BIEN 500 Systems Physiology

Blood cell, immunity and blood clotting

Chapter 37 in 14th Edition (and Prior)

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Part 2

Fall 2023

Lecture 07

Hemostasis

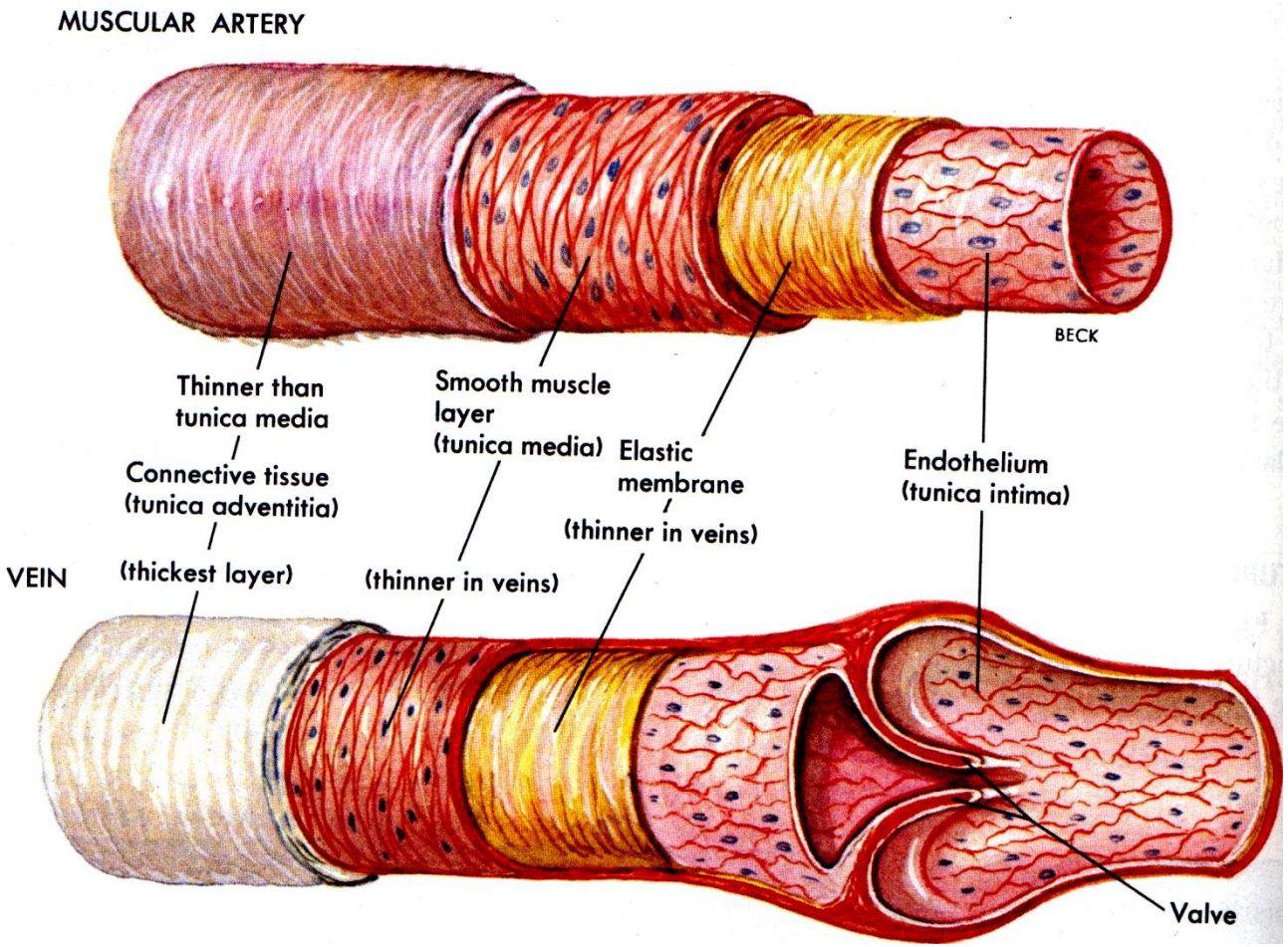
- Definition: the arrest of bleeding

- Primary hemostasis
  – Formation of platelet plugs
  – Blood vessels (endothelium)

- Secondary hemostasis
  – Formation of fibrin through the coagulation cascade

- Tertiary hemostasis
  – Formation of plasmin for the breakdown of the clot

Anatomy of a Blood Vessel (Artery and Vein differences)



Extracellular Matrix Proteins

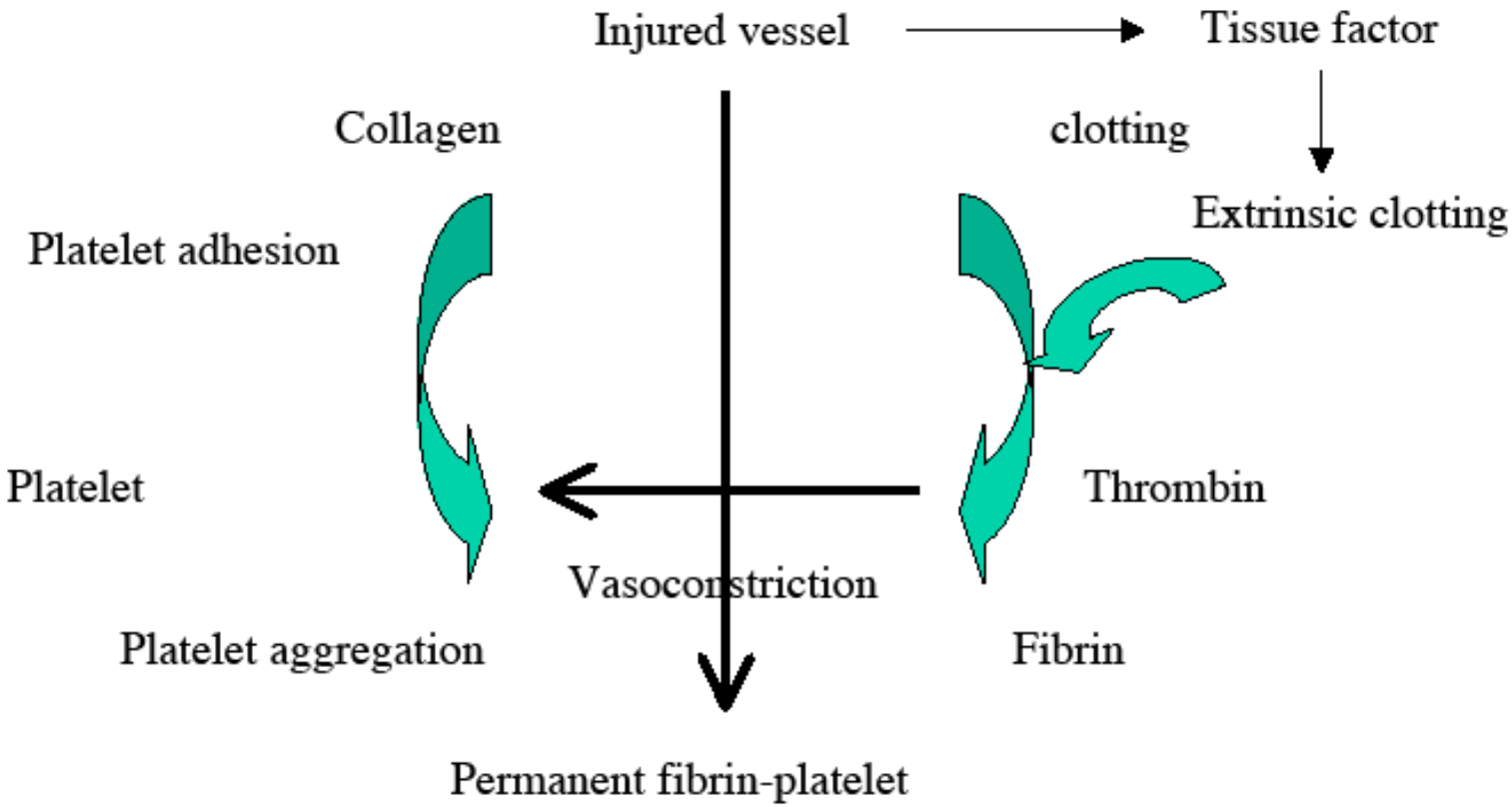
- Fibronectin
  - An important protein in terms of cell adhesion and matrix stability
  - Has binding sites (e.g., RGD peptide) for integrins, as well as other ECM molecules such as collagen and fibrin

