

# HOMEOSTASIS

Ideal balance of several systems:

- endothelium of vessel wall
- collagen below endothelium
- tonus of the vessels
- number and quality of platelets
- clotting and fibrinolytic systems
- character of blood flow in the vessel

prevents ***bleeding*** on one side and ***intravascular blood clotting*** on the other side.

# **HEMOSTASIS** (blood clotting, stop of bleeding)

= set of mechanisms which prevent bleeding on one side and stop already existing bleeding on the other side.

- Reaction of vessels
- Actions of platelets
- Blood clotting

# REACTION OF VESSELS

Vasoconstriction.

Vasoconstriction depends on the severity of vascular injury.

Serotonin (granules in platelets).

Adrenalin.

Fibrinopeptides.

# PLATELETS (THROMBOCYTES)

Nucleus-less, colorless, granulated, the smallest formed elements in blood.

**Origin:** megakaryocytes of bone marrow under the effect of colony stimulating factors – interleukins (*IL-1, IL-3, IL-6*) and granulocytes and macrophages stimulating factor (*GM-CSF*)

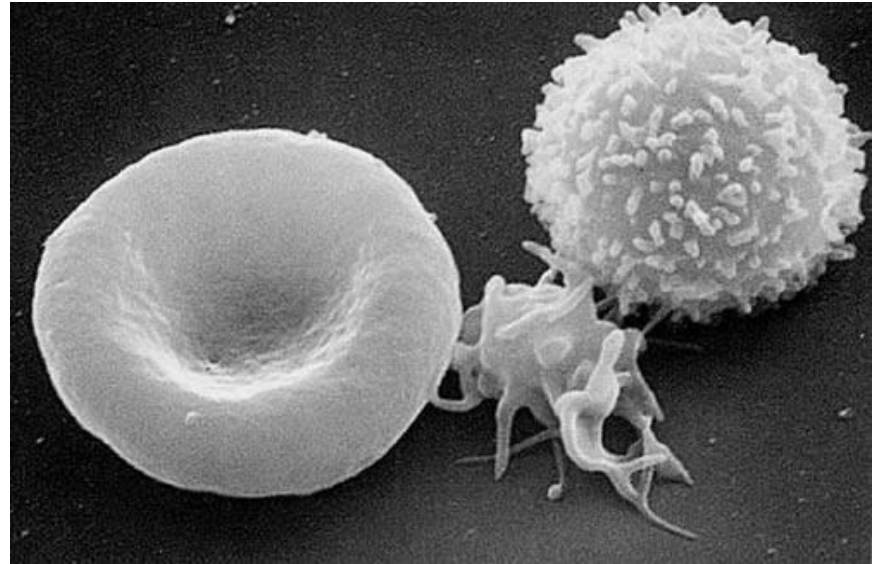
**Number:** 200 000 – 500 000 in  $\mu\text{l}$ , one third in spleen and two thirds in peripheral blood

No age and gender differences in platelet count.

Trombocytosis – after splenectomy.

**Size:** 2 – 4  $\mu\text{m}$  in diameter, 0,5 – 1  $\mu\text{m}$  thickness, 4 – 8 fl volume

**Shape:** smooth, round discs



The shape is kept by cytoskeleton (disk of microtubules around the periphery, invaginated membrane, canalicular system connected to extracellular space).

