggregation
• Induces vasodilation
• Inhibits the proliferation of vascular smooth-muscle cells
• Antiatherogenic
• Gut protection
• Regulation of renal blood flow

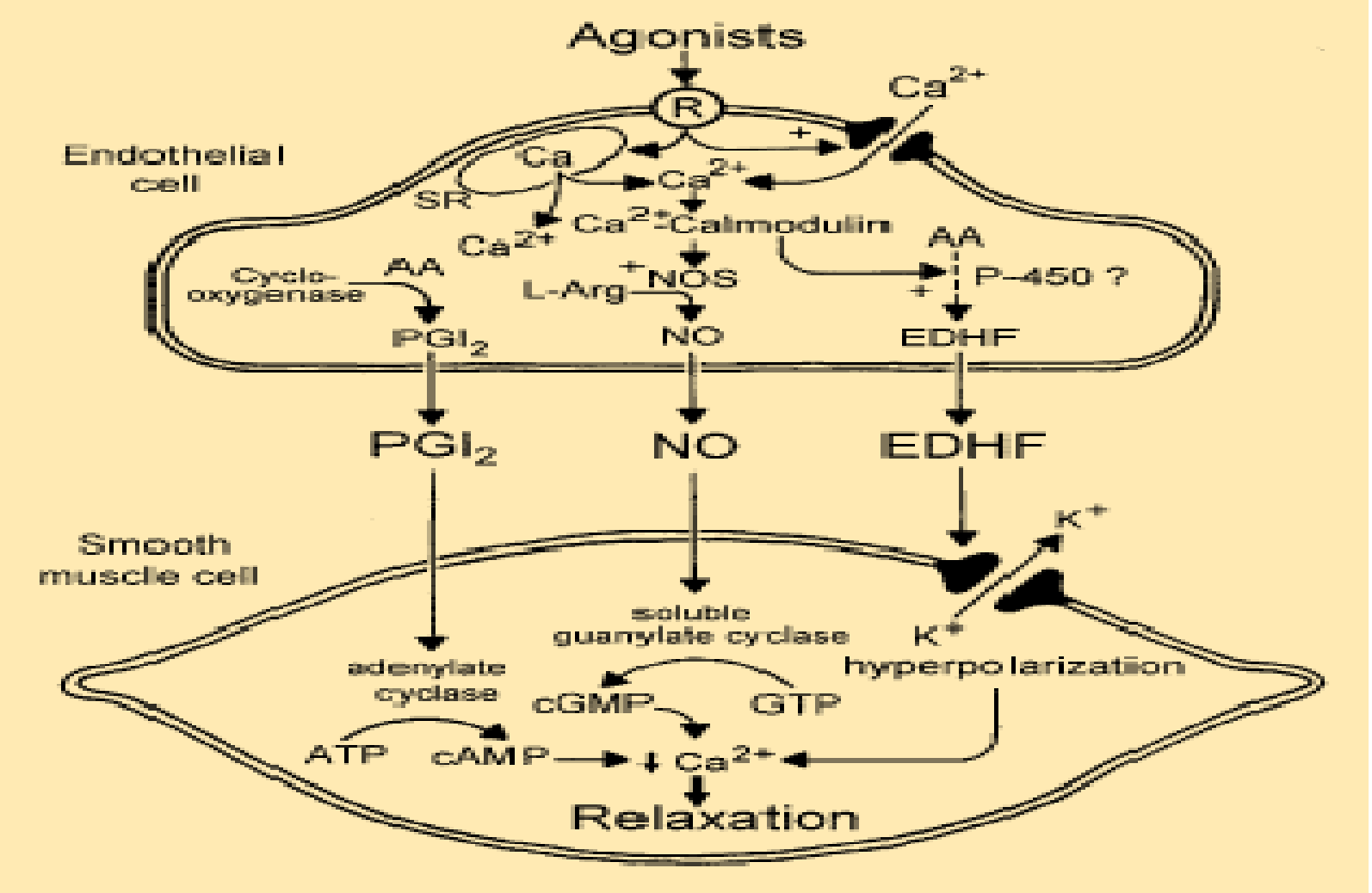
Vasodilatory Pathways



Endothelium-derived hyperpolarizing factor

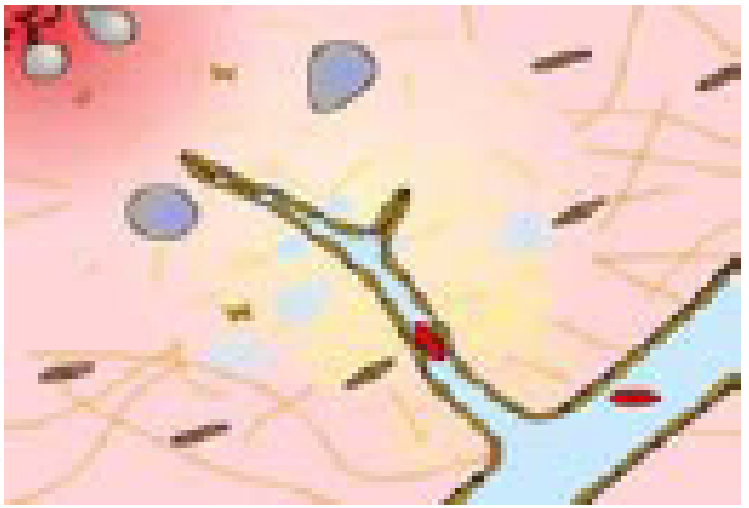
- Is a vasodilator, produced in endothelium.
- is distinct from both endothelium-derived nitric oxide or COX metabolites (i.e. , PGI2).
- dilates by hyperpolarizing the vascular smooth muscle;
- And involves potassium channel activation, most often calcium-activated potassium channels (K^{Ca})

Mediators of Vascular relaxation

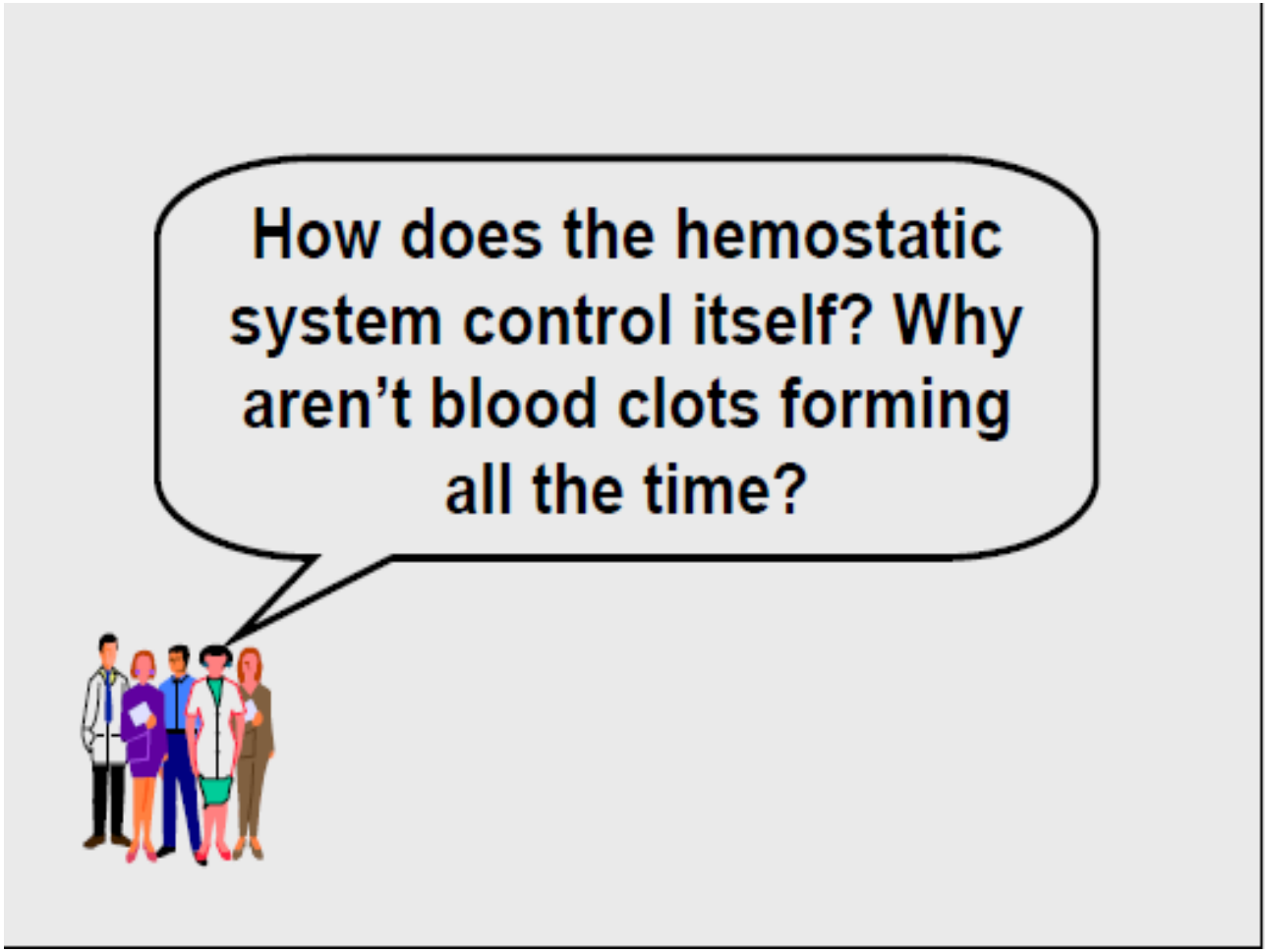


Endothelial mediators: Repair

- Platelet-derived growth factor (PDGF)
  - Mitogenesis of smooth muscle and fibroblasts
- Fibroblast growth factor (FGF)
  - Fibroblast proliferation
- Transforming growth factor – β (TGF-β)
  - Modulation of vascular repair (cell proliferation inhibition)
- Epidermal growth factor (EGF)
- Endothelial cell growth factor (ECGF)



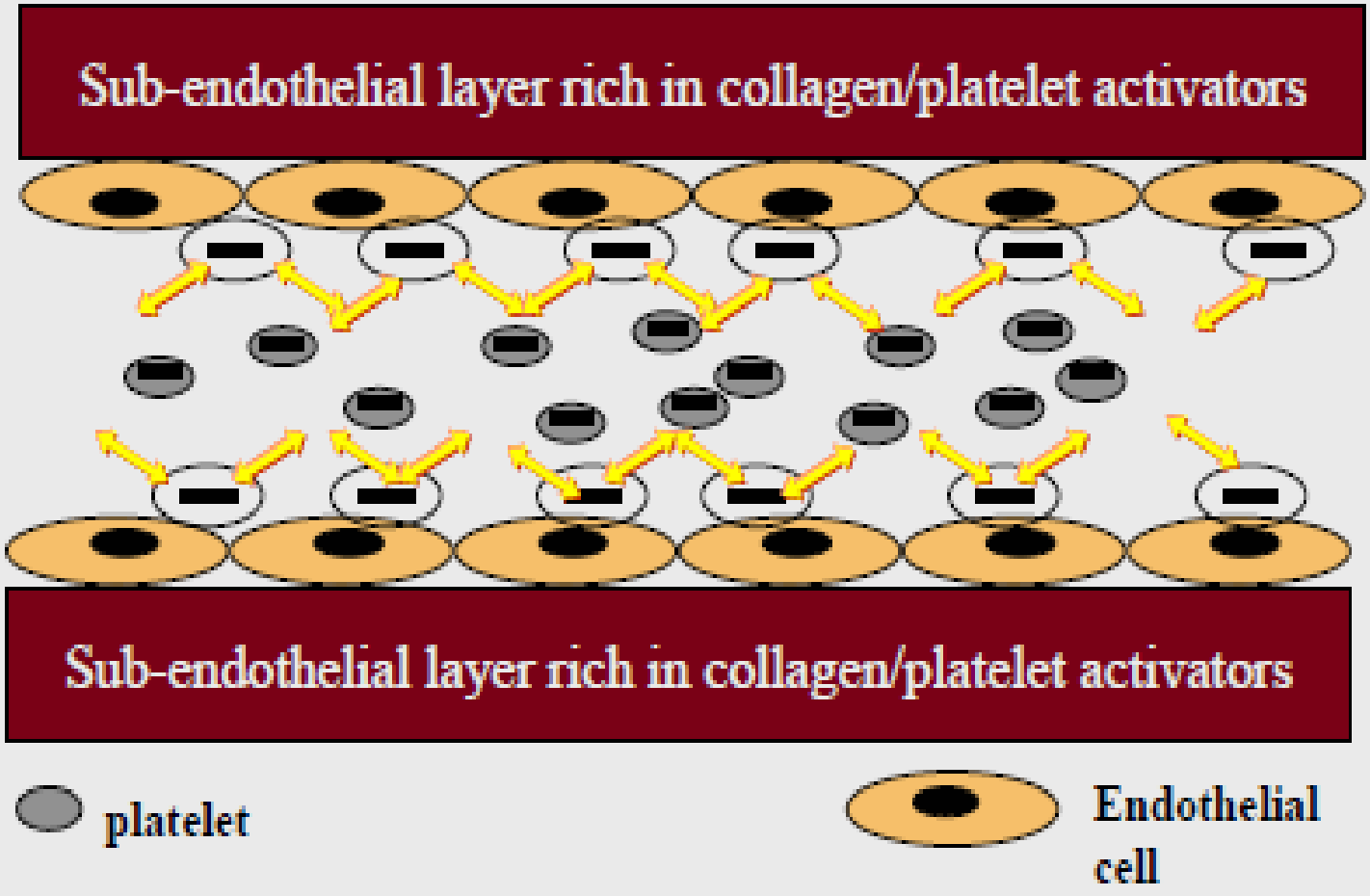
Upon conclusion one can ask the following questions



Physical Properties That Help Retard Clot Formation in Uninjured Blood Vessels

- Lamellar flow of blood in vessel-cellular components flow in center of stream and away from endothelial surface
- Negatively charged endothelial cells and platelets - negative charges repel platelets away from endothelium
- Endothelial cells shield platelets from collagen and other pro-aggregatory components of sub-endothelial layer

INTACT BLOOD VESSEL



Endothelial Cell - Properties that Prevent Initiation of Clotting

- Negative charged surface
- Production of Prostaglandin I_2 (PGI_2)- Prevents platelet aggregation
- Thrombomodulin expressed on surface of non-injured cells - binds thrombin and activates Protein C
- Heparan sulfate on surface - catalysis Antithrombin (ATIII) inactivation of coagulation enzymes
- Tissue plasminogen activator (tPA) released from endothelium - breaks down inadvertently formed fibrin that is NOT required for vessel repair

Platelets and Coagulation Proteins - Properties that Inhibit Clot Formation

- Platelets circulate in an inactive state- with injury, activating substances are exposed or produced
- PGI_2 produced by endothelial cells is a strong anti-aggregatory agent - helps keep platelets from activating
- Clotting factors circulate as ZYMOGENS-must be converted to an active enzyme by another enzyme, i.e., the "cascade of enzymatic events"
- Tissue factor is NOT present on the surface of uninjured endothelium- when tissue factor is exposed, the "Coagulation Cascade" begins

Next lecture

Primary Hemostasis: Part 2

Vo

Lecture 3: Primary Hemostasis Part-2

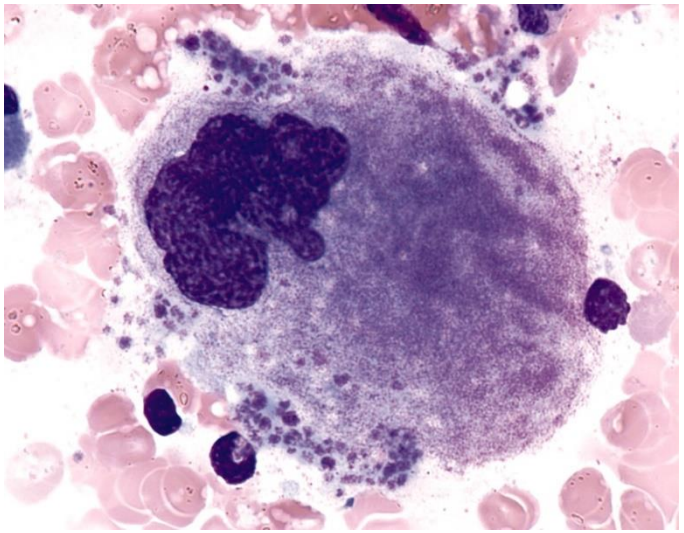
Miss: Amany S. AlHindi

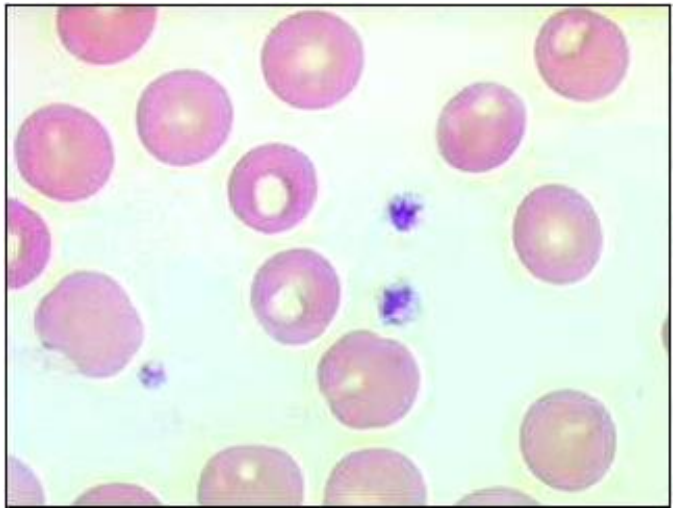
M.Sc. in Biological sciences from Islamic University of Gaza

Now we will talk about the second major component of primary hemostasis in more details

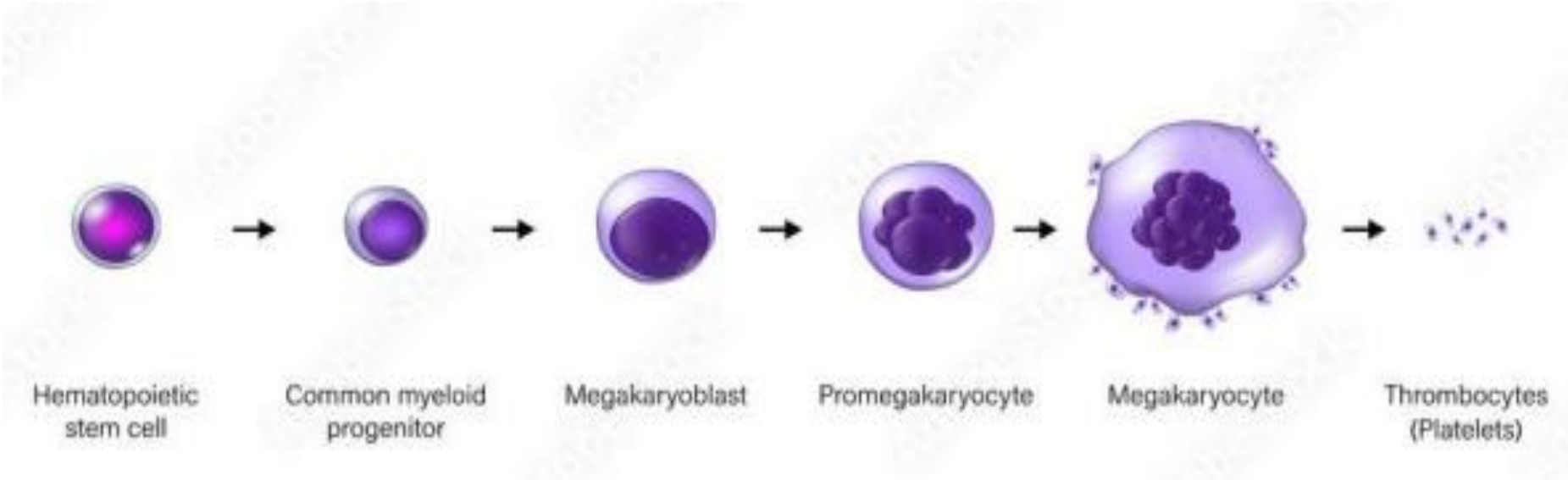
Platelets

- Second major component of the hemostatic system.
- Platelets are produced from the cytoplasm of bone marrow megakaryocytes.
- Disc-shaped, non-nucleated cells, 2-3 µm
- Appear blue-gray particles with purple-red granules about one tenth the size of red cells.
- Lifespan is 7–10 days.
- The normal platelet count for all age groups is 150–450 $\times$ 10^9/L.
- About one third of all platelets are sequestered in the spleen.





Thrombopoiesis



Platelets Production

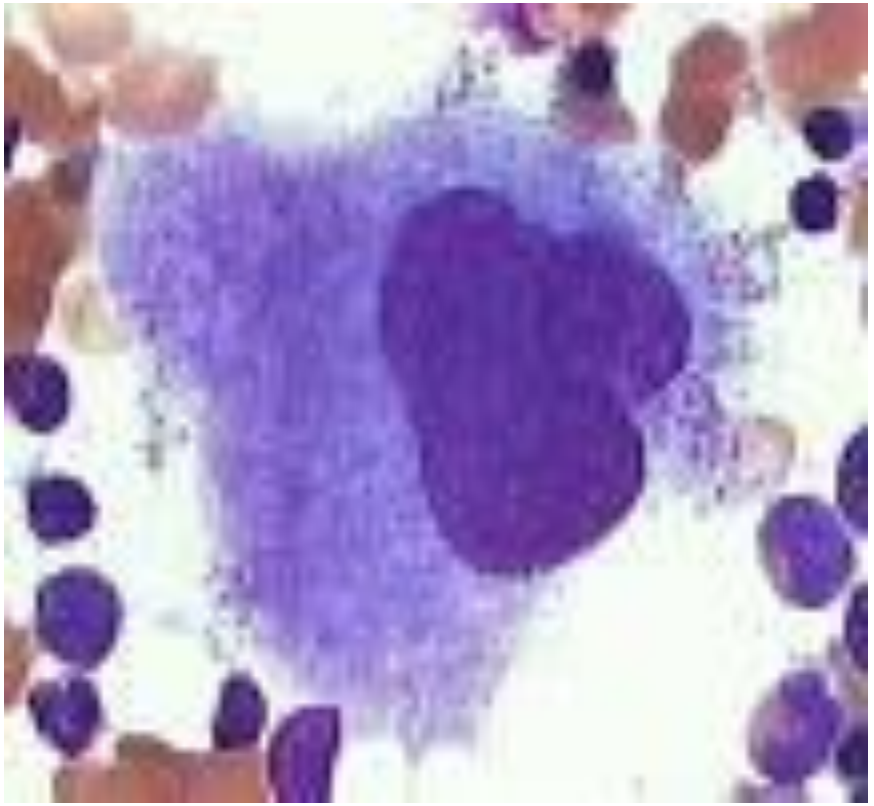
- Produced predominantly by the bone marrow megakaryocytes as a result of budding of the cytoplasmic membrane.
- Megakaryocytes are derived from the haemopoietic stem cell.
- Originate from CFU-GEMM to form CFU-Meg
- Cytokines and growth factors such as IL-3 and GM-CSF influences progenitor stages.
- Stimulated to differentiate to mature megakaryocytes under the influence of various cytokines, including thrombopoietin.
- Platelets play a key role in securing primary haemostasis.

Platelet Development

- Megakaryoblast
  - 10-15 µm
  - Increased nuclear: cytoplasmic ratio
- Promegakaryocyte
  - 80 µm
  - Dense, alpha and lysosomal granules
- Basophilic megakaryocyte
- Megakaryocyte

Production of Platelets

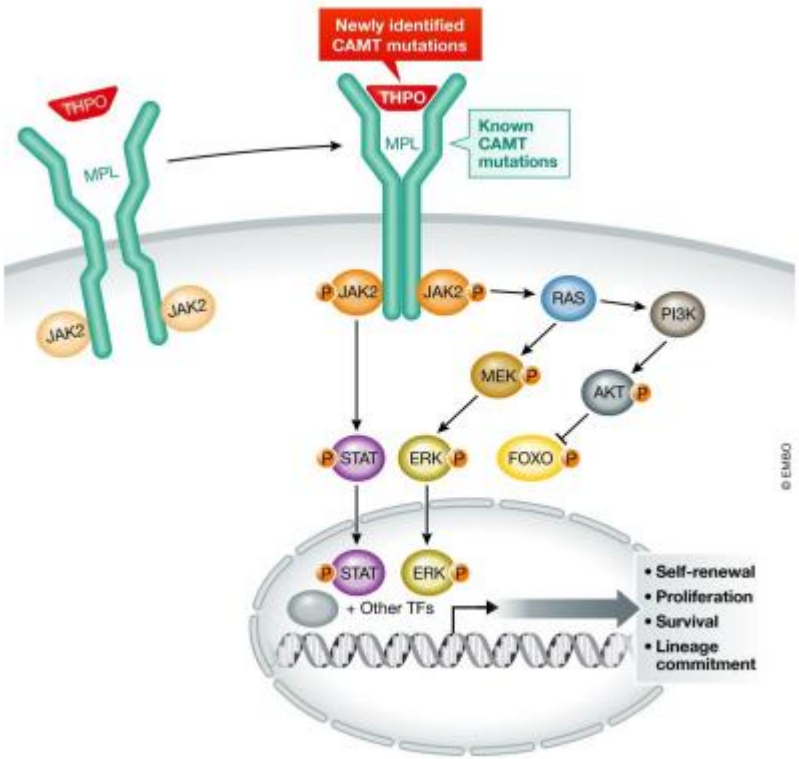
- Precursor Cell= Megakaryocyte
- Produces about 2000-4000 platelets
- Platelets are released via sinuses of bone marrow



Platelets Production

- Megakaryocyte growth and platelet production are largely controlled by **thrombopoietin (TPO),**
- TPO : a growth factor produced by the liver. The TPO level is constant and does not vary with platelet count.
- The **TPO receptor (c-mpl)** is expressed on both megakaryocytes and platelets. Because under normal conditions the platelet mass greatly exceeds the megakaryocyte mass, the amount of TPO available for binding to megakaryocytes is limited.
- In situations in which the platelet mass is reduced, such as thrombocytopenia, more TPO is available for binding to megakaryocytes, which stimulates megakaryocyte growth and increased platelet production.

Thrombopoietin



- Growth Factor of Thrombopoiesis is Thrombopoietin act on cMPL receptors through JAK2 signals.
- In essential thrombocythemia, JAK2 mutation occurs in about 50%, and cMPL mutation in about 3%

Thought question…

- If a patient had a low platelet count what will happen?

Answer…

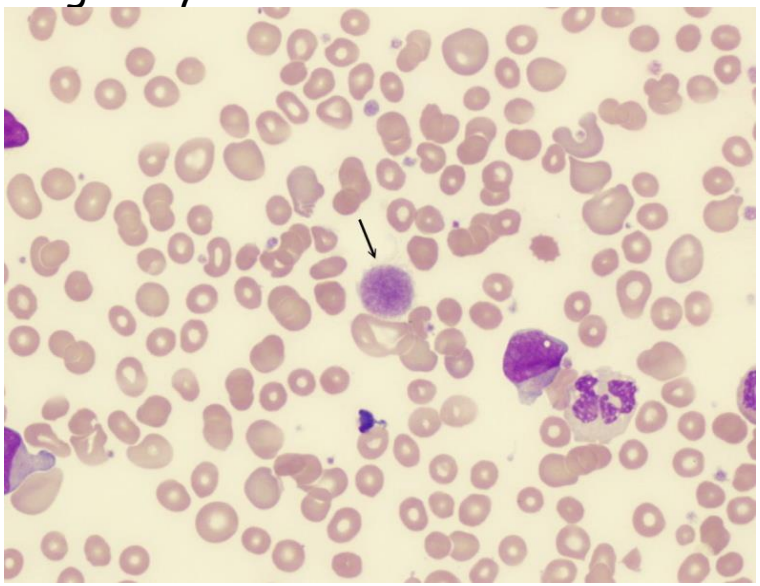
- TPO increases the number of megakaryocytes in the bone marrow, increases size and DNA count of megakaryocytes and increases maturation rate

Platelet

Giant platelets and micromegakaryocytes : Seen in conditions with increased need and/or destruction

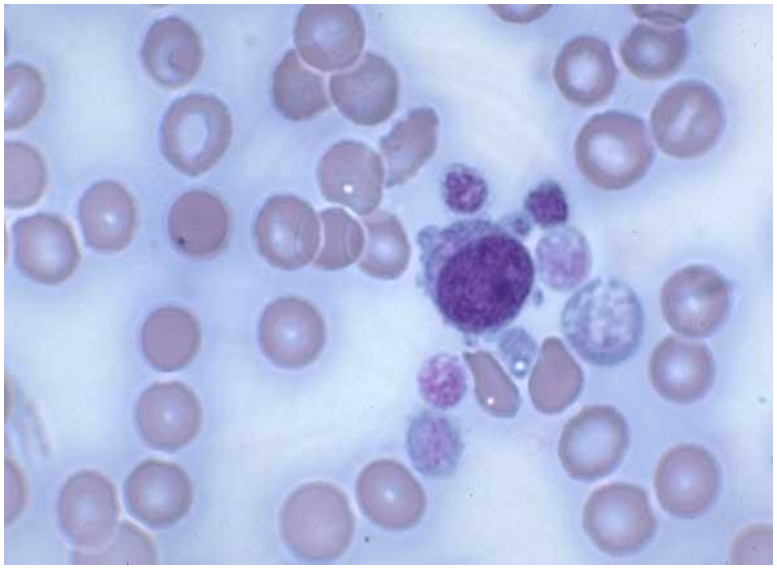
Giant platelets

- May Hegglin anomaly, Bernard-Soulier syndrome, pregnancy, malignancy

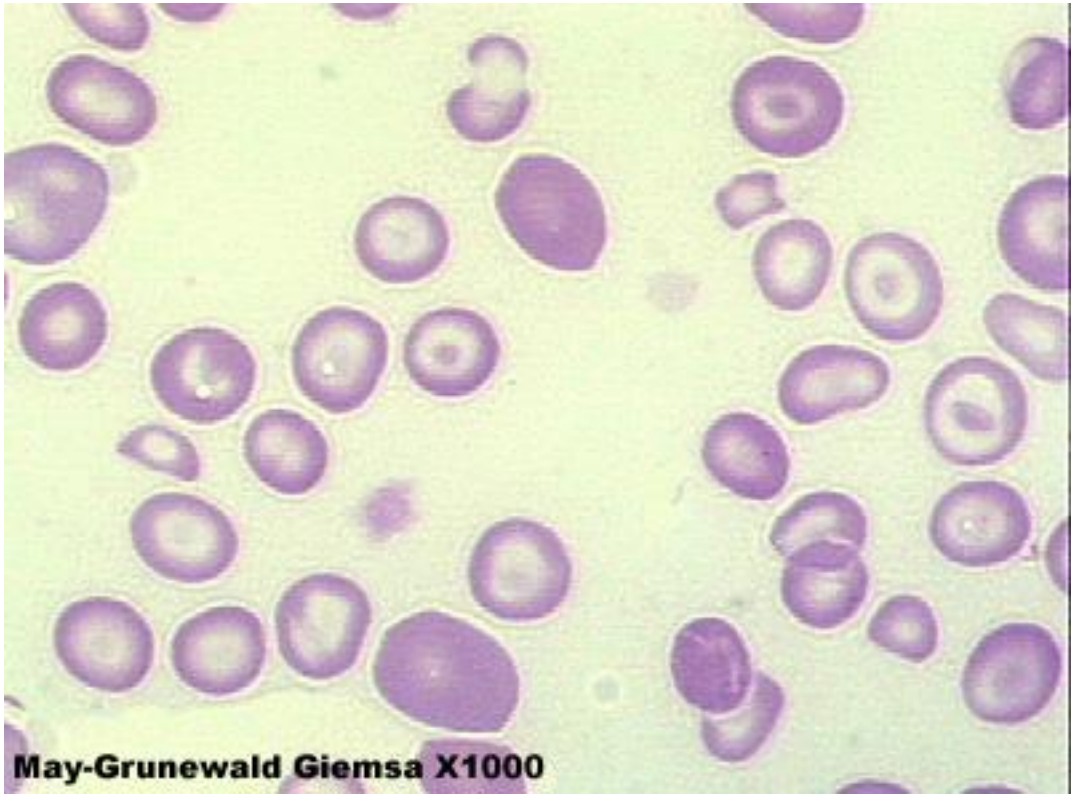


Micromegakaryocytes= Dwarf Megs

- Seen in malignant disorders such as CML and MDS

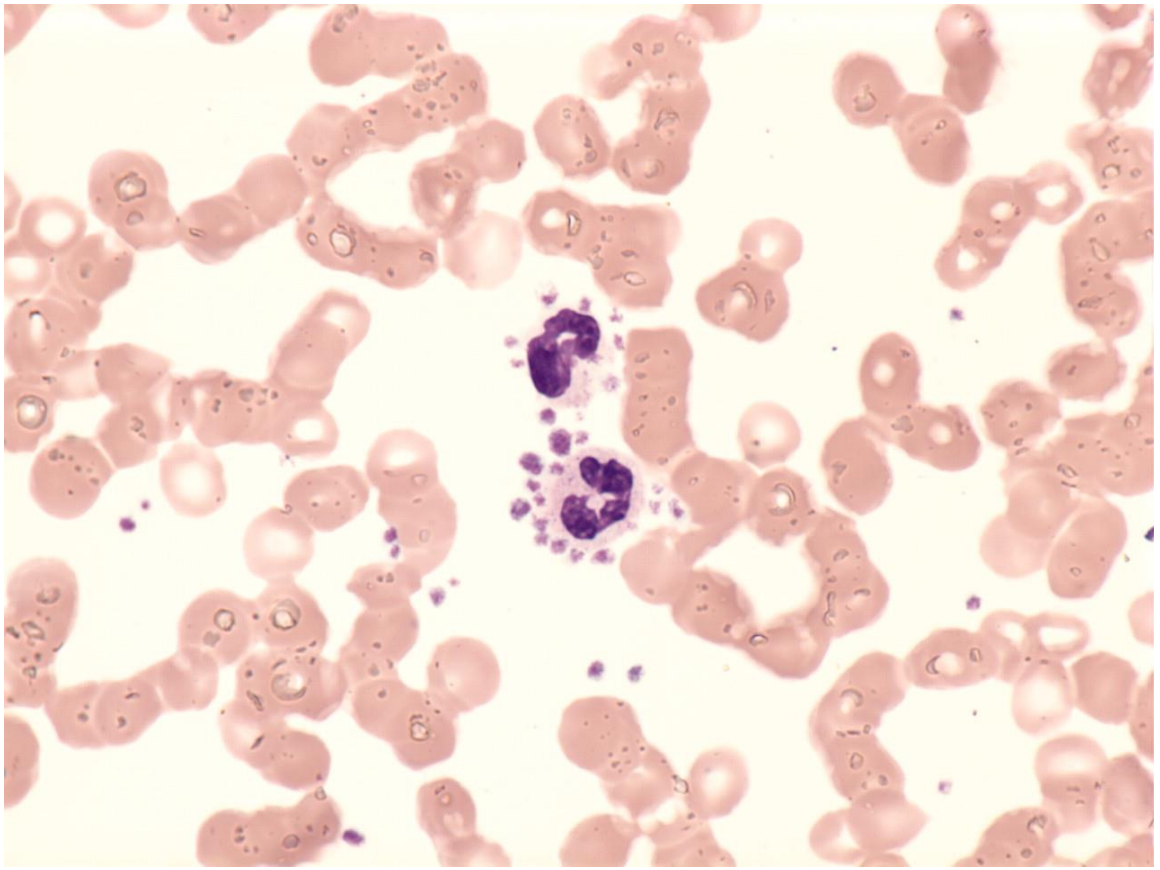


Gray Platelet Syndrome



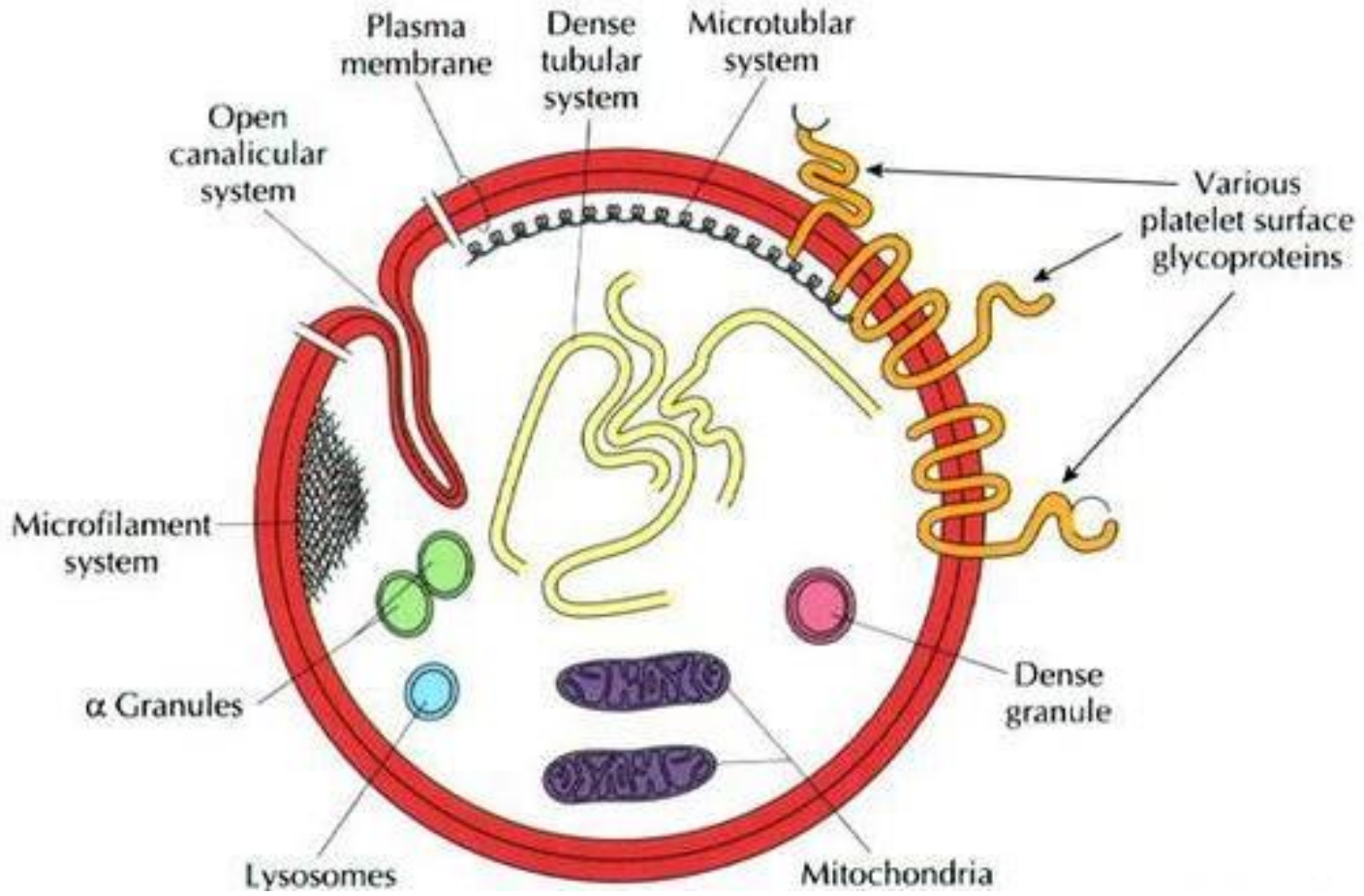
The platelets lack alpha granules

Adhesion of platelets to a neutrophil (platelet satellitism)



Platelet satellitism refers to the EDTA-anticoagulant induced formation of a platelet rosette which is characterized by four or more platelets around a neutrophil or band neutrophil. Platelet satellitism falsely decreases platelet counts.

Structure of platelets



Structure of platelets

The structure of platelets is divided into 4 zones or regions.

1- Peripheral zone
2- Structural zone
3- Organelle zone
4- Membrane systems

Structure of platelets

- **Peripheral zone:**

Responsible for platelet adhesion and aggregation

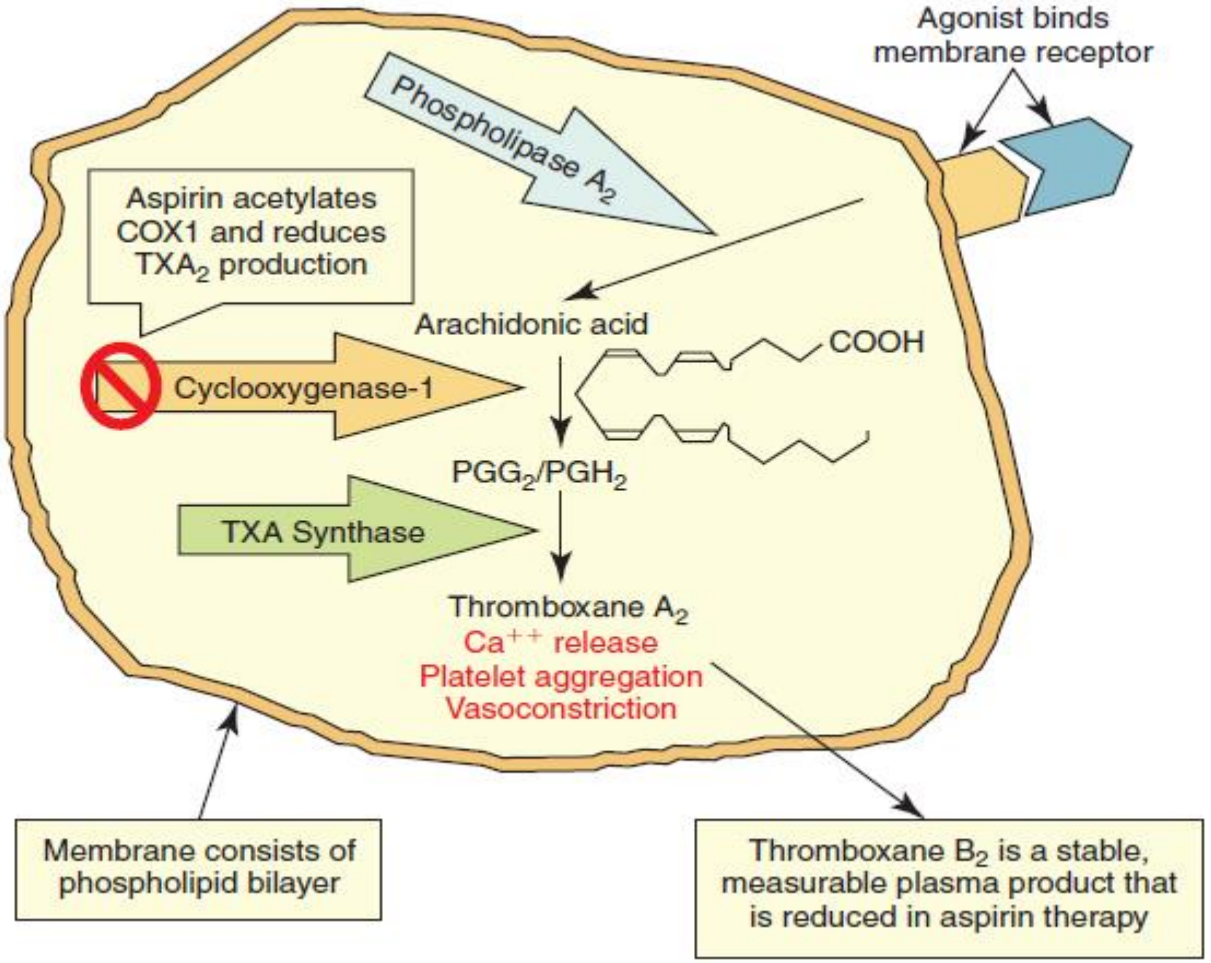
1. **Glycocalyx:**
   - **Negatively charged to repel other platelets and to repel endothelial cells.**
   - **Site of PLT functional environment:**
     - Blood Group specificity
     - Tissue compatibility
     - PLT antigenicity
     - Adhesion receptors
   - **Contains glycoprotein receptors:**
     - GPIb binds von Willebrand's factor needed for platelet adhesion to collagen
     - GPIIb/IIIa bind fibrinogen needed for aggregation
     - Bind ADP and thrombin, promoting aggregation
     - Factors I, V, VIII on surface, involved in 2° hemostasis

- Peripheral zone:

2. Plasma membrane:

- Exposed on platelet activation
- a source of phospholipids, in which secondary hemostasis occur on the phospholipids rich platelet surface. Layer called PF3 (platelet factor) surface for interaction of plasma coagulation factors.
- The plasma membrane is also a sources of arachidonic acid in which its metabolism will yield a platelet activating substances and agents. These agents are utilized in platelet aggregation and vascular vasoconstriction, especially thromboxane A2 a potent PLT aggregator and vasoconstrictor.

Thromboxane A2 a potent PLT aggregator and vasoconstrictor.