- Laminin
  - A large group of cross-shaped glycoproteins
  - Serves primarily a structural role in basement membranes, anchoring cells to the basal lamina
  - Important in cell migration, especially in neuron outgrowth

- Collagen
  - A large family of proteins that represent the majority of proteins in mammalian tissue (~25%)
  - Many different types of collagen: fibrillar, fibril-associated, and network forming collagen types

Global Scheme of Hemostasis

Platelet system Vascular system Coagulation system



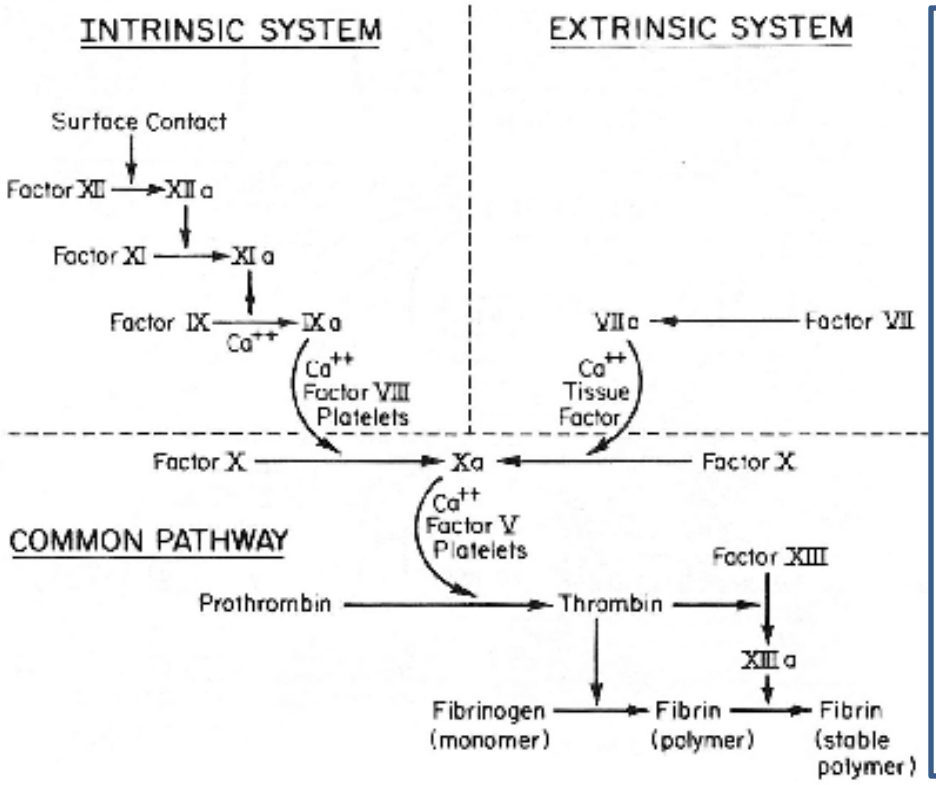
Balance Between Hemostasis, Bleeding and Thrombosis

(Heparin)

↓Coagulation	Coagulation
↑Fibrinolysis	↓Fibrinolysis
↑Coag. inhibitors	↓Coag. inhibitors
Primary hemostasis	↑Primary hemostasis

Bleeding disorder Hemostasis Thrombotic disorder

Coagulation Cascade



1. Extrinsic pathway begins with a traumatized vascular wall or traumatized extravascular tissues that come in contact with the blood. This includes a tissue factor composed of phospholipids from the membranes of the tissue.

2. The intrinsic pathway begins with trauma to the blood itself or exposure of the blood to collagen from a traumatized blood vessel wall.

Activation of Coagulation Pathway

- Intrinsic pathway
  - Initiated in the absence of Ca^{2+}
  - Initiated by the exposure of blood to material surface (subendothelium, polymers) and negatively charged surfaces (glass, clay)

- fXII adheres to negatively charged surfaces (other cells?) and undergoes conformational changes

- Highly susceptible to cleavage by trace amount of fXIIa

- Extrinsic pathway
  - Thromboplastin (Tissue Factor)
  - Transmembrane protein
    - Found in high levels in brain, lung and placenta
    - Found in blood vessel intima
  - fVII

Clotting Factors

I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Labile factor
VI	Proconvertin
VIII	Anti-hemophilic factor (AHF)
IX	Christmas factor
X	Stuart-Prower factor
XI	Plasma thromboplastin antecendent (PTA)
XII	Hageman factor
XIII	Fibrin stabilizing factor