**Membrane:** contains receptors for adhesion to certain surfaces, e.g. collagen, von Willebrand factor, fibrinogen

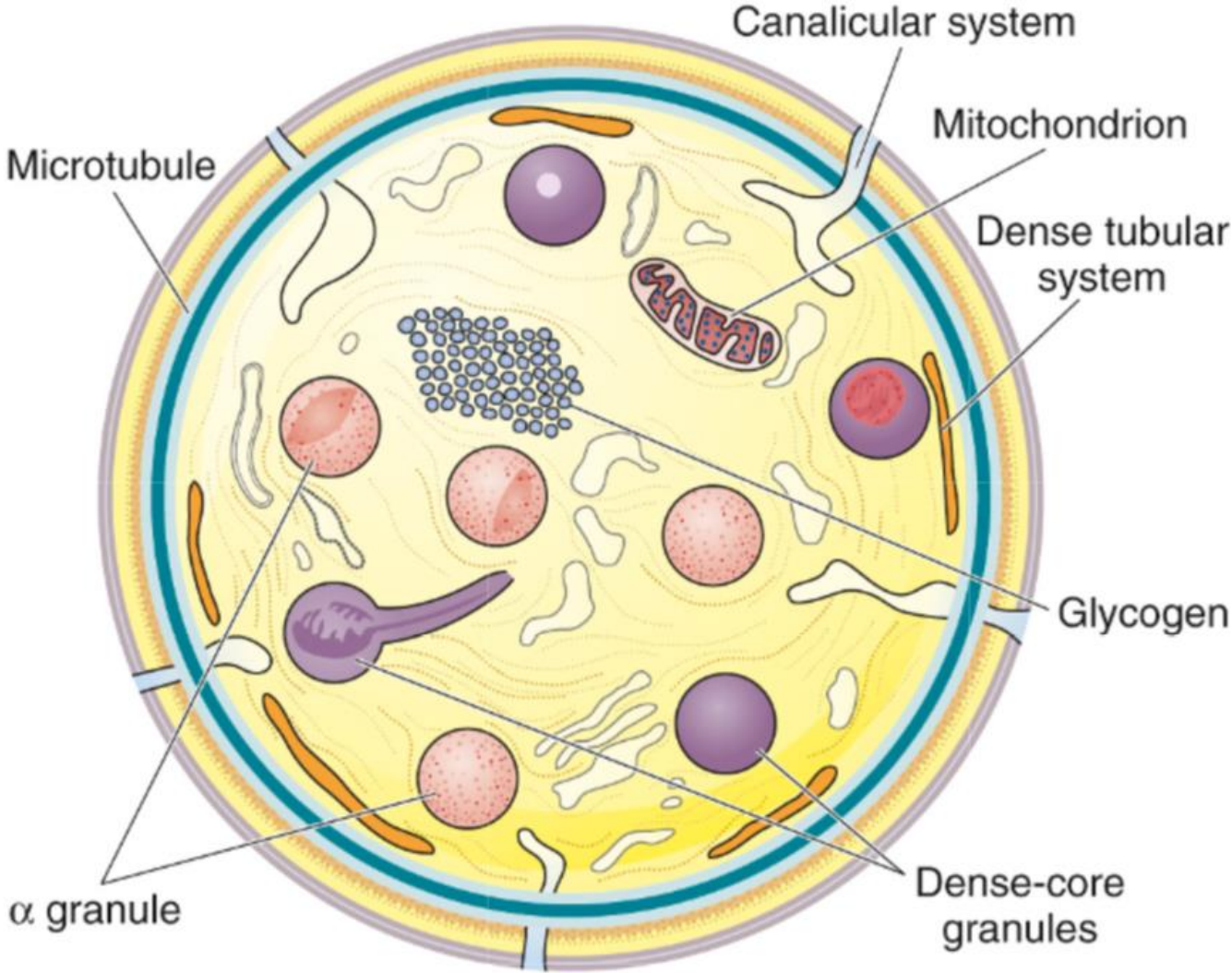
**Cytoplasm:** contains actin, myosin, glycogen, lysosomes and