- Structural or Sol-Gel zone: Responsible for platelet retraction/contraction functions and platelet shape.
  - Microtubules: Tubulin
  - Cytoskeleton: Actin/Myosin
  - Binding protein

- Organelle zone:
  - Is responsible for the metabolic activities of the platelet.
  - Responsible for storage and platelet release functions
  - Granules
    - Dense bodies, alpha granules, lysosomal granules and microperoxisomes
  - Mitochondria
  - Glycogen

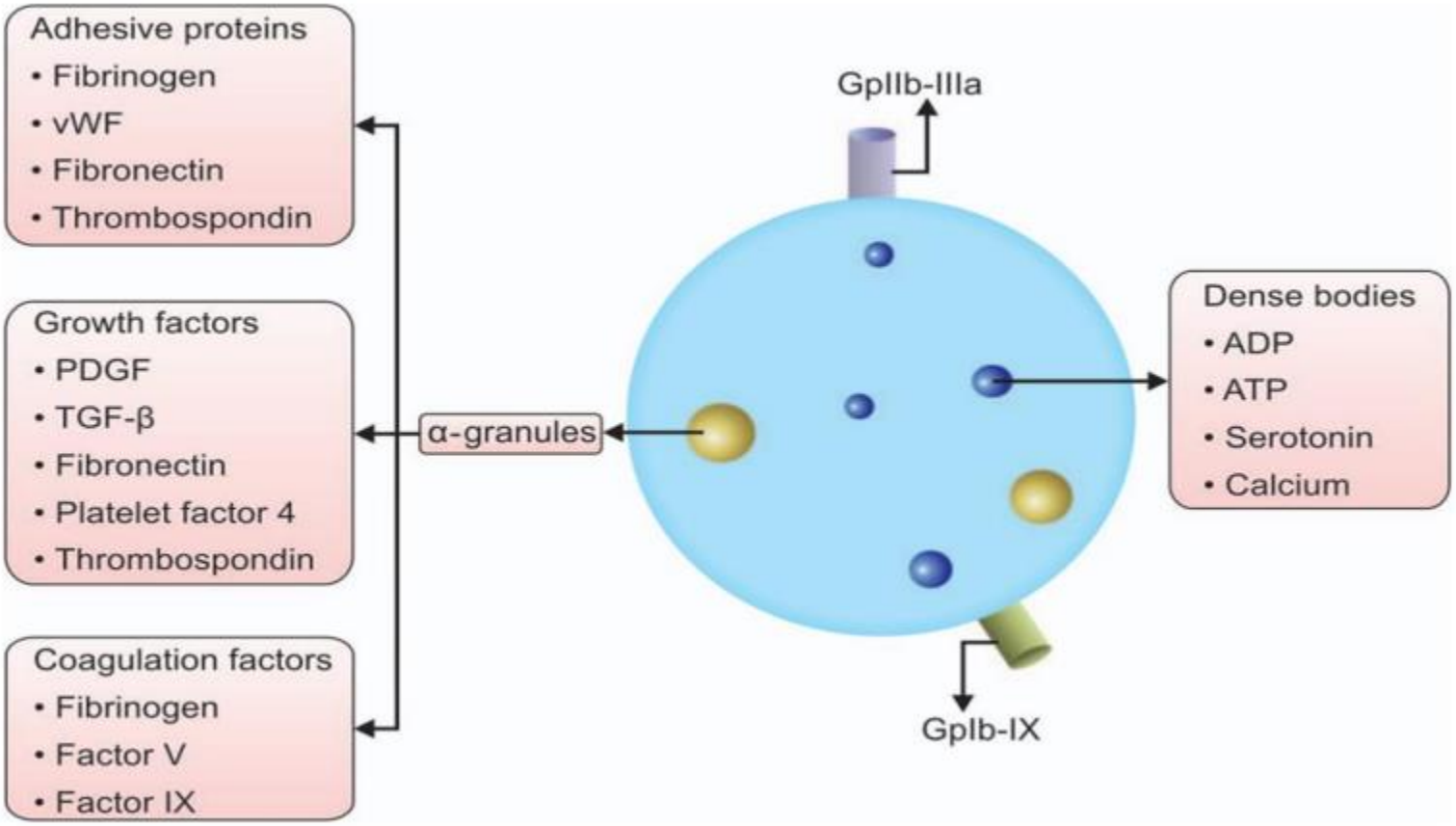
- Membrane zone:
  - Canalicular System – derived from DMS of megakaryocyte
  - Dense Tubular System – calcium storage

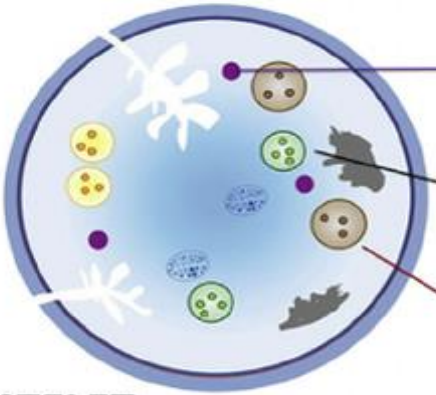
Platelet Receptors

- GPIb/IX – vWF
  - Required for PLT adhesion
- GPIIb/IIIa – Fibrinogen
  - Required for PLT aggregation
- Phospholipid (Pl)
  - Bind vitamin K dependent proteins , Ca^{++} dependent
  - Bind Va and VIIIa (called "PF3")

PLT Granules

Granule	Function
Alpha granule	
Thromboglobulin	Inhibit heparin; vessel repair
PF4 (Platelet factor 4)	Inhibit heparin, neutralize heparin
PDGF (PLT derived growth factor)	Vessel repair
Fibrinogen, Factors V & VIII	Fibrin formation
vWF	PLT Adhesion and carrier of factor VIII
Plasminogen	Precursor of plasmin (fibrinolysis)
a1-antiplasmin	Plasmin inhibitor
Fibronectin	Promotes PLT spreading
Dense granule	
ADP/ATP	PLT agonist (activator)
Calcium	Regulates PLT activation
Serotonin (5HT)	Promotes vasoconstriction
Lysosomes	
Proteolytic, hydrolytic enzymes	Digest vessel wall matrix and debris


Adhesive proteins
• Fibrinogen
• vWF
• Fibronectin
• Thrombospondin
Growth factors
• PDGF
• TGF-β
• Fibronectin
• Platelet factor 4
• Thrombospondin
Coagulation factors
• Fibrinogen
• Factor V
• Factor IX
α-granules
GpIIb-IIIa
GpIb-IX
Dense bodies
• ADP
• ATP
• Serotonin
• Calcium



PLATELET

LYSOSOMES

- α-Arabinoside
- β-Galactosidase
- β-Glucuronidase
- n-Acetylglucosaminidase
- Elastase
- Collagenase
- Cathepsin

DENSE GRANULES

- ADP, ATP
- Serotonin
- Ca^{++}
- Epinephrine
- Histamine

α-GRANULES

Coagulation Factors

- Factor V
- Factor XI
- Factor XIII
- Prothrombin
- Antithrombin
- α_2-Macroglobulin
- α_2-Antiplasmin
- Plasmin, Plasminogen
- Protein S
- PAI-1, TFPI

Chemokines

- IL-8
- NAP-2
- RANTES
- MCP-1,-3
- MIP-1α
- β-Thromboglobulin

Regulators of Growth and Angiogenesis

- bFGF
- HGF
- IGF-1
- VEGF-A, -C
- PDGF-AA,-AB,-BB
- BDNF
- Angiostatin
- PF4
- Thrombospondin
- EGF
- CTGF
- TGF-β
- Angiopoietin-1
- SDF-1
- MMP-1,-2,-9
- Endostatin
- TIMP-1,-4
- BMP-2,-4,-6

Adhesion Molecules

- P-Selectin
- Von Willebrand factor
- Vitronectin
- Fibrinogen
- Integrin αIIbβ3
- Integrin αVβ3
- Fibronectin

Immunologic Molecules

- Complement factors
- Platelet factor H
- β1H Globulin
- Factor D
- C1 Inhibitor
- IgG
- Thymosin-β4

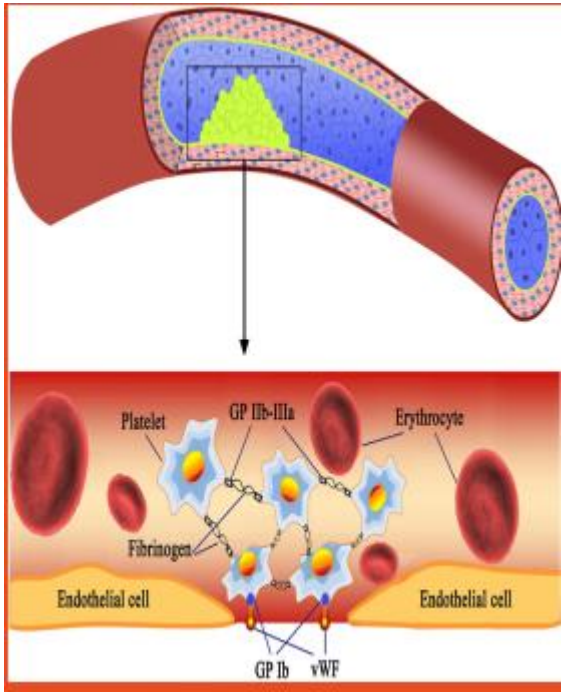
Function of Platelets

1. Surveillance of blood vessel continuity
   - Checks endothelial lining for gaps and breaks
   - Fill-in small gaps caused by separation of endothelial cells
2. Formation of primary hemostatic plug
3. Provides the reaction surface for some coagulation system reactions, as well as platelet factor 3 (PF3) which is platelet phospholipid.
4. Aid in healing injured tissue

Formation of Primary Hemostatic Plug

- Once the platelets "normal" environment is changed, they become activated or adhesive.

- Four stages of plug formation:
  - Adhesion
  - Change shape
  - Aggregation
  - Platelet Secretion& Release

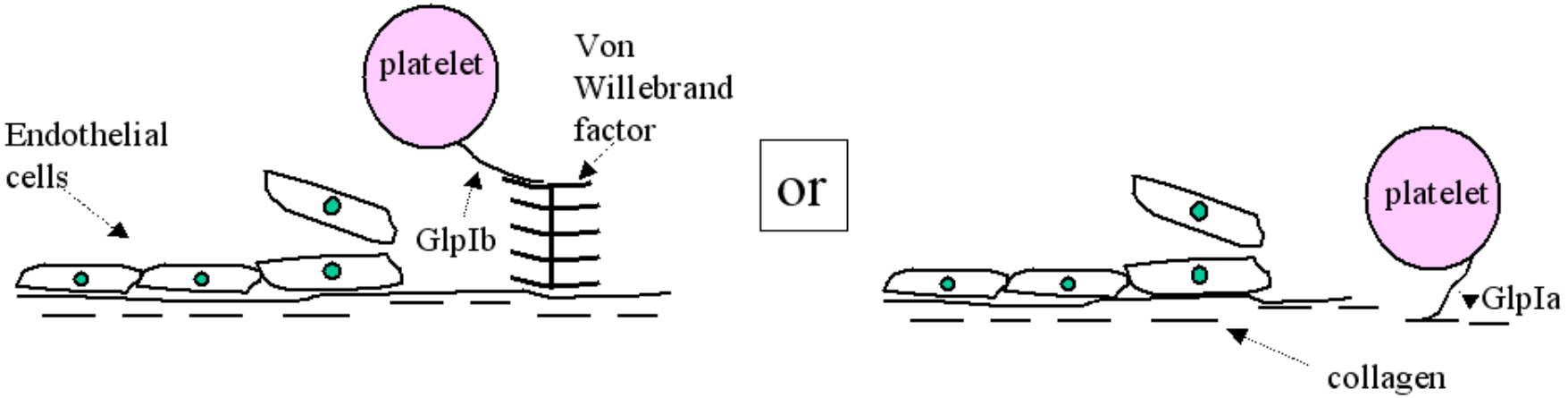


Stage 1: Platelet Adhesion

- It is the binding of platelet to non platelet surface such as sub endothelial collagen.
- Involves changes from a disc shape to a slightly broader, plate like form to increase surface area.
- a number of plasma proteins are required for normal platelet adhesion.
  - **Thrombin**
  - **Fibronectin**
  - **vWF**
- vWF is the largest component of factor VIII and secreted by platelets and by vascular endothelial cells.

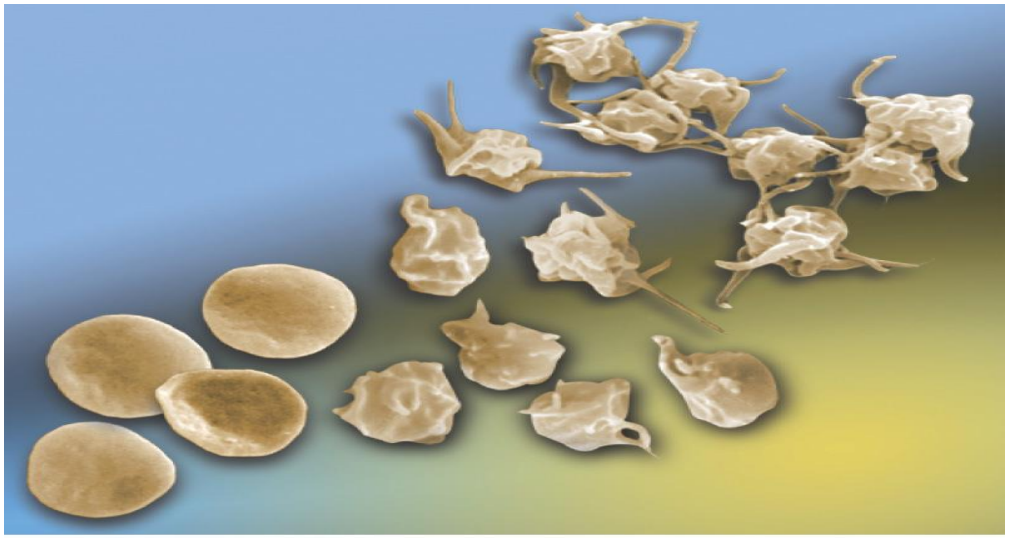
- Mechanism components
  - PLT receptor GP Ib/IX/V
  - vWF: links PLT to endothelial binding site
  - Collagen fibers

- vWF bridge physical distance between platelet and sub endothelial collagen
  - Increase bond that seal platelet to the vessel wall
  - Adhesion is Reversible & No ADP is released



Stage 2: Platelet shape change

- Shape change: from flattened disc-shaped to spiny spheres with long projections pseudopods.
- Actin contracts & pseudopods form.
- REVERSIBLE
  - Facilitates activation
- After change shape each plt has large surface area for biochemical Reactions, and large area to contact with injured tissue and other plts.



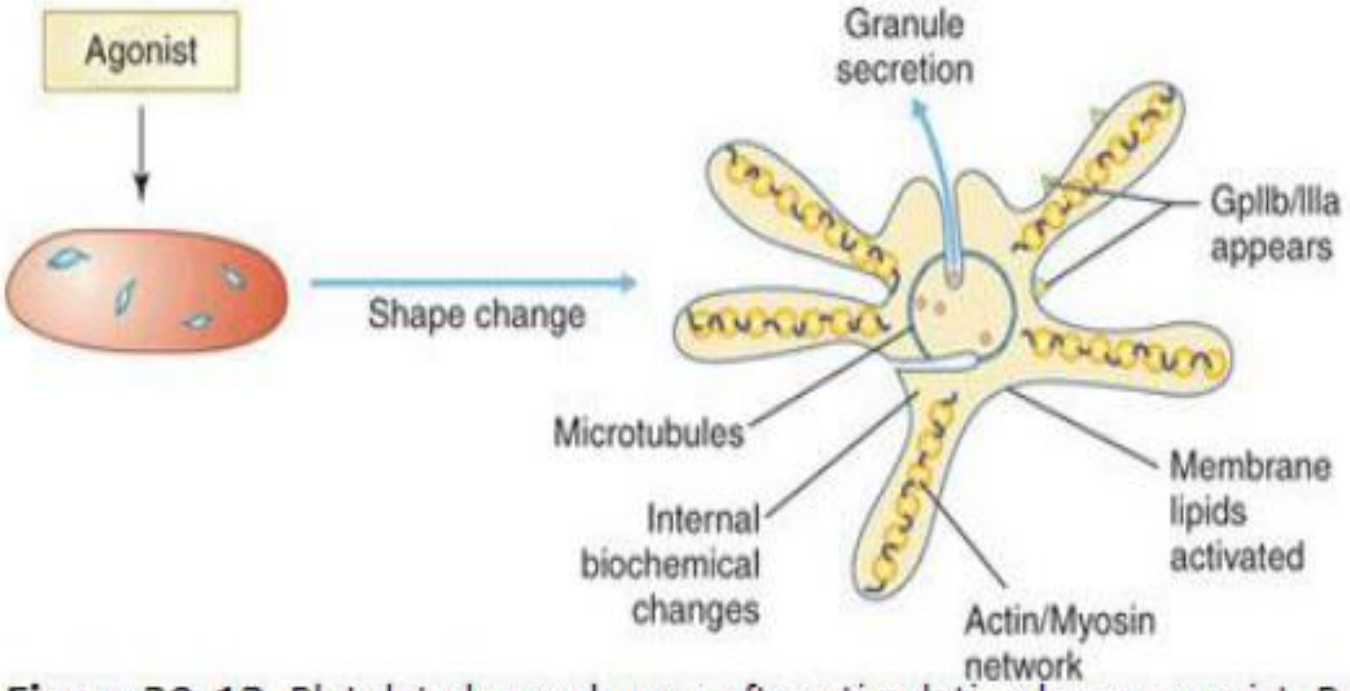


Figure 29-12. Platelet shape change after stimulation by an agonist. Pseudopods develop on the platelet surface and contain a network of actin and myosin; the microtubule circumferential ring contracts; the membrane phospholipids are activated; glycoprotein IIb/IIIa receptors appear; internal biochemical changes occur; and granule secretion follows.

Platelet Agonists

- An agent that induces platelet activation.
- Some agonists generated by platelets themselves and some by cells or molecules at the site of injury.
- Agonists are grouped as strong or weak.
- Strong agonists (collagen, thrombin) can activate the full range of platelet function themselves (shape change, secretion, aggregation).
- Weak agonists initiate platelet activation but require platelet synthesis and release of endogenous TXA_2 to drive full activation through secretion and aggregation.
- Thrombin is the most potent activator of plts in vivo.

Platelet Agonists

Table 29-9. Platelet Agonists

Platelet-derived agonists

- ADP
- Serotonin
- Platelet-activating factor (PAF)
- Thromboxane A_2 (TXA_2)

Other (nonplatelet-derived) agonists

- Collagen*
- Thrombin*
- Epinephrine

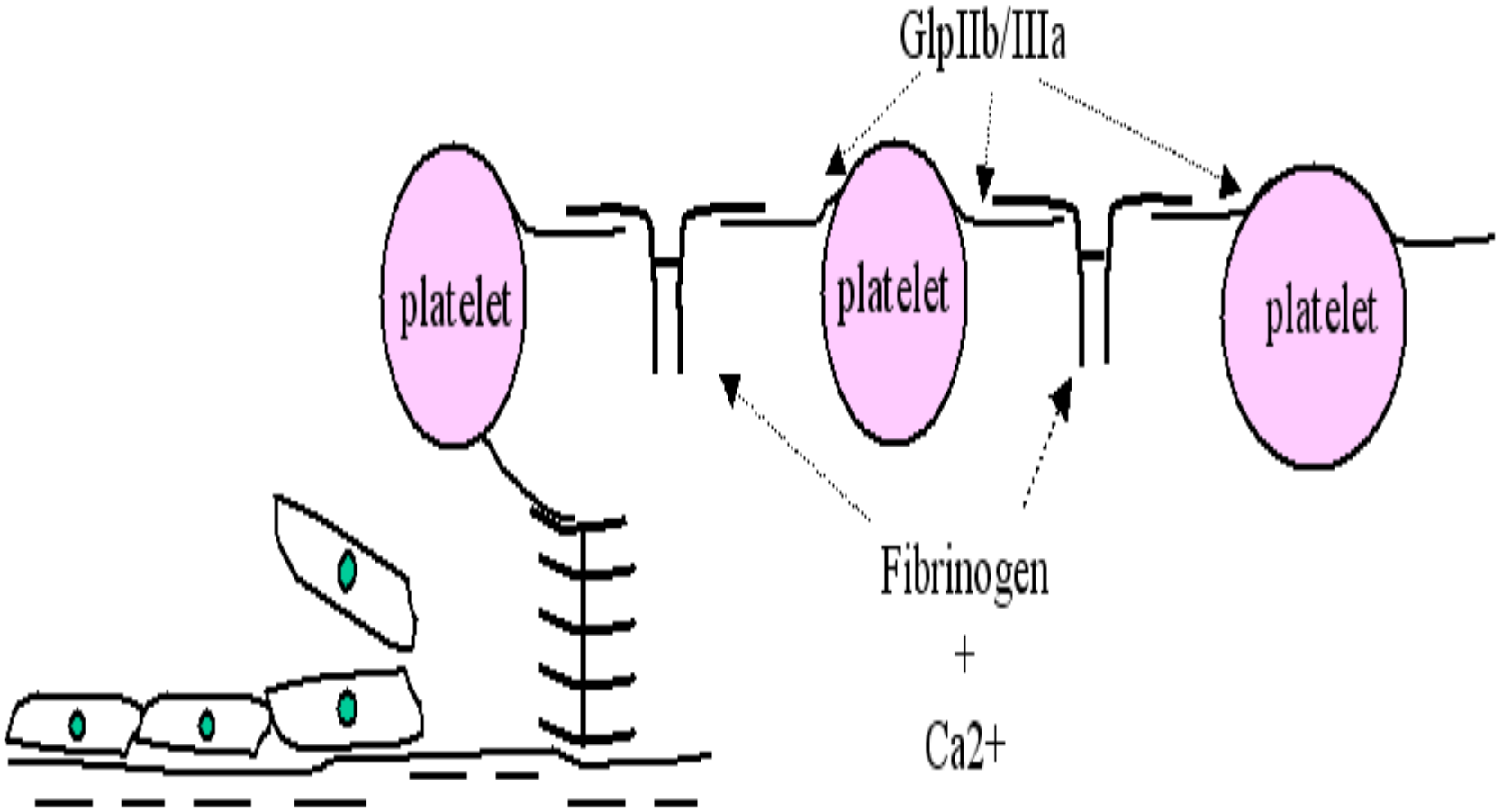
* strong agonists (full activation of platelet does not require cyclooxygenase activity)

Stage 3: Platelet Aggregation

- Aggregation is the property by which platelets bind to one another.
- When platelets are activated, a change in the GP IIb/IIIa receptor allows binding of fibrinogen, as well as VWF and fibronectin.
- Fibrinogen binds to GP IIb/ IIIa receptors on adjacent platelets and joins them together in the presence of ionized calcium (Ca^{2+}).
- Aggregation is triggered by:
  - PLT factor 3, adenosine diphosphate (ADP), Thrombin and Thromboxane A2
- Mechanism components
  - ATP, Ionized calcium, Fibrinogen, PLT receptor GPIIb/IIIa
- Aggregation: irreversible = white clot, platelet plug formed.

Platelet Aggregation

- Exposure of GPIIb/IIIa sites bind fibrinogen
- Fibrinogen + activated platelets serves as a bridge between two platelets
- Calcium must be present
- Activated platelet membrane generates TXA_2
- TXA_2 stimulates release
- Irreversible once platelet aggregate they do not disaggregate.
- Platelates shed membranes rich in phospholipid (appearance of PLT factor 3 on the PLT membrane).
- this happens during PLT plug formation.
- serves as a catalytic site for the coagulation proteins.
- Aggregation is also a response to helps initiation of the coagulation mechanism.


GlpIIb/IIIa
platelet
platelet
platelet
Fibrinogen
+
Ca2+

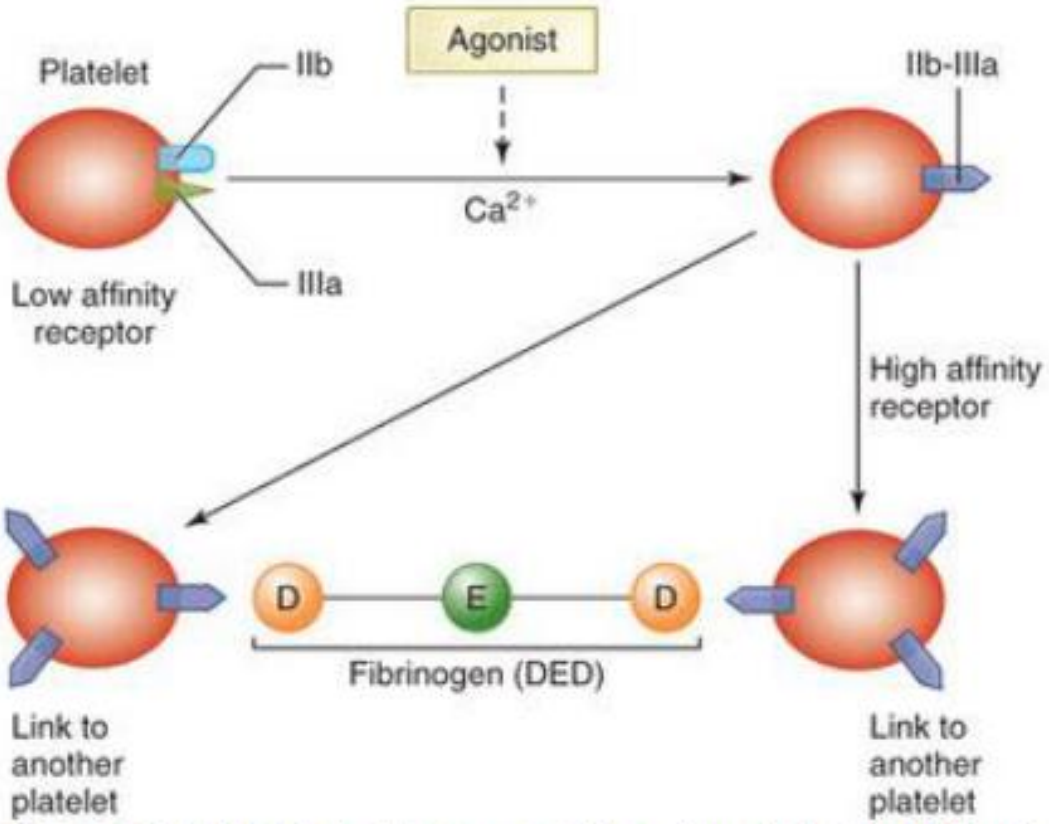
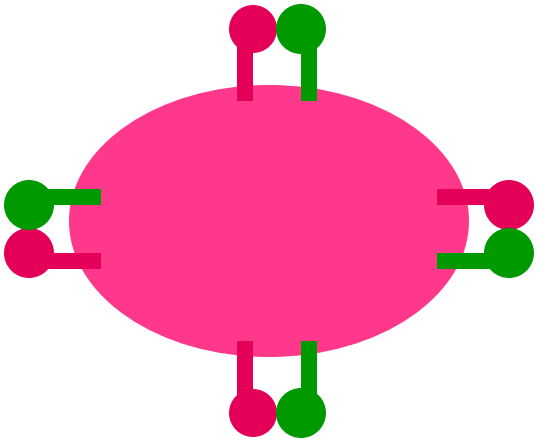


Figure 29-13. Platelet aggregation. Agonist stimulation in the presence of Ca^{+2} causes high-affinity fibrinogen receptors, activated glycoprotein IIb/IIIa, to appear on the platelet surface. Fibrinogen binds horizontally to two platelets by peptide sequences at the terminal end of its gamma and alpha chains in the D domains, one gamma chain to GPIIb/IIIa receptors on each platelet. Fibrinogen thus becomes a bridge between the two platelets.

Resting Platelet

Aggregated Platelets

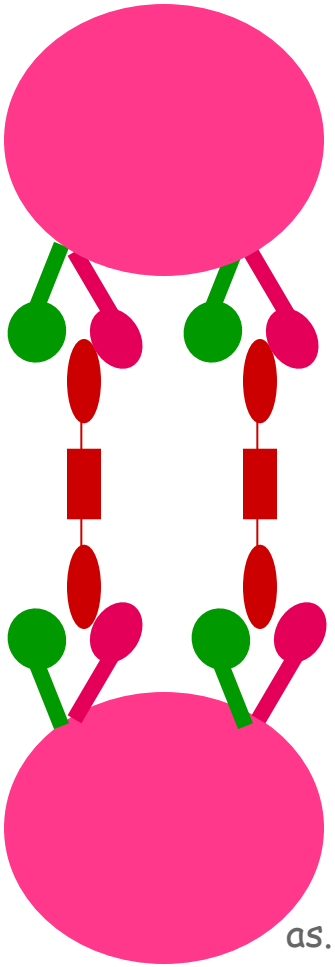
GPIIb/IIIa
(inactive)

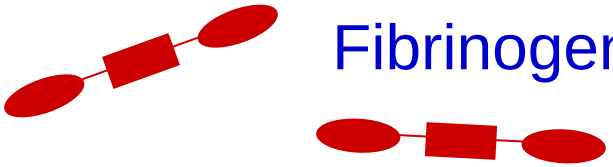


Agonist

e.g. ADP
TXA_2
thrombin

GPIIb/IIIa
(activated)





Fibrinogen

high shear: von Willebrand factor substitutes for fibrinogen

Stage 4: Platelet Secretion & Release

- Platelets secrete the contents of their granules during adhesion and aggregation, with most secretion occurring late in the platelet activation process.

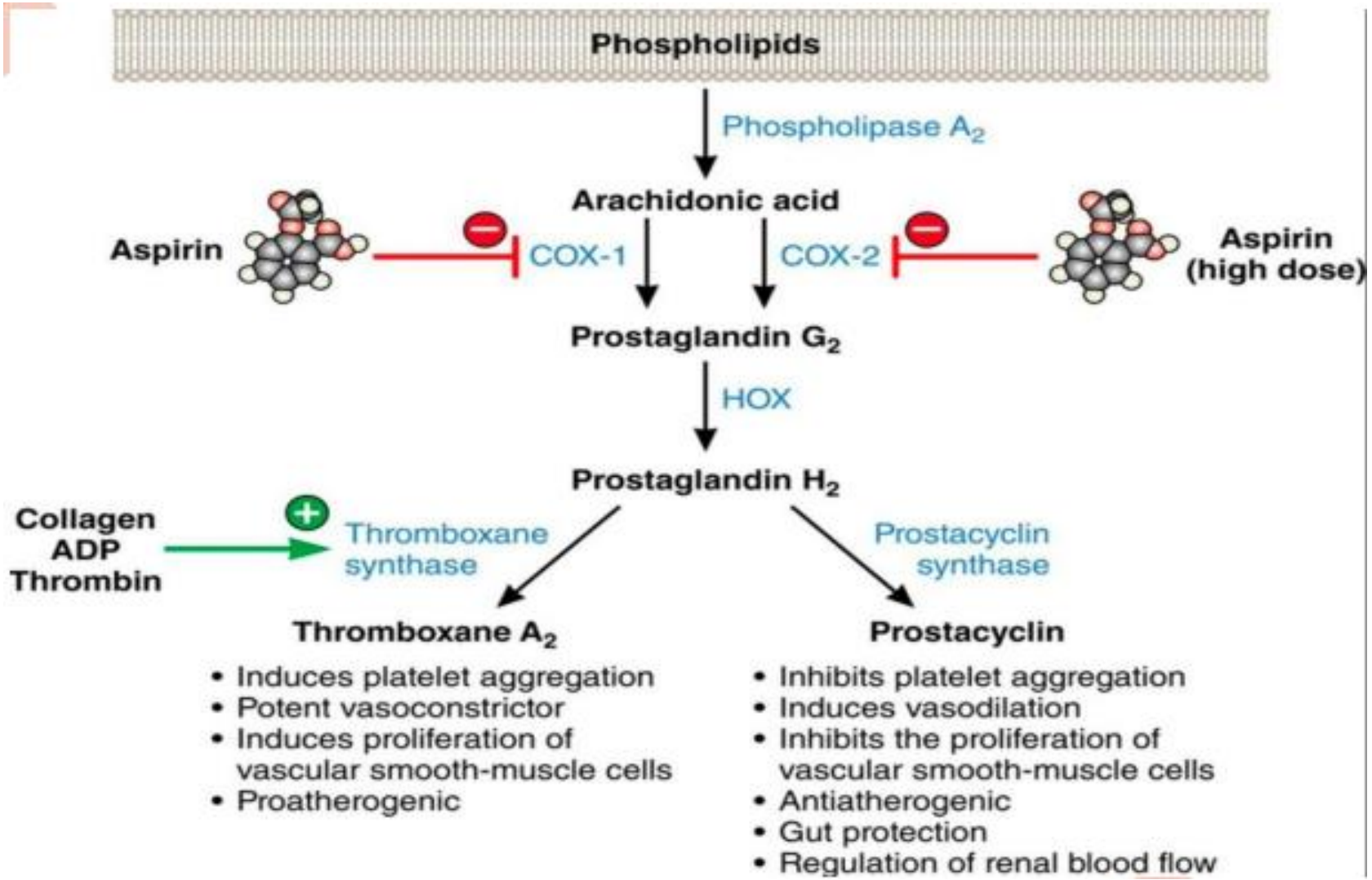
- Irreversible, platelet contents are essential to coagulation.

- Promote & Amplify PLT activities
  - Primary hemostasis
  - Secondary hemostasis

- Platelets secrete procoagulants, such as factor V, VWF, factor VIII, and fibrinogen, as well as control proteins, Ca^{2+}, ADP, and other hemostatic molecules.

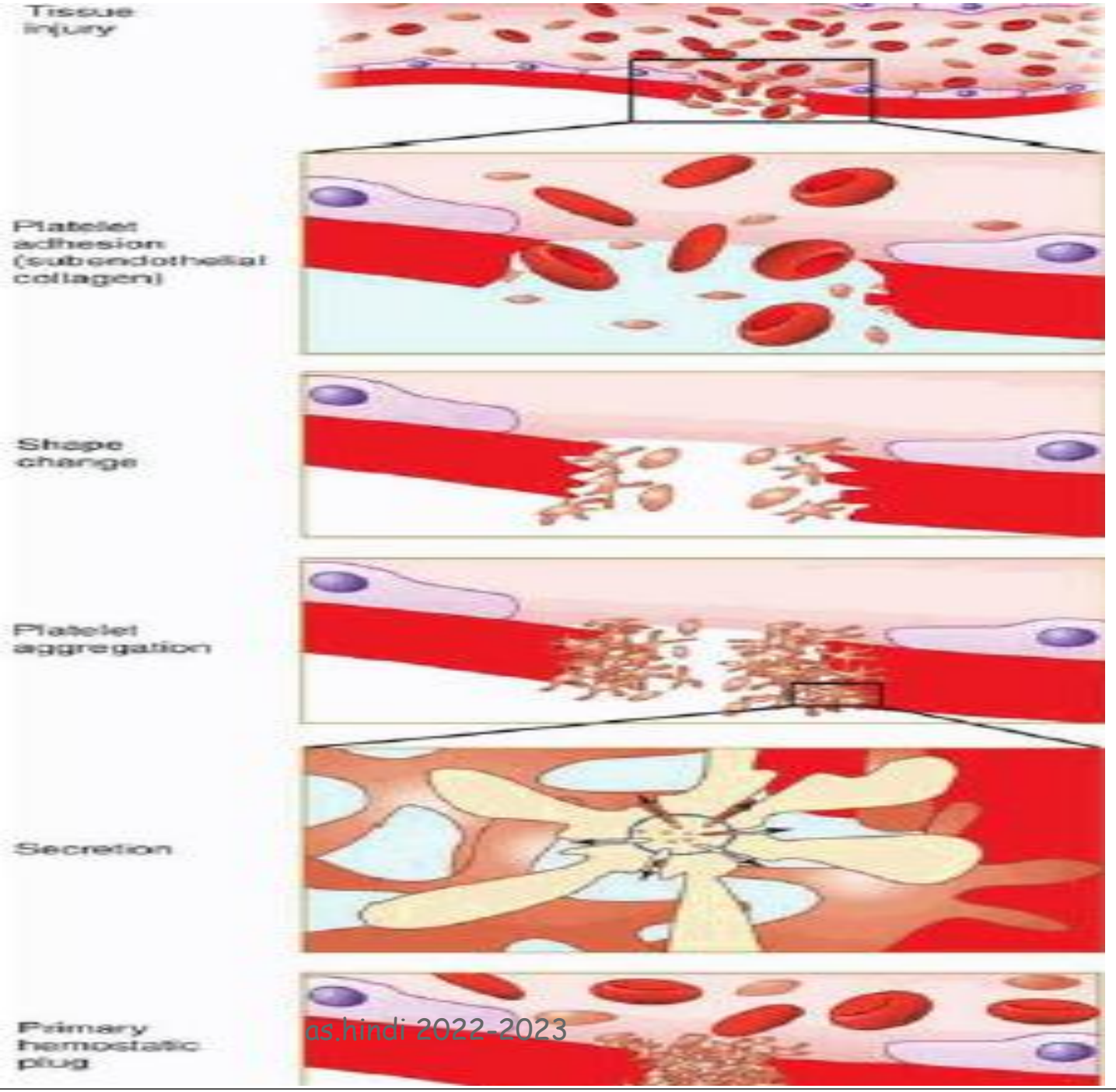
Primary Hemostatic Plug

- Injury of BV ➡ collagen appears ➡ platelet adhesion (See before) ➡ stimulation of platelet ➡ activate phospholipase A2 ➡ convert membrane phospholipids into Arachidonic acid ➡then into prostaglandin by cyclooxygenase ➡ then into Thromboxane A2 (TX A2) by thromboxane synthase (with ADP) ➡ platelet contraction ➡ release of granules as ADP from platelet which causes platelet aggregation


Phospholipids
Phospholipase A_2
Arachidonic acid
Aspirin
COX-1
COX-2
Aspirin (high dose)
Prostaglandin G_2
HOX
Prostaglandin H_2
Collagen
ADP
Thrombin
Thromboxane synthase
Prostacyclin synthase
Thromboxane A_2
• Induces platelet aggregation
• Potent vasoconstrictor
• Induces proliferation of vascular smooth-muscle cells
• Proatherogenic
Prostacyclin
• Inhibits platelet aggregation
• Induces vasodilation
• Inhibits the proliferation of vascular smooth-muscle cells
• Antiatherogenic
• Gut protection
• Regulation of renal blood flow

Erythrocytes, monocytes, and lymphocytes also participate in hemostasis.

- Erythrocytes add bulk and structural integrity to the fibrin clot; there is a tendency to bleed in anemia.
- In inflammatory conditions, monocytes and lymphocytes, and possibly ECs, provide surface-borne tissue factor that may trigger coagulation.
- Leukocytes also have a series of membrane integrins and selectins that bind adhesion molecules and help stimulate the production of inflammatory cytokines that promote the wound-healing process.



Next Lecture: Secondary Hemostasis

Coagulation Factors

Lecture 4: Secondary Hemostasis- Coagulation Factors

Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Introduction

- The classic coagulation factors are designated by Roman numerals I-XIII.

- In their active form, factors are referred to by the numeral followed by the letter a, as in factor Xa, Which means that the enzyme has transformed from zymogen form (inactive form, or pro-enzyme) to its activated form and now can act as an enzyme.

- Some factors are referred to more often by their common names than by their Roman numerals, such as fibrinogen (factor I), prothrombin (factor II), tissue factor (factor III), calcium (factor IV).

Introduction

- Some of the common names were derived from the original patients in whom symptoms leading to the determination of the factor deficiency were found. Examples are the Christmas factor (FIX) and Hageman factor (FXII).

- Prekallikrein (pre-K), also called Fletcher factor, and high-molecular-weight kininogen (HMWK), also called Fitzgerald factor, have never received Roman numerals because they belong to the kallikrein and kinin systems, respectively, and their primary functions lie within these systems.

- Platelet phospholipids (phosphatidylserine) were given no Roman numeral; instead they were once called collectively platelet factor 3.

Coagulation Factors

- Nearly all are glycoproteins synthesized in the liver, although monocytes, ECs, and megakaryocytes produce a few.

- Most coagulation factors are zymogens, proteins synthesized by hepatocytes that circulate in plasma as inactive precursors.

- Following limited proteolytic cleavage, zymogens are converted to serine proteases (so named for the serine-binding site located within the proteolytic domain) that activate other coagulation zymogens.

Coagulation factors in intrinsic, extrinsic and common pathways

Intrinsic	Extrinsic	Common
Prekallikrein	VII	X
HMWK	Tissue Factor	V
XII		II
XI		I
IX		
VIII		

Factor	Common Name	Function	Plasma Concentration
I*	Fibrinogen	Fibrin precursor	200 – 400 mg/dL
II*	Prothrombin	Thrombin precursor (serine protease)	10 mg/dL
III*	Tissue Factor	Cofactor	None
IV*	Ionized calcium	Essential Mineral	8 – 10 mg/dL
V	Labile Factor- Acclerin	Cofactor	1 mg/dL
VII	Stable Factor- Proconvertin	Serine Protease	0.05 mg/dL
VIII	Antihemophilic Factor	Cofactor	0.01 mg/dL
vWF	Von Willebrand Factor	VIII Carrier & PLT Adhesion	1 mg/dL
IX	Christmas Factor	Serine Protease	0.3 mg/dL
X	Stuart-Prower Factor	Serine Protease	1 mg/dL
XI	Plasma Thromboplastin Antecedent	Serine Protease	0.5 mg/dL
XII	Hageman Factor	Serine Protease	3 mg/dL
XIII	Fibrin-stabilizing Factor	Transglutaminase	2 mg/dL
Prekallikren	Fletcher Factor	Serine Protease	35 – 50 mg/dL
HMWK	Fitzgerald Factor	Cofactor	5 mg/dL
PLT Factor 3	Phosphotidyl Serine	Assembly Molecule	None

Classification of Clotting Factors

- The coagulation factor may be categorized into substrates, cofactors, and enzymes.

Classification of Clotting Factors

Substrate	Transglutaminase	Serine protease	Cofactor
Fibrinogen	Factor XIII	Factor XII	High MW Kinnogen
		Prekallekrein	Factor VIII
		Factor XI	Factor V
		Factor IX	Tissue factor
		Factor X	
		Factor VII	
		Factor II	
		Protein C	

Cofactors

- Factors V, VIII, HMWK, and Tissue factor are all considered cofactors.
- Factors Va and VIIIa have no enzymatic activity of their own, but with others yes.
- HMWK act as a cofactor for the contact activation phase of coagulation.
- Tissue factor or as also called tissue thromboplastin (originally called factor III) is considered as a cofactor for factor VIIa.

Substrate

- Fibrinogen is classified as a substrate because it is acted upon by the enzyme, thrombin.
- Fibrin has no enzymatic activity.

Enzymes

- They include factors thrombin (IIa), VIIa, IXa, Xa, XIa, and XIIa.
- They are all secreted as zymogens
- Zymogens are proenzymes or inactive precursors that must be modified to become active.
- As enzymes, coagulation factors enzymes are serine proteases, except factor XIII which act as transglutaminase.

Serine Proteases

- They are so called because they have serine in their active site.
- They include: thrombin, VIIa, IXa, Xa, XIa, and XIIa.
- They hydrolyze arginine or lysine containing peptide bonds of other zymogens converting them to serine proteases.

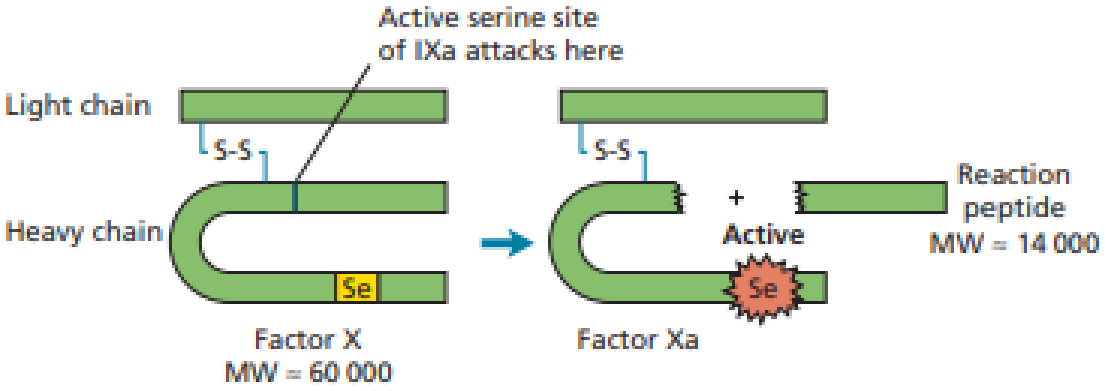


Figure 24.9 Serine (Se) protease activity. This example shows the activation of the serine site of factor X by factor IX.

Transglutaminase

- Only factor **XIIIa** is a transglutaminase.
- It catalyzes the formation of peptide bonds between glutamine and lysine on fibrin converting fibrin to a stable cross-linked fibrin.
- This is why factor XIII is called fibrin stabilizing factor.

Properties of the coagulation factors

Because of the similarities between coagulation factor in their structure and/or functional properties we can divide coagulation factors into 3 groups:

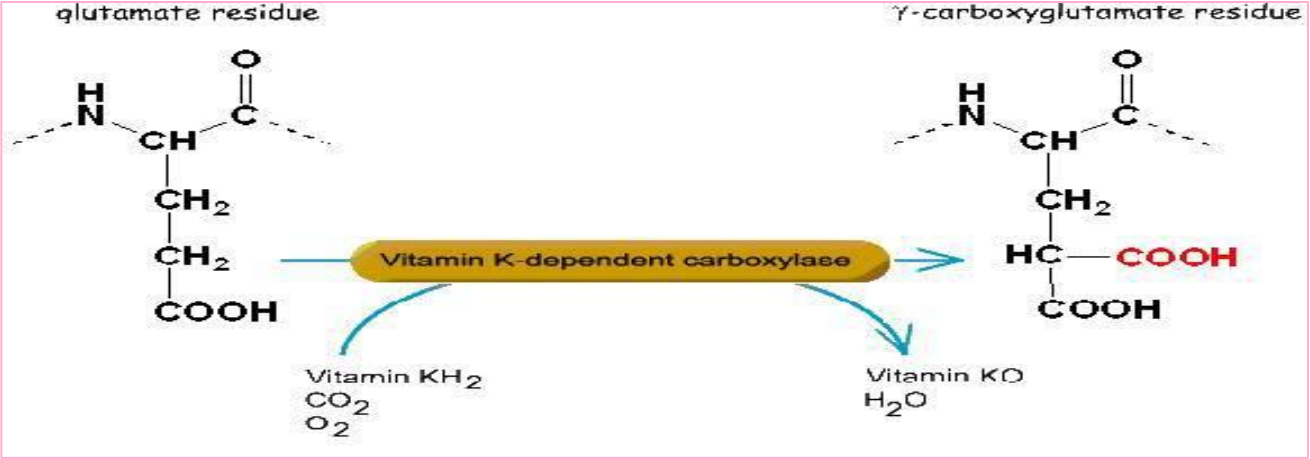
1. Prothrombin group or Vitamin K dependent group
2. Fibrinogen group
3. Contact group

1- Prothrombin group

- Includes factors II, VII, IX, and X (1972).
- All produced in the liver
- All contain γ carboxyglutamic acid rich region called GLA domain that is critical for calcium binding.
- All require Vit. K to be functional, this is why they are also called Vitamin K dependent factors.
- Precipitated with barium sulfate - absent in this "adsorbed plasma"
- Other Vitamin K dependent proteins: protein c, protein s and protein z.