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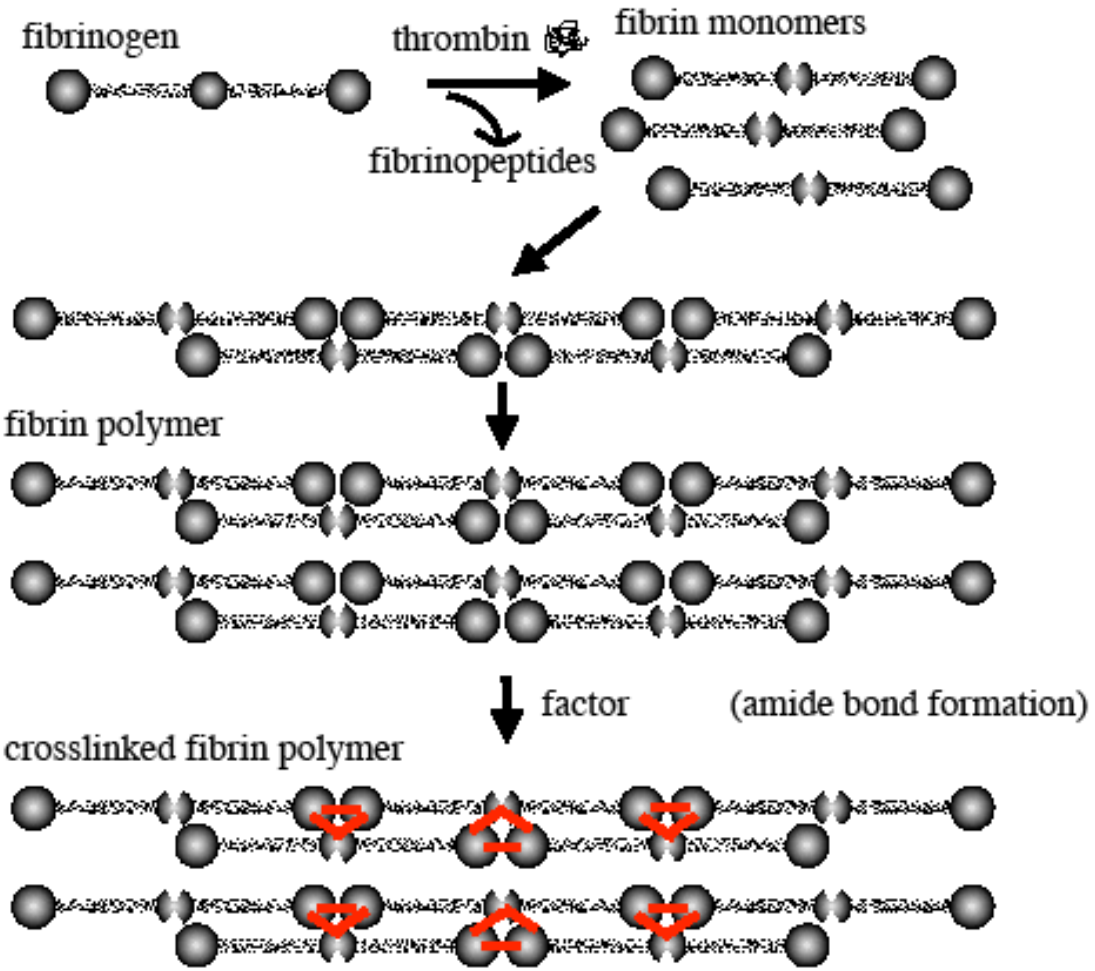
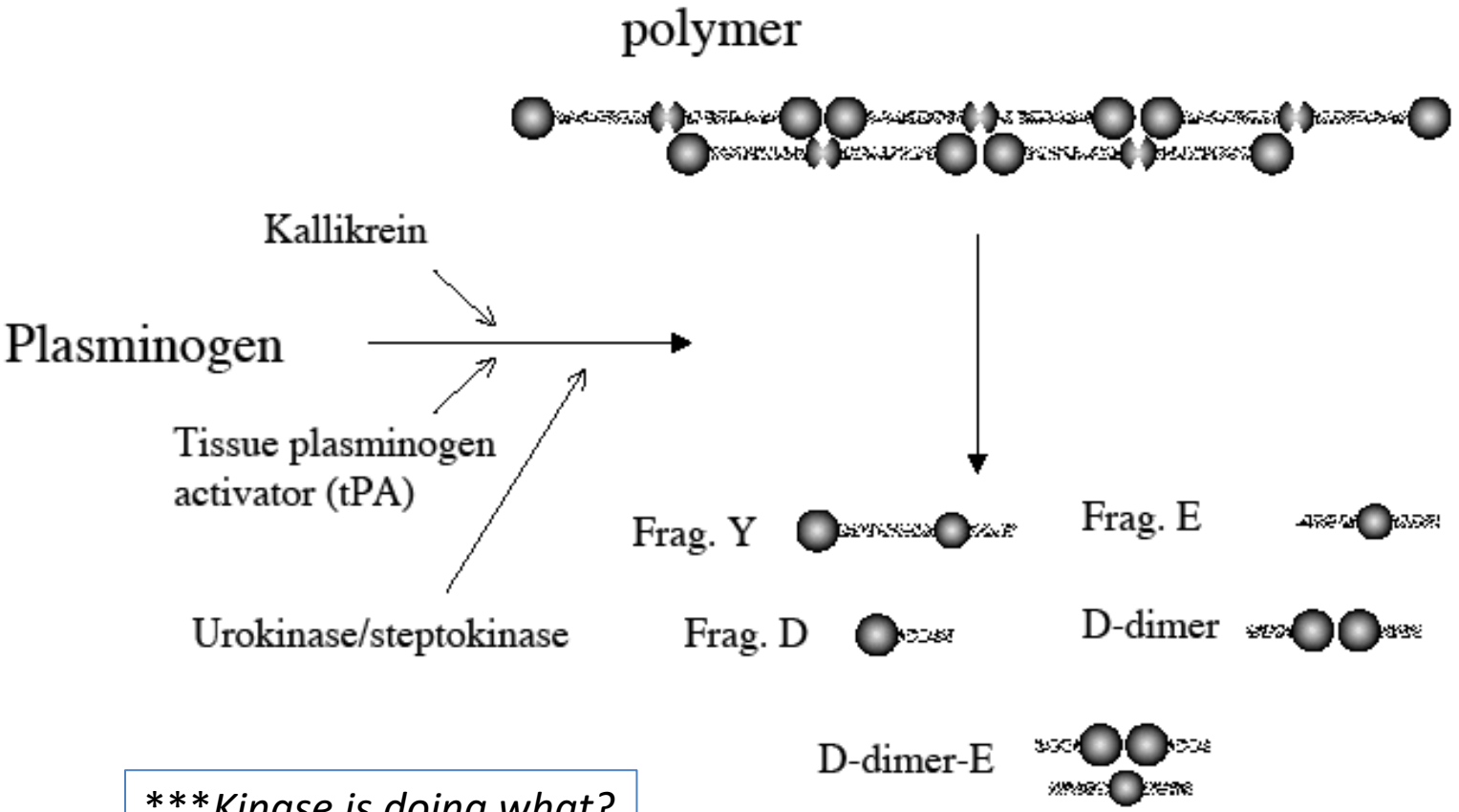
***Origin of Christmas Factor???