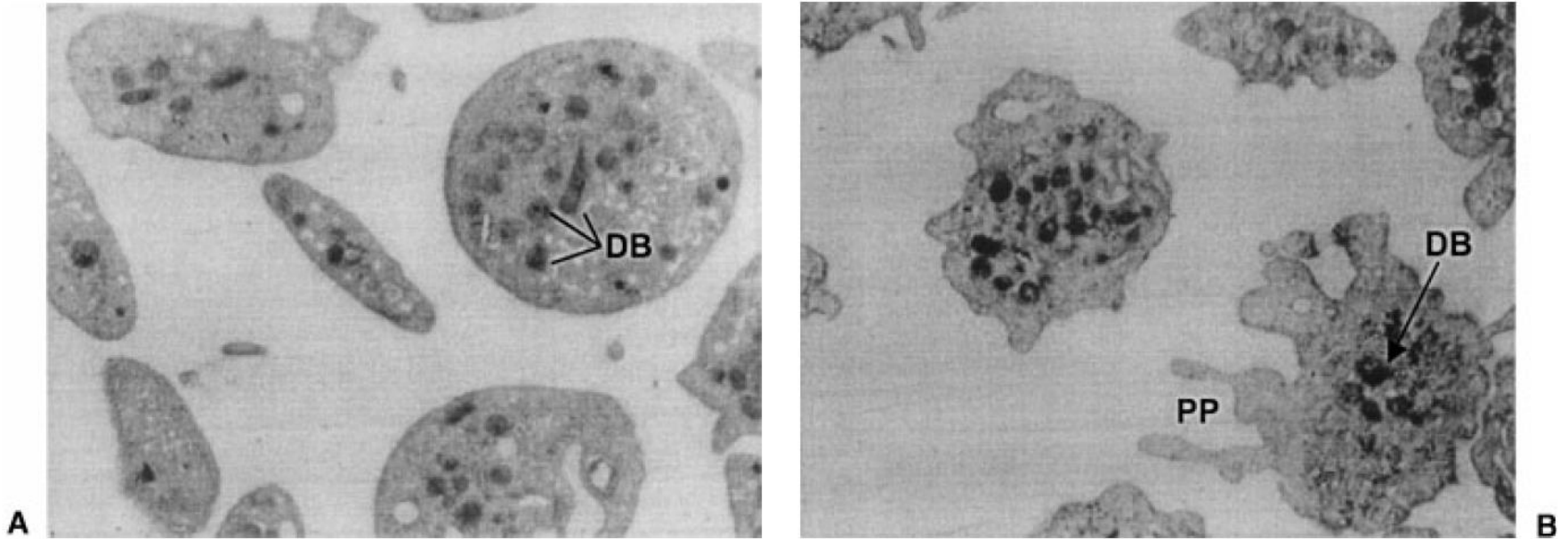
**Granules:** *dense granules* (non-protein substances – serotonin, ADP, adenonucleotides) and  *$\alpha$  granules* (protein substances - clotting factors, platelet derived growth factor – PDGF)

**Glycocalyx:** 10 – 50nm, mixture of proteins and mucopolysaccharides (clotting factors, ions, amino acids, histamin, drugs...)

**Life span:** 9 – 12 days, biological half-time – about 4 days

# Structure of thrombocyte





**Figure 1** Morphology of human platelets. (A) Thin section of discoid resting platelets with evenly distributed granules. (B) Thin section of stimulated platelets, showing formation of pseudopodia and centralization of granules. DB, dense body; PP, pseudopodium. Magnification  $\times 21,000$ .