VITAMIN K DEPENDENCY: 1972

- Lipophilic, hydrophobic vitamin
- needed for the posttranslational modification of coagulation factors II, VII, IX and X
  - Quinone found in green, leafy vegetables, fish, liver and produced by intestinal organisms *B. fragilis* and *E. coli*.
- Addition of second carboxyl group to the γ carbon of glutamic acid residues near the terminal end of II, VII, IX, and X
  - Creates a pocket for Ca^{++} that promotes phospholipid binding
- Vitamin K deficiency or presence of warfarin (vitamin K antagonist) renders II, VII, IX, X unable to participate in coagulation reactions.



2- Fibrinogen group

- Includes factors fibrinogen (I), V, VIII, and XIII.
- All are activated by thrombin.
- Have the highest molecular weight ranging from 300,000- 350,000 Da
- All are not found in serum because they are consumed during clotting.

3- Contact group

- Includes factors XI, XII, prekallikrein (PK), high molecular weight kininogen (HMWK).
- All are involved in the initial activation of the intrinsic pathway.
- All require contact with a negatively charged surfaces for activation.
- In vivo, except for factor XI, they do not appear to play essential role in coagulation.
- The activated forms of these factors activate fibrinolytic system, kinin, and complement systems as well as coagulation system.
- Found in serum after coagulation has taken place.

Factor I: Fibrinogen

- Customarily called fibrinogen
- MWT = 350 K(D)
- Half-life 4 – 6 days
- Mean plasma concentration = 200 – 400 mg/dl
- Absent in serum
- Glycoprotein, Manufactured in the liver.
- Increased in acute phase response by IL-6.
- Found in PLT α-granules
- Primary substrate for thrombin
- Active in intrinsic and extrinsic pathways
- Substrate for plasmin
- Mirror image dimer: α, β, g polypeptides linked by di-sulfide bonds

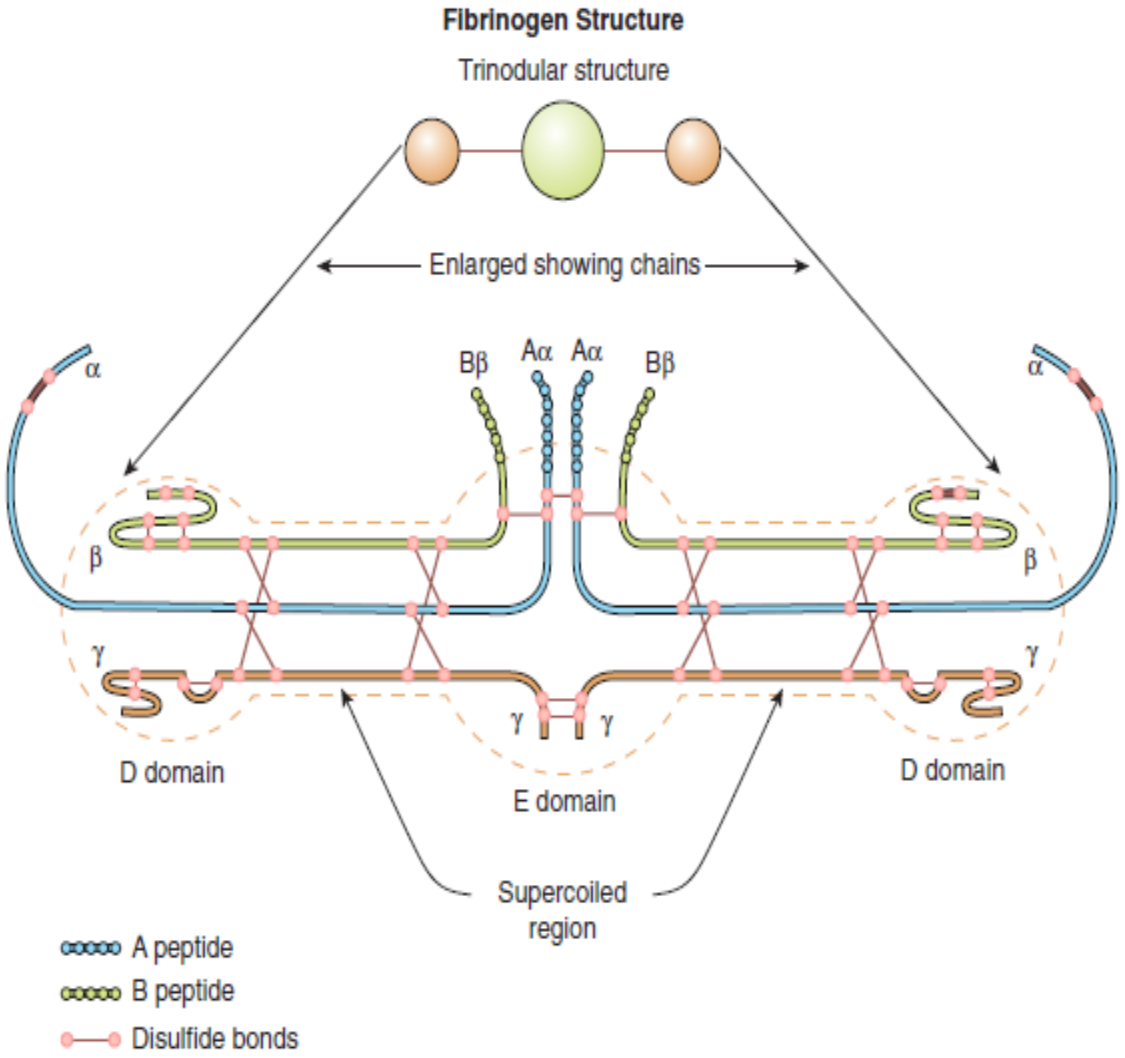
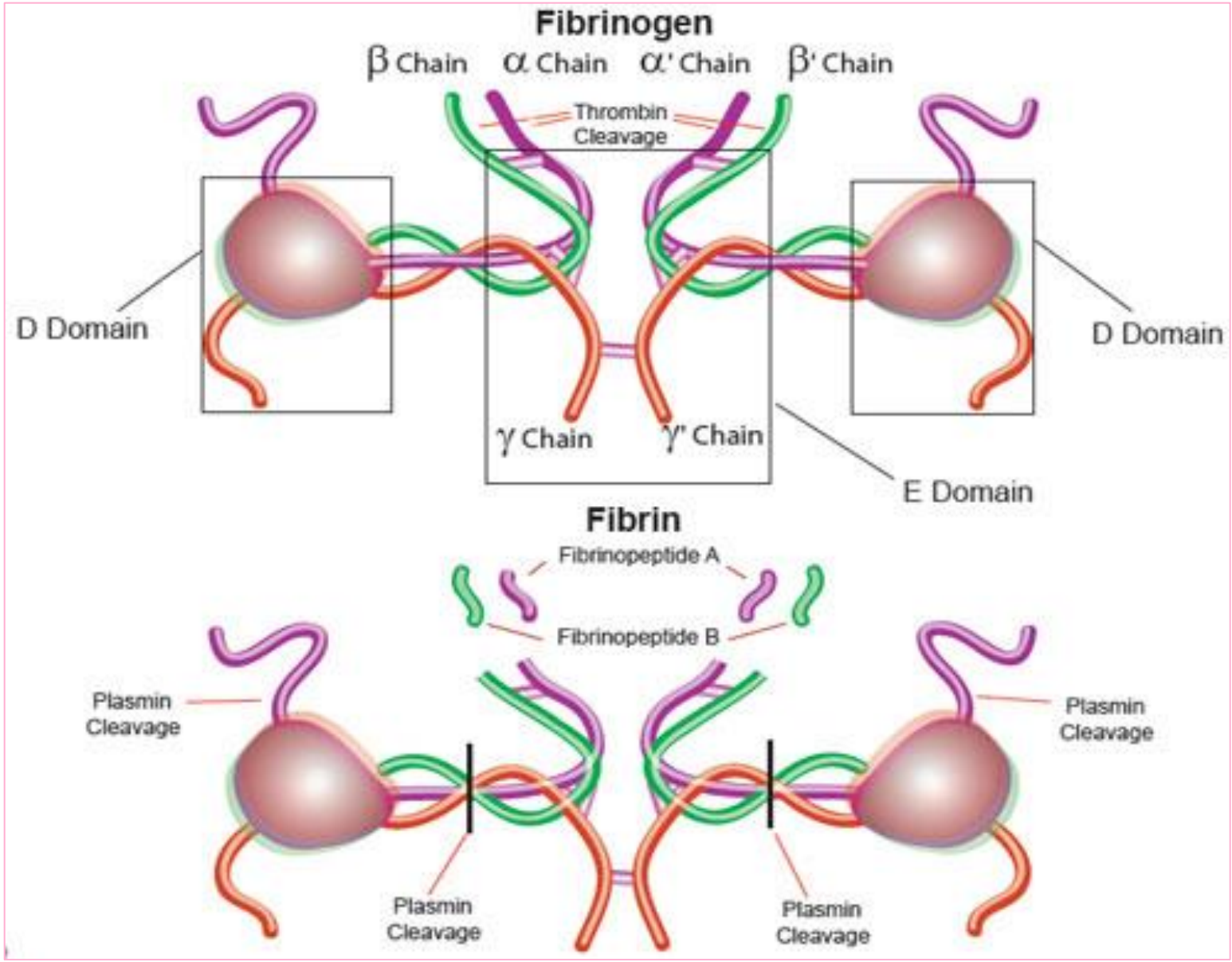
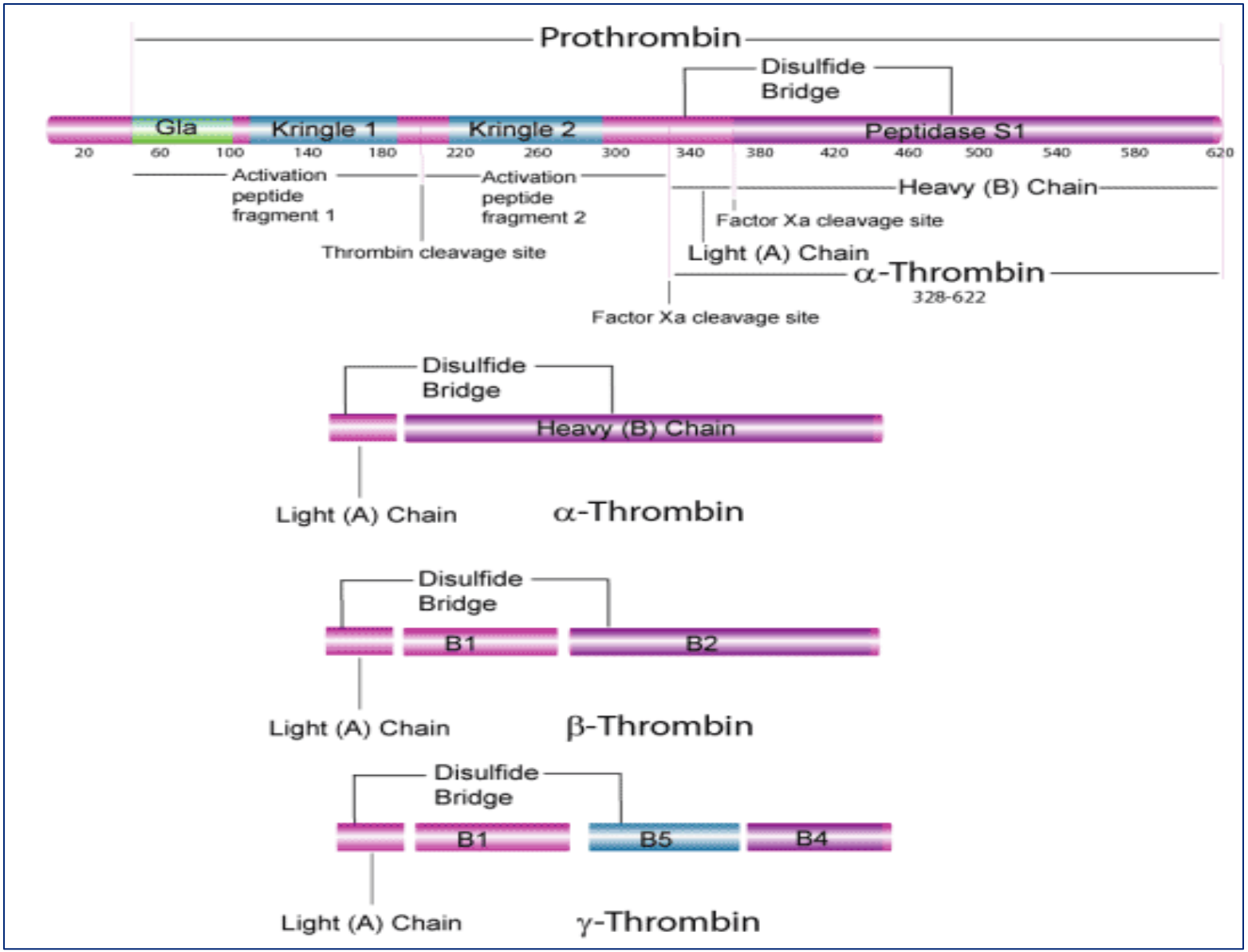


Figure 37-13 Structure of fibrinogen. Fibrinogen is a trinodular structure composed of three pairs (Aα, Bβ, and γ) of disulfide-bonded polypeptide chains. The central node is known as the *E domain*. Thrombin cleaves small peptides, A and B, from the α and β chains in this region to form fibrin. The central nodule is joined by supercoiled α-helices to the terminal nodules known as the *D domains*. (From McKenzie SB, Williams JL: *Clinical laboratory hematology*, ed 2, Upper Saddle River, NJ, 2009, Pearson, p 653.)

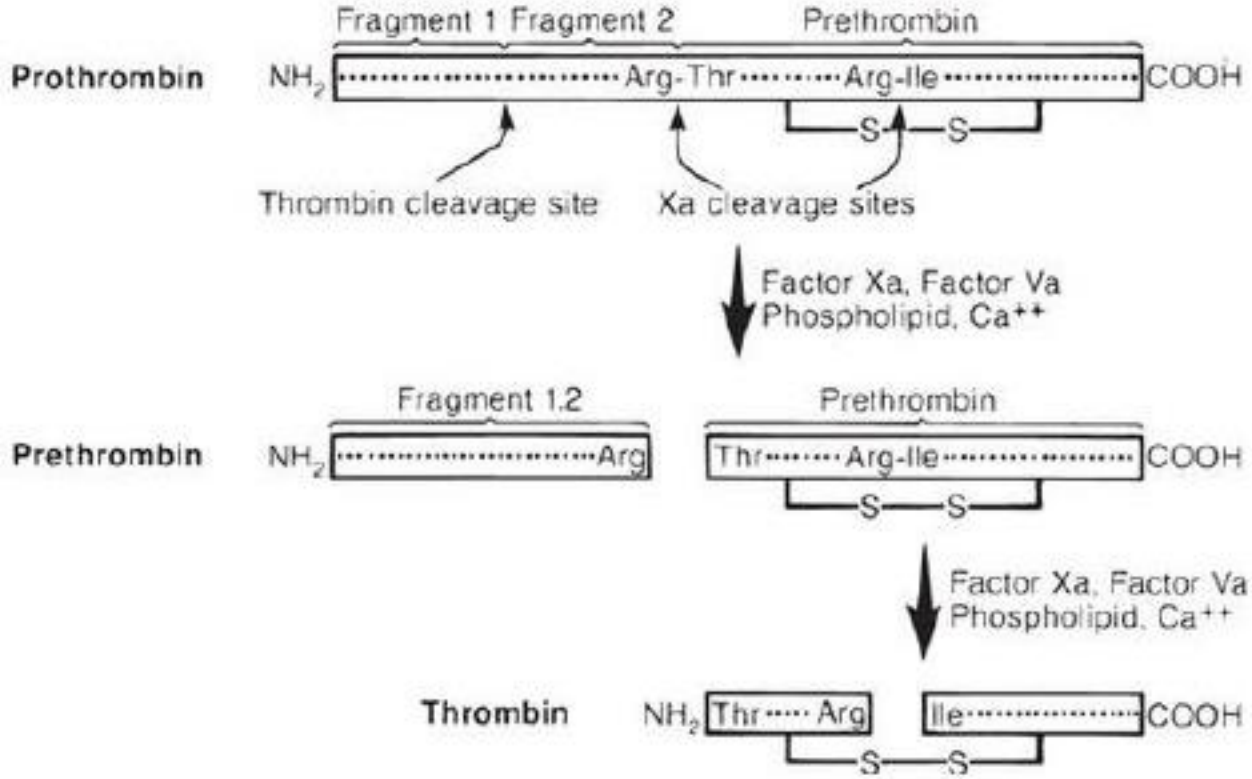

Fibrinogen
β Chain
α Chain
α' Chain
β' Chain
Thrombin Cleavage
D Domain
D Domain
γ Chain
γ' Chain
E Domain
Fibrin
Fibrinopeptide A
Fibrinopeptide B
Plasmin Cleavage
Plasmin Cleavage
Plasmin Cleavage
Plasmin Cleavage

Factor II – Prothrombin

- MW = 63K (D)
- Manufactured in liver
- Thrombin zymogen
- Serine protease
- Vitamin K dependent
- Active in intrinsic and extrinsic


Prothrombin
Disulfide Bridge
Gla
Kringle 1
Kringle 2
Peptidase S1
20 60 100 140 180 220 260 300 340 380 420 460 500 540 580 620
Activation peptide fragment 1
Activation peptide fragment 2
Heavy (B) Chain
Factor Xa cleavage site
Thrombin cleavage site
Light (A) Chain
α-Thrombin
328-622
Factor Xa cleavage site
Disulfide Bridge
Heavy (B) Chain
Light (A) Chain
α-Thrombin
Disulfide Bridge
B1
B2
Light (A) Chain
β-Thrombin
Disulfide Bridge
B1
B5
B4
Light (A) Chain
γ-Thrombin

Prothrombin Conversion to Thrombin



[Figure 30-8. The prothrombin molecule is a single-chain glycoprotein that can be divided into the "pro" portion (fragments 1 and 2 or fragment 1.2) and the thrombin portion (prethrombin). Factor Xa proteolytically cleaves the molecule between the "pro" and thrombin portions releasing fragment 1.2 and prethrombin. Prethrombin is further cleaved into two disulfide bonded chains forming the potent enzyme thrombin.]

Thrombin Functions

- Thrombin is an unique molecule that functions both as a pro-coagulant and anticoagulant.

1. In its pro-coagulant role it activates platelets through its receptor on the platelets as (agonist).
2. It regulates its own generation by activating coagulation factors V, VIII and even XI resulting in a burst of thrombin formation (when thrombin is low in concentration).
3. Inhibits factors V, VIII (when thrombin in high concentration).
4. It activates factor XI, thus preventing fibrin clots from undergoing fibrinolysis.
5. It activates factor XIII effects the cross-linking of fibrin monomers to produce a firm fibrin clot.

Thrombin Functions

6. Thrombin's role as an anticoagulant is mediated through binding to thrombomodulin, a receptor protein on the endothelial membrane of the blood vessel, initiating a series of reactions that leads to fibrinolysis (by activating protein C).

7. Thrombin has chemotactic properties enabling it to exert its effects during inflammation and vascular injury.

8. It has a mitogenic effect stimulating growth of mammalian cells, fibroblasts and macrophage-like tumor cell lines. It has also been implicated in brain development.

Factor III – Tissue Factor or Tissue Thromboplastin

- MWT = 44 K (D)
- Mean Plasma concentration = Non-circulating
- Lipoprotein
- Constitutive lipoprotein of subendothelium
- Procoagulant
- Manufactured in most body tissues – especially lungs, brain, placenta, RBC membranes. Does not circulate unless damage occurs to tissues
- Only active in extrinsic system.

Coagulation Factor IV

- ionic calcium, an essential mineral
- MWT = 40 (D)
- Mean plasma concentration 8 – 10 mg/dl
- Essential for all coagulation steps except the initiation & conversion of fibrinogen to fibrin.
- Purpose – acts as a cofactor in many reactions of the coagulation cascade
- Obtained through diet and bone storage
- Needed in intrinsic and extrinsic pathways

Coagulation Factor V

- labile factor, proaccelerin
- At one time identified as factor VI
- Substrate for thrombin and Xa
- MWT = 330K
- Half-life = 0.5 – 1.5 days
- Mean plasma concentration = 1 mg/dl
- 25% of available concentration stored in PLT a-granules
- Constitutional deficiency of the factor results in Owren's disease also known as parahemophilia.
- Cofactor for prothrombinase complex
- Deteriorates rapidly in stored blood (along with Factor VIII)
- Absent in aged plasma

Coagulation Factor VI

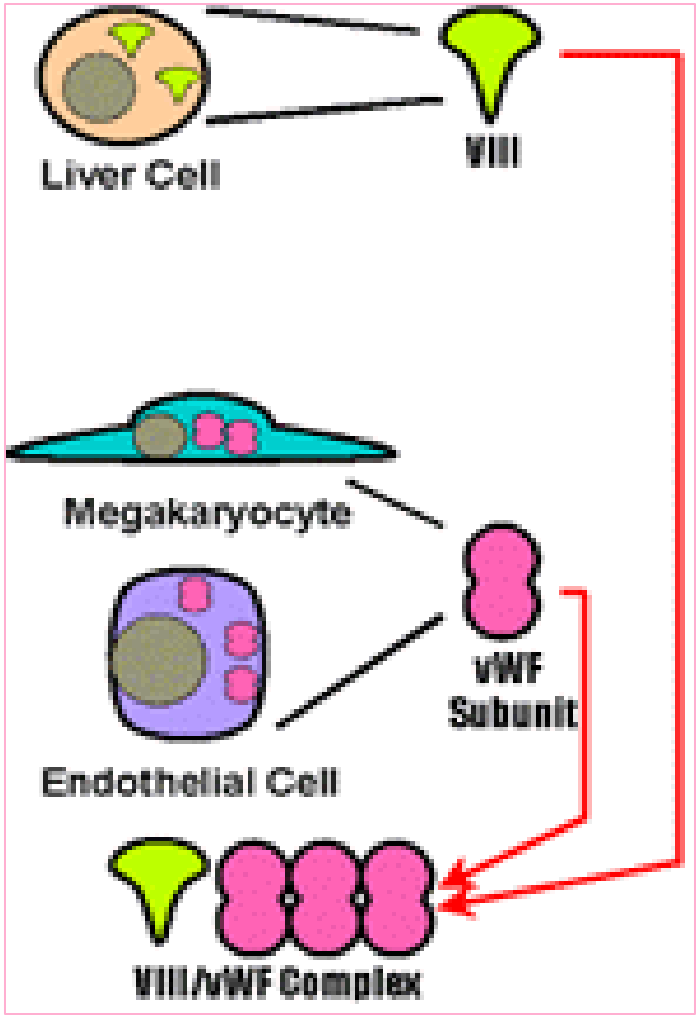
- DOES NOT EXIST!!!!!!

Factor VII – Proconvertin

- Stable Factor, Customarily called Factor VII
- MWT = 50K (D)
- Half-life 5 – 8 hours
- Mean plasma concentration = 0.05 mg/dL
- Vitamin K dependent Glycoprotein
- Circulates as a zymogen and in low quantities of active form
- Serine protease!!!!!!!!!!
- Active only in extrinsic pathway
- Is especially sensitive to Coumadin anticoagulant therapy.

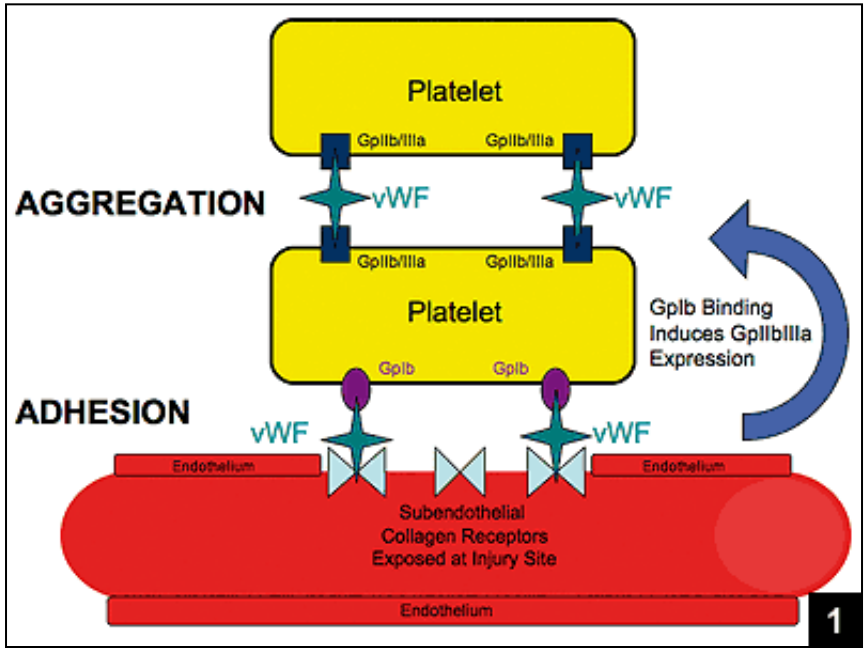
Coagulation Factor VIII

- Antihemophilic Factor (AHF)
- MWT 330 K (D)
- Plasma half-life = 8 – 12 hours
  - Very labile unless bound to vWF
  - Individuals with von Willebrand disease have both diminished vWF and VIII activity levels
  - Individuals with hemophilia A have low VIII activity but normal vWF levels
- Plasma concentration = 0.01 mg/dL
  - Travels in plasma as a heterodimer glycoprotein complexed to vWF
- Thrombin substrate
- Cofactor for TENASE complex



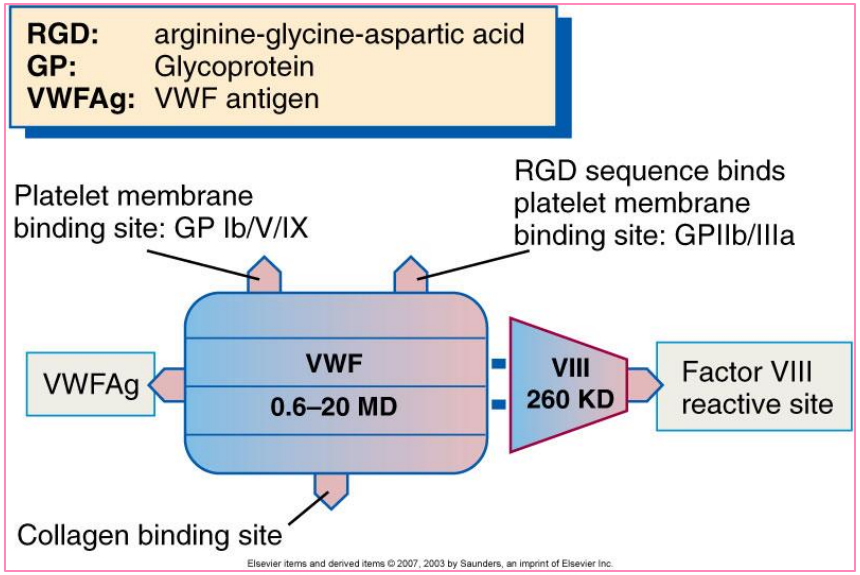
von Willebrand Factor (vWF)

- Multimeric glycoprotein
- Parts made in endothelial cells and megakaryocytes
- Stored in endothelial cells (Weibel-Palade Bodies) and PLT a-granules
- Circulate in plasma concentrations 7 – 10 mg/mL
- Ligand for PLT receptor Ib/IX (plt adhesion)



vWF/FACTOR VIII COMPLEX

- VWF binds platelet glycoprotein (GP) Ib/V/IX to provide platelet adhesion.
- RGD (arginine-glycine-aspartic acid) sequences bind GP IIb/IIIa ($\alpha_{IIb}\beta_3$) to promote platelet aggregation.
- GP IIb/IIIa also binds RGD sequences of fibrinogen.
- A third VWF site binds collagen.
- VWF also provides a binding site for coagulation factor VIII.



VIII

Coagulation Factor IX

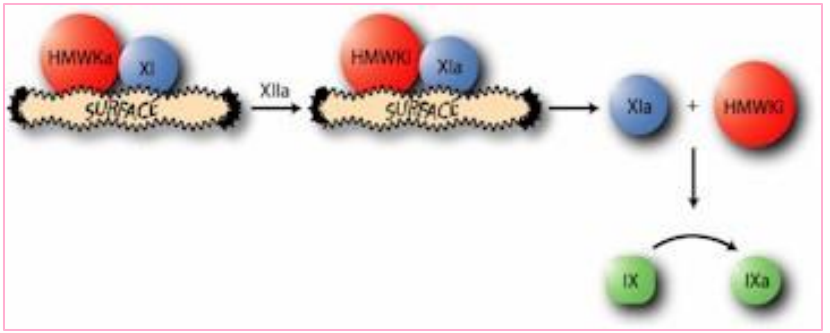
- Christmas Factor
- Deficiency results in Hemophilia B
- MWT = 57K (D)
- Half-life 1 - 3 days
- Mean plasma concentration = 0.3 mg/dL
- Activated by TF:VIIa complex and XIa
- Active in intrinsic system
- Vitamin K Dependent!!!!!!!!
- SERINE PROTEASE!!!!!!!!!!

Coagulation Factor X

- Stuart-Prower factor
- MWT = 58.8K (D)
- Half-life 2 – 2.5 days
- Mean plasma concentration = 1 mg/dL
- Activated by TENASE complex and TF:VIIa complex
- Participate in prothrombinase complex
- Vitamin K Dependent !!!!!!!!
- SERINE PROTEASE !!!!!!!!!!

Factor XI – Plasma Thromboplastin Antecedent (PTA)

- Produced in liver
- Deficiency results in Hemophilia C
- MWT = 143K (D)
- Half-life 2 – 3.5 days
- Mean plasma concentration = 0.5 mg/dL
- One of the contact factors of the intrinsic system
- Activated by Thrombin and contact factor complex (XIIa, HMWK, and PK)
- SERINE PROTEASE!!!!!!!!!!



Factor XII – Hageman Factor

- Produced in liver
- Contact factor in the intrinsic system – activated by negatively charged substances such as exposed collagen. In vitro it is also activated by glass
- SERINE PROTEASE!!!!!!!!!!

- XIIa has several functions (which are aided by cofactor HMWK)
  1) activates XI
  2) converts prekallikrein (PK) to kallikrein
  3) activates plasminogen in the fibrinolytic system

- A deficiency of Factor XII is the **only** factor deficiency that causes **no** coagulation problems (though lab testing is abnormal, a prolonged PTT).

Factor XIII – Fibrin Stabilizing Factor

- Produced in liver and platelets (a unit manufactured by megakaryocytes; β unit manufactured by hepatocytes).
- Active in intrinsic and extrinsic
- Necessary to form a stable clot – causes a crosslinking of fibrin monomers
- MWT = 320K (D)
- Half-life 3 - 6 days
- Mean plasma concentration = 1 - 2 mg/DL
- Transglutaminase
- A substrate for thrombin.
- FXIII is found in platelets, placenta and may found in monocytes.
- FXIII is involved in wound healing, fibroblast proliferation and maintenance of normal gestation

High molecular weight kininogen (HMWK), Fitzgerald Factor

- Produced in liver
- Part of the contact group of factors and involved in the feedback loop at the beginning of the intrinsic pathway
- HMWK has several functions

  1) Acts as a cofactor with Factor XII to
     a) activate Factor XI
     b) convert Prekallikrein (PK) to kallikrein
     c) activating plasminogen

  2) Is converted to kinins (bradykinin) by kallikrein which cause
     a) inflammatory reactions
     b) pain

Bradykinin Functions

- Generated from plasma HMWK
- Vasodilator
- Increased vascular permeability
- Produce pain
- Stimulate release of histamine
- Profibrinolytic properties by stimulation release of tPA by endothelial cells.
- Antithrombotic by stimulation synthesis of nitric oxide and prostacyclin.

Prekallikrein (PK), Fletcher Factor

- Produced in liver
- Part of the contact group of factors and involved in the feedback loop at the beginning of the intrinsic pathway
- PK is converted to kallekrein and has several functions

  1) when converted to kallekrein by factor XII it then loops back and accelerates the activation of larger amounts of factor XII

  2) converts HMWK to kinins

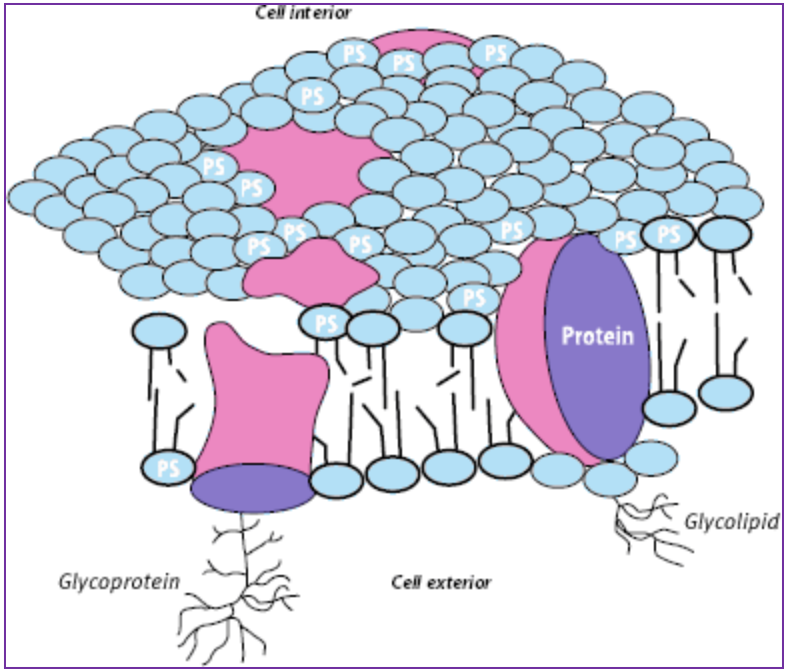
  3) activates plasminogen

  4) activates the complement system

  5) acts as a chemotactic factor to attract macrophages

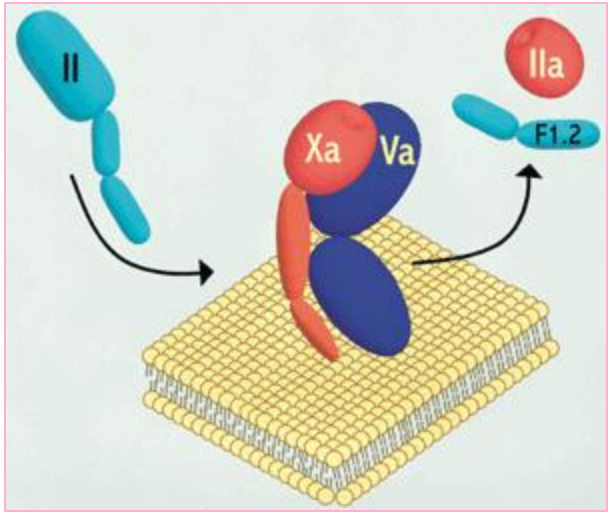
Platelet Factor 3 (PF 3)

- a.k.a. Phosphotidyl serine (phospholipid)
- Functions as an assembly molecule for coagulation
- Platelet derived



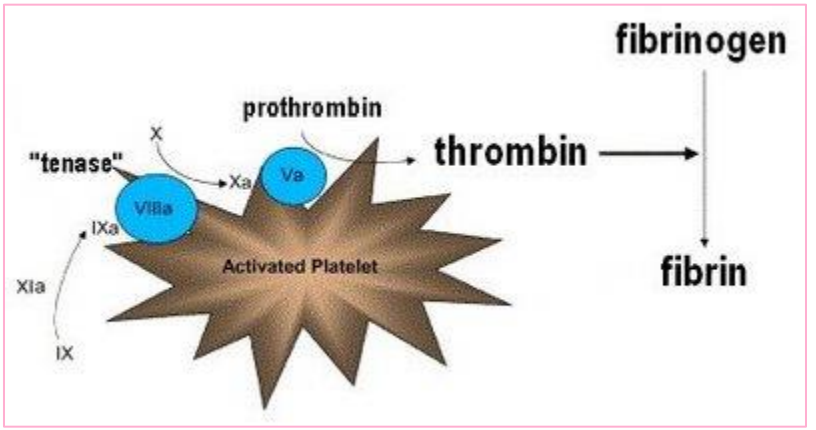
PROTHROMBINASE COMPLEX

- Important coagulation cascade complex that cleaves ("activates") prothrombin
- Composed of Xa, Va, phospholipid (PLT-derived) and ionic calcium
- The first step in the 'common' pathway of coagulation



Tenase complex

- Important coagulation cascade complex that cleaves ("activates") Factor X
- Composed of VIIIa, IXa, phospholipid (PLT-derived) and ionic calcium
- The end product of the 'intrinsic ' pathway of coagulation



Factors absent in laboratory testing reagents

1. Aged serum – absent = I, V, VIII, XIII
2. Aged plasma – absent = V, VIII
3. Adsorbed plasma (barium sulfate) – absent = II, VII, IX, X
4. Normal plasma contain all coagulation factors except tissue factor.

- These reagents are used with a test called Mixing Studies that we will take latter on.

Next Lecture: Coagulation cascade

Lecture 5: Coagulation Cascade & Coagulation Regulatory Mechanisms

Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Coagulation Cascade

- A set of reactions in which blood is transformed from a liquid to a gel.

- Is composed of four interacting sets of reactions :
  - Complex formation on phospholipid membranes
  - Intrinsic pathway
  - Extrinsic pathway
  - Common pathway

- Plasma-Based (In Vitro) Classical Coagulation: Extrinsic, Intrinsic, and Common Pathways.

- The pathways were characterized as cascades in that as one enzyme became activated, it in turn activated the next enzyme in sequence.

Complex formation on phospholipid membranes

- The coagulation cascade occurs on cell surface membranes.
- Clotting factors bind the phospholipid membrane surface and act on each other until fibrin is formed.
- The critical membranes for coagulation are the sub-endothelium that is exposed after injury and the platelet surface membrane (platelet factor 3 [PF3])

Classical Coagulation Cascade (In vitro)

1. The Intrinsic Pathway

- contact activation: The 4 contact factors: XII, XI, prekallikrein (PK) and HMWK.

- Activated in vitro when we expose them in LAB to and adsorbed to negatively charged surfaces such as glass, kaolin, celite, silica, or ellagic acid.

- Activation of contact factors does not require calcium.

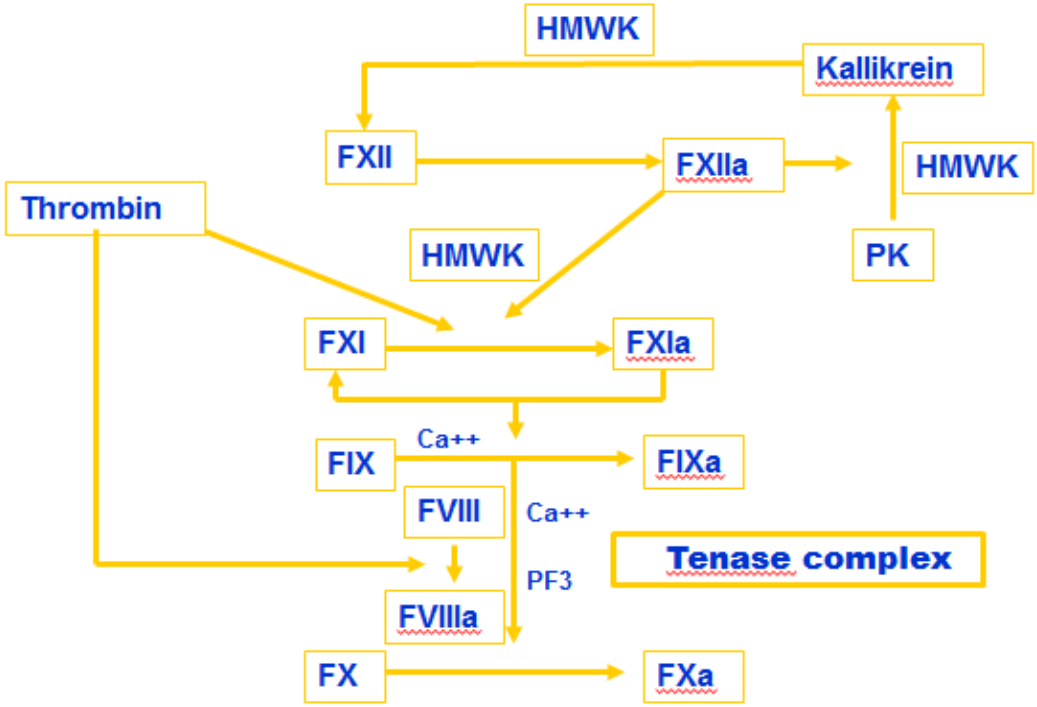
Intrinsic Pathway

1. Factor XII is activated to Factor XIIa by contact with negatively charged surfaces.
2. FXIIa will activate prekallikrein (PK) to kallikrein with HMWK as a cofactor.
3. Kallikrein in turn activates more FXII itself to FXIIa to amplify the reaction.
4. Factor XIIa activates factor XI to XIa.
5. Factor IX is the definite substrate of FXIa.
6. FVIII is activated by thrombin to FVIIIa. When activated it dissociates from its carrier the vWF.
7. The surface of activated platelets (PF3), FIXa together with calcium and activated FVIIIa form the tenase complex that activates FX to FXa.

Intrinsic Pathway

- The coagulation factors of the intrinsic pathway, in order of reaction are: XII, pre-K, HMWK, XI, IX, VIII, X, V, prothrombin (II), and fibrinogen.

- The laboratory test that detects the absence of one or more of these factors is the activated partial thromboplastin time (APTT or PTT).



Note:

- Most coagulation experts identified the activation of factor XII as the primary step in coagulation because this factor could be found in blood, whereas tissue factor could not.

- Patients deficient in FXII, PK, or HMWK do not bleed abnormally despite a prolonged APTT, so it seems that these factors do not play a critical role in vivo hemostasis.

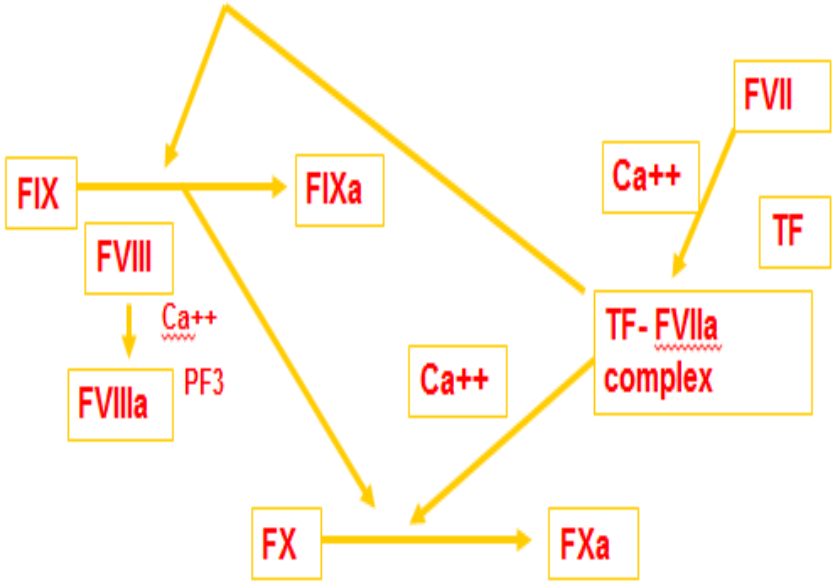
- But they contribute to fibrinolysis, inflammation, complement activation and kinin formation.

Extrinsic Pathway

- The extrinsic activation involves factor VII and the cofactor, tissue factor (TF) or what is also called Tissue thromboplastin.
- Upon injury cells with TF on their surfaces (monocytes, endothelial cells) are exposed to the blood.
- TF binds FVII and FVIIa in the presence of Ca++ forms FVIIA/TF complex.
- Once this complex is formed, it initiates the extrinsic pathway of blood coagulation.
- Also this complex activates factor IX, bypassing the need for contact activation.
- FVIIa can activate more FVII.
- Also, FXa can feed back and activate more FVIIa.

Extrinsic Pathway

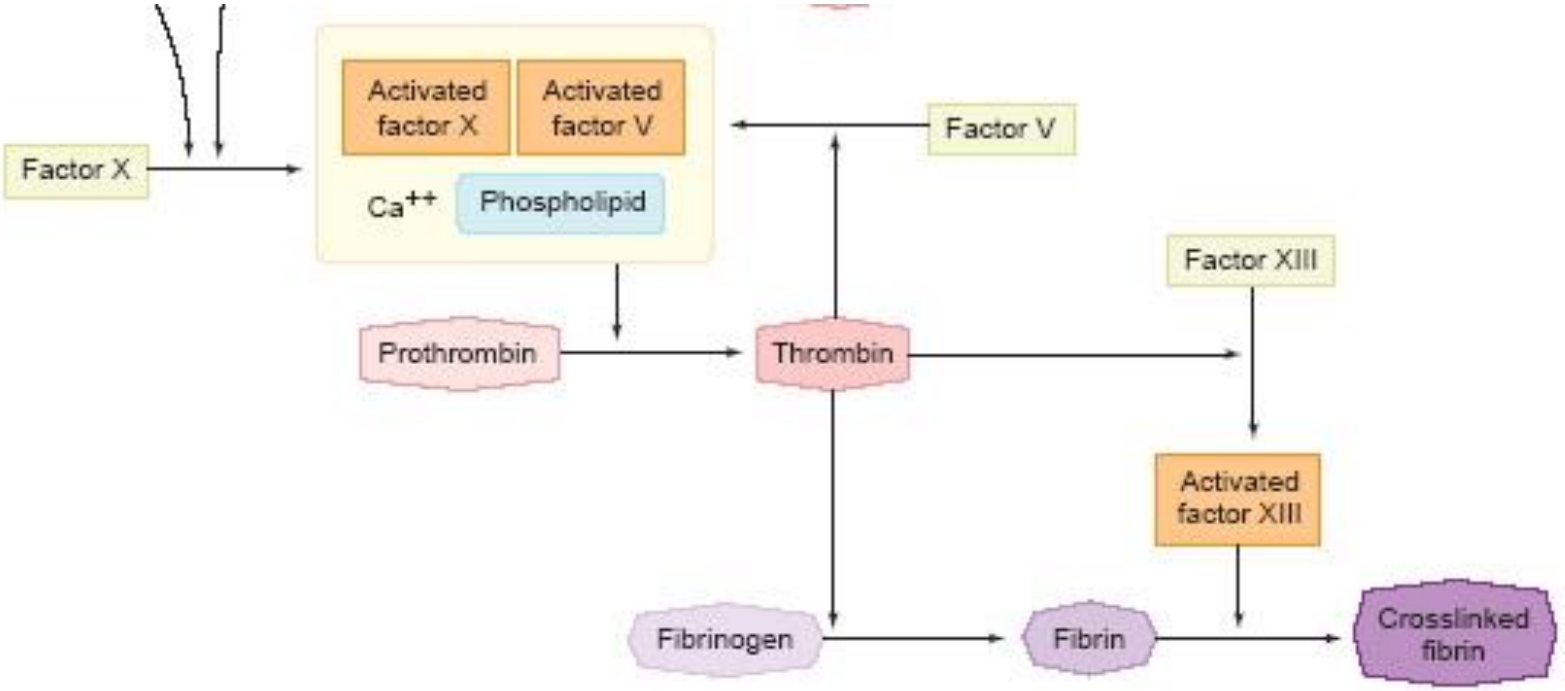
- Formation of TF:VIIa has since proven to be the primary in vivo initiation mechanism for coagulation.
- Because tissue factor is not present in blood, the tissue factor pathway has been called the extrinsic pathway.
- This pathway includes the factors VII, X, V, prothrombin, and fibrinogen.
- The test used to measure the integrity of the extrinsic pathway is the prothrombin
- time test (PT).