Fibrin Assembly



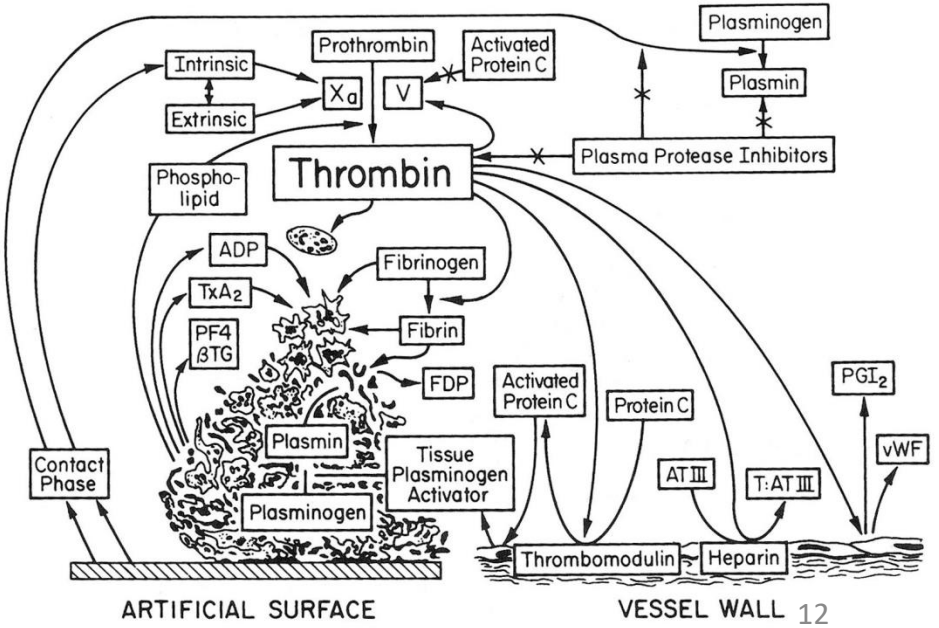
Fibrinolysis: Breakdown of Fibrin Clots



****Kinase is doing what?*

Control Mechanisms

- Required to prevent massive clotting throughout body
- Blood flow reduces local concentration of activated factors
- Rate increases with surface area
- Natural coagulation inhibitors and feedback proteins remove activated proteins
  - antithrombin; protein C system; heparin; thrombomodulin
- Fibrinolysis
  - Activation of plasmin from plasminogen; catalyzed by plasminogen activators such as tissue plasminogen activator (tPA) and urokinase
  - Plasmin in turn cleaves fibrin into different degradation products



Platelets- 1

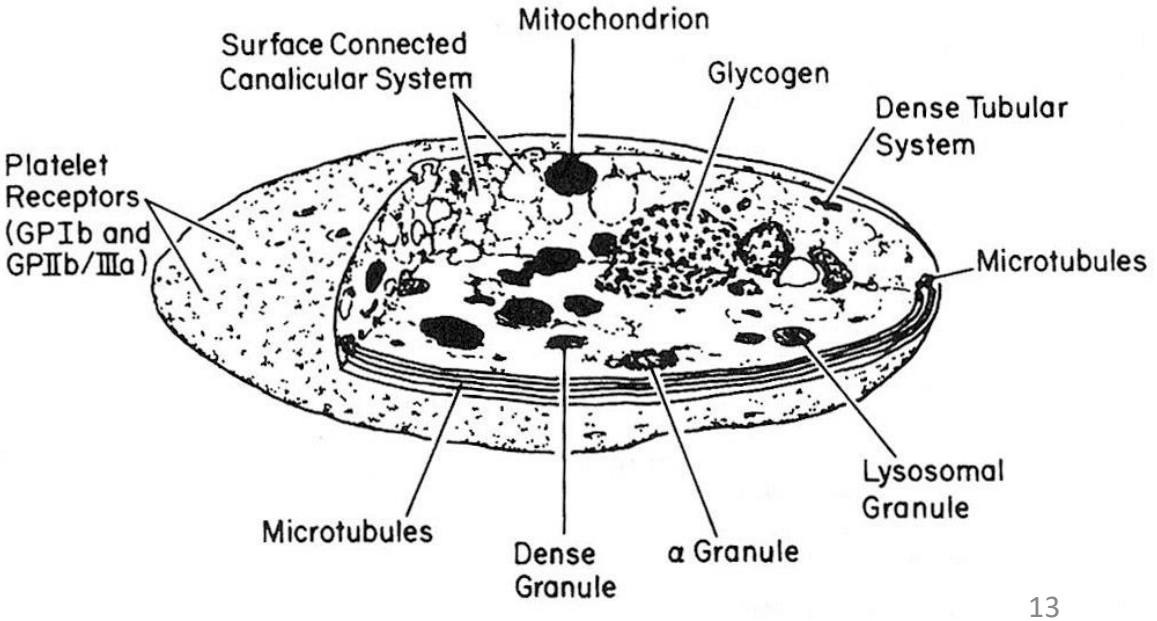
- Non-nucleated, disk-shaped cells having a diameter 3-4 microns

•Platelets are formed in the bone marrow from megakaryocytes; the megakaryocytes fragment into minute platelets either in the bone marrow or soon after entering the blood, especially as they squeeze through the capillaries.

- Functions
  - -Bleeding arrest and stabilize blood clots by
    - •catalyzing thrombin production from prothrombin
    - •forming rapid assembly
- 3 types of cytoplasmic storage granules whose contents are released upon platelet activation (shown below)

Receptors and Binding Proteins

: heparin binding proteins, platelet factor 4 (PF4), b-thromboglobulin, platelet derived growth factor (PDGF), coagulation proteins such as fibrinogen, von Willebrand factor, factor V and factor VIII, and ECM proteins such as fibronectin