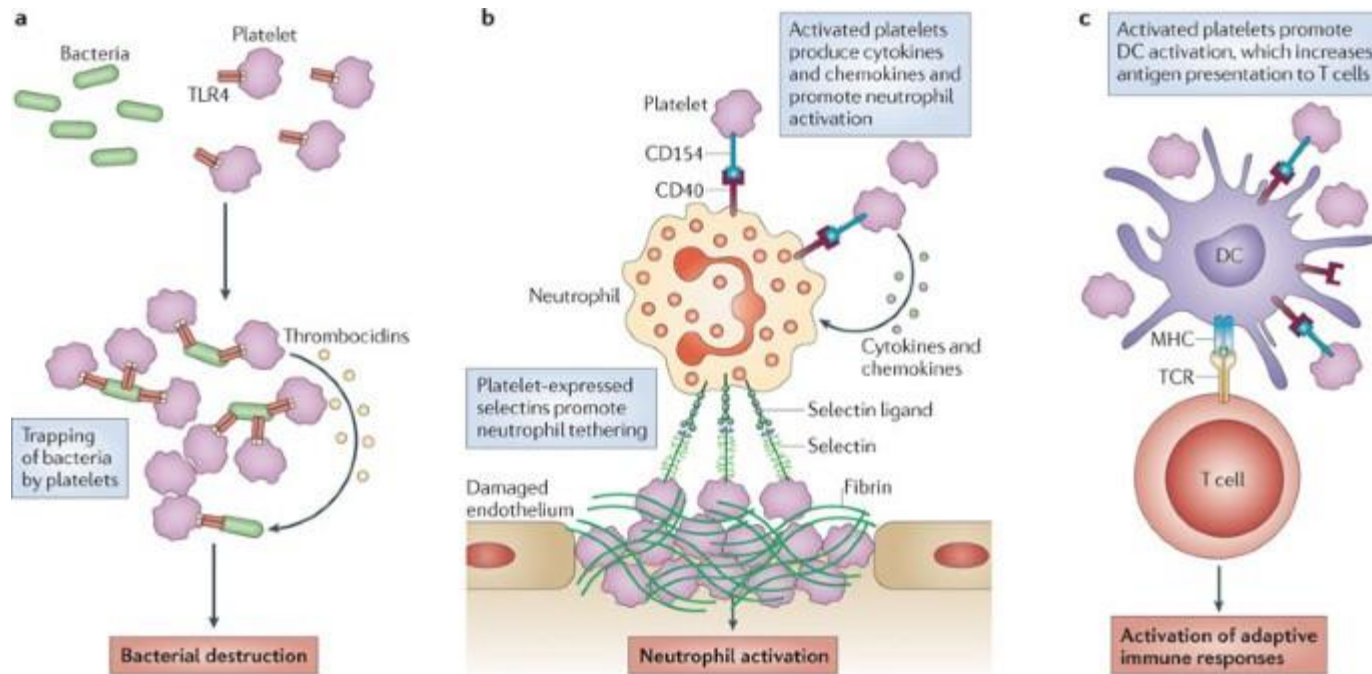
Jurk K, Kehrel BE: **Platelets: Physiology and biochemistry. *Seminars in Thrombosis and Hemostasis* 2005, 31(4):381-392.**