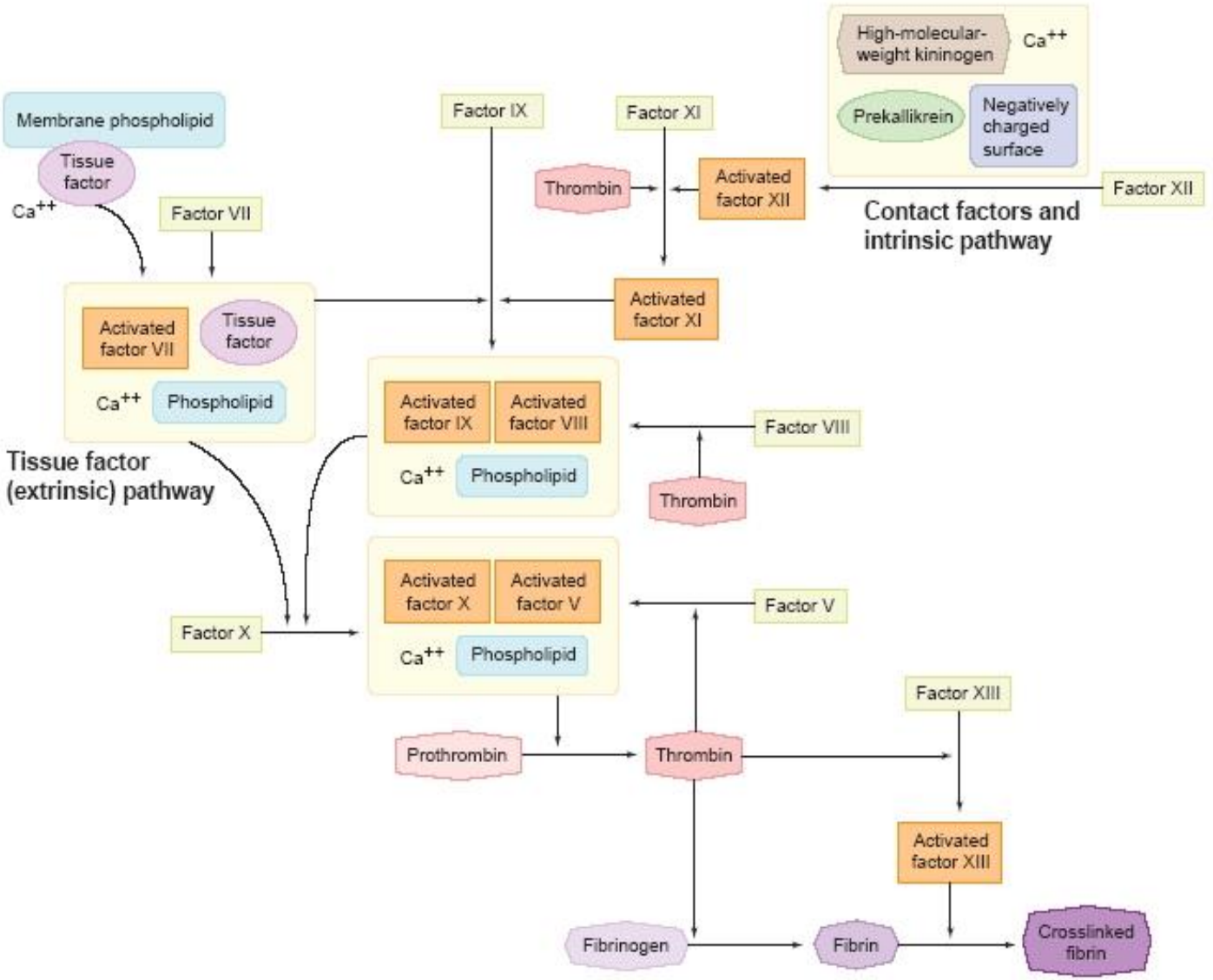
The common pathway

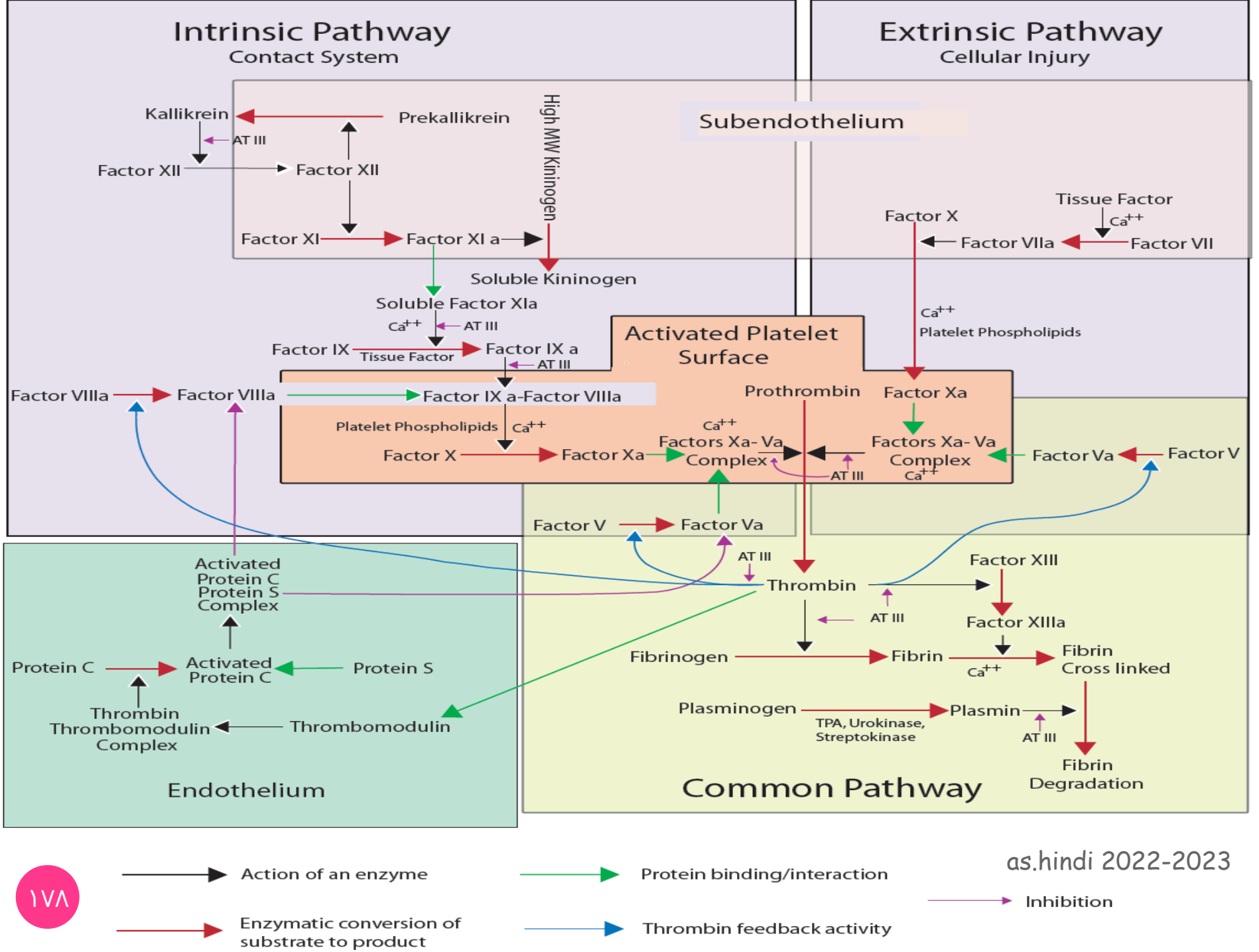
- The common pathway converge with the intrinsic and extrinsic pathways, in which both of them activates factor X.
- As seen factor X can be activated by FIXa/FVIIIa/Ca++/PL or VIIa/TF/Ca++
- FXa then forms a complex with cofactor V, PF3 and Ca++ (Prothrombinase complex). This complex acts to optimally activate prothrombin to thrombin.

The common pathway



Coagulation Cascade





Current Concept of coagulation

- Coagulation can be described as occurring in two phases:
  - initiation, which occurs on tissue factor–expressing cells and produces 3% to 5% of the total thrombin generated, and
  - propagation, occurring on platelets, which produces 95% or more of the total thrombin.

Current Concept of coagulation

- Coagulation depends on the presence of both tissue factor–bearing cells and activated platelets, clotting is localized to the site of injury.
- Protease inhibitors and intact endothelium prevent clotting from spreading to other parts of the body.
- The extrinsic or tissue factor pathway as occurring on the tissue factor–bearing cell and the intrinsic pathway (minus factors XII, HMWK, and pre-K) as occurring on the platelet surface.
- These are not separate and redundant pathways; they are interdependent and occur in parallel until blood flow has ceased and termination by control mechanisms takes place.

Current Concept of coagulation

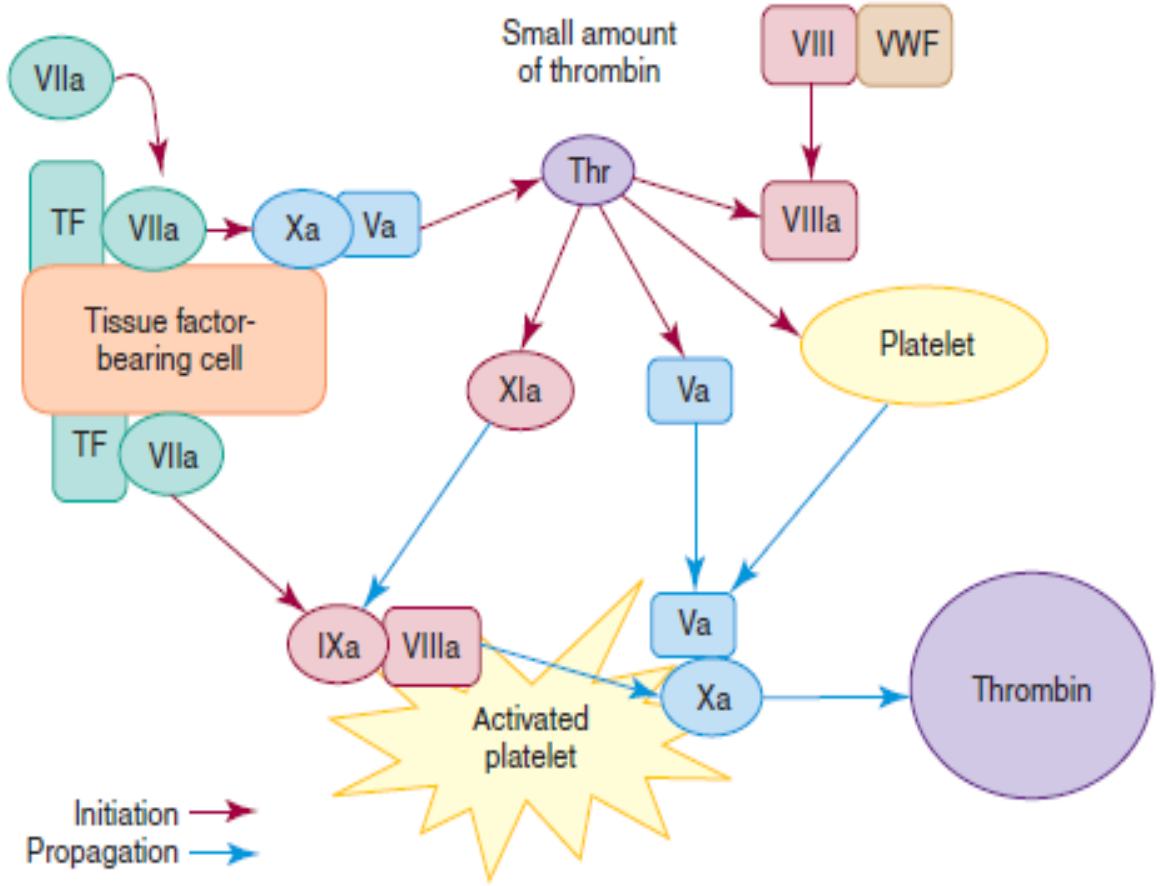


Figure 37-16 Cell-based in vivo physiologic coagulation. VIIa binds to tissue factor (*TF*) and activates both factors X and IX. Cell-bound factor Xa combines with Va and generates a small amount of thrombin (Thr), which activates platelets, V, VIII, and XI and begins fibrin formation. Factor IXa, activated by both TF:VIIa and XIa, combines with factor VIIIa on the platelet surface to activate X, which forms prothrombinase (Xa:Va) and produces a burst of thrombin.

Initiation phase:

- About 1% to 2% of factor VIIa is present normally in blood in the activated form.
- Fibroblasts and other subendothelial cells provide tissue factor, a cofactor to factor VIIa.
- Factor VIIa binds to tissue factor on the membrane of subendothelial cells, and the extrinsic complex TF:VIIa is formed.
- TF:VIIa activates low levels of both factor IX and factor X.
- Minute amounts of thrombin are generated by membrane-bound Xa and Xa:Va prothrombinase complexes.
- Factor Va comes from the activation of plasma factor V by thrombin.
- The amount of thrombin generated in this phase is minute, platelets, cofactors, and procoagulants become activated; fibrin formation begins; and the initial platelet plug is formed.

The low level of thrombin generated in the initiation phase

1. activates platelets through cleavage of protease activated receptors PAR-1 and PAR-4;
2. activates factor V released from platelet a-granules;
3. activates factor VIII and dissociates it from VWF;
4. activates factor XI, the intrinsic accessory pro-coagulant that activates more factor IX;
5. And splits fibrinogen peptides A and B from fibrinogen and forms a preliminary fibrin network.

Propagation phase:

- More than 95% of thrombin generation occurs during propagation.
- In this phase the reactions occur on the surface of the activated platelet, which now has all the components needed for coagulation.
- Platelets are activated at the site of injury by both the low-level thrombin generated in the initiation phase and by adhering to exposed collagen.
- They are sometimes referred to as COAT-platelets: platelets partially activated by collagen and thrombin.
- They also provide a surface for formation and amplification of intrinsic tenase and prothrombinase complexes.

Propagation phase:

- The cofactors Va and VIIIa activated by thrombin in the initiation phase bind to platelet membranes and become receptors for Xa and IXa.

- IXa generated in the initiation phase binds to VIIIa on the platelet membrane to form the intrinsic tenase complex IXa:VIIIa. More factor IXa is also generated by platelet-bound factor XIa. This intrinsic tenase complex activates factor X at a 50- to 100-fold higher rate than the extrinsic tenase complex.

- Factor Xa binds to Va to form the prothrombinase complex, which activates prothrombin and generates a burst of thrombin. Thrombin cleaves fibrinogen into a fibrin clot, activates factor XIII to stabilize the clot, binds to thrombomodulin to activate the protein C control pathway, and activates TAFI to inhibit fibrinolysis.

Important Notes:

- Both platelets and tissue factor–bearing cells are essential for physiologic coagulation.
- Deficiencies of any of the key proteins of coagulation complex formation and activity (VII, IX, VIII, X, V, or prothrombin) compromise thrombin generation and manifest as significant bleeding disorders.

Coagulation Regulatory Mechanisms

Natural Inhibitors/Anticoagulants

1. Tissue Factor Pathway Inhibitor TFPI:

- TFPI inhibits coagulation in a two-step process by first binding and inactivating Xa.
- The TFPI:Xa complex then binds to TF:VIIa, forming a quaternary complex and preventing further activation of X and IX.

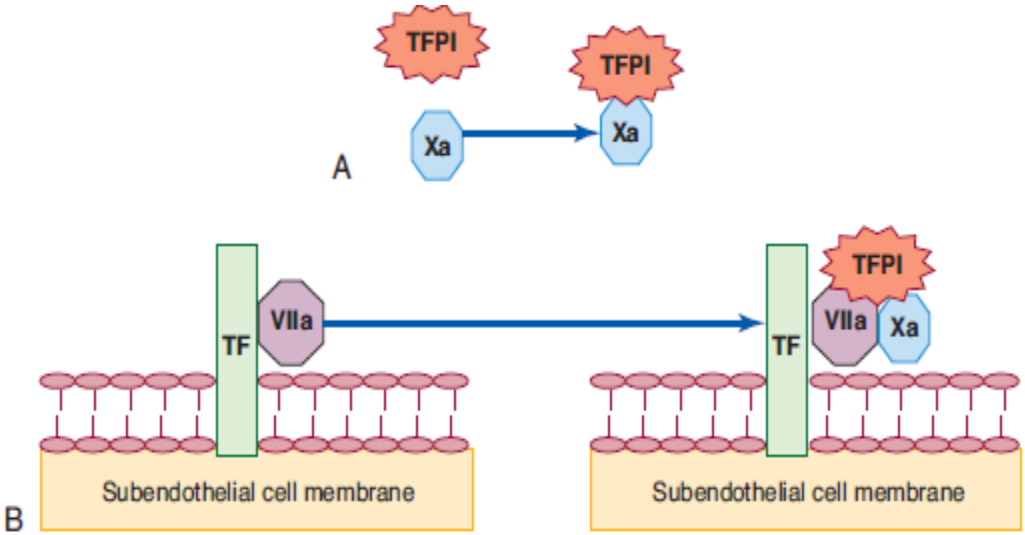


Figure 37-18 Tissue factor pathway inhibitor. *TFPI* binds the complex of tissue factor (*TF*) and factors VIIa and Xa in a Xa-dependent feedback mechanism. **A,** TFPI first binds to factor Xa and inactivates it. **B,** The TFPI:Xa complex then binds and inactivates TF:VIIa, preventing more activation of Xa. Alternatively, TFPI may bind directly to Xa and VIIa in the TF:VIIa:Xa complex.

2. Activated Protein C Pathway

- At damaged endothelial tissue site, *thrombin (T)* binds *thrombomodulin (T) generated from endothelium.*
- The TT complex activates plasma zymogen Protein C
- Activated Protein C (APC) with its cofactor Protein S inactivates Va and VIIIa
  - Deficiency of PC or PS leads to thrombosis
  - PC and PS are Vit K dependent.

Activated Protein C Pathway

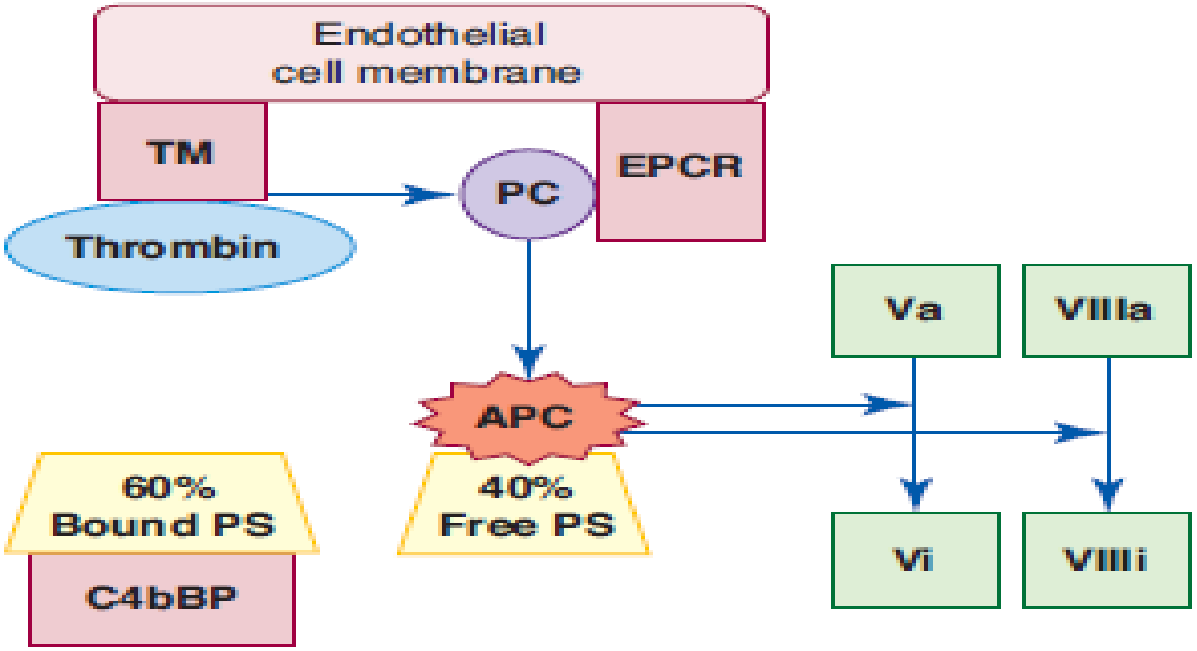


Figure 37-19 Protein C pathway. After binding thrombomodulin (*TM*), thrombin activates protein C (*PC*), bound by endothelial cell protein C receptor (*EPCR*). Free protein S (*PS*) [not bound to C4b binding protein (*C4bBP*)] binds and stabilizes activated protein C (*APC*). The APC/protein S complex digests and inactivates factors Va (Vi, inhibited factor V) and VIIIa (VIIIi, inhibited factor VIII).

Protein S

- Protein S, the cofactor that binds and stabilizes APC, is synthesized in the liver and circulates in the plasma in two forms.
- About 40% of protein S is free, but 60% is covalently bound to the complement control protein C4b-binding protein (C4bBP).
- Bound protein S cannot participate in the protein C anticoagulant pathway; only free plasma protein S can serve as the APC cofactor.
- Protein S-C4bBP binding is of particular interest in inflammatory conditions because C4bBP is an acute phase reactant.
- When the plasma C4bBP level increases, additional protein S is bound, and free protein S levels become proportionally decreased, which may increase the risk of thrombosis.

Protein C Regulatory Pathway

- Chronic acquired or inherited protein C or protein S deficiency or mutations of protein C, protein S, or factor V compromise the normal downregulation of factors Va and VIIIa and may be associated with recurrent venous thromboembolic disease.

- Neonates who completely lack protein C have a massive thrombotic condition called *purpura fulminans* and die in infancy unless treated with protein C replacement and anticoagulation.

3. Antithrombin and Other Serine Protease Inhibitors (Serpins)

1. Antithrombin (AT) was the first of the coagulation regulatory proteins to be identified.
2. Other members of the serpin family include:
   a) heparin cofactor II,
   b) protein Z-dependent protease inhibitor (ZPI),
   c) protein C inhibitor,
   d) C1 inhibitor
   e) a1-protease inhibitor (a1-antitrypsin),
   f) a2-macroglobulin,
   g) a2-antiplasmin,
   h) and PAI-1.

Antithrombin (AT)

- AT is a serine protease inhibitor (serpin) that binds and neutralizes serine proteases, including thrombin and factors IXa, Xa, XIa, XIIa, prekallikrein and plasmin.

- AT's activity is accelerated 2000-fold by binding to heparin and is the basis for the anticoagulant activity of pharmaceutical heparin.

- In vivo, heparin is available from endothelium associated mast cell granules or as EC heparan sulfate, a natural glycosaminoglycan that activates AT, although not to the same intensity as therapeutic unfractionated heparin.

Antithrombin (AT)

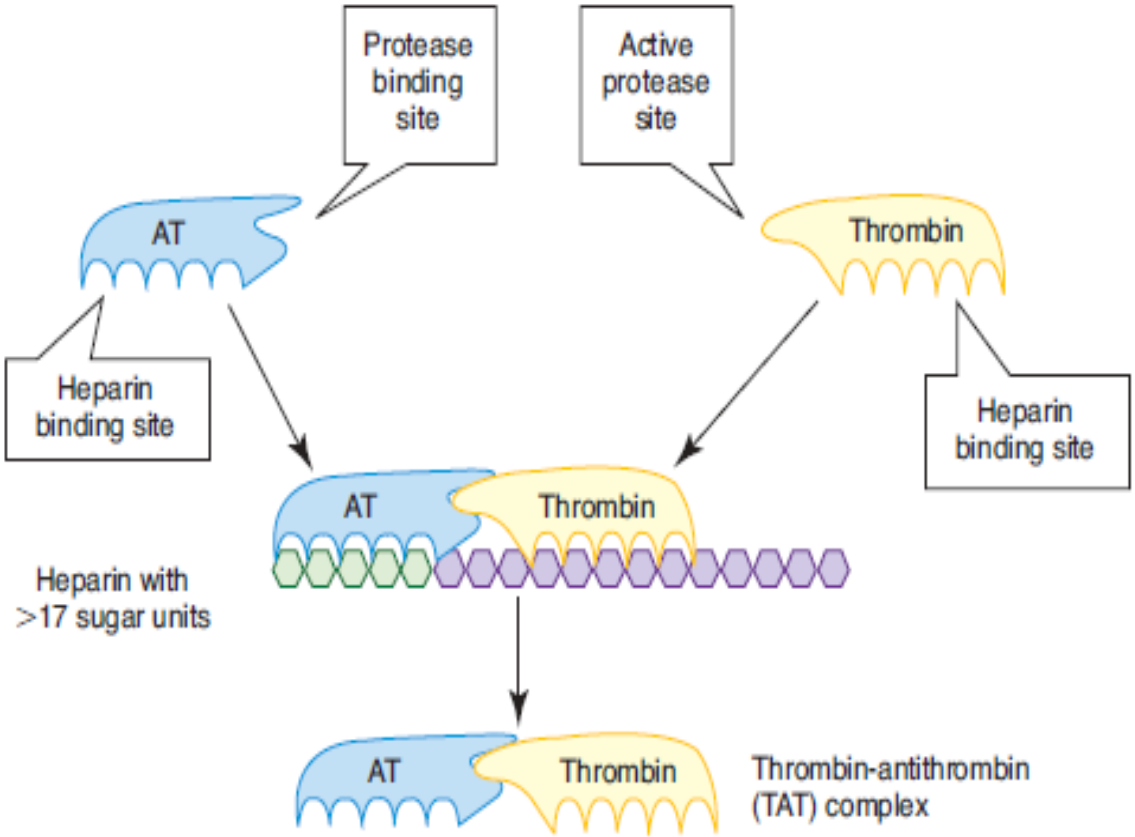


Figure 37-20 Unfractionated heparin potentiates antithrombin-thrombin reaction. Antithrombin (*AT*) attaches to a specific pentasaccharide sequence in unfractionated heparin. The thrombin binding site for heparin is adjacent to the AT site. The AT is sterically modified to covalently bind and inactivate the thrombin active protease site. Thrombin and AT, covalently bound, release from heparin and form measurable plasma thrombin-antithrombin (*TAT*) complexes, useful as a marker of coagulation activation.

Protein z-dependent protease inhibitor (ZPI)

- ZPI, in the presence of its cofactor, protein Z, is a potent inhibitor of factor Xa.
- ZPI covalently binds protein Z and factor Xa in a complex with Ca^{2+} and phospholipid.
- Protein Z is a vitamin K–dependent plasma glycoprotein that is synthesized in the liver.
- Protein Z increases the ability of ZPI to inhibit factor Xa 2000-fold.
- ZPI also inhibits factor XIa, in a separate reaction that does not require protein Z, phospholipid, and Ca^{2+} .

Other Serine Protease Inhibitors (Serpins)

- Heparin cofactor II is a serpin that primarily inactivates thrombin.
- AT and heparin cofactor II both require heparin for effective anticoagulant activity.
- C1 inhibitor is a major inhibitor of factor XIIa.
- a1-antitrypsin is a major inhibitor of factor XIa.
- a2-macroglobulin is capable of inhibiting several serine proteases: thrombin, Xa, kallikrein and plasmin.

Next Lecture: Fibrinolysis System

Lecture 6: Fibrinolysis

Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Introduction

- The final stage of coagulation, begins a few hours after fibrin polymerization and cross-linking.

- Two activators of fibrinolysis: TPA and UPA, are released in response to inflammation and coagulation.

- Fibrinolytic proteins assemble on fibrin during clotting. Plasminogen, plasmin, TPA, UPA, and PAI-1 become incorporated into the fibrin clot as they bind to lysine through their "kringle" loops, thereby concentrating and localizing them to the fibrin clot.

Fibrinolysis

- Fibrinolysis is the systematic, accelerating hydrolysis of fibrin by bound plasmin.
- TPA and UPA activate fibrin-bound plasminogen several hours after thrombus formation, degrading fibrin and **restoring normal blood flow during vascular repair.**
- Again, there is a delicate balance between activators and inhibitors.
- Excessive fibrinolysis can cause bleeding due to premature clot lysis before wound healing is established, whereas inadequate fibrinolysis can lead to clot extension and thrombosis.

TABLE 37-11 Proteins of the Fibrinolysis Pathway

Name	Function	Molecular Mass (Daltons)	Half-Life	Mean Plasma Concentration
Plasminogen	Plasma serine protease, plasmin digests fibrin/fibrinogen	92,000	24–26 hr	15–21 mg/dL
Tissue plasminogen activator	Serine protease secreted by activated endothelium, activates plasminogen	68,000	Unknown	4–7 μg/dL
Urokinase	Serine protease secreted by kidney, activates plasminogen	54,000	Unknown	—
Plasminogen activator inhibitor-1	Secreted by endothelium, inhibits tissue plasminogen activator	52,000	1 hr	14–28 mg/dL
α_2-Antiplasmin	Inhibits plasmin	51,000	Unknown	7 mg/dL
Thrombin-activatable fibrinolysis inhibitor	Suppresses fibrinolysis by removing fibrin C-terminal lysine binding sites	55,000	8–10 min	5 μg/mL

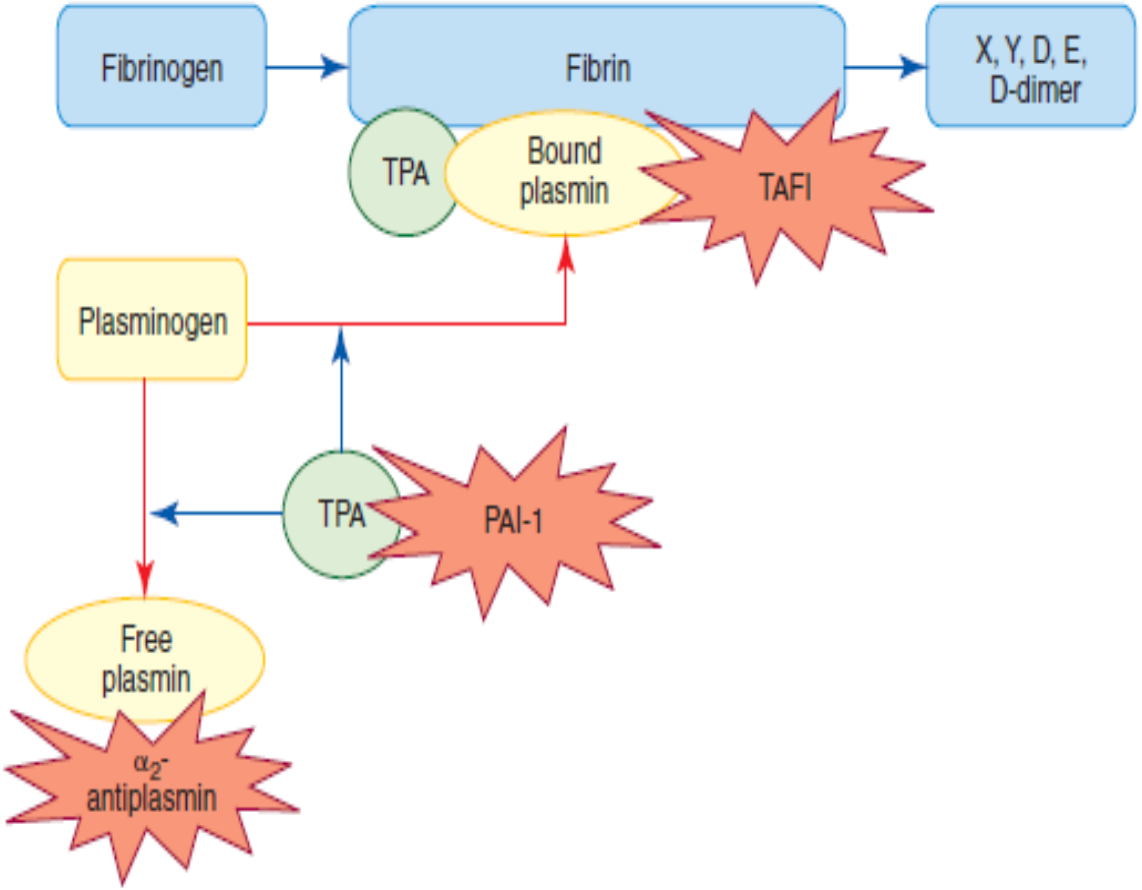


Figure 37-21 Fibrinolysis pathway and inhibitors. Plasminogen and tissue plasminogen activator (*TPA*) are bound to fibrin during coagulation. TPA converts bound plasminogen to plasmin, which slowly digests fibrin to form fibrin degradation products (FDPs) X, Y, D, E, and D-D (D-dimer). D-dimer is produced from cross-linked fibrin. Free plasmin is neutralized by α_2-antiplasmin. TPA is neutralized by plasminogen activator inhibitor-1 (*PAI-1*). Thrombin-activatable fibrinolysis inhibitor (*TAFI*) inhibits fibrinolysis by cleaving lysine residues on fibrin, preventing the binding of plasminogen, plasmin, and TPA.

Plasminogen

- **Plasminogen** is a 92,000 Dalton plasma zymogen produced by the **liver.**
- It is a single-chain protein possessing five glycosylated loops termed ***kringles.***
- Kringles enable plasminogen, along with activators TPA and UPA, to bind fibrin lysine molecules during polymerization .
- This fibrin-binding step is essential to fibrinolysis. Fibrin-bound plasminogen becomes converted into a two-chain active plasmin molecule when cleaved between arginine at position 561 and valine at position 562 by neighboring fibrin-bound TPA or UPA.

Plasmin

- **Plasmin** is a serine protease that systematically digests fibrin polymer by the hydrolysis of arginine-related and lysine-related peptide bonds. Bound plasmin digests clots and restores blood vessel patency.

- Its localization to fibrin through lysine binding prevents systemic activity. As fibrin becomes digested, the exposed carboxy-terminal lysine residues bind additional plasminogen and TPA, which further accelerates clot digestion.

- Free plasmin is capable of digesting plasma fibrinogen, factor V, factor VIII, and fibronectin.

- However, **plasma α2-antiplasmin** rapidly binds and inactivates any free plasmin in the circulation.

Plasminogen Activation

1. Tissue Plasminogen Activator (TPA)

- ECs secrete TPA, which hydrolyzes fibrin-bound plasminogen and initiates fibrinolysis.

- TPA, with two glycosylated kringle regions, forms covalent lysine bonds with fibrin during polymerization and localizes at the surface of the thrombus with plasminogen, where it begins the digestion process by converting plasminogen to plasmin.

- Circulating TPA is bound to inhibitors such as PAI-1 and is cleared from plasma.

- Synthetic recombinant TPAs mimic intrinsic TPA and are a family of drugs used to dissolve pathologic clots that form in venous and arterial thrombotic disease.

Plasminogen Activation

2. Urokinase Plasminogen Activator (UPA)

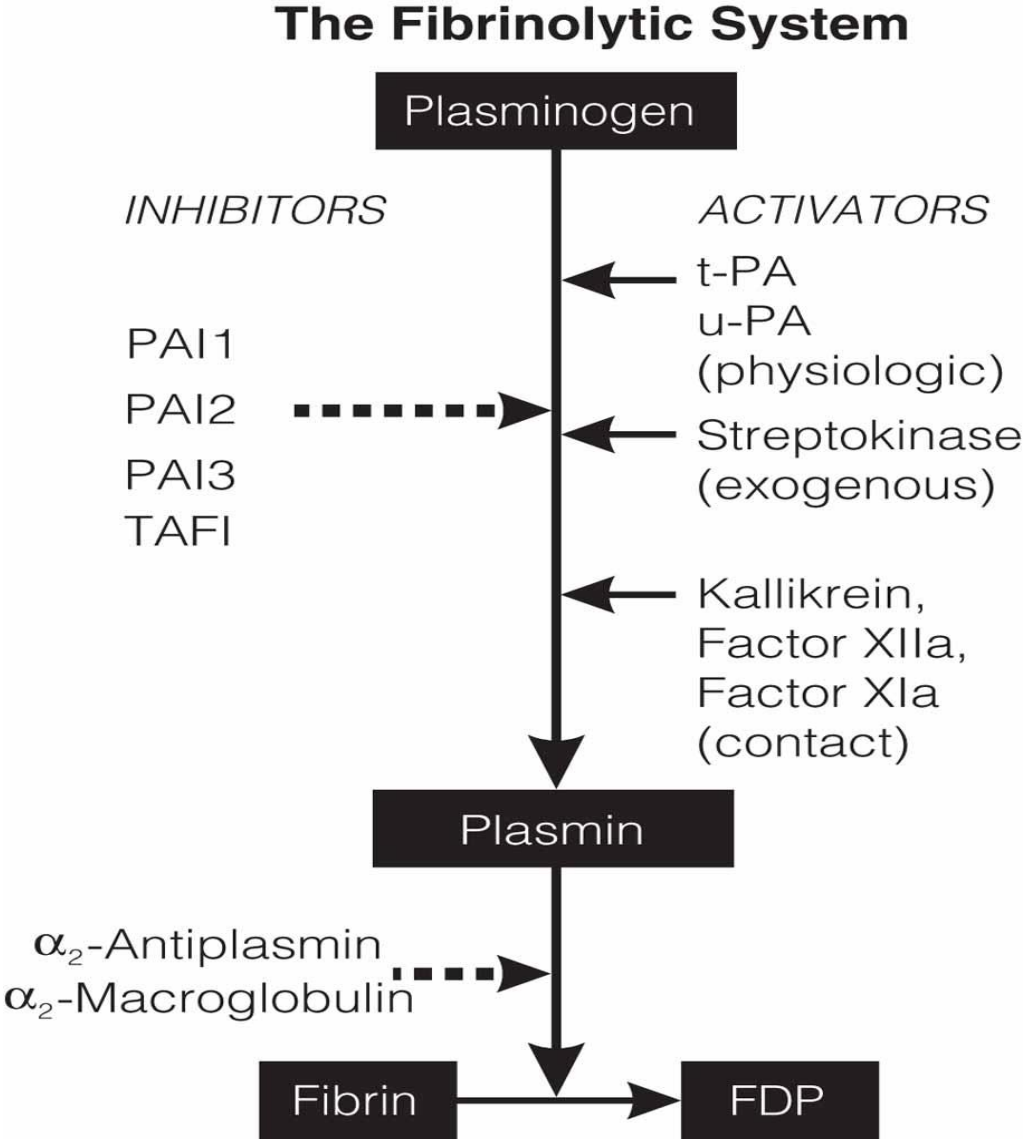
- Secreted by Urinary tract epithelial cells, monocytes, and macrophages.

- UPA circulates in plasma at a concentration of 2 to 4 ng/mL and becomes incorporated into the mix of fibrin-bound plasminogen and TPA at the time of thrombus formation.

- UPA has only one kringle region, does not bind firmly to fibrin, and has a relatively minor physiologic effect.

-

- Like TPA, purified UPA preparations are used to dissolve thrombi in myocardial infarction, stroke, and deep vein thrombosis.


The Fibrinolytic System
Plasminogen
INHIBITORS
ACTIVATORS
t-PA
u-PA
(physiologic)
PAI1
PAI2
PAI3
TAFI
Streptokinase
(exogenous)
Kallikrein,
Factor XIIa,
Factor XIa
(contact)
Plasmin
α_2-Antiplasmin
α_2-Macroglobulin
Fibrin
FDP

Activators

Fibrin Degradation Products and D-Dimer

- Plasmin cleaves fibrin and produces a series of identifiable fibrin fragments: X, Y, D, E, and D-D.
- Several of these fragments inhibit hemostasis and contribute to hemorrhage by preventing platelet activation and by hindering fibrin polymerization.
- Fragment X is described as the central E domain with the two D domains (D-E-D), minus some peptides cleaved by plasmin.
- Fragment Y is the E domain after cleavage of one D domain (D-E).
- Eventually these fragments are further digested to individual D and E domains.

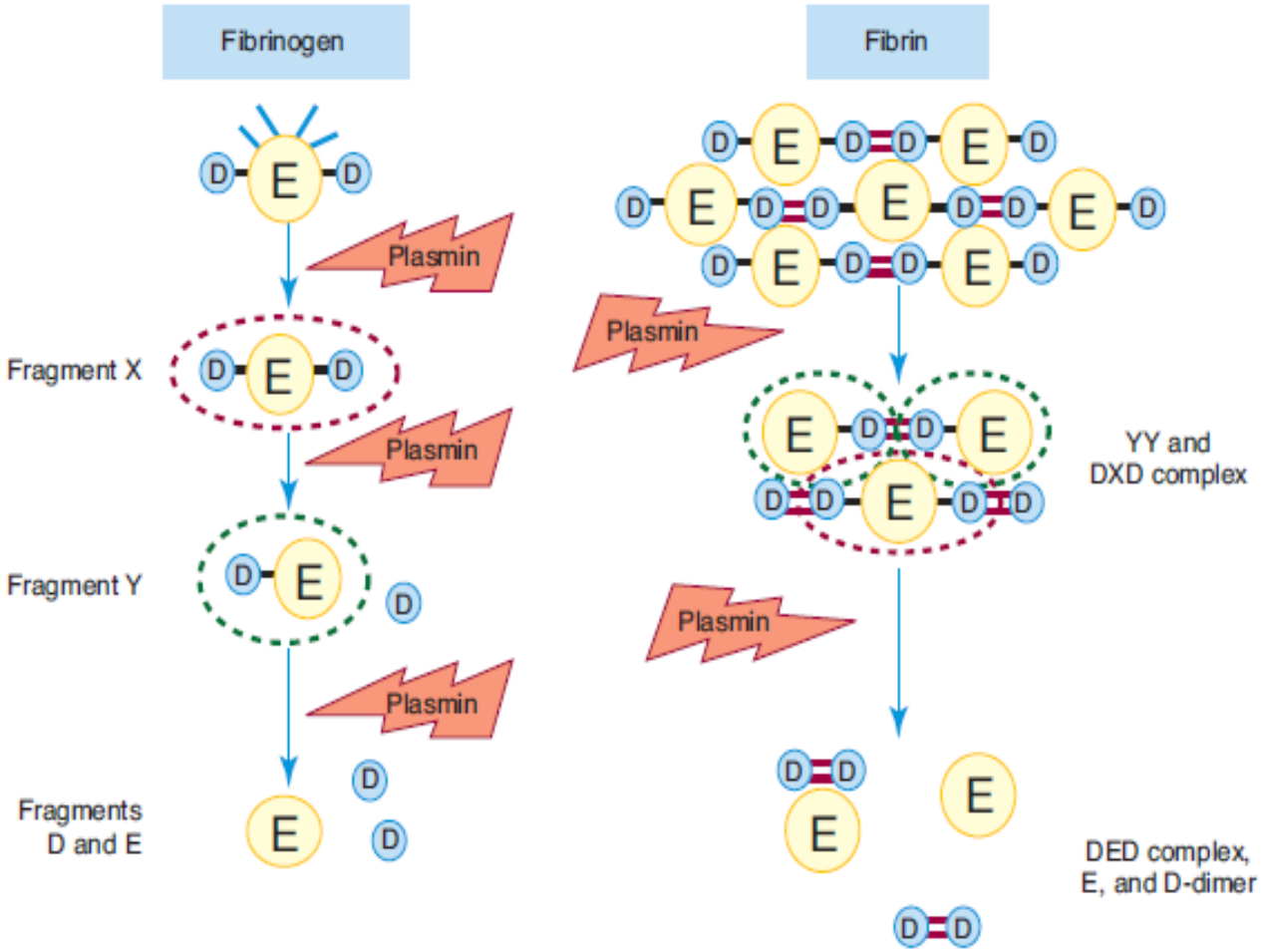


Figure 37-23 Degradation of fibrinogen and fibrin by plasmin. Plasmin systematically degrades fibrinogen and fibrin by digestion of small peptides and cleavage of D-E domains. From fibrinogen, fragment X consists of a central E domain with two D domains (D-E-D); further cleavage produces fragment Y (D-E), with eventual degradation to D and E domains. From cross-linked fibrin, plasmin digestion produces fragment complexes from one or more monomers. D-dimer consists of two D domains from adjacent monomers that have been cross-linked by factor XIIIa in the process of fibrin formation (thrombosis).

D-Dimer

- The D-D fragment, called D-dimer, is composed of two D domains from separate fibrin molecules cross-linked by the action of factor XIIIa.