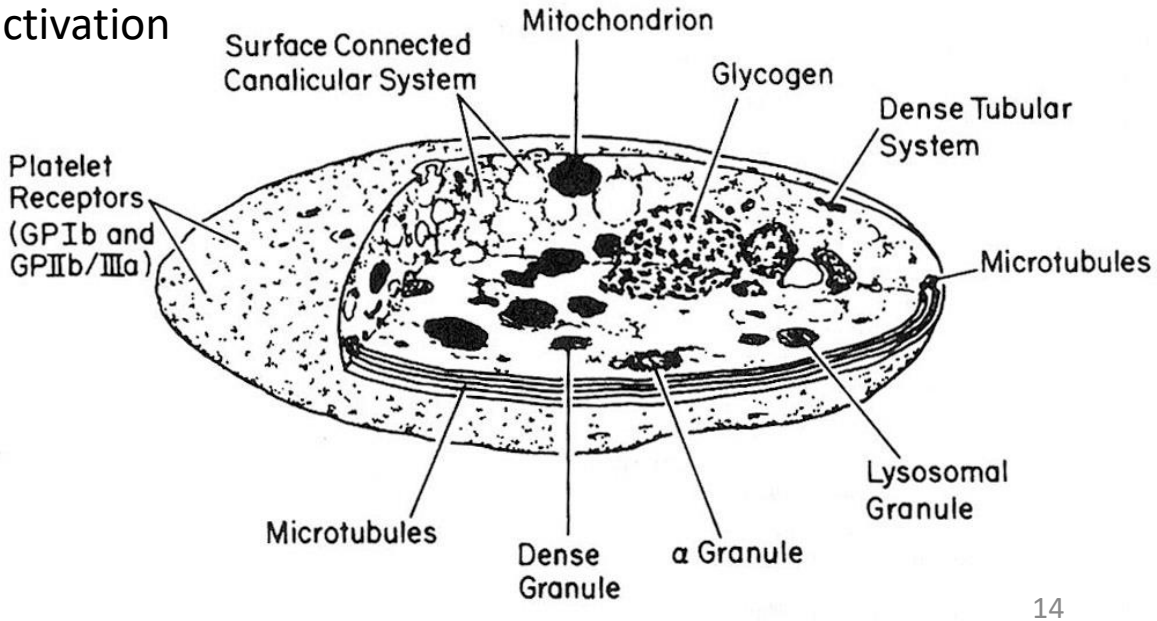


Platelets- 2

- **<u>Dense</u> granules**: adenosine diphosphate (ADP), serotonin, and Ca^{2+}
- **<u>Lysosomal</u> granules**: enzymes such as acid hydrolase
- Platelets have a short half-life= 8-12 days.

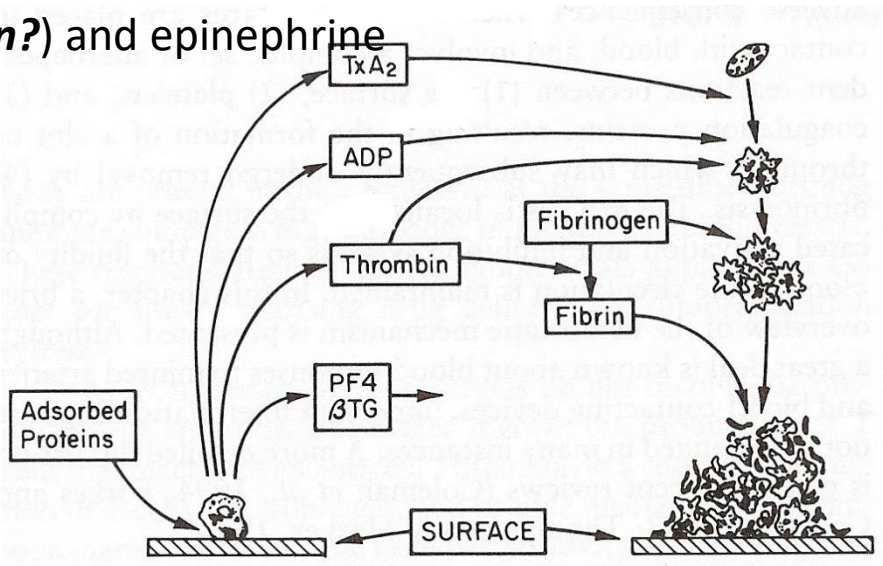
- Activated by ADP, thrombin, fibrinogen binding etc

- Adhesion, aggregation and activation

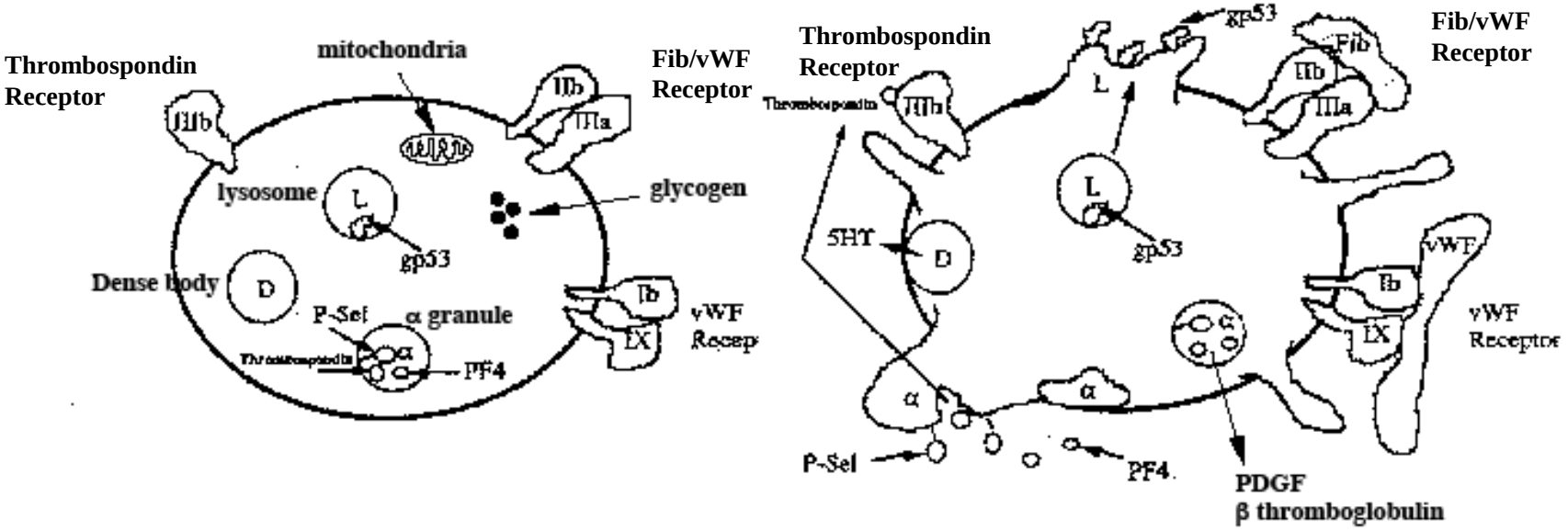


Platelet Adhesion, Aggregation and Activation

- Adhere to both artificial (implants) and natural surfaces (ECM, vascular injuries)
- Interaction of surface receptors
  - GPIIb/IIIa (most abundant) to RGD of fibrinogen, fibronectin, vitronectin, von Willebrand factor etc
  - GP1b to von Willebrand factor
  - GPII/I to collagen
  - Mediated by Ca^{2+}
- Aggregation through the binding with "bridging molecules" such as fibrinogen
- Recruitment of more platelets by cytokines and other mediators such as thromboxane A2, arachidonic acid (***Aspirin?***) and epinephrine
- Activation
  - Initiation of contractile processes lead to shape change; from discoid to pseudopodium formation
  - Release of granule contents
- ADP, thrombin: platelet activators
- TxA2: recruitment of more platelets
- Ca^{2+}