- Fragments X, Y, D, and E are produced by digestion of either fibrin or fibrinogen by plasmin, but *D-dimer is a specific product of digestion of cross-linked fibrin only* and

- is therefore a marker of thrombosis and fibrinolysis - that is, thrombin, factor XIIIa and plasmin activation.

Fibrin Degradation Products and D-Dimer

- The various fragments may be detected by quantitative or semiquantitative immunoassay to reveal fibrinolytic activity.

- D-dimer is separately detectable by monoclonal antibody for D-dimer antigen, using a wide variety of automated quantitative laboratory immunoassays and other formats including point-of-care tests performed on whole blood.

- The D-dimer immunoassay is used to identify chronic and acute DIC and to rule out venous thromboembolism in suspected cases of deep venous thrombosis or pulmonary embolism.

Haemostatic Effect of FDPs

The FDPs are significant because of their haemostatic effects, which include;

- Anti-thrombin activity
- Interference with polymerization of fibrin monomer
- Interference with platelet activity

Control of Fibrinolysis

1. Plasminogen Activator Inhibitor 1 (PAI-1)

- Inactivating both TPA and UPA and thus preventing them from converting plasminogen to plasmin.
- Is a single-chain glycoprotein serine protease inhibitor and is produced by ECs, megakaryocytes, smooth muscle cells, fibroblasts, monocytes, adipocytes, hepatocytes, and other cell types.
- Platelets store a pool of PAI-1, accounting for more than half of its availability and for its delivery to the fibrin clot.
- PAI-1 is present in excess of the TPA concentration in plasma, and circulating TPA normally becomes bound to PAI-1.

Plasminogen Activator Inhibitor 1 (PAI-1)

- Only at times of EC activation, such as after trauma, does the level of TPA secretion exceed that of PAI-1 to initiate fibrinolysis. Binding of TPA to fibrin protects TPA from PAI-1 inhibition.

- Plasma PAI-1 levels vary widely. PAI-1 deficiency has been associated with chronic mild bleeding due to increased fibrinolysis. PAI-1 is an acute phase reactant and is increased in many conditions, including metabolic syndrome, obesity, atherosclerosis, sepsis, and stroke.

- Increased PAI-1 levels correlate with reduced fibrinolytic activity and increased risk of thrombosis.

2. a2-Antiplasmin

- Is a serine protease inhibitor of free plasmin, synthesized in the liver.

- The therapeutic lysine analogues, tranexamic acid and e-aminocaproic acid, are similarly antifibrinolytic through their affinity for kringles in plasminogen and TPA. Both inhibit the proteolytic activity of plasmin.

3. Thrombin-Activatable Fibrinolysis Inhibitor

- TAFI is a plasma procarboxypeptidase synthesized in the liver that becomes activated by the thrombin-thrombomodulin complex.

- Activated TAFI functions as an antifibrinolytic enzyme. It inhibits fibrinolysis by cleaving exposed carboxy-terminal lysine residues from partially degraded fibrin, thereby preventing the binding of TPA and plasminogen to fibrin and blocking the formation of plasmin.

- In coagulation factor–deficient states, such as hemophilia, decreased thrombin production may reduce the activation of TAFI, resulting in increased fibrinolysis that contributes to more bleeding.

- Conversely, in thrombotic disorders, increased thrombin generation may increase the activation of TAFI. The resulting decreased fibrinolysis may contribute further to thrombosis.

- TAFI also may play a role in regulating inflammation and wound healing.

Other Inhibitors

- Anti-thrombin III, inhibits fibrinolysis by inhibiting plasmin and kallikrein
- The C1 in-activator also inhibits plasmin.

Next Lecture: Vitamin K & Coagulation

Lecture 7: Vitamin K & Coagulation

Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Vitamin K

- Vitamin K is a group of lipophilic, hydrophobic vitamins.
- They are needed for the post-translation modification of proteins required for blood coagulation.
- They are involved in metabolism pathways, in bone mineralization, cell growth, metabolism of blood vessel wall.

Vitamin K

Types:

1) Vit K 1 (Phylloquinone): used mainly for blood clotting
   - natural form, found in plants
   - provides the primary source of vitamin K to humans through dietary consumption

2) Vitamin K2 compounds (Menaquinones): important in non-coagulation actions - as in metabolism and bone mineralization, in cell growth, metabolism of blood vessel walls cells.
- made by bacteria in the human gut (*Bacteroides fragilis* and *Escherichia coli).*

Dietary Sources

- Green leafy vegetables
- vegetable oil
- broccoli
- cereals

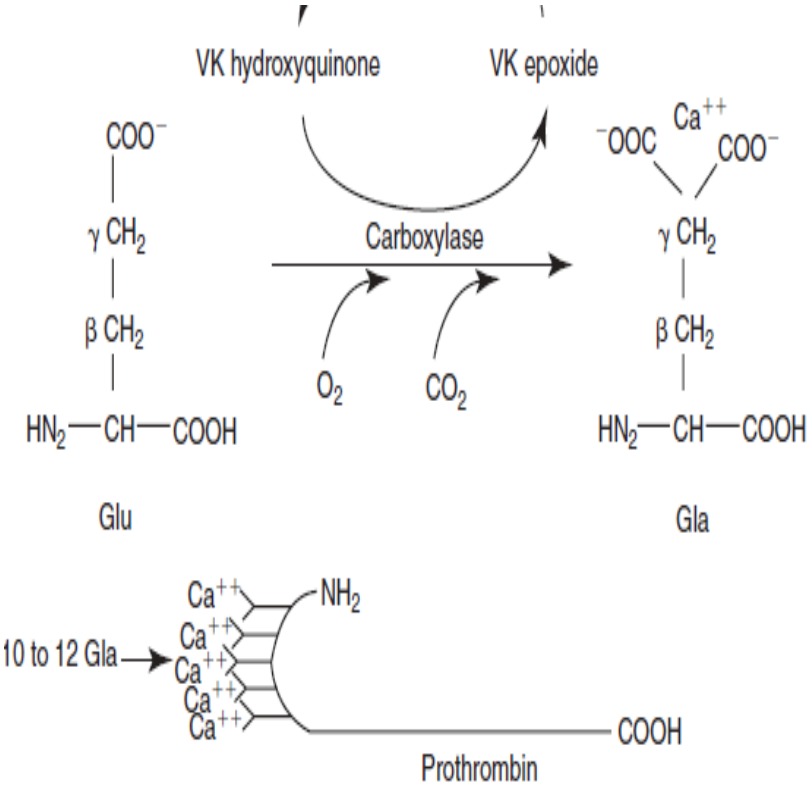
Vitamin K Function

- Vitamin K serves as an essential cofactor, catalyzes an essential posttranslational modification of the prothrombin group proteins: γ-carboxylation of amino-terminal glutamic acids.

- Glutamic acid is modified to γ - carboxyglutamic acid when a second carboxyl group is added to the γ carbon.

- With two ionized carboxyl groups, the γ - carboxyglutamic acids gain a net negative charge, which enables them to bind ionic calcium (Ca^{2+}).

- The bound calcium enables the vitamin K-dependent proteins to bind to negatively charged phospholipids to form coagulation complexes.



Vitamin K Deficiency

- Deficiencies are very rare in humans except in newborns due to:
  - insufficient gut bacteria
  - poor placental transport of vitamin K
  - low prothrombin synthetic capacity of neonatal liver

- Newborns routinely receive vitamin K injection (0.5 -1 mg vitamin K) or 2 mg orally, because human milk is very low in vitamin K (2.5 µg/L).

- Bleeding episodes may occur in patients with low vitamin K status on long-term antibiotic treatment (loss of colonic bacteria).

Vitamin K Deficiency

- Deficiency is caused by fat malabsorption or by the liver failure.
- Blood clotting disorders – dangerous in newborns, life-threatening bleeding (hemorrhagic disease of the newborn).
- Osteoporosis due to failed carboxylation of osteocalcin and decreased activity of osteoblasts.
- Under normal circumstances there is not a shortage, vit. K is abundant in the diet.

Vitamin K Dependent Coagulation

- Certain clotting factors/proteins require calcium to bind for activation.
- Calcium can only bind after gamma carboxylation of specific glutamic acid residues in these proteins.
- The reduced form of vitamin K2 (vitamin KH2) acts as a cofactor for this carboxylation reaction.
- These proteins are known as "Vitamin K dependent" proteins

Vitamin K Dependent Proteins

- factor II
- factor VII
- factor IX
- factor X
- protein C & protein S
- Protein Z

PIVKA

- Deficiency of vitamin K is associated with a decrease of the functional activity of these factors.

- These non-functional proteins are released into the circulation in normal levels & are called Protein Induced by Vitamin K Absence or Antagonism (PIVKA).

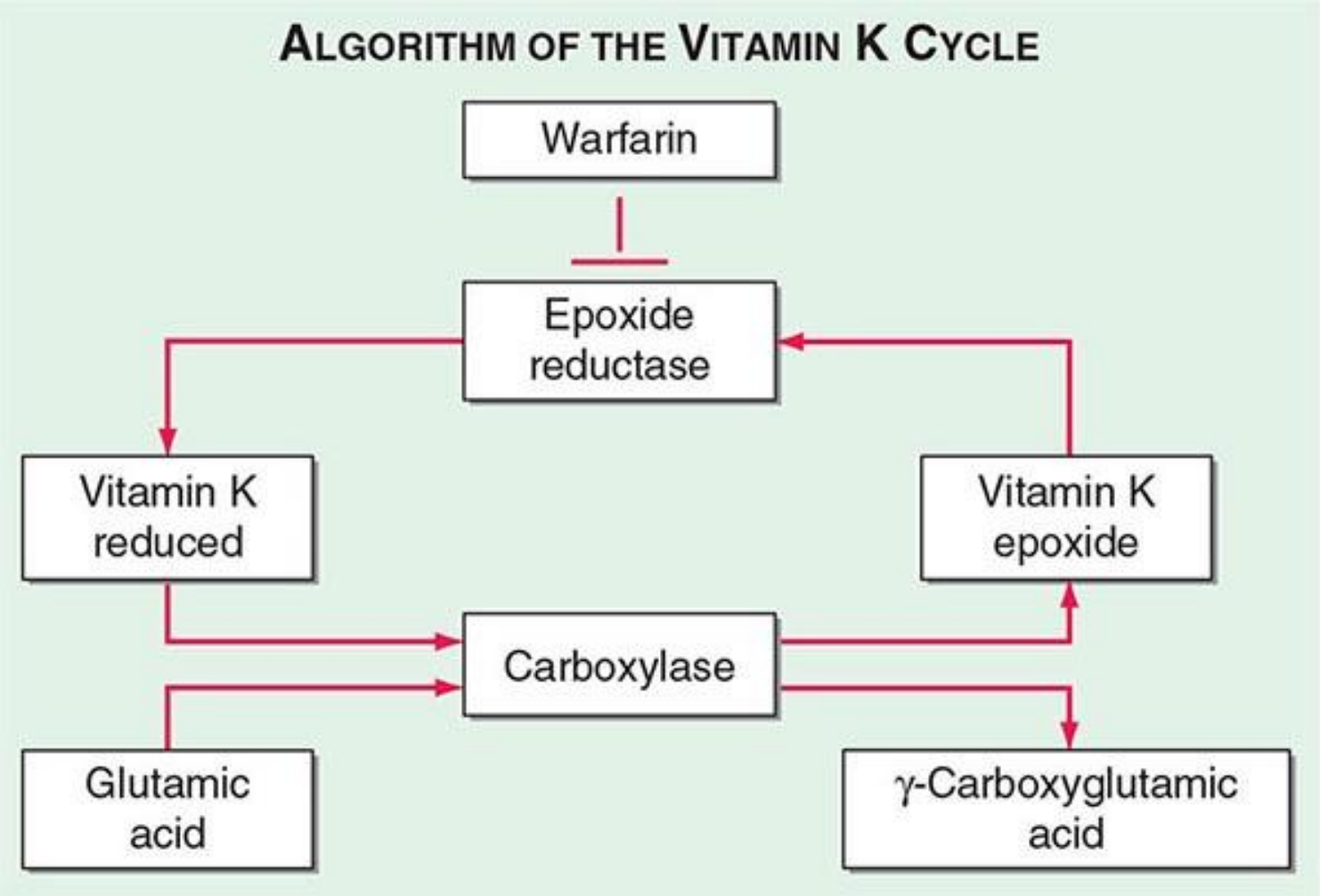
PIVKA Properties

- Can not bind Calcium ions.
- Are not adsorbed on aluminum hydroxide & barium salts.
- Can be activated in vitro with venom of certain snakes (Echis Carinatus).
- This Ecarin characteristic is the basis of their laboratory measurement.

Vitamin K Cycle

- Vitamin K is a fat-soluble vitamin,
- the body stores very little of it, and its stores are rapidly depleted without regular dietary intake.
- Because of its limited ability to store vitamin K, the body recycles it through a process called the vitamin K cycle.
- The vitamin K cycle allows a small amount of vitamin K to function in the gamma-carboxylation of proteins many times, decreasing the dietary requirement.
- The two enzymes important in vitamin K cycle:
    1. γ-glutamylcarboxylase and
    2. epoxide reductase are critical for the metabolism and regeneration of vitamin K.

Vitamin K Cycle



Vitamin K Cycle

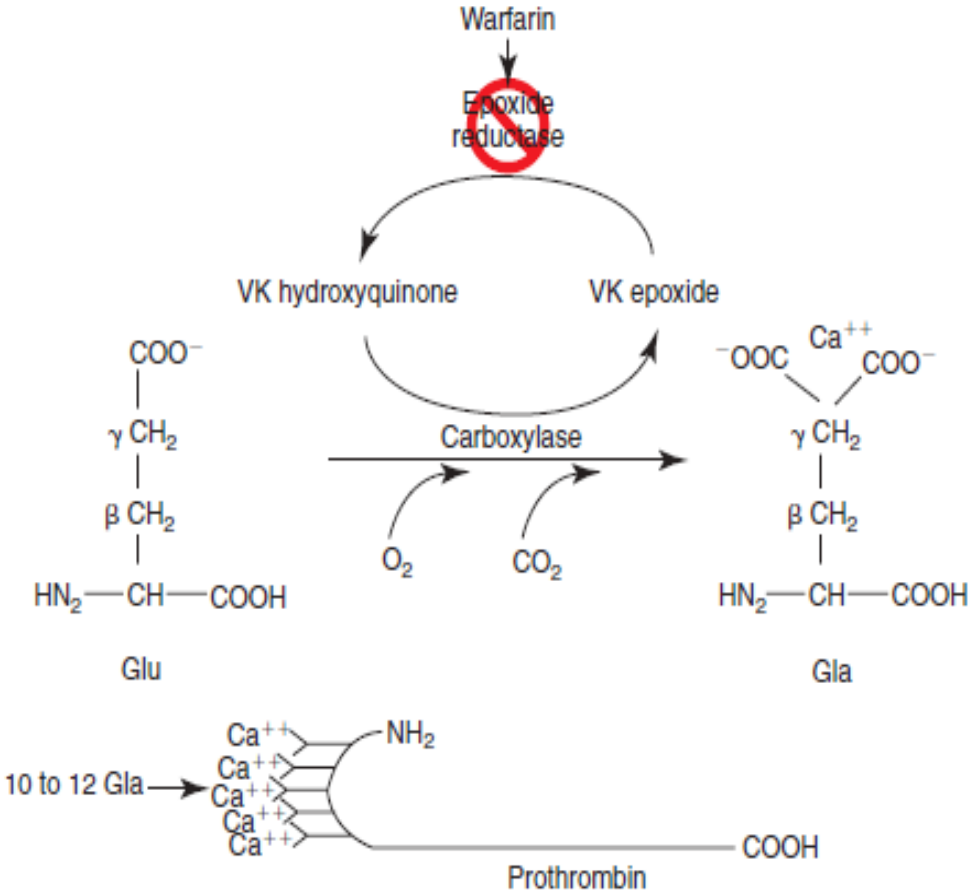
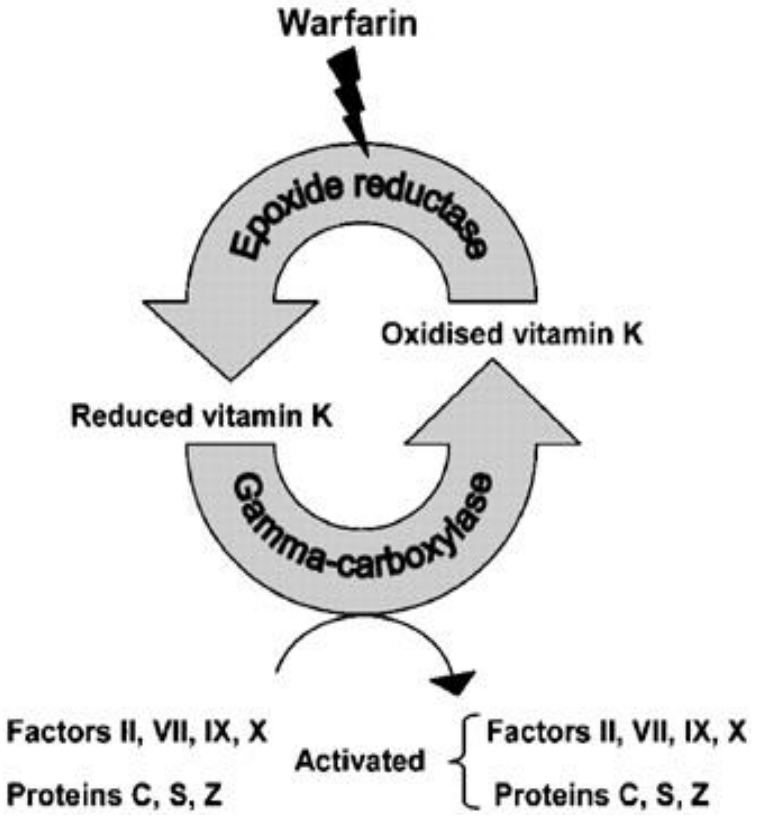


Figure 37-9 Vitamin K (*K*) posttranslational γ-carboxylation of coagulation factors II (prothrombin), VII, IX, and X, and control proteins C, S, and Z. Vitamin K hydroxyquinone transfers a carboxyl (COO^-) group to the γ carbon of glutamic acid (*Glu*), creating γ-carboxyglutamic acid (*Gla*). The negatively charged pocket formed by the two carboxyl groups attracts ionic calcium, which enables the molecule to bind to phosphatidylserine. Vitamin K hydroxyquinone is oxidized to vitamin K epoxide by *carboxylase* in the process of transferring the carboxyl group but is subsequently reduced to the hydroxyquinone form by *epoxide reductase*. Warfarin suppresses *epoxide reductase*, which slows the reaction and prevents γ-carboxylation. "Des-carboxy" proteins are unable to participate in coagulation. There are typically 10 to 12 γ-carboxyglutamic acid molecules near the amino terminus of the vitamin K–dependent factors.

Vitamin K antagonism

- Warfarin blocks the action of the reductase and competitively inhibits the effects of vitamin K.
- Warfarin prevents the recycling of vitamin K by inhibiting two important reactions and creating a functional vitamin K deficiency.
- Warfarin is a competitive inhibitor of Epoxide reductase. In the presence of Warfarin, vitamin K epoxides cannot be reduced, they accumulate and are excreted



Vitamin K antagonism

- Vitamin K Antagonists Abnormal precursor of prothrombin (preprothrombin) containing little or no carboxyglutamate, and incapable of chelating calcium, is released into the circulation.

- Thus, in the presence of Warfarin or in vitamin K deficiency the process of coagulation is inhibited,

Warfarin treatment

- Large quantities of dietary or supplemental vitamin K can overcome the anticoagulant effect of vitamin K antagonists.
- patients taking these drugs are cautioned against consuming very large or highly variable quantities of vitamin K in their diets.
- Like all anticoagulants, the major side effect of Warfarin is bleeding.
- Treatment of pregnant women with Warfarin can lead to fetal bone abnormalities (Fetal Warfarin syndrome)

Vitamin K deficiency

- Lack of vitamin K in the diet
- Fat malabsorption and that thus reduce the absorption of vitamin K
- Disease or surgical interventions that affect the ability of the intestinal tract to absorb vitamin K
- Chronic liver diseases

Vitamin K deficiency in the newborn

- Transplacental transfer of vitamin K is very limited during pregnancy
- The storage of vitamin K in neonatal liver is also limited.
- Breast milk is a poor source of vitamin K.
- Maternal medications that interfere with vitamin K stores or function (e.g., carbamazepine, phenytoin, barbiturates, some Cephalosporins, rifampin, Isoniazid, Warfarin or Warfarin like drugs) can result in vitamin K deficiency bleeding in the infant.

Clinical Manifestations

- The main symptom is bleeding (hemorrhage)—into the skin (causing bruises), from the nose, from a wound, in the stomach, or in the intestine.
- Blood may be seen in the urine or stool.
- In newborns, life-threatening bleeding within or around the brain may occur.
- Having a liver disorder increases the risk of bleeding because proteins that help blood clot (clotting factors) are made in the liver.
- Vitamin K deficiency may also weaken bones.

Prevention/Treatment

- Vitamin K can be given orally
- In the case of someone who improperly absorbs fat or is at high risk of bleeding, Vitamin K can be injected under the skin.
- If a drug is causing Vitamin K deficiency, the dose is altered or extra Vitamin K is given
- In people who suffer from both severe liver disorders and Vitamin K deficiency, Vitamin K injections may be insufficient so blood transfusions may be necessary to replenish clotting factors.
- It is recommended that all newborns are given an injection of phylloquinone (Vitamin K1) into the muscle to prevent intracranial bleeding after delivery.
- Formulas for infants contain Vitamin K.

Laboratory Studies

- A Prothrombin time (PT), activated partial Thromboplastin time (aPTT), fibrinogen levels, and a platelet count should be included in the initial workup for vitamin K deficiency bleeding (VKDB) in a newborn.

- A prolonged PT is usually the first laboratory test result to be abnormal in vitamin K deficiency bleeding due to reduction in Prothrombin, FVII, FIX, and FX levels.

- The diagnosis of vitamin K deficiency bleeding is confirmed if administration of vitamin K halts the bleeding and reduces the PT value.

- Infants with vitamin K deficiency bleeding typically have a prolonged PT with platelet counts and fibrinogen levels within the normal range for newborns.

Next Lecture: Bleeding Disorders

Lecture 8: Bleeding Disorders

Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Classification of Hemostatic Disorders

1. Bleeding disorders (hemorrhagic disorders/hemorrhagic diathesis): bleeding disorders have an abnormal tendency to bleed (hemorrhage) due to failure of hemostasis.

2. Thrombotic disorders: they cause thrombus formation.

Clinical manifestations of bleeding disorders

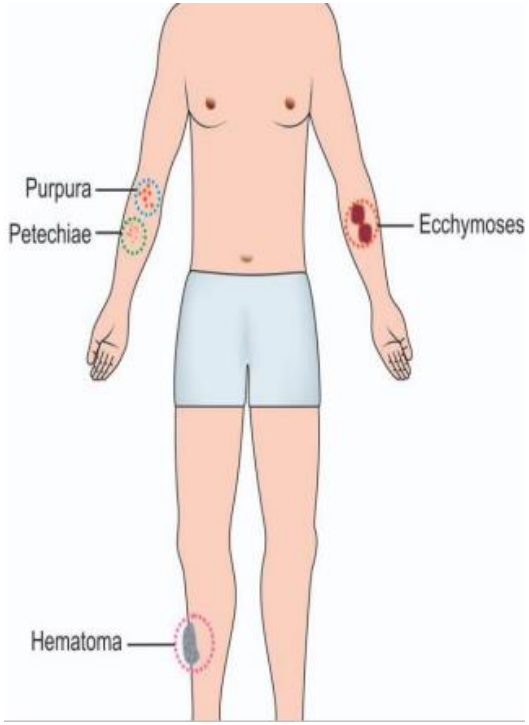
- Any patient with a clinically significant bleeding disorder will present to his/her physician with hemorrhagic symptoms.
- Ranges form the presence of more bruises than usual to hemorrhaging that is life threatening (localized or generalized).
- The severity of the bleed is generally in proportion to the severity and the type of defect.
- Acquired or congenital.

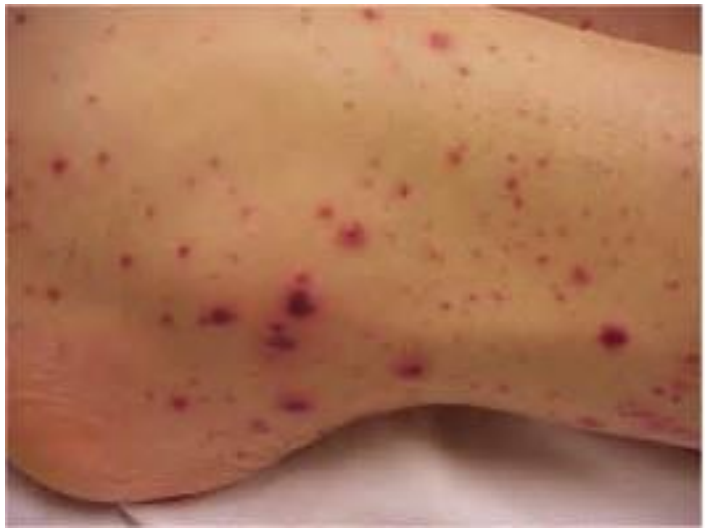
Type of bleeding

- Type of bleeding may indicate which component of hemostasis is defected:
  - In primary hemostasis defects usually results in bleeding from the skin or mucous membranes such as nose or gingiva (superficial).
  - While in patients with coagulation factor abnormalities the bleeding is usually internal into deeper tissues and joints (deep).

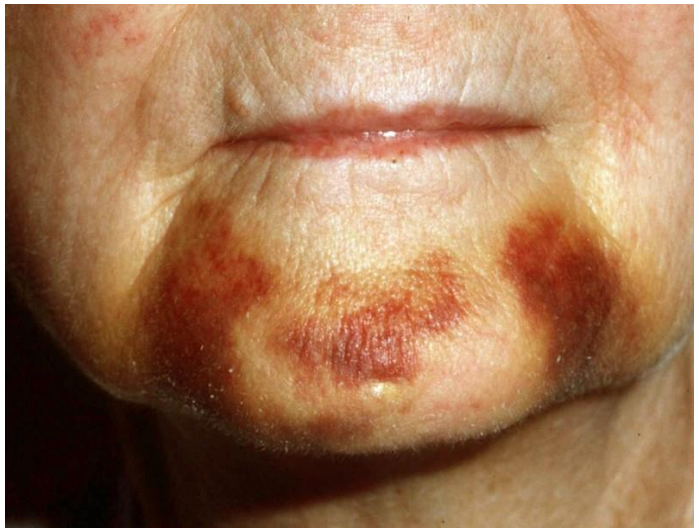
Bleeding Disorder Terms

- Petechiae-pinpoint size/pinhead size hemorrhagic spots in skin less than 3mm.
- Purpura-hemorrhage under the skin, varying in color and duration
- Ecchymosis-purplish patch caused by extravasation of blood into skin, larger than petechiae
- Epistaxis-nosebleed
- Menorrhagia-excessive menses
- Hematuria-blood in urine
- Hemarthrosis-bleeding into joint
- Hematemesis-vomiting blood
- Hemoptysis-spitting blood
- Melena-blood in stool (occult blood)

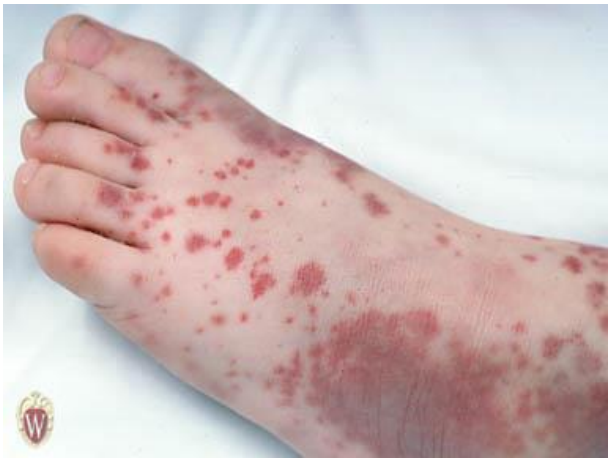




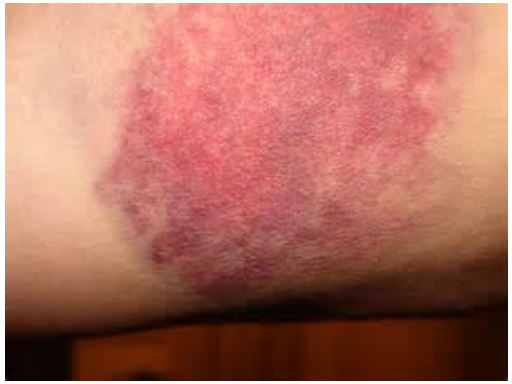
Petechiae



Ecchymosis

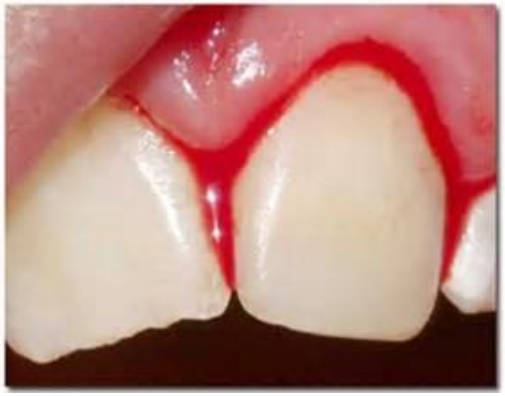


Purpura



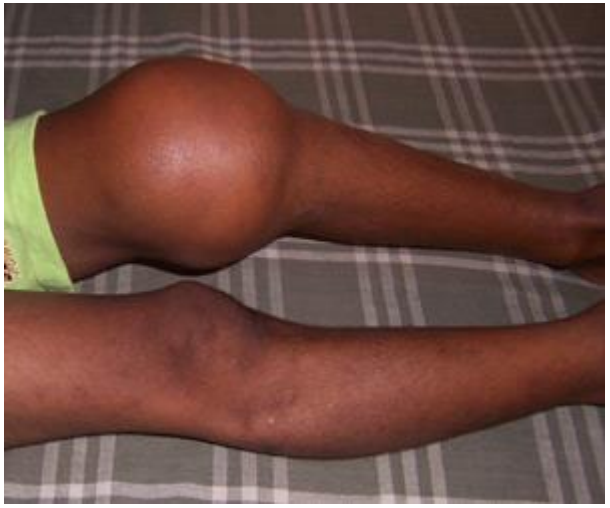
Hematoma

Epistaxis



Gingival bleeding

Secondary hemostasis disorders joint bleeding or Hemarthrosis



Diagnosis of bleeding disorders

1. Patient bleeding history
2. Family History
3. Physical examination
4. Laboratory testing

Classification of Bleeding Disorders

1. Disorders of primary hemostasis
   a. Vessel wall abnormalities
   b. Platelet abnormalities

2. Disorders of coagulation system (disorders of secondary hemostasis)

Screening tests for Hemostatic disorders

Test	Assesses
Hemoglobin, hematocrit; reticulocyte count	Anemia associated with chronic bleeding; bone marrow response
Platelet count	Thrombocytopenia
Prothrombin time (PT)	Deficiencies of factors II (prothrombin), V, VII, or X (clotting time prolonged)
Partial thromboplastin time (PTT)	Deficiencies of all factors except VII and XIII (clotting time prolonged)
Thrombin time or fibrinogen assay	Hypofibrinogenemia and dysfibrinogenemia

Disorders of primary hemostasis

a. Vessel wall abnormalities
   - Congenital, e.g. Ehlers-Danlos syndrome
   - Acquired, e.g. Henoch-Schonlein purpura

b. Platelet abnormalities
   - Quantitative: thrombocytopenia (e.g. ITP, drug-induced, congenital)
   - Qualitative: platelet function disorders
     - Inherited, e.g. Glanzmann thrombasthenia, Bernard Soulier syndrome
     - Acquired, e.g. Uremia, drugs

Disorders of coagulation system (disorders of secondary hemostasis)

- Congenital: hemophilia A, B; von Willebrand disease; other coagulation factor deficiencies [XI, VII, II, V, X].

- Acquired: vitamin K deficiency, liver disease, disseminated intravascular coagulation.

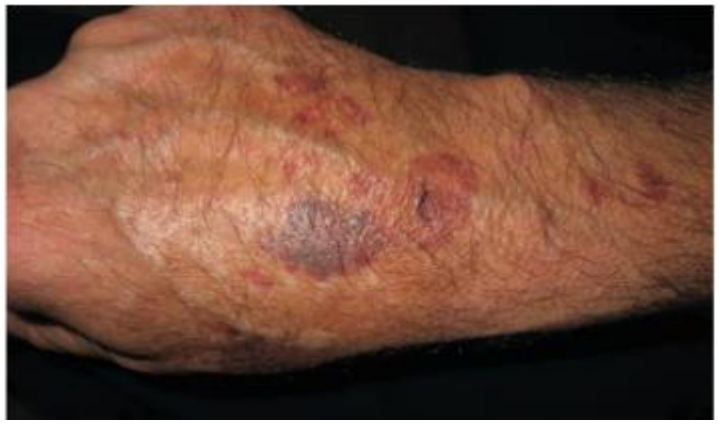
Table: Distinguishing patterns of bleeding in platelet/vascular and coagulation disorders

Characteristics	*Platelet/vascular disorders*	*Coagulation disorders*
Onset	Spontaneous and develops immediately after trauma/surgery	Delayed bleeding after trauma/ surgery
Type of lesion	Petechiae, ecchymoses	Hematomas
Sites	Skin, mucous membrane	Deep tissues
• Mucous membrane	Common from nose, mouth, gastrointestinal and genitourinary tracts	Uncommon except from gastrointestinal or genitourinary tract
• Into the joint	Absent	Common in severe factor deficiencies
• Into the muscle	Following trauma	Spontaneous

Vascular diseases

- Symptoms are usually the superficial ones.
- Usually these diseases are diagnosed by exclusion. After ruling out PLT disorders, coagulation or fibrinolytic disorders in patient who has bleeding symptoms.
- In vascular diseases, PLt count and screening tests for coagulation factors are usually normal.
- Bleeding time may be prolonged in some vascular diseases.

Inherited vascular disorders

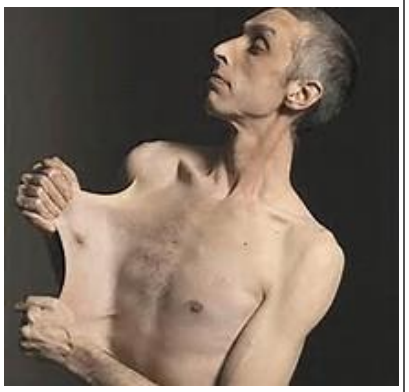


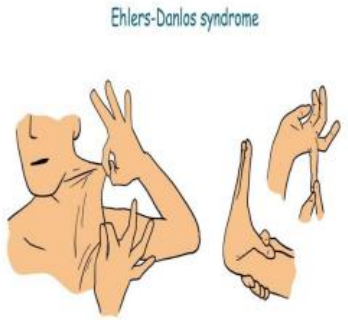
Hereditary Hemorrhagic Telangiectasia (HHT)

- Autosomal dominant trait
- Defect of angiogenesis
- Involves bleeding from abnormally dilated vessels "telangiectasias"
- Vessels involved do not contract normally and collapse easily
- Patient has pinpoint lesions (tiny areas of bleeding)
- Lesions occur on face, hands and feet
- May develop at all ages
- Blood loss may cause anemia
- Diagnosis based on physical appearance

Ehlers-Danlos Syndrome

- Lack of structural tissue support (collagen disorders)
- Skin elasticity and fragility.
- Hypermobility of joints
- Evidenced by bleeding/bruising
- Recurrent joint problems & scarring of the face.
- The most serious is deficient of type III collagen (blood vessel type). Which leads to Acute & sever Internal bleeding & sudden death.





Hemangioma-Thrombocytopenia Syndrome (Kasabach-Merritt Syndrome)

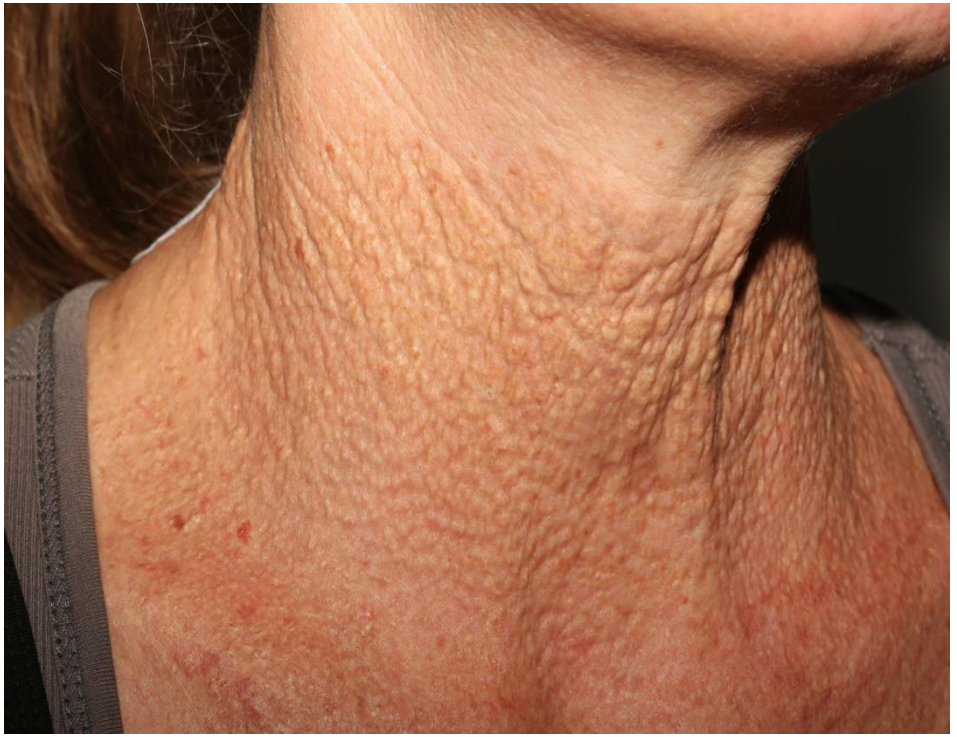
- Benign tumour of vascular tissue
- Grow rapidly to giant proportion.
- Threaten the function of neighbouring tissues.
- Mechanical injury may result in sever bleeding.
- May trigger a localized DIC with thrombocytopeia & consumption coagulopathy, thereby worsening bleeding.
- Tumor composed of many blood vessels (blood-filled)

Cavernous Hemangioma

- Lesion may swell and bleed
- Tumor site may form clots, hemolyzed RBCs and vessel obstruction
- Present at birth
- Treatment is by surgical removal, if possible, or localized radiotherapy with injection of fibrinolytic inhibitors.

Pseudoxanthoma Elasticum

- Autosomal recessive trait
- Lack of skin elasticity
- Some connective tissue calcified
- Bleeding and bruising evident



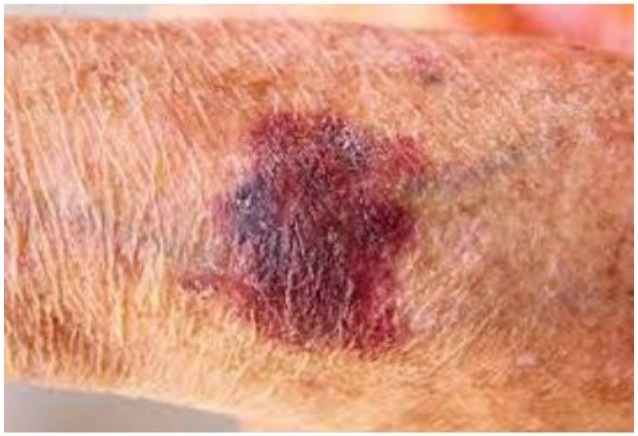
Marfan syndrome

- In addition to vascular defects,
- is characterized by skeletal and ocular defects.

Acquired Vascular Disorders

Senile Purpura

- Occurs in elderly population
- Usually benign
- Collagen degradation/loss affects vessel integrity
- superficial, persistent purpuric spots on the forearms and other sun-exposed areas of the skin.
- No treatment/therapy available



Scurvy

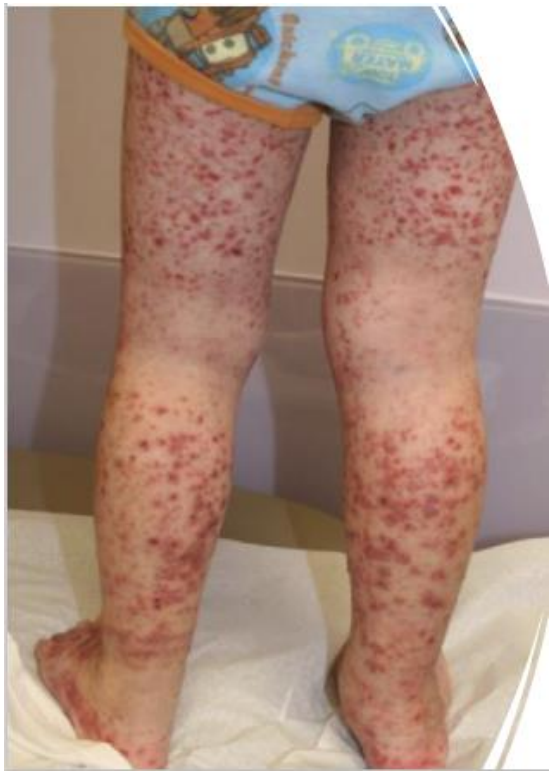
- Caused deficient Vitamin C
- Vitamin C required for vessel collagen integrity, Acts as "cement" holding endothelial cells together
- Lack of Vitamin C prevents proper collagen formation
- Result: bleed and vessel fragility
- Symptoms include gum bleeding, petechiae and bleeding into tissues and muscles
- Treated with Vitamin C

Cushing syndrome and steroid therapy

- In **Cushing syndrome**, protein-wasting due to excessive corticosteroid production causes loss of perivascular supporting tissue.

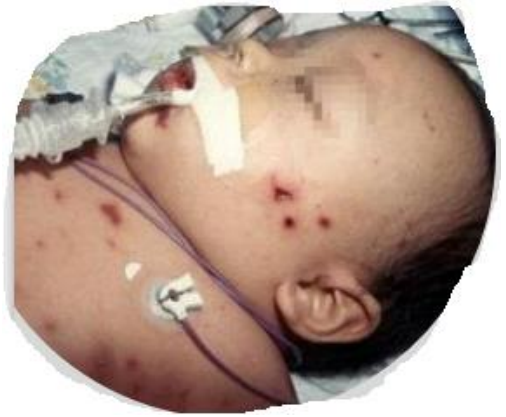
Allergic Purpura (Henoch-Schönlein Purpura)

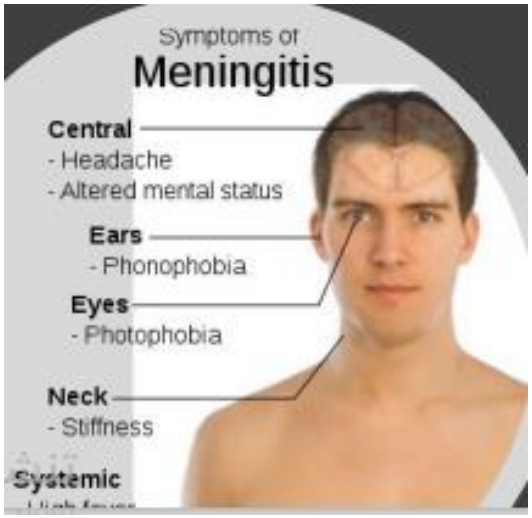
- Self-produced "autoantibodies" damage to vessels
  - Caused by drugs resulting in purpura
  - Caused by allergic/immune disturbance
  - Evidenced by swelling, ulcers, purpura and lesions and other symptoms
  - Affects children
- Other allergic purpura-Henoch-Schonlein variety
  - Accompanied by joint and abdominal pain
  - Avoidance of allergen aids recovery



Infectious purpura

- Observe petechiae and purpura
- Results from
  - Inflammatory response to agent
  - Autoimmune/autoantibody response
  - Bacterial products or toxins especially meningococcemia, septicemia, infective endocarditis and several of the rickettsioses.
  - Injury caused by agent
- Low platelets observed and DIC
- Cure is to treat infection





Amyloidosis

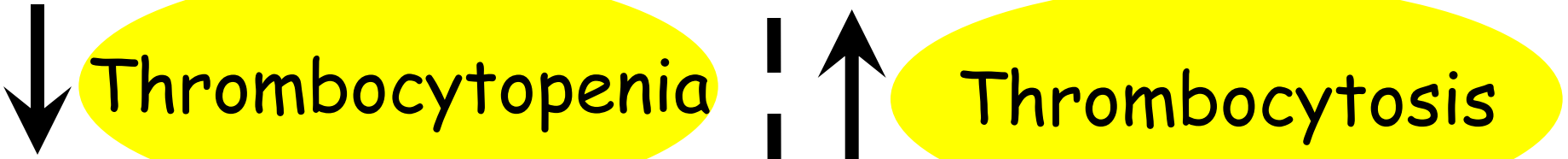
- In systemic amyloidosis perivascular deposition of amyloid may weaken the blood vessel wall and cause purpura.

- This is most commonly seen in plasma cell neoplasms and presents as mucocutaneous petechiae.