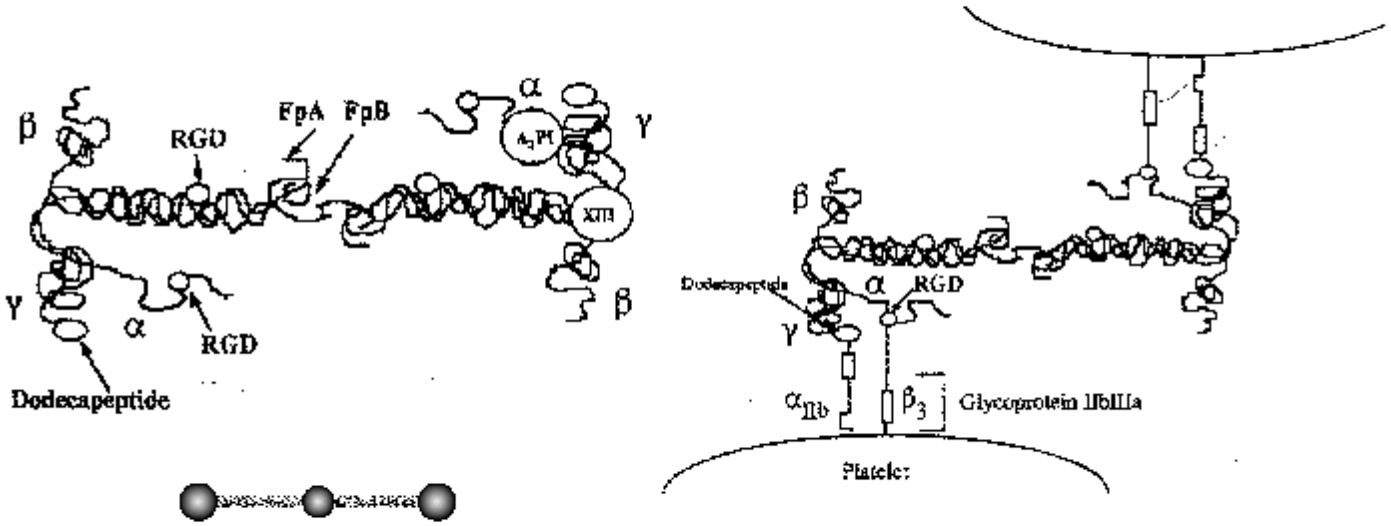
Platelet Activation



Resting

Activated

Platelet-Fibrinogen Bridging



Drugs that Inhibit Platelet Functions

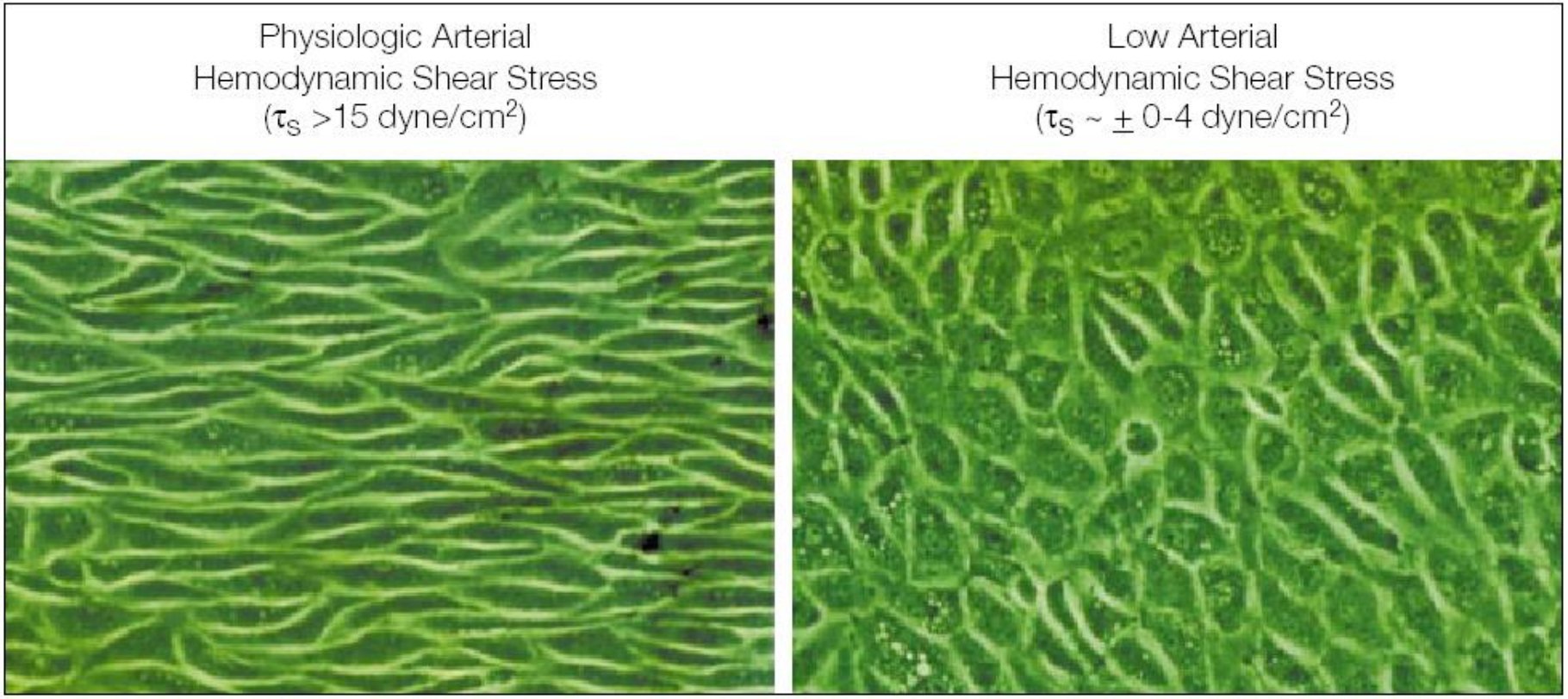
- Inhibitors of prostaglandin and thromboxane synthesis
  – Inhibit phospholipase activity: hydrocortisone
  – Cyclo-oxygenase inhibitors: aspirin (irreversible)
  – Ibuprofen (reversible)

- Substances that decrease Ca^{2+} availability
  – Inhibit phosphoesterase: diphyridamole
  – Activate adenylate cyclase: iloprost

- Drugs that act on biological membranes
  – Ticlopidine (Ticlid), Clopidogrel: unknown mechanism

- Drugs that block platelet aggregation
  – Antibody against GPIIb-IIIa
  – RGD peptide analogs (non)peptide-based: Block GPIIb-IIIa

Effect of Blood Flow on Endothelial Cell Shape



Functions of Endothelial Cell

- A semipermeable barrier for the transfer of substances between blood and surrounding tissues (tight junctions in brain make a tighter barrier = blood brain barrier [BBB]).
- Mediation of vascular repair processes
- Processing of antigen immunity
- Maintenance of thromboresistance
- Synthesis of mediators that regulate interactions between vessel wall and blood components
  - Factor VIII, von Willebrand's factor
  - Fibronectin
  - Collagen
  - Thrombomodulin
  - Tissue plasminogen activator (tPA)
  - Plasminogen activation inhibitor (PAI-1)

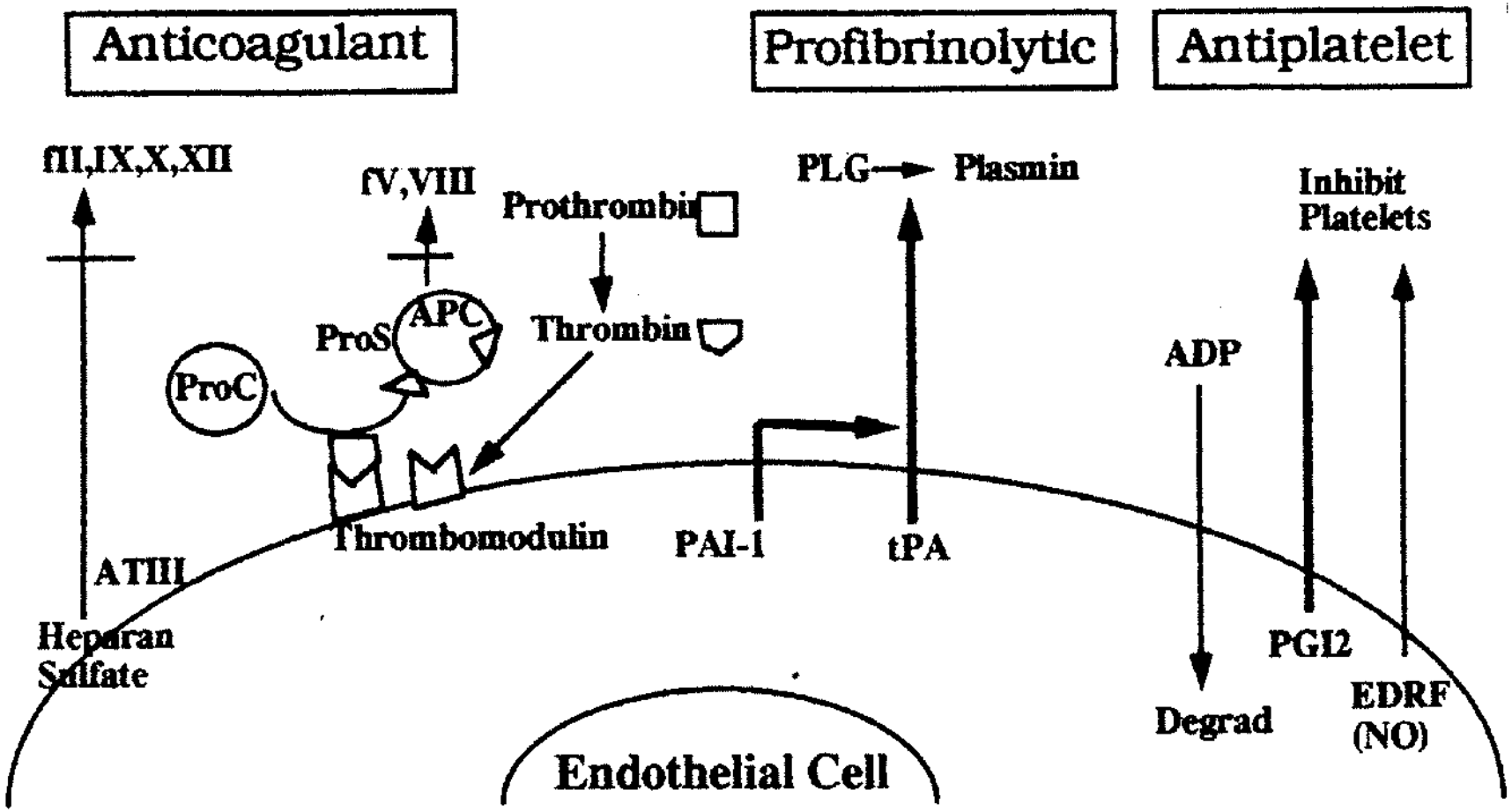
Thromboresistance of Endothelial Cell

- Antiplatelet
  - Prostacyclin (PGI_2): inhibits platelet adhesion and aggregation
  - Degradation of ADP, a platelet activator

- Anticoagulant
  - Thrombomodulin
    - Uptake, inactivation and clearance of thrombin
    - Participate in Protein C activation
  - Heparan sulfate
    - Antithrombin III binding

- Profibrinolytic
  - Plasminogen activators: plasmin production for fibrin degradation (e.g., tissue plasminogen activator (tPA))

Thromboresistant Role of Endothelium



Consequences of Endothelial Disruption

- Vasoconstriction
- Platelet adhesion to subendothelium
  – Aggregation and activation
- Initiation of coagulation
  – Coagulation factor released from endothelium
    - fV, fVIII, von Willebrand factor, tissue factor
- Fibrinolysis follows the release of tissue plasminogen (tPA) from endothelium