Platelet Disorders

Platelet Disorders Classification:

Quantitative PLT Disorders



PLT Reference Range = 150 - 450 x10⁹/L

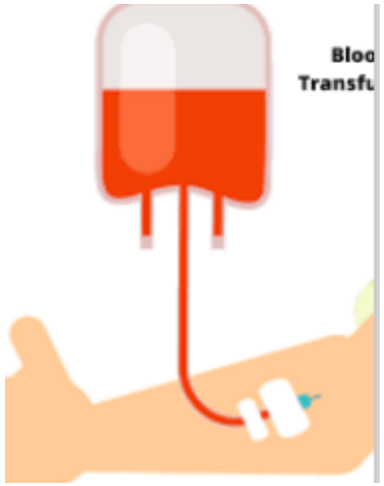
Qualitative PLT Disorders

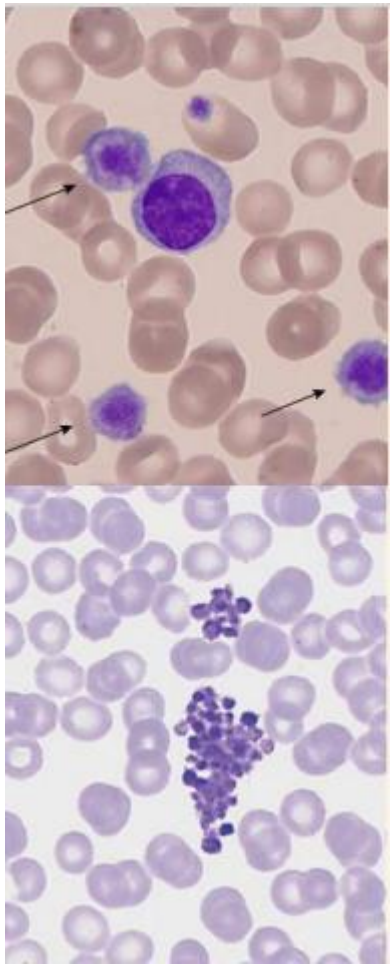
Platelet Disorders Classification:

- **Quantitative platelet disorders**
  - Thrombocytopenia
    - Increased destruction
    - Decreased production
    - Sequestration
    - Dilutional
  - Thrombocytosis
- **Qualitative platelet disorders**
  - Hereditary
    - Defective adhesion of platelets
    - Disorders of platelet secretion
    - Defective platelet aggregation
  - Acquired

We talk about real thrombocytopenia

- **Do not forget pseudo-thrombocytopenia:**
  - Platelet aggregation
  - Platelet satellitism
  - Clotted sample
  - Cold agglutinin
  - Giant platelets
  - Hemodilutional thrombocytopenia as pregnancy and IV fluids





Thrombocytopenia

- platelet count of fewer than 100,000/μl
- is the most common cause of clinically important bleeding.
- True thrombocytopenia has to be differentiated from the thrombocytopenia artifact that can result from:
  - poorly prepared blood smears or
  - automated cell counts when platelet clumping or platelet satellitosis are present.
- The primary pathophysiologic processes that result in thrombocytopenia are:
  - decreased platelet production,
  - accelerated platelet destruction, and
  - abnormal platelet distribution (sequestration)

Hemostatic Level

- Hemostatic Platelet count level is more than 50 $x10^9/L$.

- This means that normal hemostasis may occur ≥ 50 $x10^9/L$

Thrombocytopenia

Platelet count	Symptoms
50-100 $X10^9/L$	Prolonged bleeding following trauma
< $50X10^9/L$	Easy bruising Purpura following minor trauma
< 20 $X10^9/L$	Spontaneous bleeding Petechiae May suffer spontaneous internal and intracranial bleeding

Thrombocytopenia

- Bleeding due to thrombocytopenia alone is associated with a prolonged bleeding time, normal prothrombin time (PT) and activated partial thromboplastin time (APTT).

Causes of thrombocytopenia

1. Decreased production of platelets

- **Generalized primary diseases of bone marrow:** Aplastic anemia (congenital and acquired)
- **Bone marrow invasion/infiltration:** Leukemia, disseminated cancer
- **Selective impairment of platelet production**
  - Drug-induced: Alcohol, thiazides, cytotoxic drugs
  - Infections: Measles, human immunodeficiency virus (HIV)
- **Ineffective megakaryopoiesis**
  - Megaloblastic anemia: Because of impaired DNA synthesis in nutritional deficiency of vitamin B12 or folic acid.
  - Myelodysplastic syndromes

Causes of thrombocytopenia

2. *Increased platelet destruction*

- **Immune mediated**
  - **Autoimmune**
    - Primary: Immune thrombocytopenic purpura (acute and chronic)
    - Secondary: Systemic lupus erythematosus, B cell lymphoid neoplasms
  - **Alloimmune**: Post-transfusion or pregnancy
  - **Drug-induced**: Quinidine, heparin, sulfa compounds
  - **Infections**: HIV infection, infectious mononucleosis, cytomegalovirus

- **Non-immune mediated**

  a_ Disseminated intravascular coagulopathy (DIC)

  b_ Thrombotic thrombocytopenic purpura (TTP)

  c_ Hemolytic Uremic syndrome (HUS)

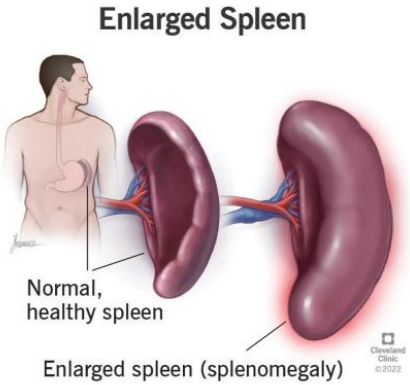
  d_ HELP syndrome in preeclampsia

  All called Microangiopathic hemolytic anemia (MAHA)
  - Mechanical destruction: Prosthetic heart valves, malignant hypertension
  - Microangiopathic hemolytic anemias

Causes of thrombocytopenia

3. *Sequestration*

- Hypersplenism: It is a syndrome which is associated with increased sequestration/pooling of platelets (up to 80%) in the spleen. It can cause moderate thrombocytopenia and when necessary, hypersplenic thrombocytopenia can be ameliorated by splenectomy.



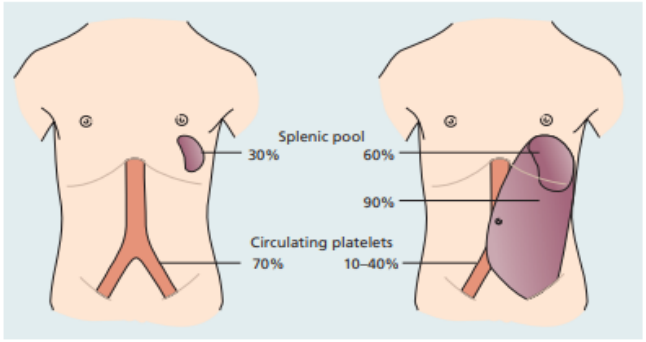
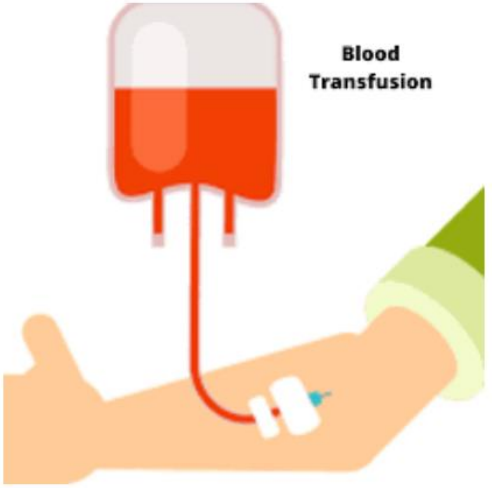


Figure 25.9 The platelet distribution between the circulation and spleen in normal individuals (left), and in patients with moderate or massive splenomegaly (right).

Causes of thrombocytopenia

4. *Dilutional*

- Massive blood transfusions can produce dilutional thrombocytopenia.
- Mild dilutional thrombocytopenia also occurs frequently during the third trimester of pregnancy.



Idiopathic (Immune) Thrombocytopenic Purpura (ITP)

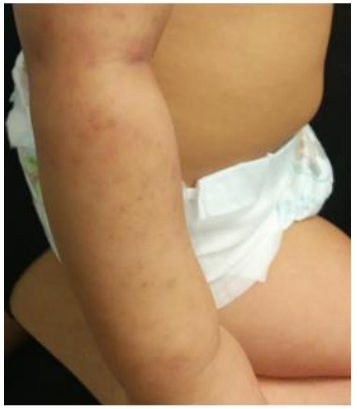
- ITP is the most common form of thrombocytopenia as well as common form of immune thrombocytopenia.

- In ITP, there is **increased destruction of platelets** by immune mechanisms and mainly by autoimmune mechanism, hence also referred to as **autoimmune thrombocytopenia.**

- There are two major subtypes of primary ITP, acute and chronic; both are autoimmune disorders in which platelet destruction results from the formation of antiplatelet autoantibodies.

Acute immune thrombocytopenic purpura

- A self-limited disease > 90% remission rate
- Most common in children 2 to 4 years and occurs with equal frequency in both sexes.
- Presents 1 to 3 weeks after viral (measles, rubella, EBV) infection.
- Platelet destruction is caused by antiplatelet autoantibodies.
- IgM type of antibodies that combine with platelets and result in destruction of platelets in the spleen.
- Platelet count is decreased, sometimes even below 10,000/cu mm (10×10^9/l).
- Supportive treatment
- Steroids are not helpful



Chronic immune thrombocytopenic purpura

- Adult disease primarily
- is more common in females (F:M ratio is 3:1) between 20 and 40 yrs.
- Insidious onset with no prodrome
- persistent thrombocytopenia lasting more than 6 to 12 months.
- Symptoms include: easy bruising, prolonged menses, mucosal bleeding
- Splenomegaly and lymphadenopathy are uncommon in primary ITP
- Bleeding complications are unpredictable
- Mortality is 1%
- Spontaneous remission is rare

Pathogenesis

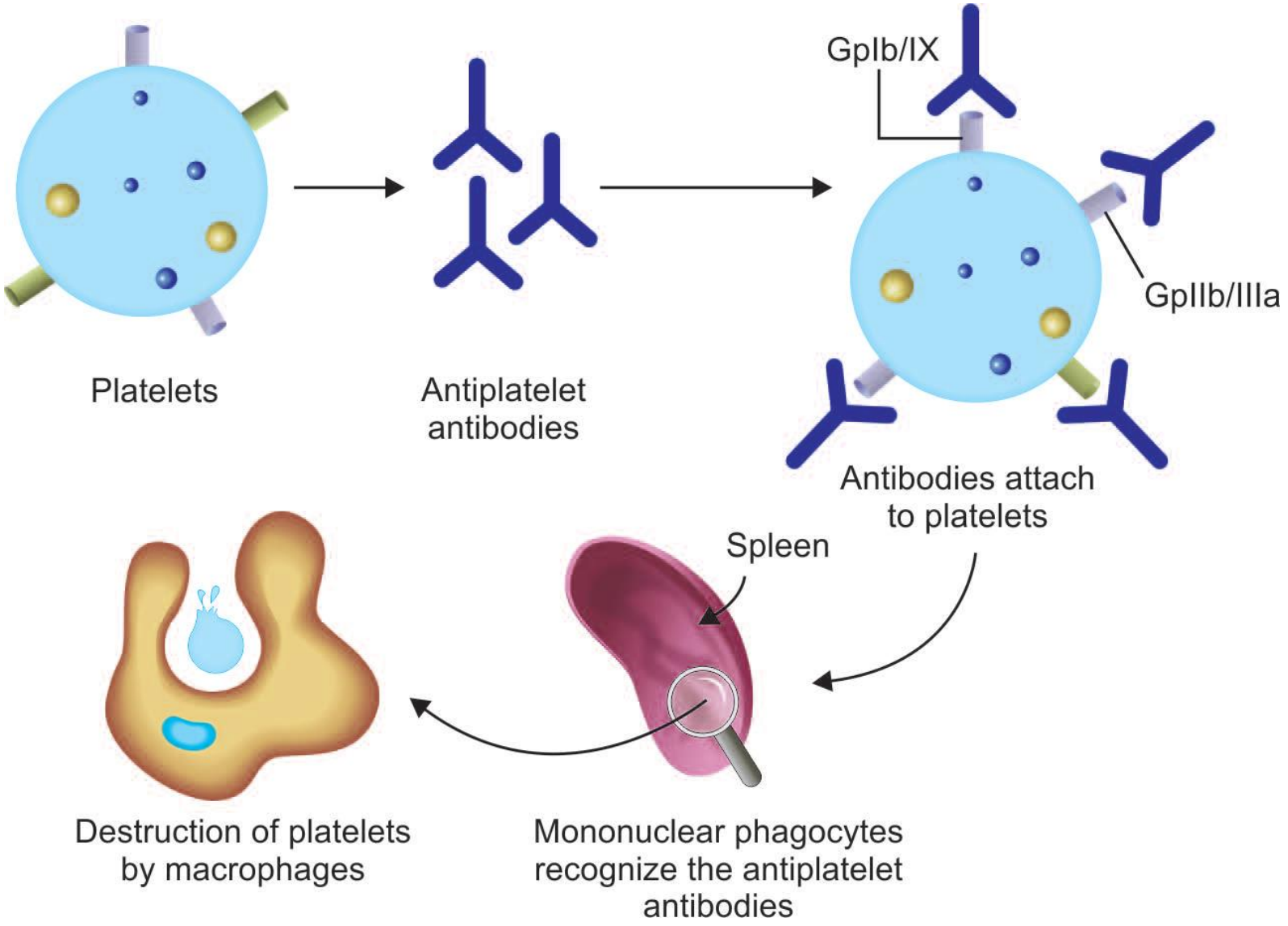
- Chronic ITP is an **autoimmune disorder** with formation of **antiplatelet antibodies**, directed against membrane glycoproteins most often IIb-IIIa or Ib-IX of platelets.

- The antiplatelet antibodies can be demonstrated in approximately 80% of patients and are of the IgG type.

- The mechanism of platelet destruction is similar to that seen in autoimmune hemolytic anemias. The antiplatelet antibodies act as opsonins and are recognized by IgG Fc receptors present on mononuclear phagocytes of RE system (mainly spleen) and are destroyed there resulting in thrombocytopenia.

- **Spleen** is not only the **major site of destruction** of platelets but also important site of autoantibody synthesis. Thus, splenectomy shows marked improvement in about 75 to 80% of patients.

Pathogenesis of chronic ITP



Chronic ITP

- Hospitalization common because of a complex differential diagnosis
- Multiple treatments
- Platelet transfusions are used only for life threatening bleeding
- Life threatening bleeding is treated with IV Immune globulin (1g/kg)

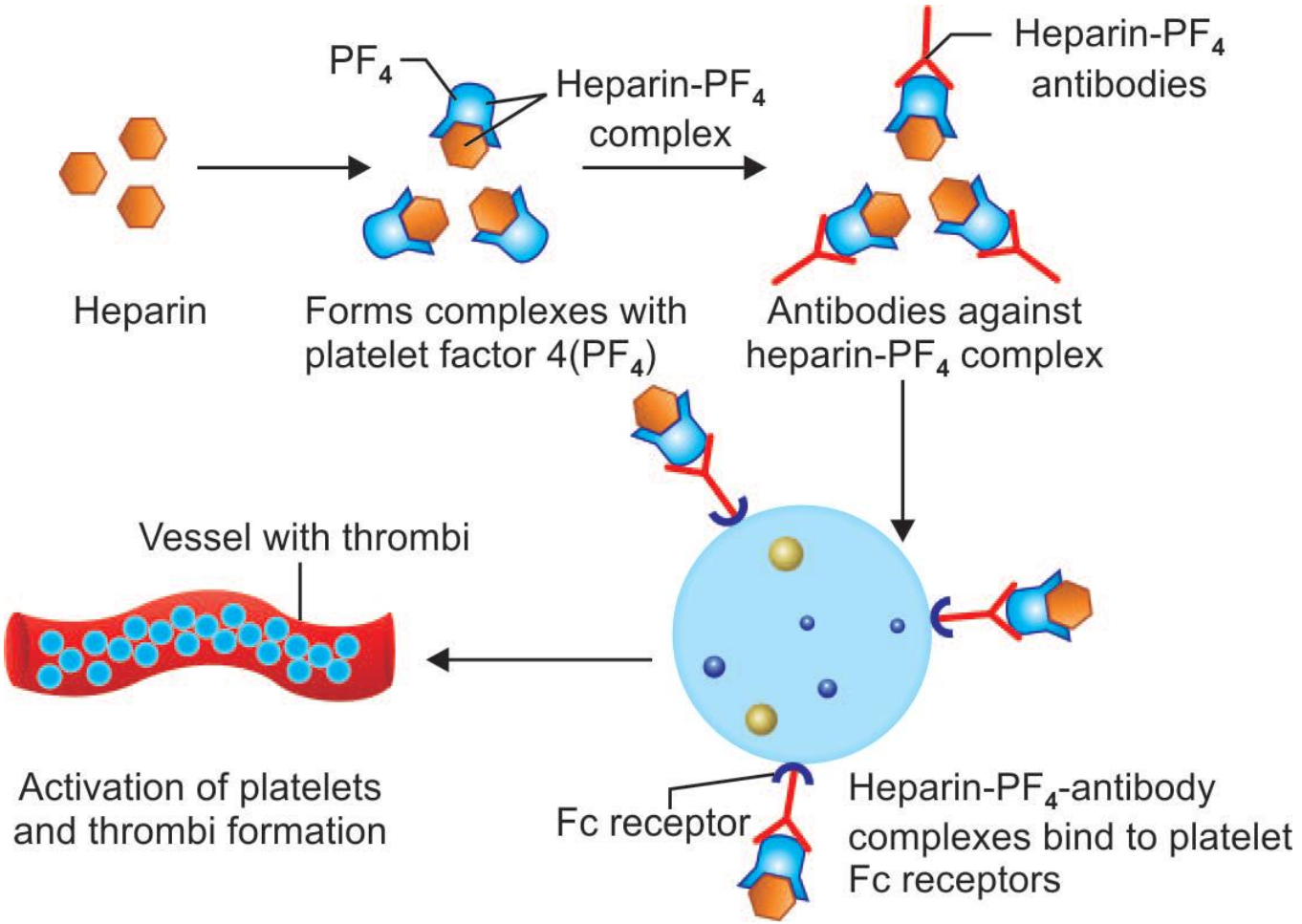
Heparin-induced thrombocytopenia (HIT)

- Heparin is the widely used anticoagulant for prevention or treatment of thrombosis.
- Thrombocytopenia occurs in about 10% of patients receiving heparin and it is important to diagnose this entity to prevent its fatal consequences.

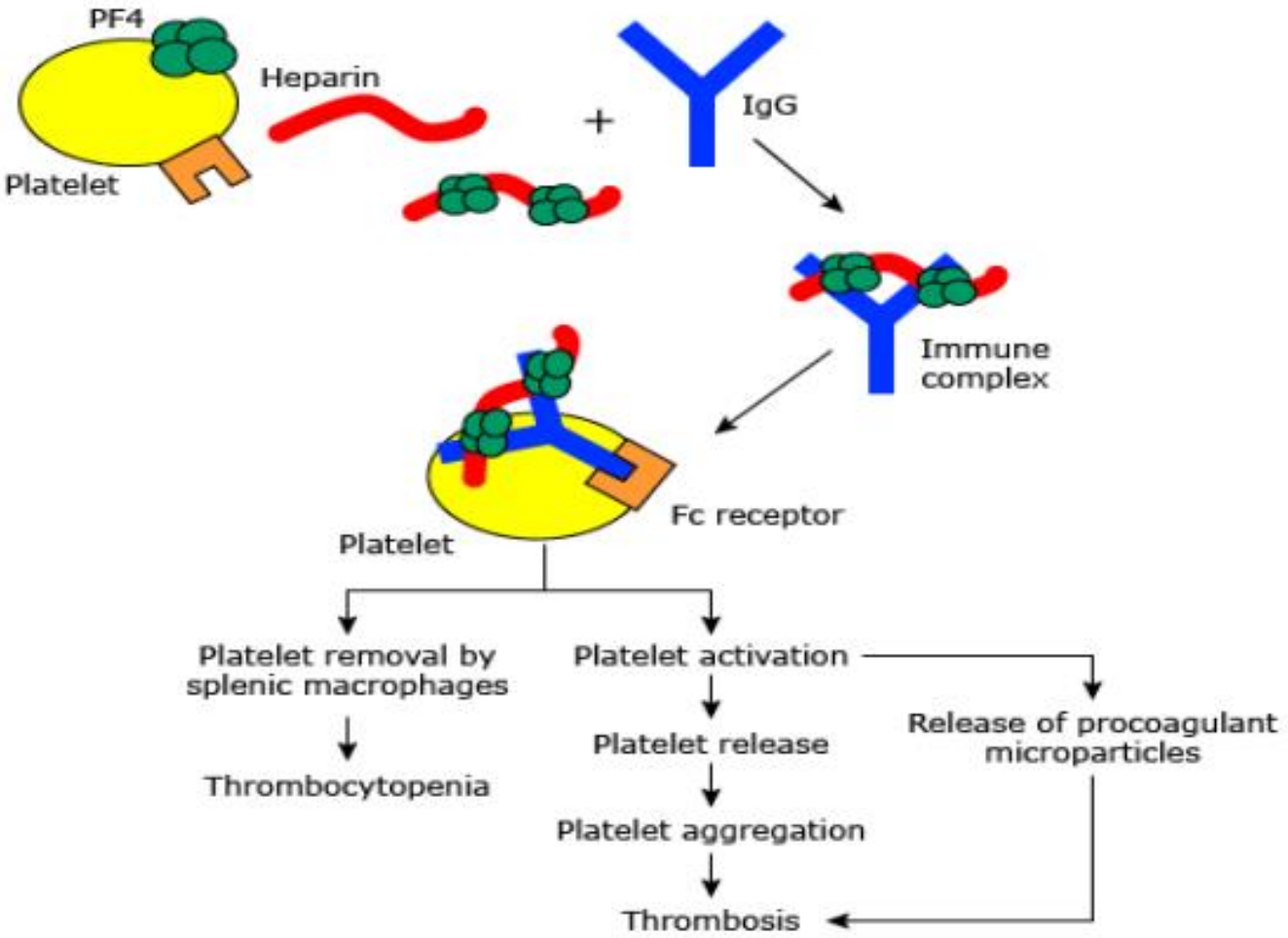
Heparin can cause two clinically distinct types of syndromes:

- **Type I:** Thrombocytopenia develops immediately following heparin therapy and is probably due to direct platelet-aggregation induced by heparin.
- **Type II:** It is associated with severe thrombocytopenia and usually develops 1 to 2 weeks after starting heparin therapy (or earlier if the patient has been previously sensitized to heparin). Surprisingly, thrombocytopenia is associated with life-threatening thrombosis in both veins and arteries, known as "white clot syndrome".

Pathogenesis of heparin induced thrombocytopenia



HIT


PF4
Heparin
Platelet
+
IgG
Immune complex
Fc receptor
Platelet
Platelet removal by splenic macrophages
Thrombocytopenia
Platelet activation
Platelet release
Platelet aggregation
Thrombosis
Release of procoagulant microparticles

HIV-associated thrombocytopenia

- Like T cells, megakaryocytes also have CD4 and CXCR4, the receptor and coreceptor respectively, for HIV and are thus liable for infection by HIV. Infected megakaryocytes have **impaired platelet production** and are also susceptible to apoptosis.

- HIV infection also stimulates B lymphocytes to produce immunoglobulins, including autoantibodies, which predispose to the **immune-mediated destruction** by the mononuclear phagocyte system.

Non-immune Thrombocytopenia: Thrombotic Microangiopathies

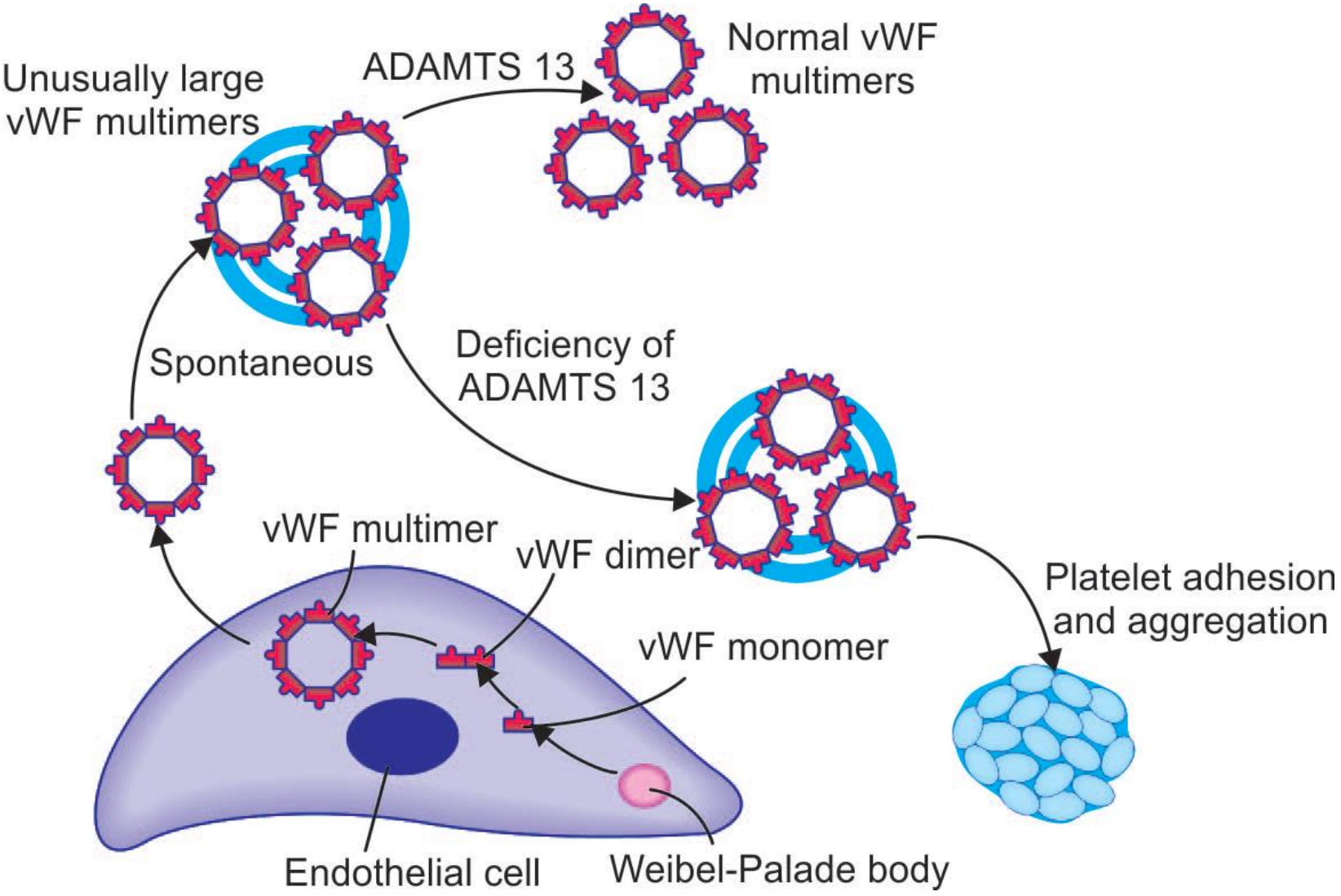
Thrombotic Thrombocytopenic Purpura (TTP)

- The classic five symptoms of TTP are:
  - Microangiopathic hemolytic anemia (MAHA),
  - Thrombocytopenia: more severe in TTP,
  - transient neurologic symptoms,
  - fever and
  - renal failure.
- More common in adults

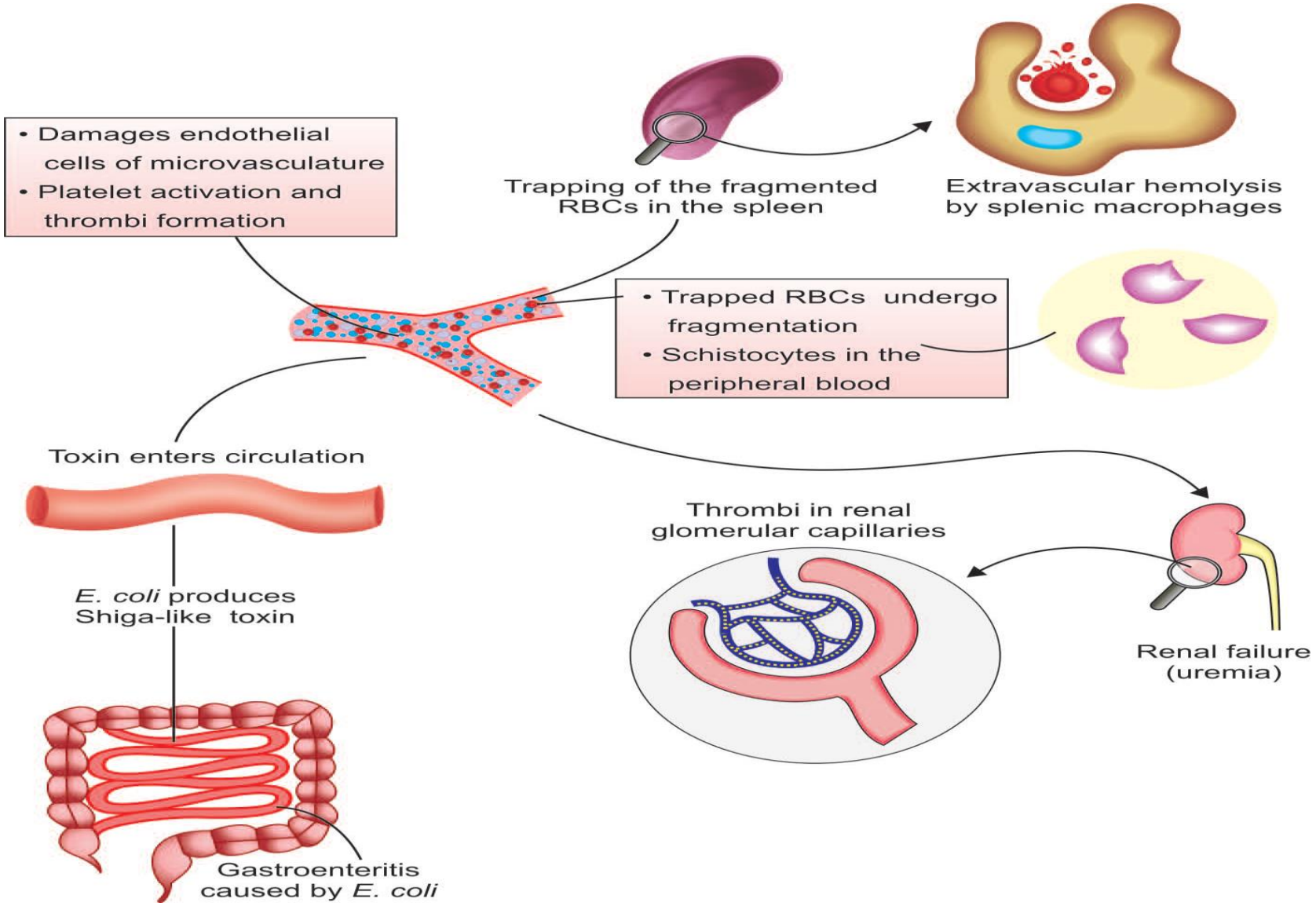
Hemolytic-Uremic Syndrome (HUS)

- HUS is distinguished from TTP by:
  - the absence of fever and neurologic symptoms,
  - the prominence of acute renal failure (uremia) include Renal insufficiency, azotemia, proteinuria, hematuria.
- primarily affects children and different pathogenesis.

Pathogenesis of TTP



Pathogenesis of HUS



Laboratory diagnosis of thrombotic microangiopathies

Peripheral blood

- **Hemoglobin:** Decreased usually less than 6 g/dL.
- **Platelet count:** Markedly reduced often below 20,000/cu mm (20×10^9/L).

Peripheral smear

RBCs: Show fragmented red cells (schistocytes), polychromatophils, nucleated RBCs and microspherocytes.

WBCs: Show mild leukocytosis with a shift to left.

Platelets: Markedly reduced.

Reticulocyte count: Increased.

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are usually normal, because the coagulation system is not activated.

Urine: Shows moderate proteinuria and both gross and microscopic hematuria.

Laboratory diagnosis of thrombotic microangiopathies

Morphology

- **Blood vessels:** Characteristic feature is the deposition of **PAS-positive platelet-fibrin microthrombi in arterioles and capillaries** throughout the body. It is mainly seen in the heart, brain and kidneys.

- Both DIC and thrombotic microangiopathies have common features like microvascular occlusion and microangiopathic hemolytic anemia, but differ in their pathogenesis.

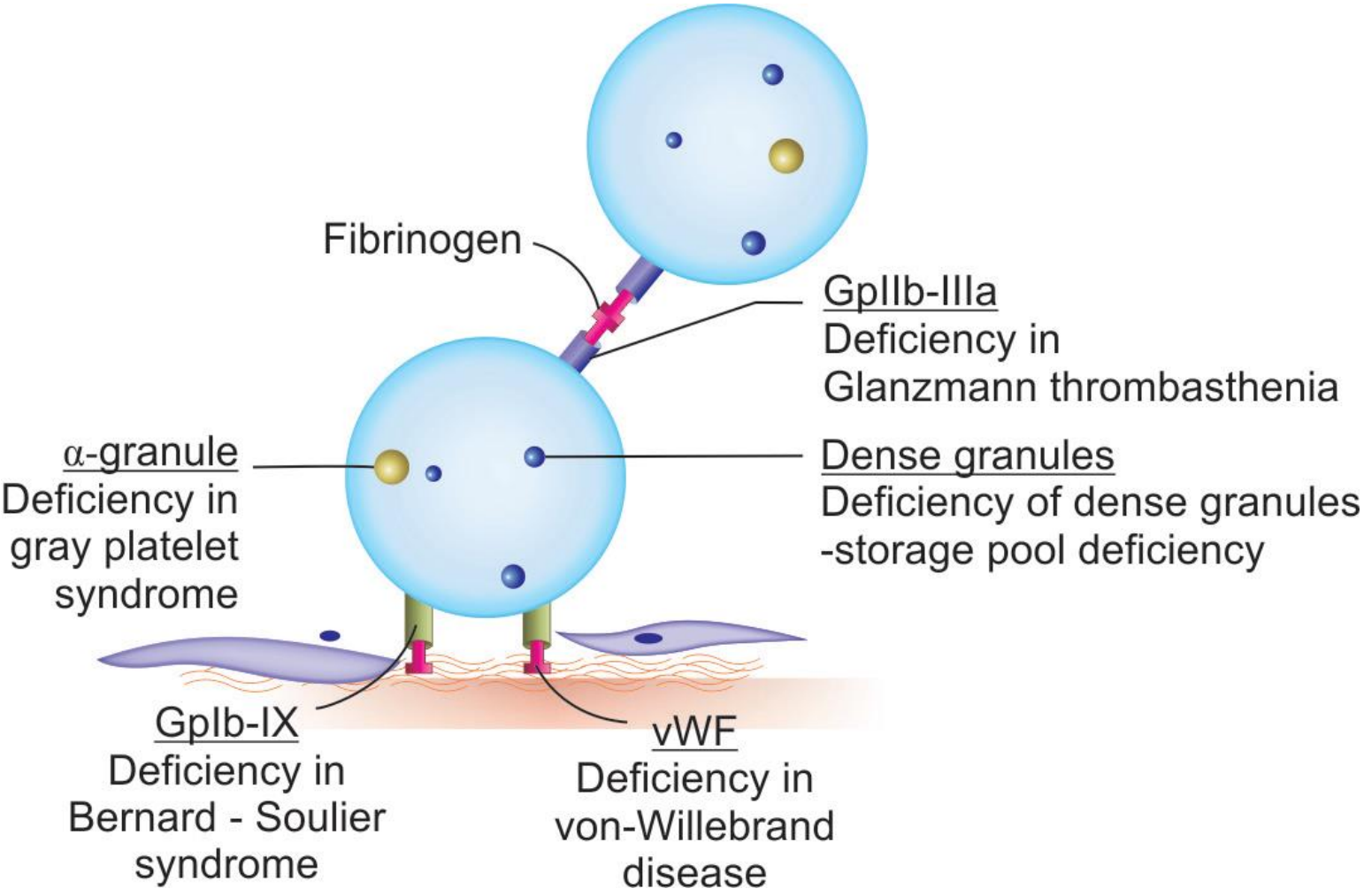
- **In TTP and HUS** (unlike DIC), activation of the coagulation cascade is not a primary event, and hence **PT** and **PTT (test for coagulation)** are usually **normal**.

Qualitative platelet disorders

- Manifestations include:
  - Petechiae
  - Easy and spontaneous bleeding from mucous membranes
  - Prolonged bleeding from trauma

- Lab diagnosis:
  - Plt count normal
  - Prolonged bleeding time
  - PT, PTT, fibrinolysis tests are normal
  - Platelet aggregation studies variable.

Classification of platelet functional (qualitative) disorders



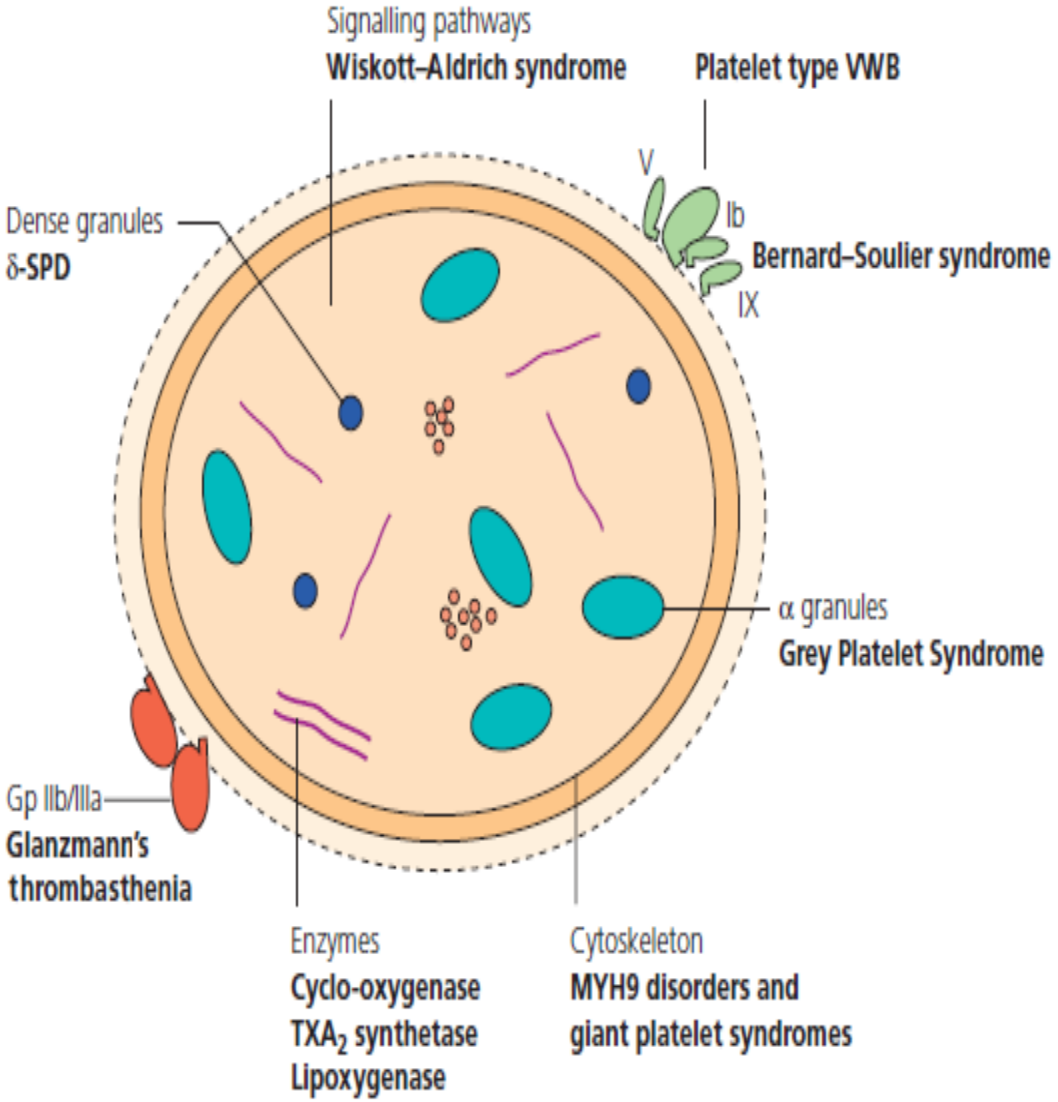
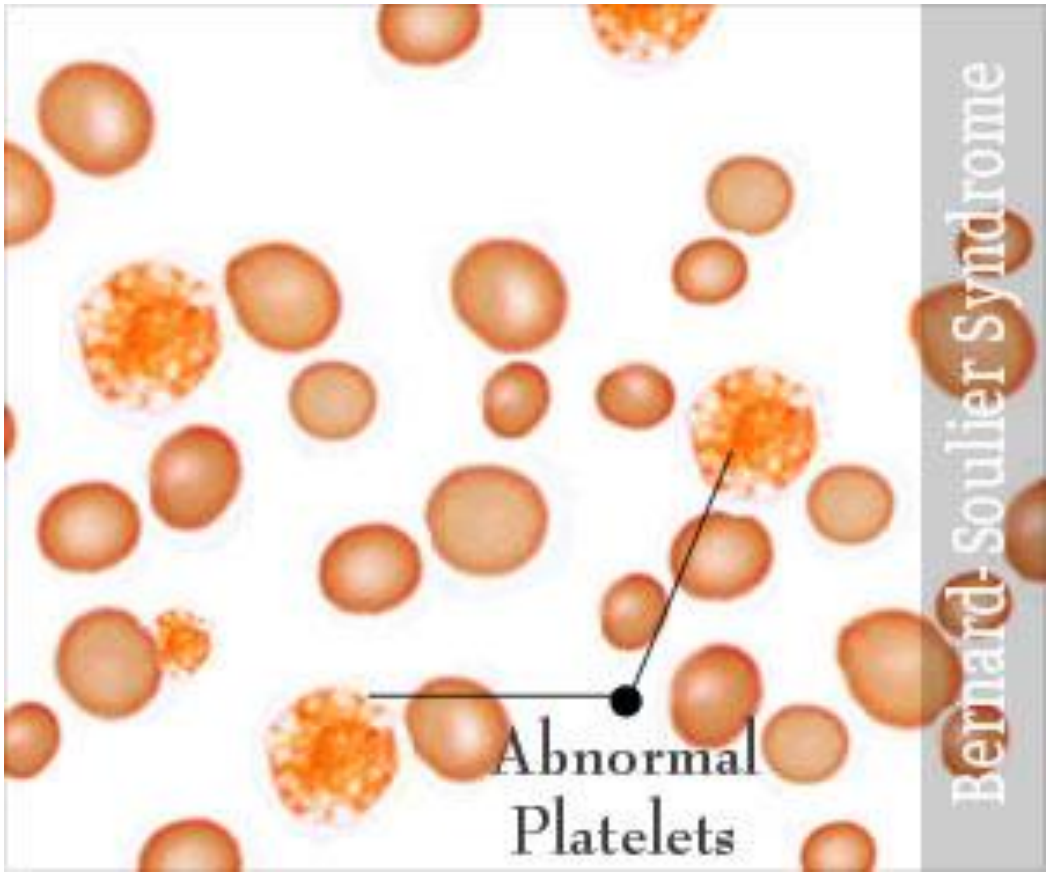


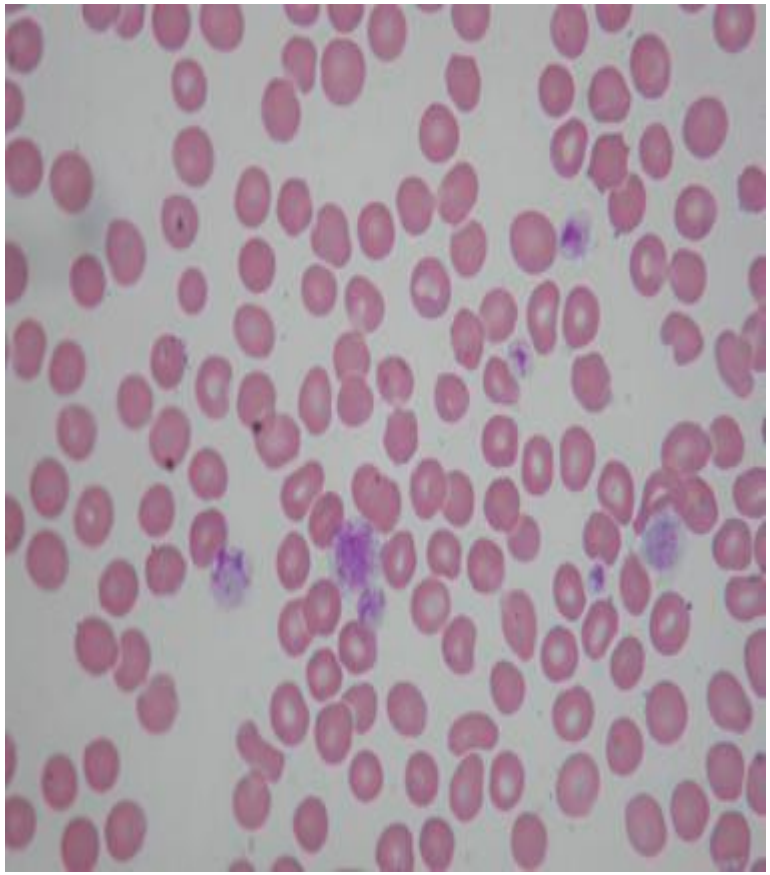
Figure 7.4 Site of abnormality in congenital platelet disorders.

Defected Adhesion: Bernard-Soulier Syndrome (BSS)

- autosomal recessive
- causes a deficiency of glycoprotein Ib (GpIb), the receptor for von Willebrand factor
- Lack of the complex prevents interaction of the PLTs with vWF and prevents subsequent adhesion to collagen.
- BSS is a giant platelet disorder, meaning that it is characterized by abnormally large platelets
- Thrombocytopenia: The degree of thrombocytopenia may be estimated incorrectly, due to the possibility that when the platelet count is performed with automatic counters, giant platelets may reach the size of red blood cells.
- Normal von Willebrand factor
- Normal aggregation with all aggregants EXCEPT Ristocetin.

Bernard-Soulier Syndrome (BSS)





Diagnosis

- Platelet aggregation studies show normal aggregation in response to all agonists except ristocetin (opposite pattern than thrombasthenia)

- Flow cytometry: decreased expression of mAbs to CD42b (GPIb), CD42a (GPIX), CD42d(GPV)

vWF

- Will be discussed with secondary hemostasis defects.

Aggregation defects: Glanzmann's Thrombasthenia

- Eduard Glanzmann (1887-1959), Swiss pediatrician
- Reported a case of a bleeding disorder starting immediately after birth.
- IIbIIIa most abundant platelet surface receptor (80,000 per platelet)
- IIbIIIa complex is a Ca++ dependent heterodimer
- Genes for both subunits are found on Chromosome 17
- Disease is caused by mutations (substitution, insertion, deletion, splicing abnormalities) in genes encoding for IIb or IIIa resulting in qualitative or quantitative abnormalities of the proteins

Glanzmann's Thrombasthenia

- Fundamental defect of thrombasthenic patients is the inability of the platelets to aggregate.
- Other problems: platelets do not spread normally on the subendothelial matrix (due to lack of IIbIIIa - vWF/fibronectin interaction)
- Also, alpha granule fibrinogen is decreased to absent

Diagnosis

- Platelet count and morphology are normal
- Bleeding time prolonged
- The hallmark of the disease is severely reduced or absent platelet aggregation in response to multiple agonists ie ADP, thrombin, or collagen (except Ristocetin)
- Flow cytometry: decreased mAb expression of CD41 (GPIIb) and CD61 (GPIIIa)

Storage Pool Defects

- Classified by type of granular deficiency or secretion defect.
- Dense body deficiency, alpha granule deficiency (gray platelet syndrome), mixed deficiency.
- Deficiencies in either the α or dense granules cause poor secondary platelet aggregation.
- Absence of α granules in Grey Platelet Syndrome, an autosomal dominant inherited condition, results in large, pale platelets on blood films

Qualitative disorders (Acquired)

- Uremia:
  - Due to toxin affect on the platelets
- Drugs:
  - Aspirin: prevents the release of TxA2, those platelets affected by aspirin still circulate but are nonfunctional.
  - Antibiotics: penicillins & cephalosporins. Drug coats the platelet membrane blocking ADP and epinephrine receptors, so platelet can not respond to agonist.
- Alcohol: mechanism unclear
- Hematological disorders:
  - Myeloproliferatine disorders, acute leukemias, myelodysplasia, multiple myeloma and macroglobulinemia. platelet dysfunction is due to intrinsic platelet defects.

Next Lecture:
Bleeding Disorders part 2

Lecture 9: Bleeding Disorders: Due to Abnormalities of Coagulation Factor

Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Secondary hemostasis defects

- Symptoms:
  - Deep bleeding
  - Joint bleeding
  - Muscle bleeding
  - Hematomas are common
  - Ecchymosis massive
  - Excessive bleeding from traumatic injury
  - Petechaie are not seen

Screening tests in Secondary hemostasis defects

- PT is prolonged (extrinsic pathway)
- APTT is prolonged (intrinsic pathway)
- Or both are prolonged
- Plts are normal in count and function
- Thrombin time is prolonged in disorders of fibrinogen
- If any test is abnormal of these screening tests, additional testing may resolve the disorder.

Additional testing

- Specific factor assays
- Fibrinogen level
- D- Dimer
- FDPs
- Antithrombin level

Table 24.3 Screening tests used in the diagnosis of coagulation disorders.

Screening tests	Abnormalities indicated by prolongation	Most common cause of coagulation disorder
Thrombin time (TT)	Deficiency or abnormality of fibrinogen or inhibition of thrombin by heparin or FDPs	DIC Heparin therapy
Prothrombin time (PT)	Deficiency or inhibition of one or more of the following coagulation factors: VII, X, V, II, fibrinogen	Liver disease Warfarin therapy DIC
Activated partial thromboplastin time (APTT or PTTK)	Deficiency or inhibition of one or more of the following coagulation factors: XII, XI, IX (Christmas disease), VIII (haemophilia), X, V, II, fibrinogen	Haemophilia, Christmas disease (+ conditions above)
Fibrinogen quantitation	Fibrinogen deficiency	DIC, liver disease

DIC, disseminated intravascular coagulation; FDPs, fibrin degradation products.
NB. Platelet count and the tests of platelet function are also used in screening patients with a bleeding disorder (p. 328).

Hereditary Type

- The genetic defect can either be the failure of synthesis of one of the proteins or the production of a malfunctioning or abnormal molecule.
  - Quantitative vs Qualitative
- But in both of these genetic defects they will result in slowing down and ineffective production of fibrin.

Types of Bleeding Disorders

- Hemophilia A (factor VIII deficiency)
- Hemophilia B (factor IX deficiency)
- von Willebrand Disease (vWD)
- Other

Hemophilia

- Hemophilia A and B are similar in both clinical and pathological features, the difference being in the deficient factor.
- Both are sex-linked recessive disorders resulting in inherited deficiency of the clotting factor or synthesis of a defective clotting factor.
- Males are affected and females are carriers.

Hemophilia Prevalence

- Hemophilia A; 1 in 5000 population
- – coagulation factor VIII deficiency

- Hemophilia B; 1 in 30000 population
- – coagulation factor IX deficiency

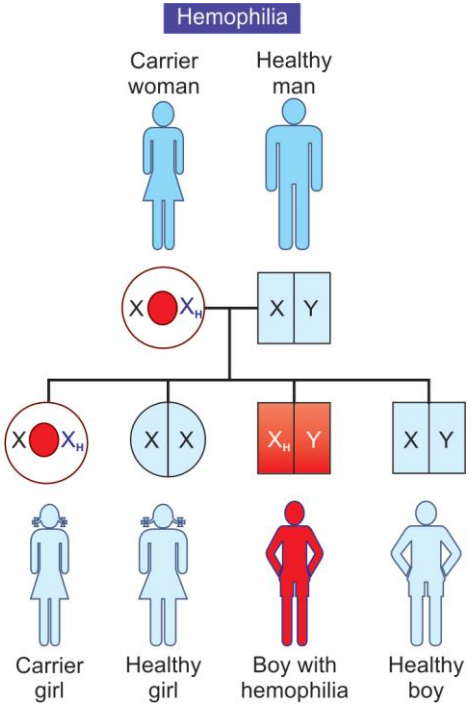
- Hemophilia A is six-fold more prevalent than hemophilia B.

HEMOPHILIA A (FACTOR VIII DEFICIENCY)

- Common hereditary X-linked recessive disease.
- About 30% of hemophilic may be due to acquired mutations.
- Reduced amount or activity of factor VIII is associated with life-threatening bleeding.
- Bleeding is due to both inadequate coagulation and inappropriate clot removal (fibrinolysis).

Mode of Inheritance

- X-linked recessive disease. Genes for factor VIII are located on the long arm of the X-chromosome.
- Does not manifest when there is a normal copy of X-chromosome.
- Males with a defective/mutant factor VIII gene (hemophiliac gene) on their single X chromosome (XH) suffer from hemophilia.
- Heterozygous females are carriers and do not express the full clinical disease because of the paired normal X-chromosome.
- However, females with two copies of the defective XH chromosome may rarely suffer from hemophilia.



Molecular Genetics

- Causative mutations include deletions, inversions, point mutations and insertions.

Clinical Features

- Clinical severity depends on the level of factor VIII activity with normal range expressed as percentage.

- Normal factor VIII or IX level = 50-150%

  - Mild hemophilia
    factor VIII or IX level = 6-50%
  - Moderate hemophilia
    factor VIII or IX level = 1-5%
  - Severe hemophilia
    factor VIII or IX level = <1%

Clinical manifestations

Common clinical presentations include:

- Frequent and spontaneous hemorrhage into the joints-hemarthrosis.
- Hemorrhage into soft tissues.
- Prolonged bleeding following trauma

Laboratory Findings of hemophilia A

- Bleeding time: normal
- Clotting time: prolonged, but is not a sensitive test
- Platelet count: normal
- Prothrombin time: normal
- Activated partial thromboplastin time (APTT): increased (normal 30–40 seconds)
- Factor VIII assay: essential for the diagnosis and to assess the levels and severity of disease
- Fibrinogen assay: normal
- FDP: negative
- Detection of carriers: by DNA markers
  - To detect female carriers
  - Prenatal diagnosis of affected fetuses.

Complications

Due to Hemophilia

- Deforming arthritis and contractures: this is due to repeated bleeding into the joints.
- Anemia: excessive, spontaneous or repeated bleeding leads to anemia.

Causes of death

- Intracranial hemorrhage
- Prolonged bleeding.

Treatment of Hemophilia

- Factor VIII concentrates
- FFP
- Cryoprecipitate
- DDAVP (Desmopressin): release of stores from endothelial cells raising factor VIII and vWD serum levels
- Tranexamic acid (Amicar, antifibrionlytic)
- Gene therapy: insertion of Factor VIII gene

Complications due to treatment

- Viral hepatitis: hepatitis B, C and D in patients who received multiple transfusions of FFP/cryoprecipitate.

- AIDS: in individuals who received fresh frozen plasma (FFP) or cryoprecipitate, when screening tests for HIV were not available.

- Factor VIII inhibitors: makes further management difficult due to alloantibodies, upon mixing studies does not normalize.

Acquired haemophilia

- ✓ Circulating antibodies against coagulation factors :
- ✓ Causes :
  1. Treatment of congenital Factor defeciency by factors may leads to stimulate antibodies against this drugs (alloantibody)
  2. Autoimmune process in haemostatic normal person stimulate Ab against specific factor.

  Causes :
  - Autoimmune disease
  - SLE
  - Rheumatoid arthritis
  - Pregnancy
  - Malignancy as lymphprolifratve disorders,.
  - Medications as penicillin, sulph,....
  - Dermatological disorders as psoriasis,..
  - Idiopathic common
- ✓ diagnosis :
- Prolonged PT or PTT without correction by mixing with normal plasma 50:50 mix and After 2 hours Incubation at 37 C.
- Prolonged PT or PTT after correction diagnosis of Antibodies against coagulation factor
- Most common inhibitors occurs to factor VIII, V and vWF

HEMOPHILIA B (CHRISTMAS DISEASE, FACTOR IX DEFICIENCY)

- Clinically indistinguishable from hemophilia A
- X-linked recessive disorder
- Variable clinical severity
- Assay of factor IX should be done to diagnose Christmas disease (named after the first patient).

Laboratory Findings

- Bleeding time: normal
- Clotting time: prolonged
- Platelet count: normal
- Prothrombin time: normal
- Activated partial thromboplastin time prolonged
- APTT is corrected by mixing studies
- Specific factor assay

Factor XI deficiency (hemophilia C)

- Autosomal recessive
- Mild to moderate bleeding symptoms
- Bleeding tendency is not as great as in factor VIII or factor IX deficiency.

Other Hereditary Coagulation Disorders

- The other coagulation disorders namely afibrinogenemia, dysfibrinogenemia and deficiency of factors like XIII, X and VII are rare.

Von willebrand disease (VWD)

Von willebrand disease (VWD)

- Is the most common inherited bleeding disorder known in human, Estimated that 1% of the population has vWD.
- Autosomal inheritance pattern
  - Males and females are affected equally
- Caused by a deficiency or an abnormality of von Willebrand Factor.
- It is synthesized and secreted mainly from the endothelial cells, also from the megakaryocytes. VWF is also stored in the a granules of platelets but this is not contribute to the plasma VWF concentration it is released when platelets are activated.

vW Factor Functions in Hemostasis

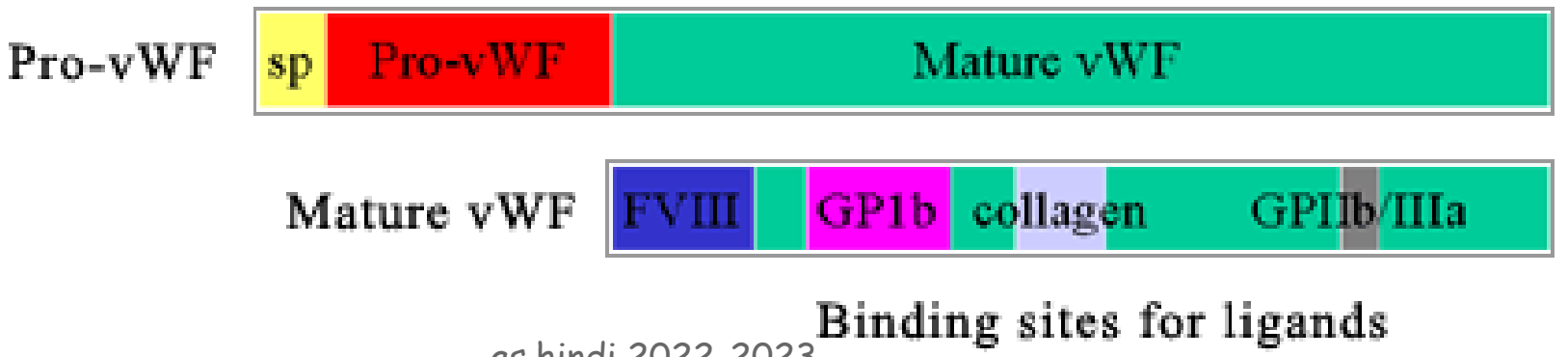
- Carrier protein for Factor VIII (FVIII)
  - Protects FVIII from proteolytic degradation
  - Localizes FVIII to the site of vascular injury
  - If VWF absent, then the half life of VIII is reduced.

- VWF plays a role in platelet adhesion and aggregation, so when it is absent or deficient then the platelet adhesion and aggregation is defected.

- So the defect is dual: primary and secondary hemostasis will be defected.

vWF

- VWF gene : short arm of chromosome 12p113
  - VWF gene is expressed in endothelial cells and megakaryocytes
- vWF is produced as a propeptide which is extensively modified to produce mature vWF
- VWF has a multistep synthesis after translation called posttranslational modification:
  - First: dimer formation
  - Second: tetramer formation
  - Then: multimer formation



vWF

- Weibel-Palade bodies are the storage granules of endothelial cells that store VWF.
- Secretion from these bodies can be activated by: histamine, epinephrine, DDAVP and acute phase reactions.
- So, VWF is an acute phase reactant, as also VIII is an acute phase reactant.

Diagnosis

- The diagnosis of VWD is based on the availability of normal VWF that must be:
  - Present in adequate amount
  - Has normal multimeric structure
  - Have intact functional domains (binding sites)

vWD Classification

- Disease is due to either a quantitative deficiency of vWF or to functional deficiencies of vWF
  - Due to vWF role as carrier protein for FVIII, inadequate amount of vWF or improperly functioning vWF can lead to a resultant decrease in the available amount of FVIII.

vWD Classification

- Type 1: Partial deficiency in VWF
- Type 2: Qualitative defect in VWF
- Type 3: complete deficiency in VWF

Type 1

- 60-80% of all vWD cases
- Partial quantitative deficiency
- Called classic VWD
- Most common type
- Autosomal dominant
- 1-2% of general population

Type 2 example: Type 2N

- Markedly decreased affinity of vWF for FVIII
- Results in FVIII levels reduced to usually around 5% of the reference range.
- Called Autosomal hemophilia
- FVIII binding region mutation
- Reduced plasma FVIII
- Platelet binding normal

vWD Type III

- Autosomal Recessive disorder
- vWF protein is virtually undetectable
  - Absence of vWF causes a secondary deficiency of FVIII and a subsequent severe combined defect in blood clotting and platelet adhesion
- Severe bleeding

Acquired vWD

- Mechanisms
  - Autoantibodies to vWF
  - Absorption of HMW vWF multimers to tumors and activated cells
  - Increased proteolysis of vWF
  - Defective synthesis and release of vWF from cellular compartments
- Most described in patients with clonal lymphoproliferative, autoimmune diseases, DIC, pancreatitis and tumors.

Table 26.3 Classification of von Willebrand disease.

Type 1	Quantitative partial deficiency
Type 2	Functional abnormality
Type 3	Complete deficiency

Secondary classification of type 2 VWD

Subtype	Platelet-associated function	Factor VIII binding capacity	High MW VWF multimers
2A	Decreased	Normal	Absent
2B	Increased affinity for GPIb	Normal	Usually reduced/absent
2M	Decreased	Normal	Normal
2N	Normal	Reduced	Normal

GPIb, glycoprotein Ib; MW, molecular weight; VWD, von Willebrand disease; VWF, von Willebrand factor.

VWF concentration by blood group

- In patients with VWD and O group you expect the disease to be more severe than other blood groups.

Clinical Manifestations

- Most cases are of mild bleeding
- Common symptoms
  - Spontaneous bleeding from mucous membranes (e.g. epistaxis)
  - Excessive bleeding from wounds or menorrhagia
- In severe cases, similar to hemophilia A.

Tests to diagnose VWF

- Platelet count: normal
- Bleeding time: prolonged
- Clotting time: prolonged
- Tourniquet test (Hess test): positive due to defect in platelet adhesion
- APTT: prolonged APTT
- PT: normal
- vWF assay: plasma level of active vWF is decreased
- Platelet function test: defective ristocetin induced platelet aggregation test is diagnostic of vWF.

vWD Treatment

- DDAVP
- Cryoprecipitate
- FVIII concentrate

Summary of laboratory tests in hereditary coagulation disorders

Table 26.2 Main clinical and laboratory findings in haemophilia A, factor IX deficiency (haemophilia B, Christmas disease) and von Willebrand disease.

	Haemophilia A	Factor IX deficiency	von Willebrand disease
Inheritance	Sex-linked	Sex-linked	Dominant (incomplete)
Main sites of haemorrhage	Muscle, joints, post-trauma or postoperative	Muscle, joints, post-trauma or postoperative	Mucous membranes, skin cuts, post-trauma or postoperative
Platelet count	Normal	Normal	Normal
PFA-100	Normal	Normal	Prolonged
Prothrombin time	Normal	Normal	Normal
Partial thromboplastin time	Prolonged	Prolonged	Prolonged or normal
Factor VIII	Low	Normal	May be moderately reduced
Factor IX	Normal	Low	Normal
VWF	Normal	Normal	Low or abnormal function (Table 26.3)
Ristocetin-induced platelet aggregation	Normal	Normal	Impaired

VWF, von Willebrand factor.

Acquired disorders

- Usually characterized by multiple clotting abnormalities
  1. Vitamin K deficiency: in neonates, low levels of vitamin K levels may produce life-threatening hemorrhage during the first week of life known as *hemorrhagic disease of the newborn.*

  2. Liver disease: liver synthesizes all the clotting factors and severe liver disease is associated with a hemorrhagic diathesis.

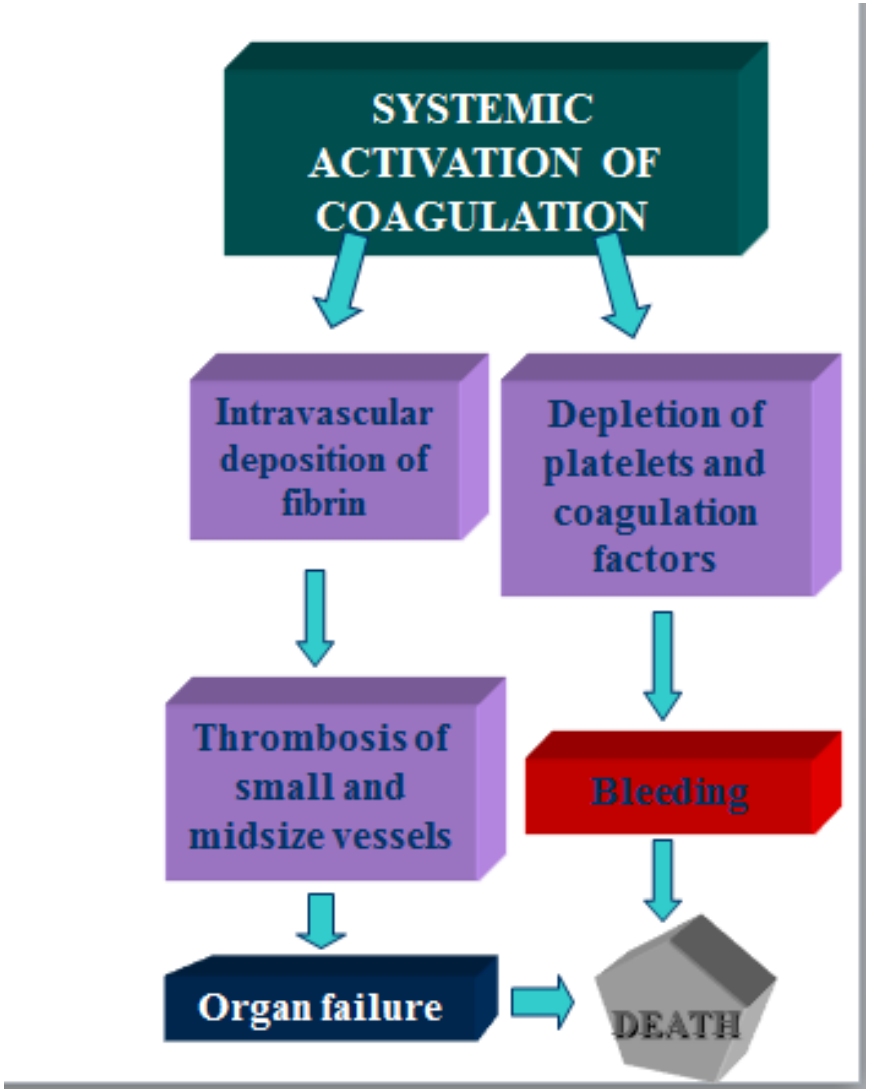
  3. Other causes: disseminated intravascular coagulation that involves deficiency of several coagulation factors.

Disseminated Intravascular Coagulation (DIC)

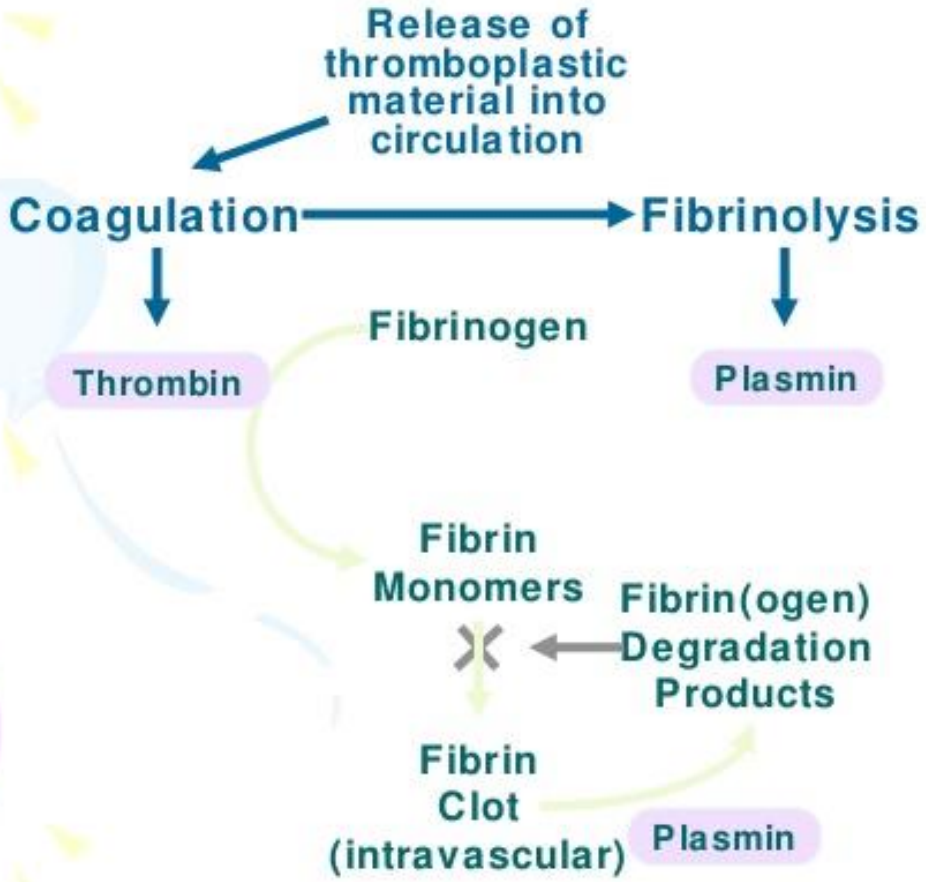
DIC

- Acquired bleeding disorder.
- Is not a disease entity but an event that can accompany various disease processes.
- Is characterized by the systemic activation of the coagulation system followed by activation of fibrinolytic system.
- This means there is high thrombin and plasmin generation.
- Coagulation/ clotting is always the initial event.
- As a result of the depletion of clotting factors, hemorrhage occurs simultaneously.

DIC mechanism



Pathogenesis of DIC



Consumption of coagulation factors; presence of FDPs
↑ aPTT
↑ PT
↑ TT
↓ Fibrinogen

Presence of plasmin
↑ FDP

Intravascular clot
↓ Platelets
Schistocytes

Pathophysiology of DIC

- Activation of Blood Coagulation
- Suppression of Physiologic Anticoagulant Pathways
- Impaired Fibrinolysis
- Cytokines

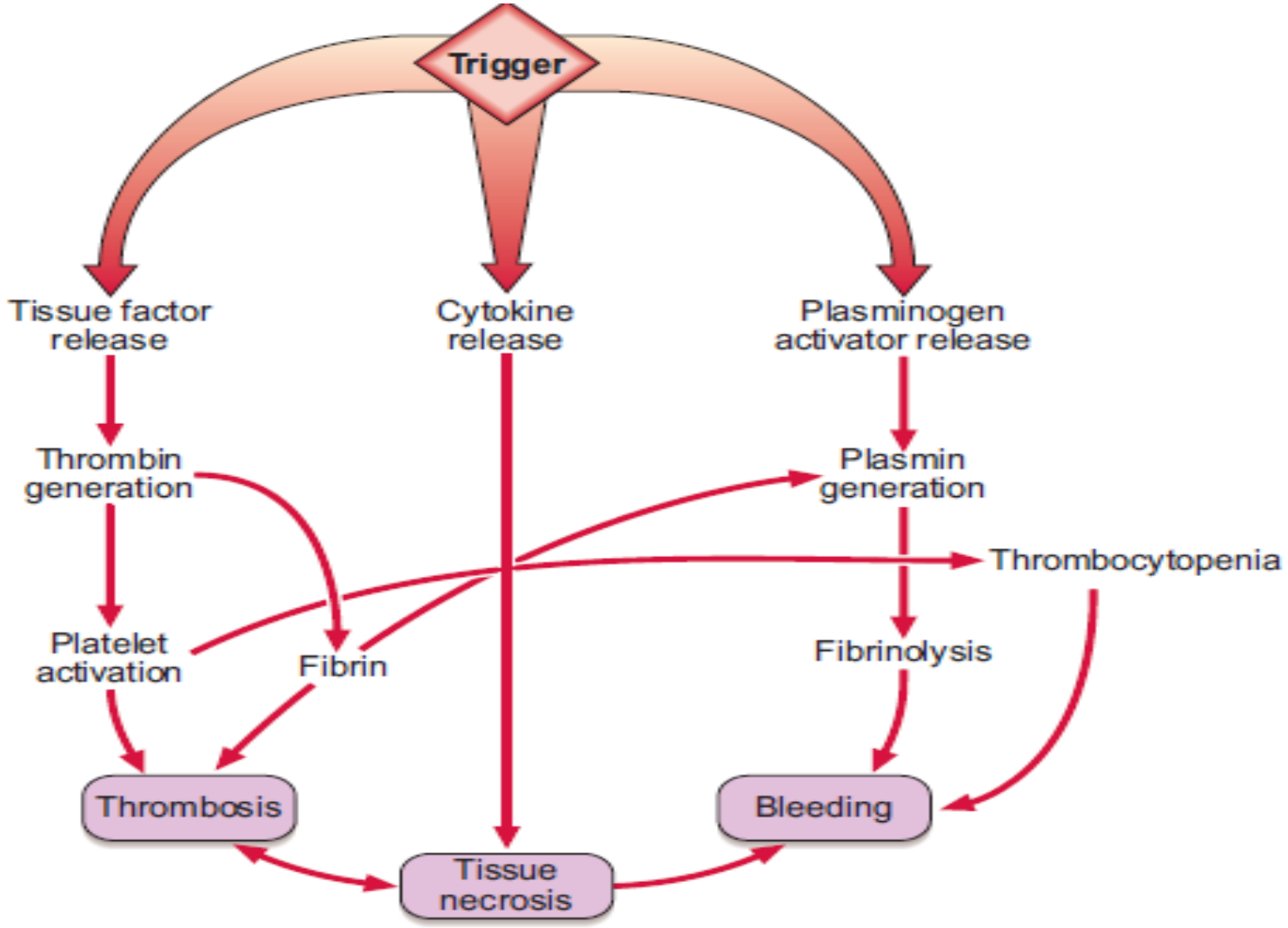


Fig 38.1 **Pathophysiology of DIC.** A simplification of the complex interactions.

Pathophysiology of DIC

- Activation of Blood Coagulation
  - Tissue factor/factor VIIa mediated thrombin generation via the extrinsic pathway
    - complex activates factor IX and X
  - TF
    - endothelial cells
    - monocytes
    - Extravascular:
      - lung
      - kidney
      - epithelial cells

Pathophysiology of DIC

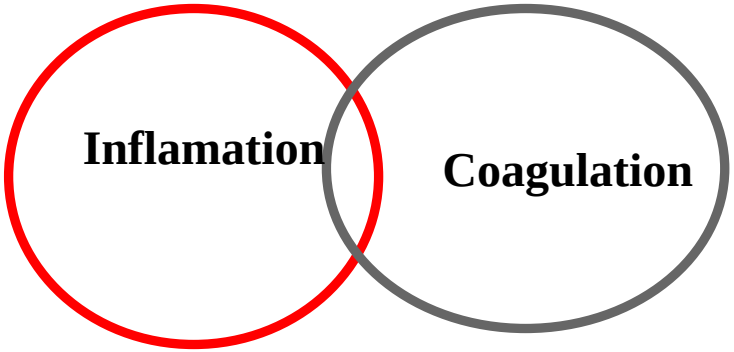
- Suppression of Physiologic Anticoagulant Pathways
  - reduced antithrombin III levels
  - reduced activity of the protein C-protein S system
  - Insufficient regulation of tissue factor activity by tissue factor pathway inhibitor (TFPI)
    - inhibits TF/FVIIa/Fxa complex activity

Pathophysiology of DIC

- Impaired Fibrinolysis
  - relatively suppressed at time of maximal activation of coagulation due to increased plasminogen activator inhibitor type 1

Pathophysiology of DIC - Cytokines

- Cytokines
  - IL-6, and IL-1 mediates coagulation activation in DIC
  - TNF-α
    - mediates dysregulation of physiologic anticoagulant pathways and fibrinolysis
    - modulates IL-6 activity
  - IL-10 may modulate the activation of coagulation



hemorrhagic/bleeding diathesis:

A. Causes of hemorrhagic/bleeding diathesis:

- Consumption of platelets
- Consumption of coagulation factors
- Activation of fibrinolytic system.

B. Mechanism of hemorrhagic diathesis fibrin-thrombi activate secondary fibrinolytic system and generate plasmin. The plasmin cleaves fibrinogen and fibrin and generates fibrin split products (FSPs) [or fibrin degradation products (FDP)]. FSPs are potent anticoagulant and antiplatelet effect and produces hemorrhagic diathesis.

Conditions Associated With DIC

- Infections and their endotoxins
- Abruption placenta
- Amniotic fluid embolism
- Dead fetus in utero
- Abortion
- Acute fatty liver of pregnancy
- Liver disease
- Metastatic cancer
- Snake venom
- Head trauma
- surgery

Clinical features

- Cerebral bleeding
- Petechiae
- Purpura
- Hematuria
- Oral mucous membrane bleeding
- GI bleeding

Diagnosis

Screening Assays

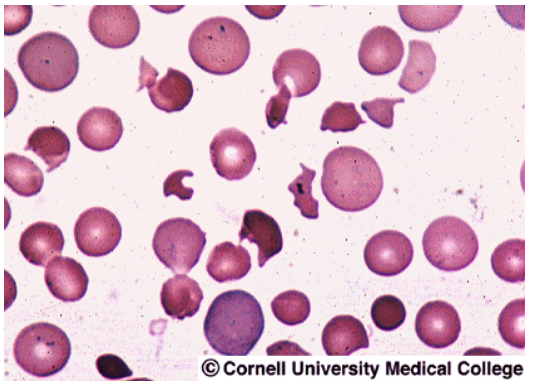
- APTT: increased as a result of consumption and inhibition of the function of clotting factors.
- Prothrombin time: increased.
- Thrombin time (TT): increased because of decreased fibrinogen.
- Fibrinogen: decreased.
- Bleeding time: increased due to decreased platelet count.
- Platelet count: decreased due to utilization of platelets in microthrombi.
- Peripheral smear: microangiopathic hemolytic anemia with schistocytes.

Confirmatory Tests

- FDP (fibrin degradation/split products): secondary fibrinolysis results in generation of FDPs, which can be measured by latex agglutination
- D-dimer test: it is specific for diagnosing DIC.

Microscopic findings in DIC
Micrangiopathic Hemolytic Anemia

- Fragments
- Schistocytes
- Paucity of platelets



Treatment

Prognosis

- Depends on the underlying disorder.
- Mortality is high in severe cases.

Treatment

- Removal of the underlying cause
- Replacement of clotting factors and platelets.

Next Lecture:
Thrombotic Disorders

Lecture 10: Thrombosis & Thrombophilia

Miss: Amany S. AlHindi

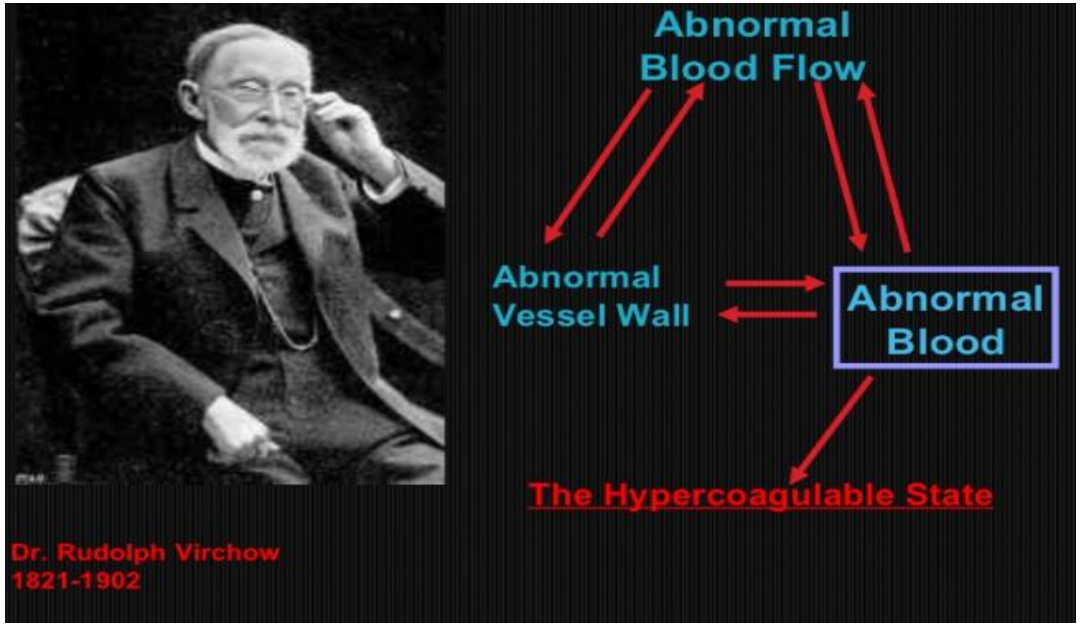
M.Sc. in Biological sciences from Islamic University of Gaza

Thrombophilia

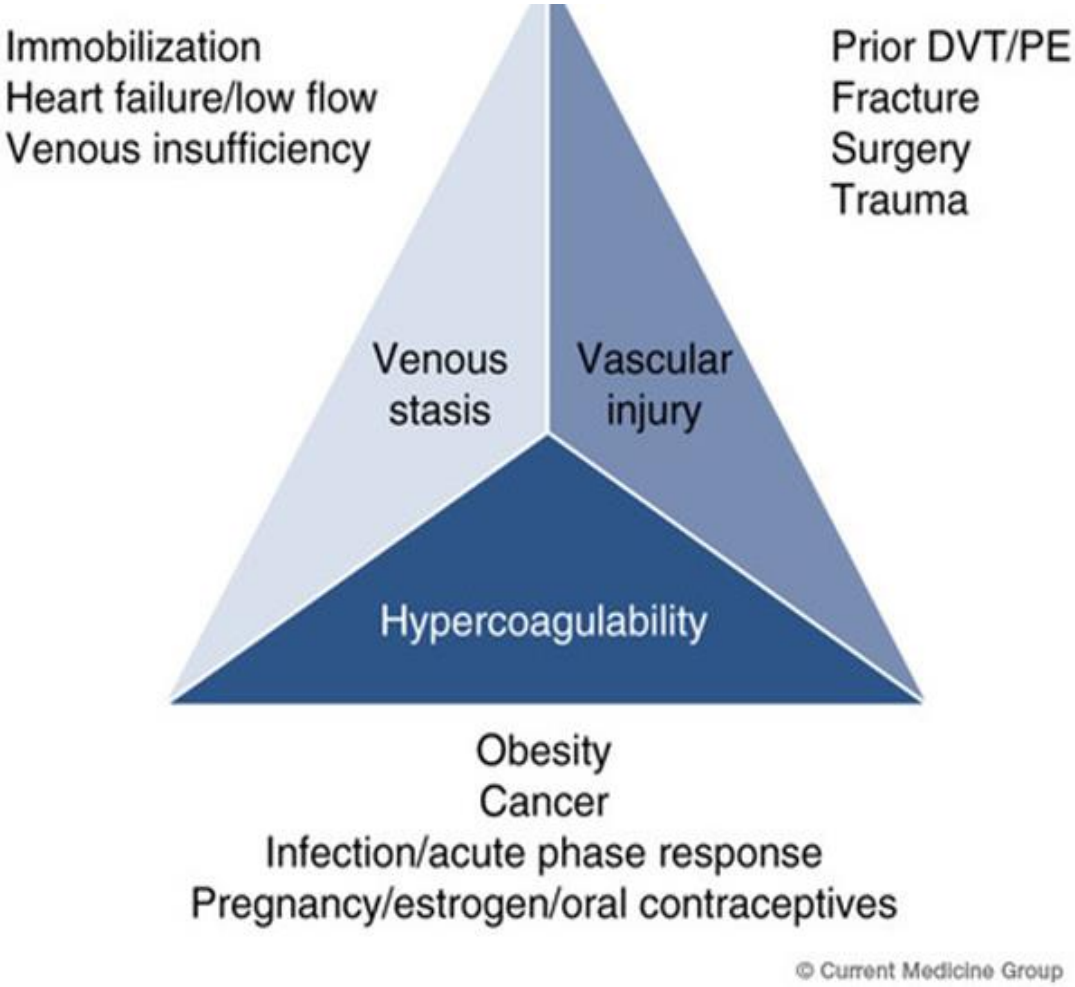
- Thrombophilia or hypercoagulability is the tendency to develop thrombosis (blood clots) due to an abnormality in the system of coagulation.

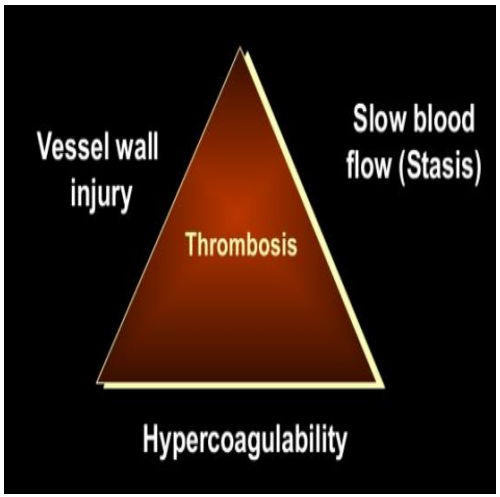
What influence thrombosis?

- Anormal Vessel wall- endothelial cells
- Abnormal blood flow
- Abnormal blood (Platelets Coagulation factors)

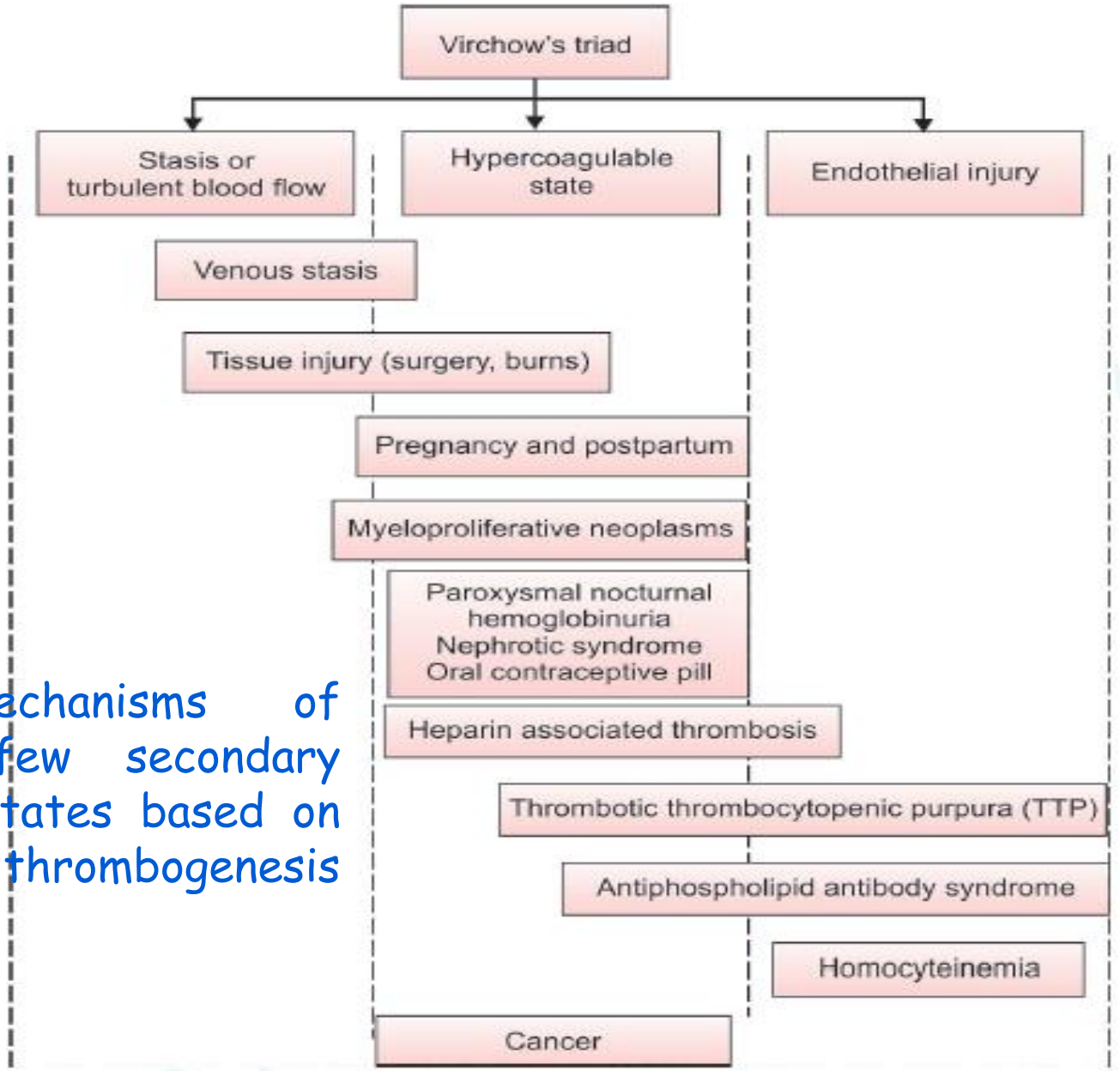


Virchow's thrombosis model





Predominant mechanisms of thrombosis in few secondary hypercoagulable states based on Virchow's triad of thrombogenesis



Risk factors of thrombosis

- Acquired
  - Advancing age
  - Prior Thrombosis
  - Immobilization
  - Major surgery
  - Malignancy
  - Estrogens
  - Antiphospholipid antibody syndrome
  - Myeloproliferative Disorders
  - Heparin-induced thrombocytopenia (HIT)
  - Prolonged air travel
- Mixed/ unknown
  - High levels of factor VIII
  - Acquired Protein C resistance in the absence of Factor V Leiden
  - High levels of Factor IX, XI

- Inherited
- Antithrombin deficiency
- Protein C deficiency
- Protein S deficiency
- Factor V Leiden mutation (Factor V-Arg506Gln)
- Prothrombin gene mutation (G A transition at position 20210)
- Dysfibrinogenemias (rare)

Where thrombi could be formed? What does it contain?

- Could be formed in:
  - Arteries
  - Veins
  - Heart
  - Microcirculation

- It contain fibrin, platelets and entrapped RBCs and WBCs.

- But the relative proportion of each thrombus is influenced by blood flow and shear forces, thus it differs in arteries from venous.

Thrombi vs Clot and Embolus

- Thrombi: it occurs intravascular (in vivo)
- Clot: occurs extravascular, in tissues (hematomas), or in vitro (in the lab).
- Embolus: piece of thrombotic material breaks off and travel through the circulatory system to a new site.

Types of Thrombus

1. Arterial thrombi (White thrombi)
   - High shear rates
   - Primarily platelet aggregates + minor fibrin strands
   - Thrombus associated with vascular abnormalities (atherosclerosis) most often
   - Arterial thrombosis usually treated by antiplatelet

2. Venous Thrombi (Red thrombi)
   - Low shear rates
   - Primarily red cells and fibrin strands (few platelets)
   - Most often occurs in cases of stasis (inadequate flow) or biochemical abnormalities
   - So DVT treated by anticoagulant.

Arterial Thrombosis

- Cerebral artery thrombosis= stroke
- Mesenteric artery thrombosis= Bowel infarction
- Coronary artery thrombosis= Myocardial infarction

Table 27.1 Risk factors for arterial thrombosis (atherosclerosis).

Positive family history
Male sex
Hyperlipidaemia
Hypertension
Diabetes mellitus
Gout
Polycythaemia
Hyperhomocysteinaemia
Cigarette smoking
ECG abnormalities
Elevated CRP, IL6, fibrinogen, lipoprotein-associated phospholipase A_2
Lupus anticoagulant
Collagen vascular diseases
Behçet's disease

CRP, C-reactive protein; ECG, electrocardiogram.