Stimuli that Change Endothelium to Procoagulant State

- Endotoxin
- Cytokines
  - TNFa, IL-1
- Thrombin
- Others
  - Substrates

Prothrombotic Roles of Endothelium

- Procoagulatant
  – Increase procoagulant (vWf/fVIII)
  – Decrease inhibitors

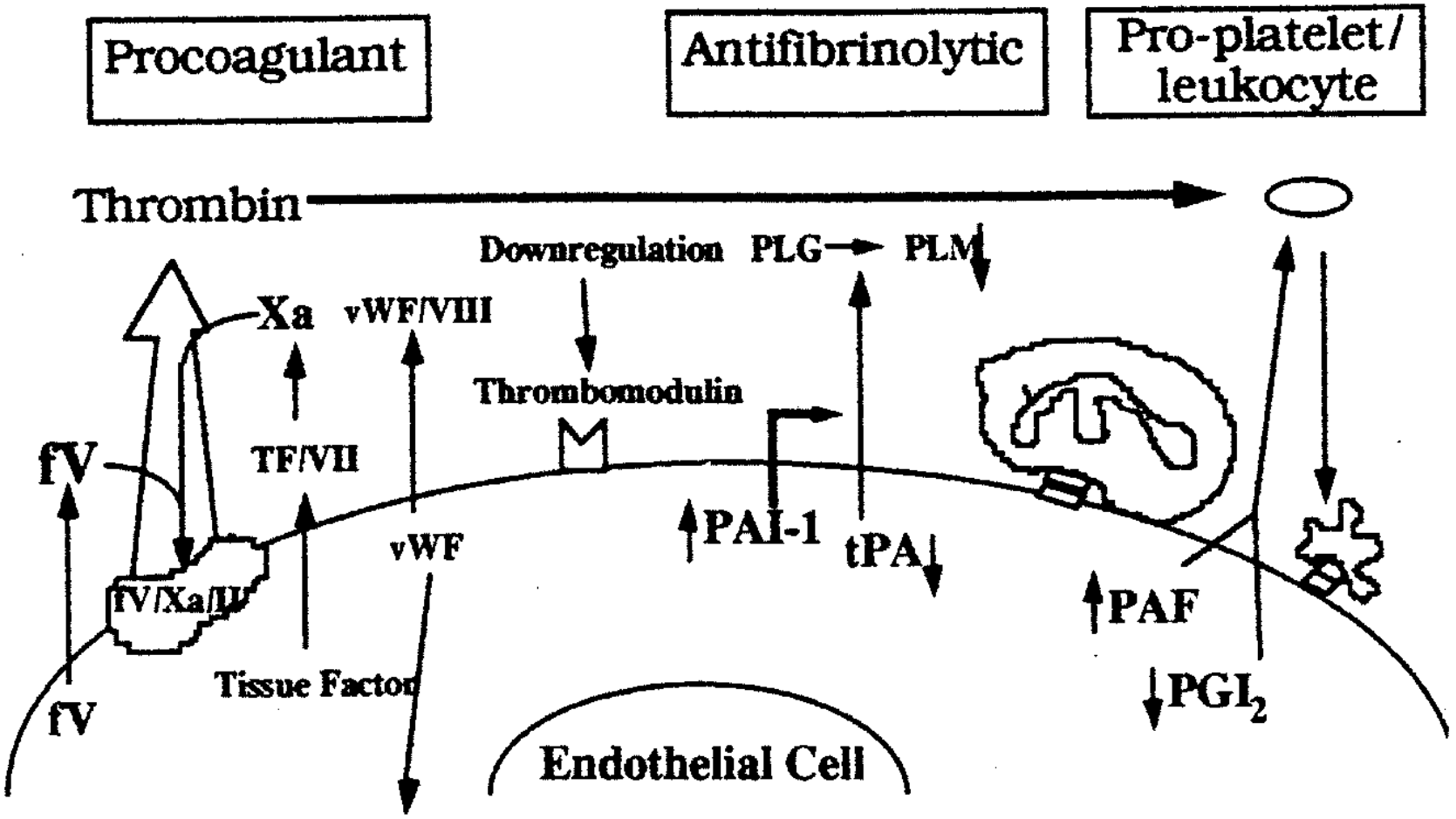
- Anti-fibrinolytic
  – Increase plasminogen activator inhibitor (PAI-1)
  – Decrease tissue plasminogen activator (tPA)

******Use of tPA after stroke****?

- Pro-platelet activating
  – Platelet activating factor (PAF)

- Increase leukocyte adhesion

Prothrombotic Role of Endothelium

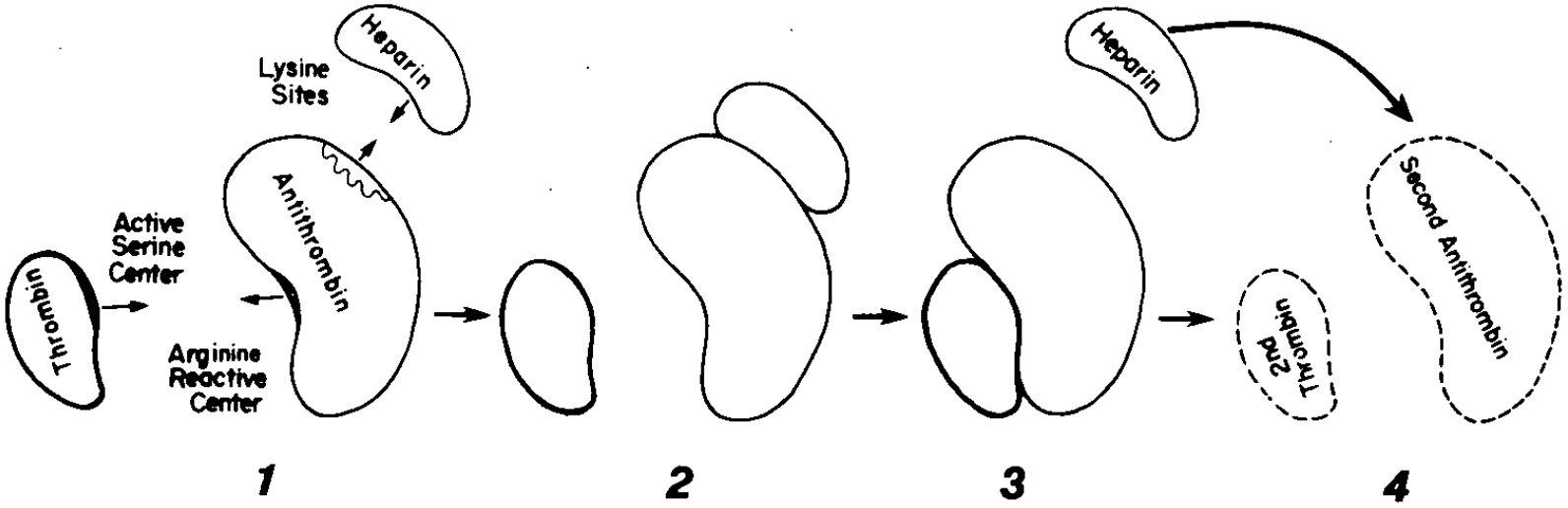


Antithrombotic Agents

- Anticoagulants
  - Heparin
  - Coumadin
  - Hirudins (produced by the leech)
  - Sodium citrate
  - Recombinant proteins of thrombomodulin, antithrombin III, tissue factor pathway inhibitor

- Antiplatelet agents
  - Inhibitors of prostaglandin and thromboxane synthesis (e.g., aspirin, ibuprofen)
  - Decrease the availability of intracellular Ca^{2+}
  - Block platelet aggregation (e.g., anti-GPIIb-IIIa antibodies)
  - Act on biological membrane

Mechanism of Heparin Action



1. Heparin binding to antithrombin III - (***What charge is Heparin***?)

2. Conformational changes of antithrombin III

3. Enhances binding of Antithrombin with thrombin (makes Antithrombin 100-1,000 x more effective). ***So what effect on blood clotting***?

4. <u>Reuse</u> of heparin

Warfarin (Coumadin) Therapy

- Vitamin K exists in two forms:
  - K1: from leafy vegetables and oils
  - K2: from bacteria of gut

- Absorbed in the presence of bile salts
- No significant body storage
- Necessary for full function of coagulation factors (II, VII, IX, X, Pro C)
- Warfarin/Coumadin competes with Vitamin K– ***so is pro- or anticoagulant***?

Weblinks

- ***Coagulation cascade (text is there, but couldn't see plugin)

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End!