Thromboembolism

- Arterial: often fragment of thrombus from heart wall or heart valve, travels downstream to smaller vessel - may lead to stroke or MI.

- Venous: fragment of venous thrombus that breaks off and travels upstream towards the heart, may lead to pulmonary embolism.

Thromboembolism

- Most inherited conditions are associated with increase in the risk of venous thromboembolism.

- Acquired conditions are associated with both arterial and venous thromboembolism.

Venous Thromboembolism (VTE)

- Venous system: low flow & pressure
- Thrombi are fibrin rich
- Function of age, biologic conditions, genetic, environmental factors and their interactions.
- Venous thromboembolism (VTE)
  - Deep vein thrombosis (DVT)
  - Pulmonary embolism (PE)
  - Superficial, portal, cerebral, or retinal vein thrombosis

Venous Thromboembolism (VTE)

- The most common is deep venous thrombosis (DVT) of the lower limbs. The main site, where there is maximum stasis and low blood flow.

- Propagation of thrombus is associated with red cell entrapment……red thrombus.

- Venus thrombi may become dislodged or fragment, resulting in the formation of circulating thrombi. This may result in pulmonary embolism

Pulmonary embolism

- Presents with acute chest pain.
- Breathlessness with shock.
- Cough & hemopysis.
- May be fatal.

Venous Thrombosis symptoms

- Typically presents with pain, swelling, discoloration & warmth in the affected area.

- However these symptoms may be absent & non of them is specific).

Inherited Hypercoagulable States

- Usually seen in young age (prior to 45 years)
- Recurrent VTE events
- Family history with VTE
- Thrombosis develops in the venous system and at unusual anatomical sites like visceral veins.

Hereditary Risk Factors for Venous Thrombosis

- Antithrombin Deficiency
- Protein C deficiency
- Protein S deficiency
- Factor V Leiden (FVL)
- Prothrombin G20210A
- Dysfibrinogenemias (rare)
- MTHFR deficiency: Hyperhomocysteinemia

Other causes of venous thromboembolism

- Elevated factor VIII
- Plasminogen deficiency
- Plasminogen activator deficiency
- Elevated PAI-1

Site of thrombosis vs Coag. defect

Abnormality	Arterial	Venous
Factor V Leiden	-	+
Prothrombin G20210A	-	+
Antithrombin deficiency	-	+
Protein C deficiency	-	+
Protein S deficiency	-	+
Hyperhomocysteinemia	+	+
Antiphospholipid syndromes	+	+

Antithrombin Deficiency

- Antithrombin III is a physiological inhibitor of thrombin in circulation and its deficiency results in thrombus formation.

- AT deficiency: Autosomal dominant disorder

- There are two types of AT deficiency.
  1. Type I is a quantitative disorder characterized by a reduction in the amount of AT.
  2. Type II is a qualitative disorder in which the concentration of AT is normal but the molecule is functionally abnormal.

- Risk of a thrombosis—20% to 80%.

Antithrombin Deficiency

- AT deficiency is associated with recurrent venous thrombosis, which may include almost every vein site.
- The thrombotic event may be primary (in the absence of triggering factors) or may be followed by another risk factor, such as pregnancy, surgery, or any other acquired factors.
- Acquired AT deficiency may be correlated with disseminated intravascular coagulation (DIC), liver disease, nephrotic syndrome, oral contraceptives and pregnancy.
- Testing by AT assay

Protein C Deficiency

- Is inherited as an autosomal dominant trait.
- Proteins C and S act as a complex, which degrades factors Va and VIIIa. When they are deficient, activated factor V and VIII are not neutralized and resulting activation of the clotting system, which promotes thrombosis.
- It may be homozygous or heterozygous type:
  - Homozygous protein C deficiency is a serious life-threatening condition. They present during neonatal period with thrombosis, purpura fulminans characterized by superficial thrombosis and necrotic skin lesions.
  - Heterozygous protein C deficiency may be asymptomatic or present with recurrent venous thrombosis at a young age.

Protein C Deficiency

- Acquired protein C deficiency may be linked with vitamin K deficiency, liver disease, malnutrition, DIC and warfarin therapy.

Protein S Deficiency

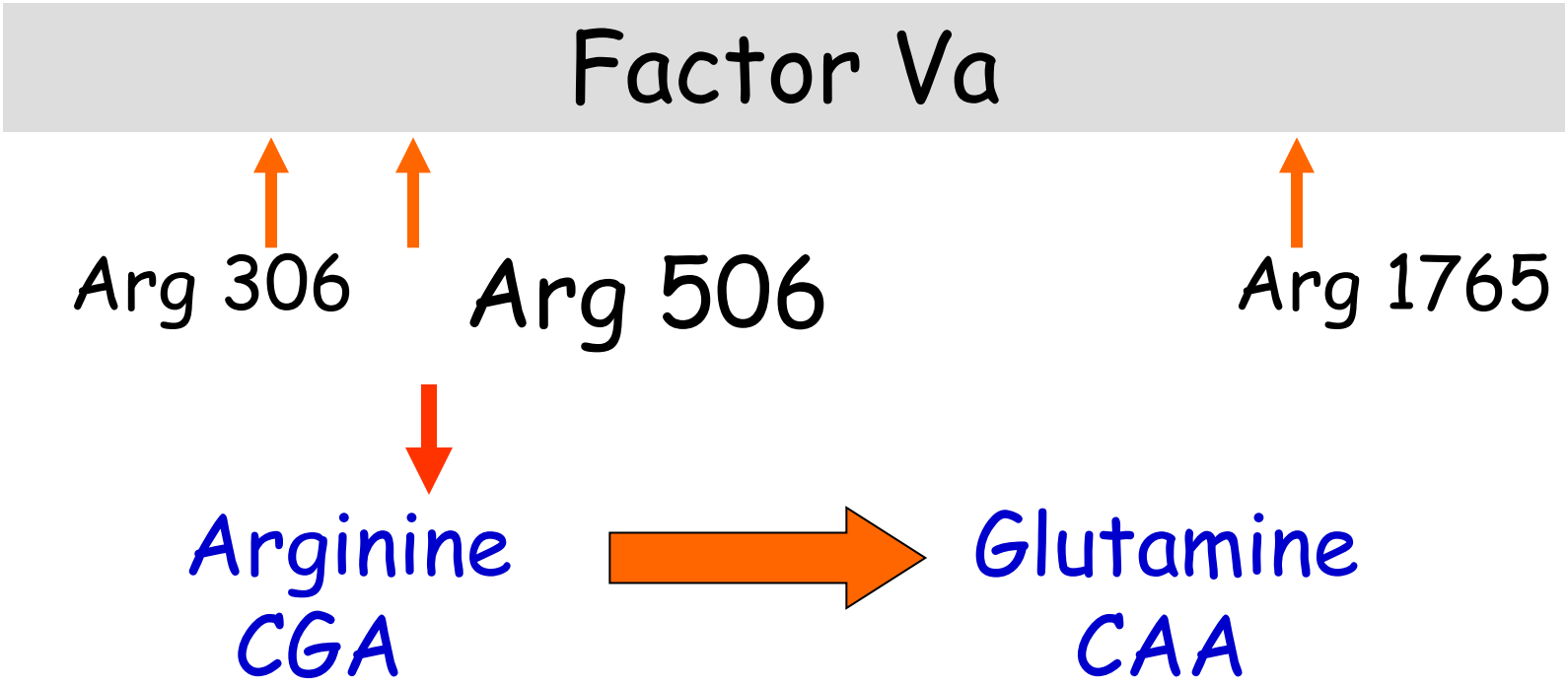
- Protein S deficiency is inherited in an autosomal dominant fashion.
- Protein S circulates in plasma in two forms: free (40%) and bound to C4bbinding protein (60%). The cofactor activity of protein S is carried primarily by free protein S.
- As with AT and protein C deficiencies, protein S deficiency is divided into two types (Quantitative & Qualitative).
- Similar to protein C deficiency, many patients with thrombosis have additional inherited or acquired risk factors.
- Most patients with protein S deficiency may experience venous thrombosis. However, arterial thrombosis has been reported in 25% of patients with protein S deficiency.
- Acquired protein S deficiency may be correlated with vitamin K deficiency, liver disease, and DIC.

Activated Protein C (APC) Resistance (Factor V Leiden)

- Most common genetic disorder associated with familial thrombophilia.
- Activated proteins C (APC) and protein S complex inhibits activated factor normal V and VIII. The variant clotting factors cannot be degraded.
- A **point mutation** (results in a glutamine to arginine substitution at position 506) in the factor V gene result in synthesis of a factor V variant. This variant is known as factor V Leiden/Leiden mutation.
- Factor V variant has normal procoagulant activity but is resistant to inhibition by activated protein C (APC).

Factor V Leiden

Factor Va resistant to APC cleavage



- A polymorphism in factor V at the APC cleavage site (Arg506 → Gln) results in a FVa molecule resistance to degradation by APC.

Factor V Leiden

- Factor V Leiden is the most common inherited cause for thrombosis in the Caucasian population of northern and western Europe.
- In the United States, factor V Leiden is seen in 6% of the Caucasian population.
- The homozygous form of factor V Leiden has a 80-fold increased risk of thrombosis, whereas heterozygous carriers have a 2-fold to 10-fold increase in thrombosis.

Factor V Leiden

- The thrombotic complications associated with factor V Leiden are
  - VTE,
  - recurrent miscarriage, and MI.
- Smoking increases the risk of thrombosis 30-fold in individuals with factor V Leiden.
- Other causes of APCR (8%) are related to pregnancy, oral contraceptive use, cancer and other acquired disorders

- The activated protein C resistance (APCr) test is the recommended screening test for detection of factor V Leiden, followed by a confirmatory test such as DNA analysis of the factor V gene, which encodes the factor V protein.

Prothrombin allele G20210A

- Prothrombin mutation (G20210A) is the second most prevalent cause of an inherited form of hypercoagulability.
- Caused by a single point mutation, G20210A is an autosomal dominant disorder that causes increased concentration of plasma prothrombin.
- The risk of VTE increases as the plasma prothrombin level increases to a level greater than 115 IU/dL.
- Similar to factor V Leiden, the thrombotic episodes develop early, before the age of 40 years.
- Diagnosed by PCR techniques.

Increased Factor VIII Activity

- Elevated activity levels of factor VIII are associated with VTE.
- If factor VIII activity is greater than 150%, the risk for VTE increases 3-fold;
- if the activity is greater than 200%, the thrombotic risk increases 11-fold.

Factor XII deficiency

- Factor XII deficiency is also linked to thrombosis.
- Factor XII, prekallikrein factor, and Fitzgerald factor, commonly referred to as contact factors, initiate activation of the intrinsic pathway.
- Patients with deficiencies of these factors have a prolonged aPTT but no bleeding problems.
- Factor XII plays a major role in the fibrinolytic system and in activating plasminogen to plasmin.
- Patients with factor XII deficiency have impaired fibrinolysis and are prone to thrombosis.

Tissue factor pathway inhibitor (TFPI) deficiency

- Tissue factor pathway inhibitor (TFPI) deficiency is another marker for thrombosis.
- TFPI is important in the prevention of clot formation. It inhibits factor Xa and factor VIIa–tissue factor complex.
- TFPI deficiency is associated with thromboembolic disorder, owing to the excessive activation of the extrinsic pathway.

Hyperhomocysteinemia

- An increased plasma concentration of homocysteine is known as homocysteinemia, which predisposes to atherosclerosis and thrombosis.
- The plasma homocysteine level may be genetically determined, but is also partly controlled by the dietary content of vitamin B12, folate and pyridoxine.
- Deficiency of these dietary vitamins causes moderate degree of homocysteinemia.
- Mechanism of thrombosis is not clearly known. It may be due to:
  - Endothelial damage induced by homocysteine.
  - Linkage between homocysteine metabolites and various proteins, including fibrinogen.

Fibrinolytic system disorders

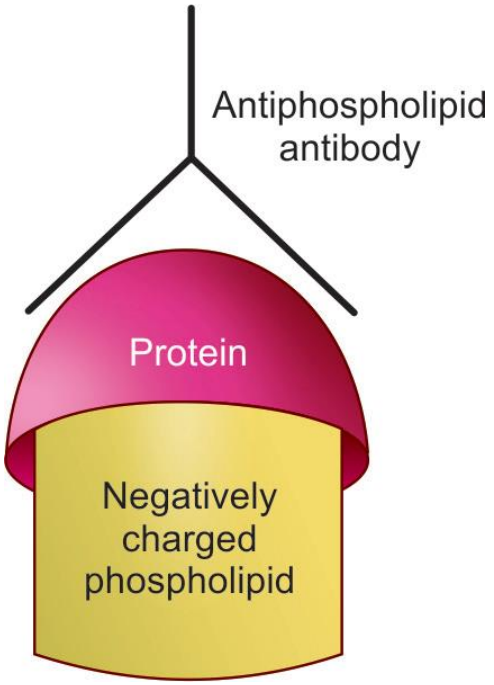
- Plasminogen deficiency
- tPA deficiency
- Excessive release of plasminogen activator inhibitor (PAI)

Acquired Thrombotic Disorders

- cancer, surgery,
- liver disease, nephrotic syndrome,
- DIC, pregnancy, and vitamin K deficiency.
- Drugs such as oral contraceptives or hormone replacement therapy may predispose to thrombosis.
- The most common causes of acquired thrombotic disorders are antiphospholipid syndrome and heparin induced thrombocytopenia.

Acquired Hypercoagulable States
Antiphospholipid Antibody Syndrome (APLA/APS)

- Presence antiphospholipid antibodies (APAs) in the plasma are associated with hypercoagulable state.
- Antiphospholipid antibody reacts with plasma proteins, which are bound to phospholipids.
- Two important antiphospholipid antibodies: lupus anticoagulant antibody and anti-β2 glycoprotein antibody.



Antiphospholipid antibody against plasma proteins bound to phospholipids

Antiphospholipid Antibody Syndrome

Types:

1. primary due to genetics
2. secondary due to :
   - Rheumatoid arthritis
   - SLE
   - infections
   - some drugs

Clinical Features:

1. Attack phospholipids of the cell membrane of the fetus ➡ recurrent abortion.

normally, tissue plasminogen activator (t-PA) is necessary for the invasion of uterine blood vessels by placental trophoblastic tissue. Recurrent spontaneous abortions develop due to antibody-mediated inhibition of t-PA activity.

2. inhibit fibrinolytic system ➡ thrombosis
3. Immune thrombocytopenia.

The diagnostic criteria require :

A. one clinical event, i.e.
   A. thrombosis
   B. pregnancy complication

B. two antibody blood tests spaced at least three months apart that confirm the presence of either
   A. lupus anticoagulant
   B. anti-β2-glycoprotein-I
   C. anti-cardiolipin antibodies

Laboratory Tests

- APTT: prolonged
- Factor VIII levels: normal
- Prothrombin time: normal
- Thrombin time: normal
- Fibrinogen level: normal.

Confirmatory test

- Test for lupus anticoagulant:
  - Dilute Russell's viper venom test (DRVVT): Russell's viper venom (RVV) activates factor X leading to fibrin clot. Lupus anticoagulant prolongs clotting time by binding to RVV and preventing the action of RVV.

- Antibodies against the phospholipid–β2-glycoprotein complex:
  - Detected by enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA).

Treatment :

1. heparin to reduce the risk of further episodes of thrombosis and improve the prognosis of pregnancy.
2. Warfarin/Coumadin is not used during pregnancy because it can cross the placenta, unlike heparin, and is teratogenic.
   - #The goal of the prophylactic treatment with warfarin is to maintain the patient's INR between 2.0 and 3.0

Heparin-Induced Thrombocytopenia

- Is an immune-mediated complication associated with heparin therapy.
- HIT may develop in 3% to 5% of patients receiving unfractionated heparin. Thrombocytopenia usually develops 5 to 14 days after heparin therapy.
- About 36% to 50% of patients with HIT develop life-threatening thrombosis.
- The thrombotic tendency can last for at least 30 days.

Heparin-Induced Thrombocytopenia

- Venous thrombosis is more common than arterial thrombosis.
- Other complications of HIT include thrombocytopenia, heparin-induced skin lesions (10% to 20% of patients), and heparin resistance.
- The pathogenesis of HIT is that antibodies are produced against heparin–platelet factor 4 complex. This immune complex binds to platelet FC receptors, causing platelet activation, formation of platelet microparticles, thrombocytopenia, and hypercoagulable state

Heparin-Induced Thrombocytopenia

- Heparin-platelet factor 4 antibodies are detected by ELISA.
- When HIT is suspected, heparin should be stopped immediately and be replaced by alternative anticoagulant drugs.

Screening Laboratory Tests for Thrombophilia

- Activated protein C resistance
- Functional assays for antithrombin, protein C, and
- protein S
- aPTT, DRVVT, mixing studies, and confirmatory test for lupus anticoagulant
- ELISA for anticardiolipin antibody
- Factor VIII activity

Conditions That Require Evaluation for Hypercoagulable States

- Recurrent thrombosis in patients younger than 45 years of age
- Patients with a positive family history
- Recurrent spontaneous abortion
- Thrombosis in unusual sites
- Heparin resistance
- Protein C and protein S deficiency
- Thrombosis associated with pregnancy and estrogen therapy
- Unexplained recurrent pregnancy loss

Next Lecture:

Drugs that affect Hemostasis

Lecture 11: Drugs that affect Hemostasis

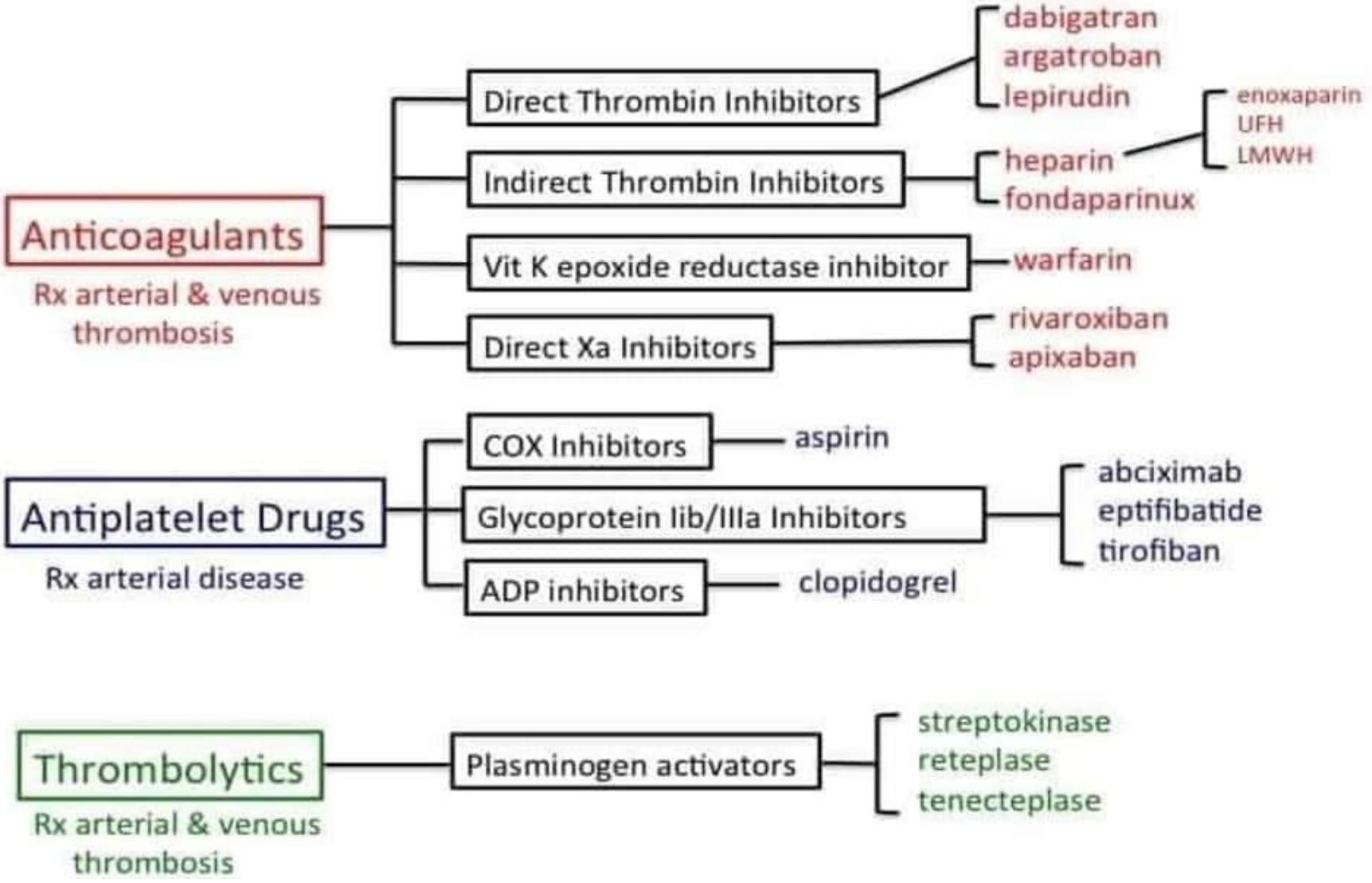
Miss: Amany S. AlHindi

M.Sc. in Biological sciences from Islamic University of Gaza

Hemostasis Drugs

1. Anticoagulant Drugs
2. Antiplatelet Drugs
3. Thrombolytic Drugs
4. Antifibrinolytic Drugs

Drugs Used to Treat Clotting Disorders



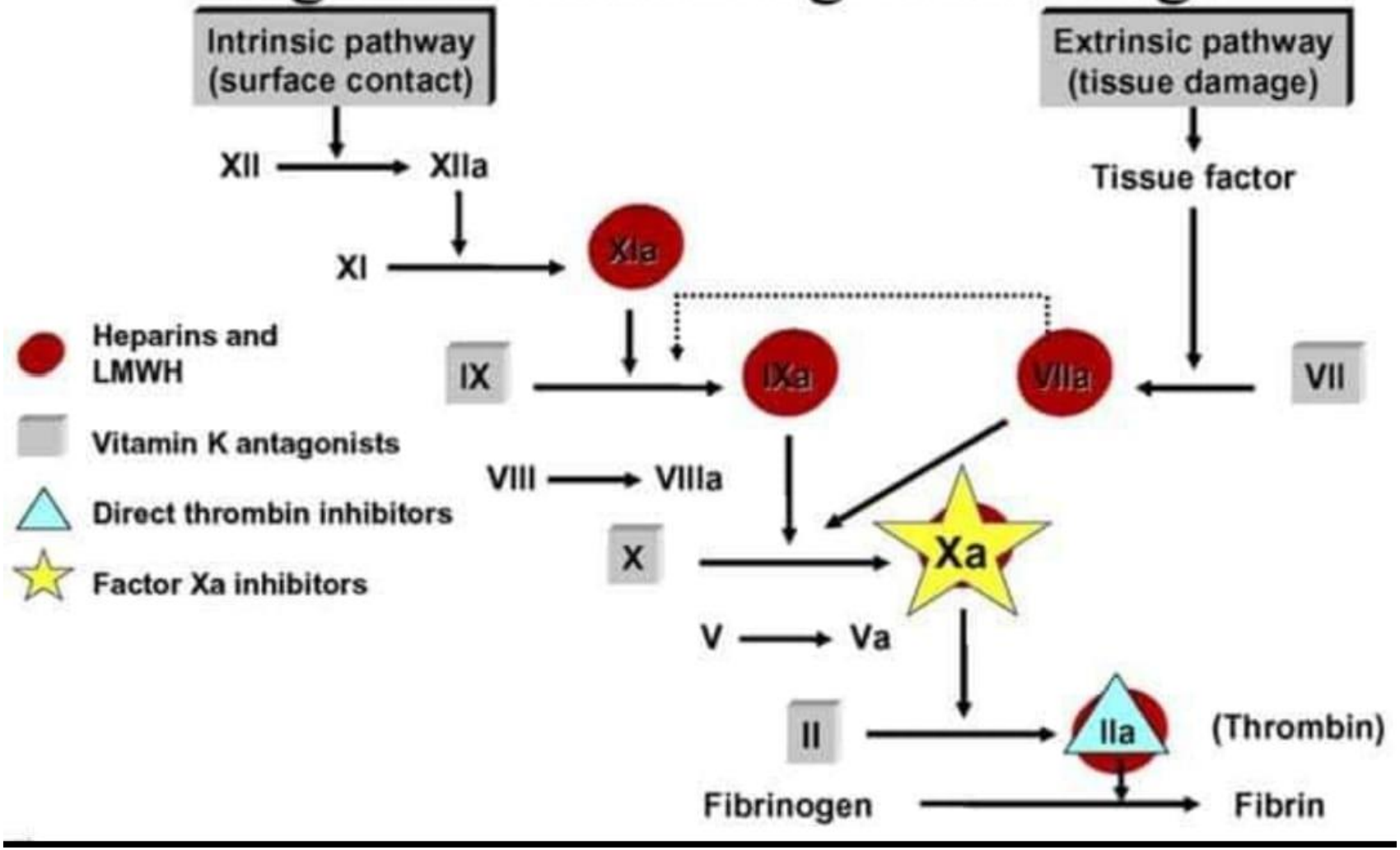
Anticoagulant Drugs
Reasons for Administration

- Used prophylactically to prevent:
  - Clot formation (thrombus)
  - An embolus (dislodged clot)
  - Disrupt the blood's natural clotting mechanisms when the patient is at risk for a clot.

- Prevent the formation of a thrombus in an immobile or post- op patent

- Prevent subsequent thrombosis but does not dissolve already formed thrombus, but intercept the extension of a thrombus once formed.

Targets for Anticoagulant Drugs



Anticoagulant Drugs

- Short-term anticoagulant drugs such as:
  - heparin are administered by IV infusion or subcutaneous injection.

- Long-term anticoagulant drugs such as:
  - warfarin (Coumadin) are administered orally.

Heparin

- Unfractionated Heparin Therapy
- Low-Molecular-Weight Heparin Therapy

Unfractionated Heparin Therapy

- Is a naturally occurring, Heparin sulfate is a heparin like substance made by the vascular endothelium.
- Commercially UFH is isolated from bovine lung or porcine intestine.
- Is a mixture of polysaccharide chains with a molecular weight of 4,000 to 30,000 daltons.
- The anticoagulant activity of heparin is enhanced by binding to AT. Heparin-AT complex inactivates thrombin and factor Xa. Other clotting factors affected by heparin are factors IXa, XIa, and XIIa.
- Do not cross placenta, given to pregnant.
- The half-life of heparin is dose-dependent.
- Heparin is cleared from the circulation by the reticuloendothelial system and metabolized by the liver.
- Unfractionated is monitored by APTT ratio

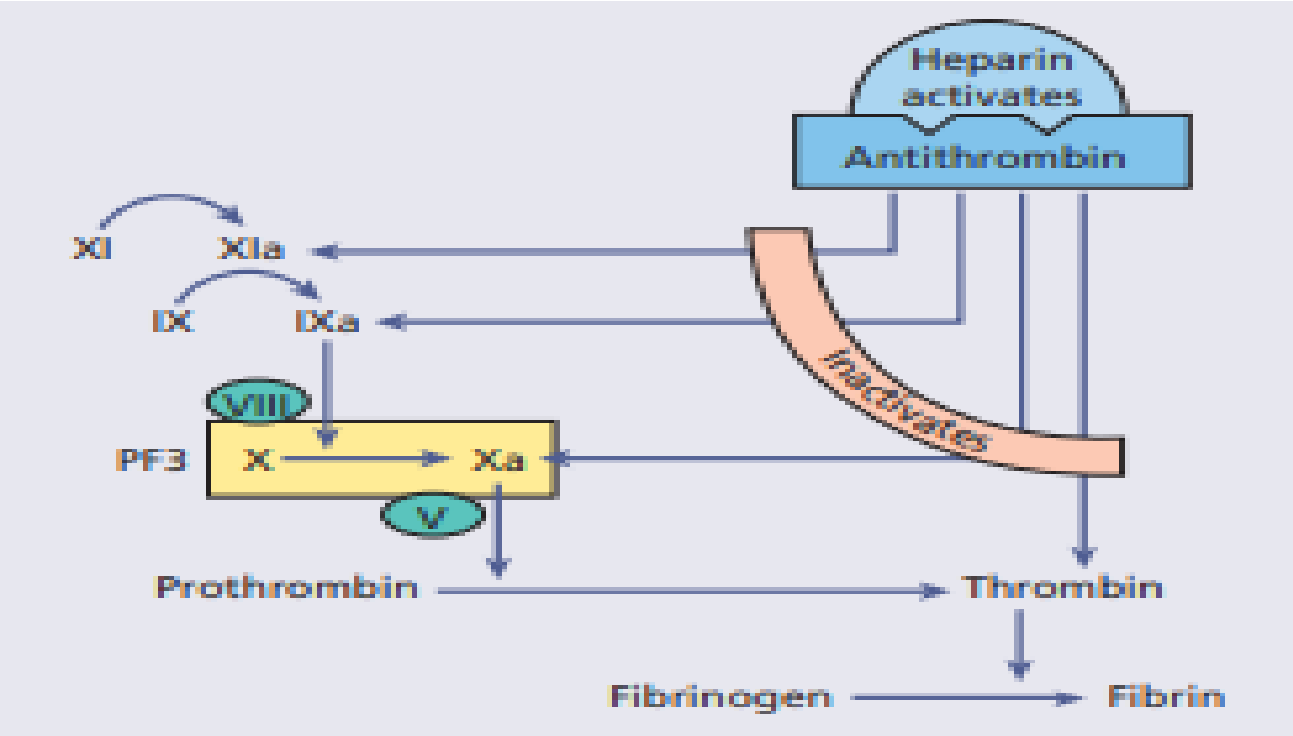


Figure 27.6 The action of heparin. This activates antithrombin which then forms complexes with activated serine protease coagulation factors (thrombin, Xa, IXa and XIa) and so inactivates them.

Unfractionated Heparin Therapy

- Heparin is given in a weight-adjusted dosage with an initial bolus (5,000 to 10,000 U) followed by continuous low-dose infusion (1,300 U).
- The therapeutic range of heparin is extremely narrow.
- The baseline APTT and platelet count should be performed for each patient before heparin therapy.
- A prolonged APTT baseline may be associated with LA or factor deficiencies that could interfere with the laboratory result interpretation.
- A 40% reduction in platelet count compared with the baseline platelet count is evidence of HIT and requires immediate discontinuation of heparin.

Adverse effects of heparin therapy

- Bleeding,
- HIT (Heparin induced thrombocytopenia), and
- Heparin resistance:
  - may occur as a result of nonspecific binding of heparin to plasma proteins, platelets, and endothelial cells or as a result of AT deficiency.

Low-Molecular-Weight Heparin Therapy (Clexan)

- Derived from UFH via enzymatic digestion to produce smaller low-molecular-weight glycosaminoglycan molecules.
- The mean weight of LMWH is about 5,000 daltons.
- LMWH is active in anticoagulation, has a longer half-life and low affinity to bind to plasma proteins and endothelial cells.

Low-Molecular-Weight Heparin Therapy

- The half-life of the drug is not dose-dependent.
- LMWH is administered subcutaneously once or twice daily based on body weight and does not require monitoring.
- LMWH has a higher inhibitory effect on factor Xa than on factor IIa.
- LMWH is cleared by the kidney. The adverse reaction of LMWH includes bleeding, HIT, or sensitivity to LMWH.

LMWH Monitoring

- Usually LMWH does not require monitoring, but if monitoring is required Anti-factor Xa assay is requested.
- In anti-factor Xa assay, the concentration of heparin is determined by inhibiting factor Xa by AT.
- Anti-factor Xa assay uses a reagent with a fixed concentration of factor Xa and AT reagent. Heparin forms a complex with anithrombin and factor Xa reagents.
- Excess factor Xa combines with the chromogenic substrate to form a colored product; the color intensity is inversely proportional to the concentration of heparin.

Advantages of LMWH over UFH

- Better subcutaneous bioavailability (70-90%) compared to UFH (20-30%).
- Longer half life: once daily subcutaneous administration.
- Since APTT is not prolonged, lab monitoring is not needed.
- Lower incidence of hemorrhagic complications.
- Less antiplatelet action and very less HIT.

Heparin Overdose

- Reverse effect of heparin with its antidote:

Protamine Sulfate

Alternative Anticoagulant Drugs
Direct Thrombin Inhibitors

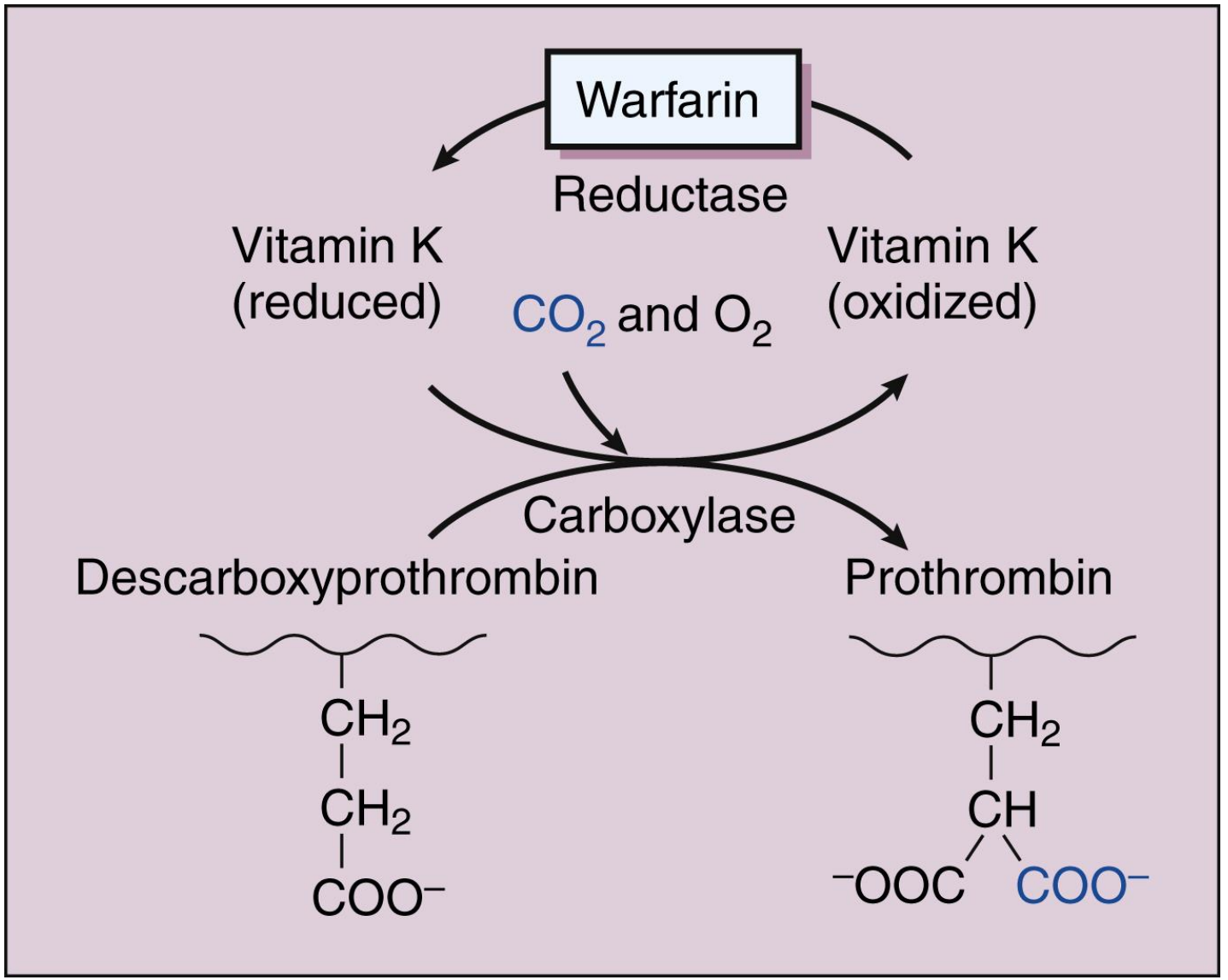
- The DTIs bind thrombin without additional binding proteins, such as antithrombin.
- Monitored by APTT
  - Hirudin
  - Lepirudin
  - Bivalirudin
  - Argatroban

Oral Anticoagulant: Coumadin (Warfarin) Therapy

- A vitamin K antagonist drug that inhibits the vitamin K-dependent coagulation factors II, VII, IX, and X.

- Warfarin inhibits gamma carboxylation of the vitamin K-dependent clotting factors, reducing their activity, and inhibits vitamin K-dependent anticoagulant proteins such as protein C and protein S.

- The half-life of warfarin is about 36 hours.


Warfarin
Reductase
Vitamin K
(reduced)
Vitamin K
(oxidized)
CO_2 and O_2
Carboxylase
Descarboxyprothrombin
Prothrombin
CH_2
CH_2
COO^-
CH_2
CH
^-OOC
COO^-

Coumadin (Warfarin) Therapy

- Warfarin is given orally as a long-term anticoagulant; the dosage varies from patient to patient and depends on dietary stores of vitamin K, liver function, preexisting medical conditions, and concurrent medications.

- Warfarin is prescribed prophylactically to prevent thrombosis after trauma or surgery or to prevent strokes in patients with atrial fibrillation.

- Therapeutic warfarin is used after acute MI to control coagulation. It is also used to prevent recurrence of DVT or PE

Coumadin (Warfarin) Therapy

- The standard warfarin regimen is 5 to 10 mg and varies from patient to patient.

- The activity of vitamin K–dependent coagulation factors decreases immediately after warfarin therapy. Because of the different half-lives of the vitamin K–dependent clotting factors, however, it takes 4 to 10 days to reach therapeutic levels.

- Warfarin therapy is monitored by PT and international normalized ratio (INR).

- The INR is calculated using the following formula:

  $INR = (PT\ ratio)^{ISI}$, where ISI refers to the international sensitivity index, which is calculated for each thromboplastin reagent against a reference thromboplastin reagent.

- Narrow therapeutic index for the drug (INR 2-3, 2.5-3.5)

Elimination Half-Lives of Vitamin K-Dependent Proteins

Protein	Half-Life
Factor VII	4–6 hours
Factor IX	24 hours
Factor II	60 hours
Factor X	48–72 hours
Protein C	8 hours
Protein S	30 hours

Adverse effect of warfarin

- Bleeding, which is directly dose-related.
  - Patients with an INR of greater than 3.0 are at higher risk of bleeding.
- Warfarin crosses the placenta and should be avoided during pregnancy.
- Urticaria
- Skin necrosis, a phenomenon that mostly occurs in patients who receive high doses of warfarin and may have heterozygous protein C deficiency.
  - Skin necrosis is caused by the rapid decrease in protein C in patients who have preexisting protein C deficiency resulting in a thrombotic state.

Warfarin Antidote

- Vitamin K (phytonadione)

Antiplatelet Drugs

- Aspirin (acetylsalicylic acid) is an antiplatelet drug that irreversibly affects platelet function by inhibiting the cyclooxygenase (COX) enzyme.
- Both PGI2 and TXA2 synthesis are inhibited.
- Baby aspirin (low dose 80-100 mg/day) inhibits TXA2 synthesis.
- Aspirin is rapidly absorbed from the gastrointestinal tract and reaches its peak concentration in plasma 1 hour after ingestion.
- The effect of aspirin on platelets starts 1 hour after ingestion and lasts for the entire platelet life span (approximately 1 week).

Aspirin (acetylsalicylic acid)

- Aspirin uses in the prophylactic of cerebral ischemia, to reduce The incidence of recurrent myocardial infraction, to decrease mortality in post-myocardial infraction patient.

- Adverse effects: Aspirin toxicity includes gastrointestinal discomfort, blood loss, and the risk of systemic bleeding.

Other antiplatelet drugs

- ADP receptors antagonist (P2Y12) : e.g. clopidogrel(plavix), Ticlopidine.
- Glycoprotein IIb /IIIa inhibitors e.g. abciximab

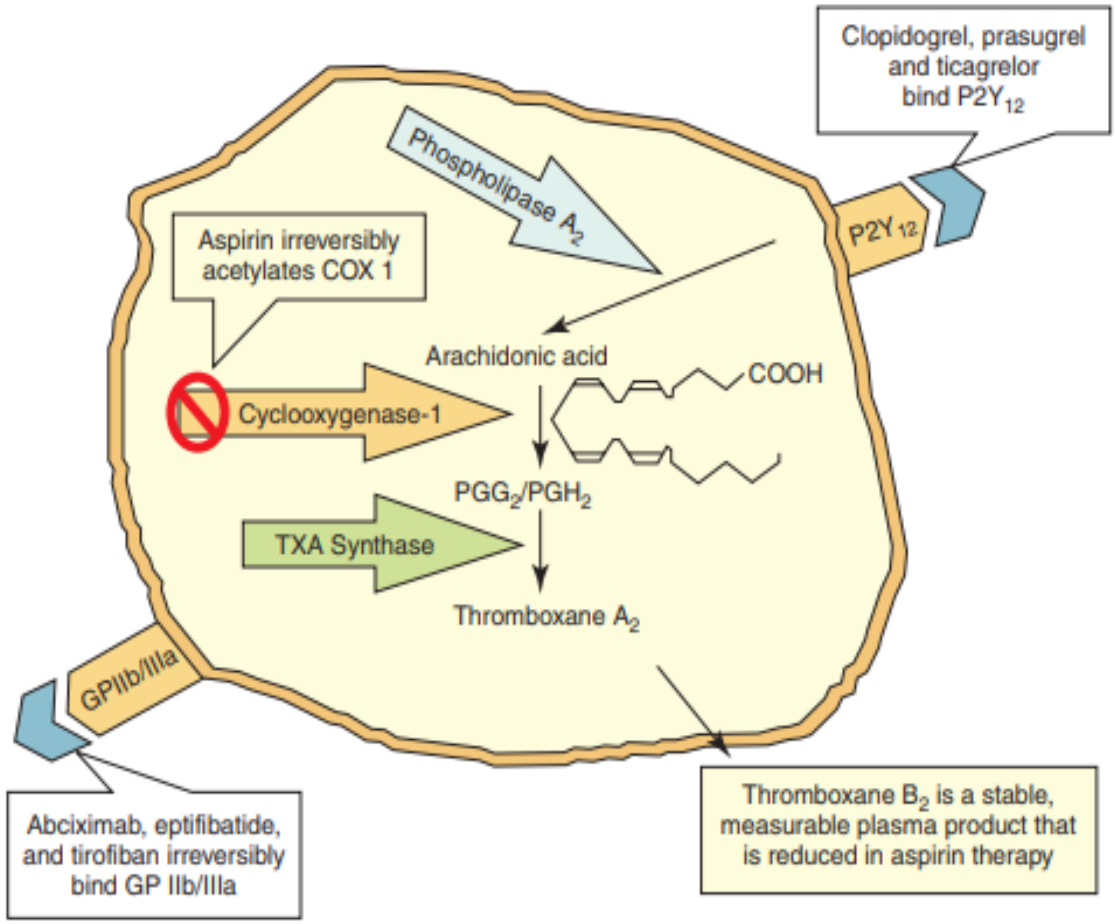


Figure 43-9 Antiplatelet drugs employ three mechanisms to inactivate platelets. Aspirin irreversibly acetylates and inactivates cyclooxygenase 1 (COX-1). Clopidogrel (irreversible), prasugrel (irreversible), and ticagrelor (reversible) bind the adenosine diphosphate (ADP) receptor, $P2Y_{12}$. Intravenous abciximab, eptifibatide, and tirofiban bind the fibrinogen binding site, glycoprotein (GP) IIb/IIIa. *PGG_2*, Prostaglandin G_2; *PGH2*, prostaglandin H_2; *TXA synthase*, thromboxane A_2 synthase.

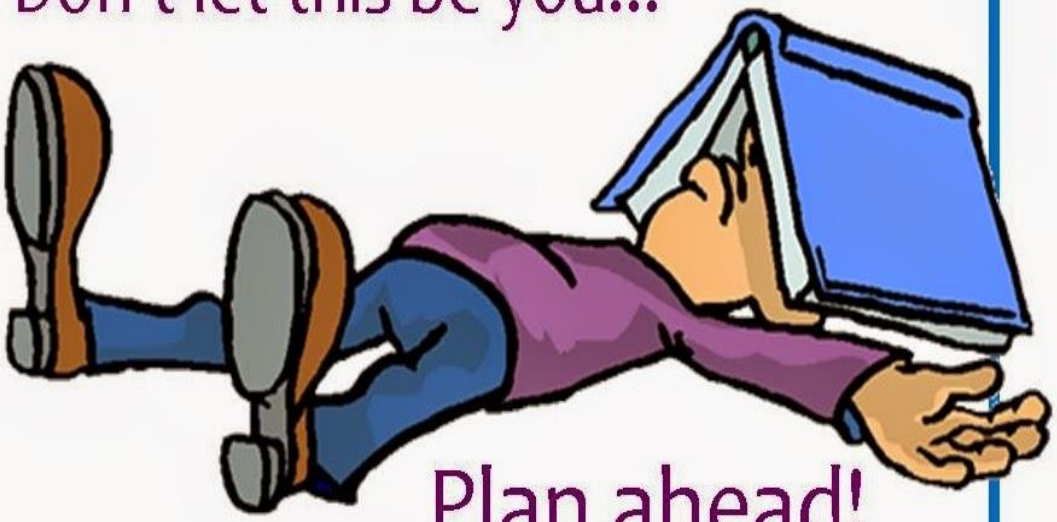
Thrombolytic Drugs (also known fibrinolytics)

- Causes rapid clot lysis (lyse an established clots).
- Are used by IV infusion or infused directly to the clogged vessel in cases of acute MI, PE and DVT.
- They act as plasminogen activators.
- Includes:
  - rtPA (Alteplase)
  - Urokinase
  - Streptokinase
- Bleeding is the most common complication associated with thrombolytic drugs.
- Monitored by measuring D-dimer levels.
- D-dimer should increases when administrating thrombolytic agents.

Antifibrinolytic Drugs

- Prevention and treatment of excessive bleeding
- Include:
  - Epsilon aminocaproic acid: specific antidote for thrombolytic drugs
  - Tranexamic acid
  - Aprotinin

Don’t let this be you...



Plan ahead!