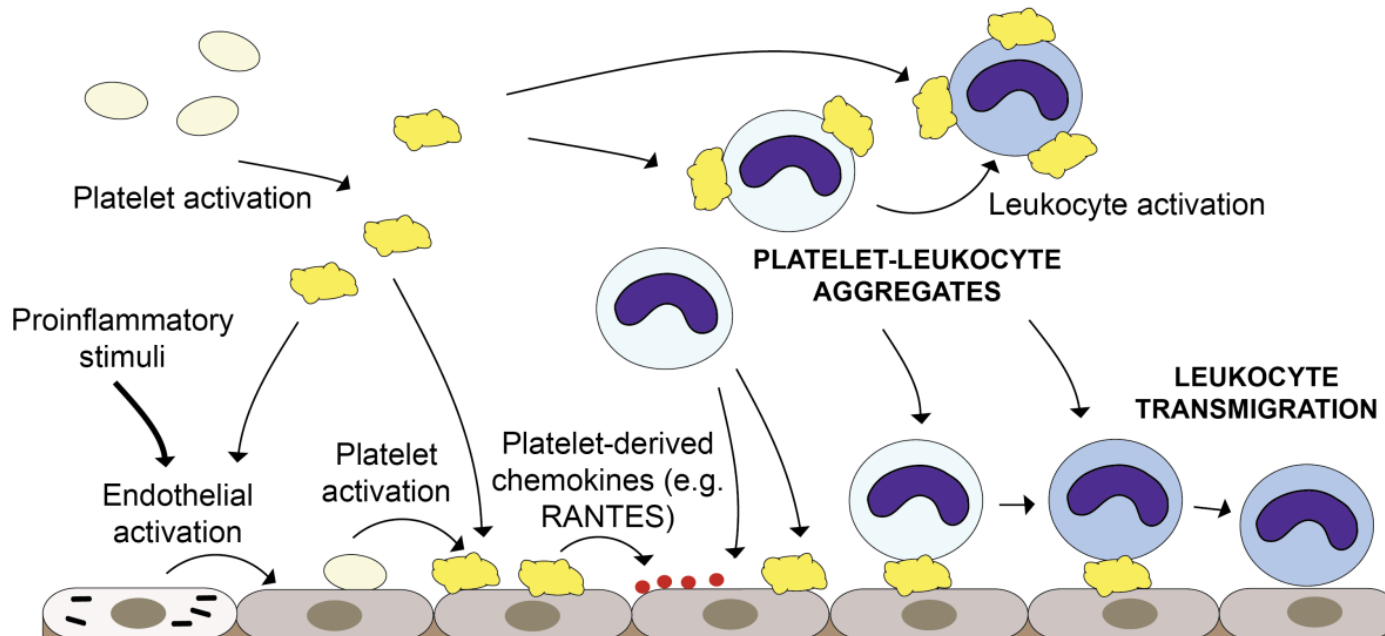
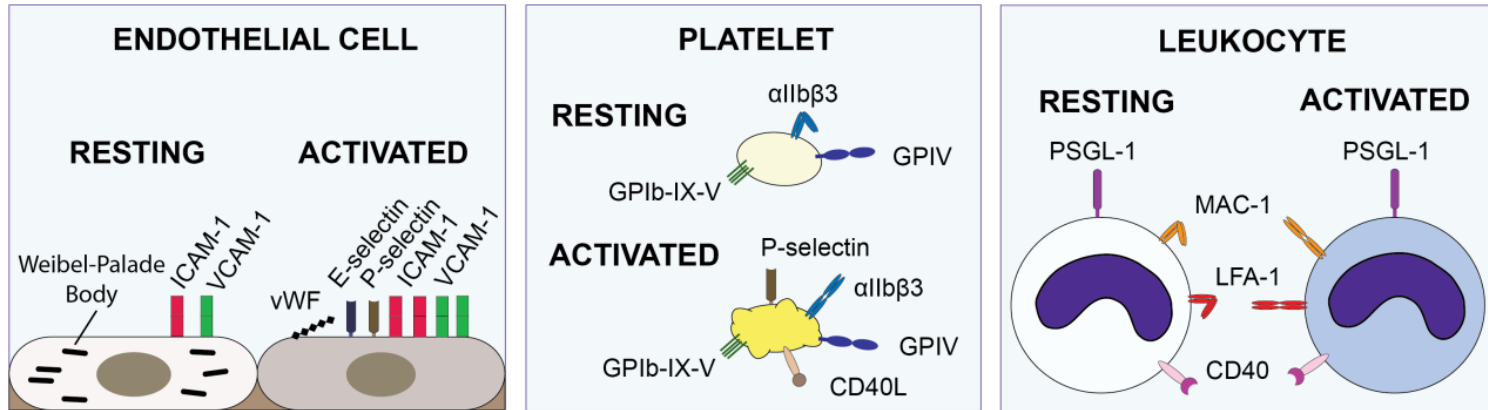
# Function of platelets

- Protection of organism from blood loss
- Keeping the integrity of vessel wall and healing of the ruptured vessel (PDGF from  $\alpha$ -granules)
- Inflammatory reactions, changes in permeability of capillaries, removing of xenogenous substances, viruses, bacteria, graft rejection ...
- Carrier for many substances absorbed to platelets surface

# Immune function of platelets



# Platelets and inflammation



# HEMOSTASIS I. – white clot

**Adhesion** (exposure of the vessel wall – collagen – receptors for collagen on platelet, laminin, von Willebrand factor).

**Activation and change of shape** – collagen, ADP, thrombin. Glycoprotein IIb/IIIa receptors.

## **Secretion (degranulation):**

Stimulation of aggregation – ADP

Stimulation of adhesion – vWF and fibronectin

Vasoconstriction – serotonin, thromboxane  $A_2$

mitogenic effects – growth factor (**PDGF**)



activation of platelets and phagocytes – **PAF** (cytokine, G-coupled receptor, phospholipase C, DAG, increase of intracellular  $Ca^{2+}$  concentration, phospholipase  $A_2$  – arachidonic acid – thromboxane  $A_2$ )!!! Therapeutic use of acetylsalicylic acid!!!

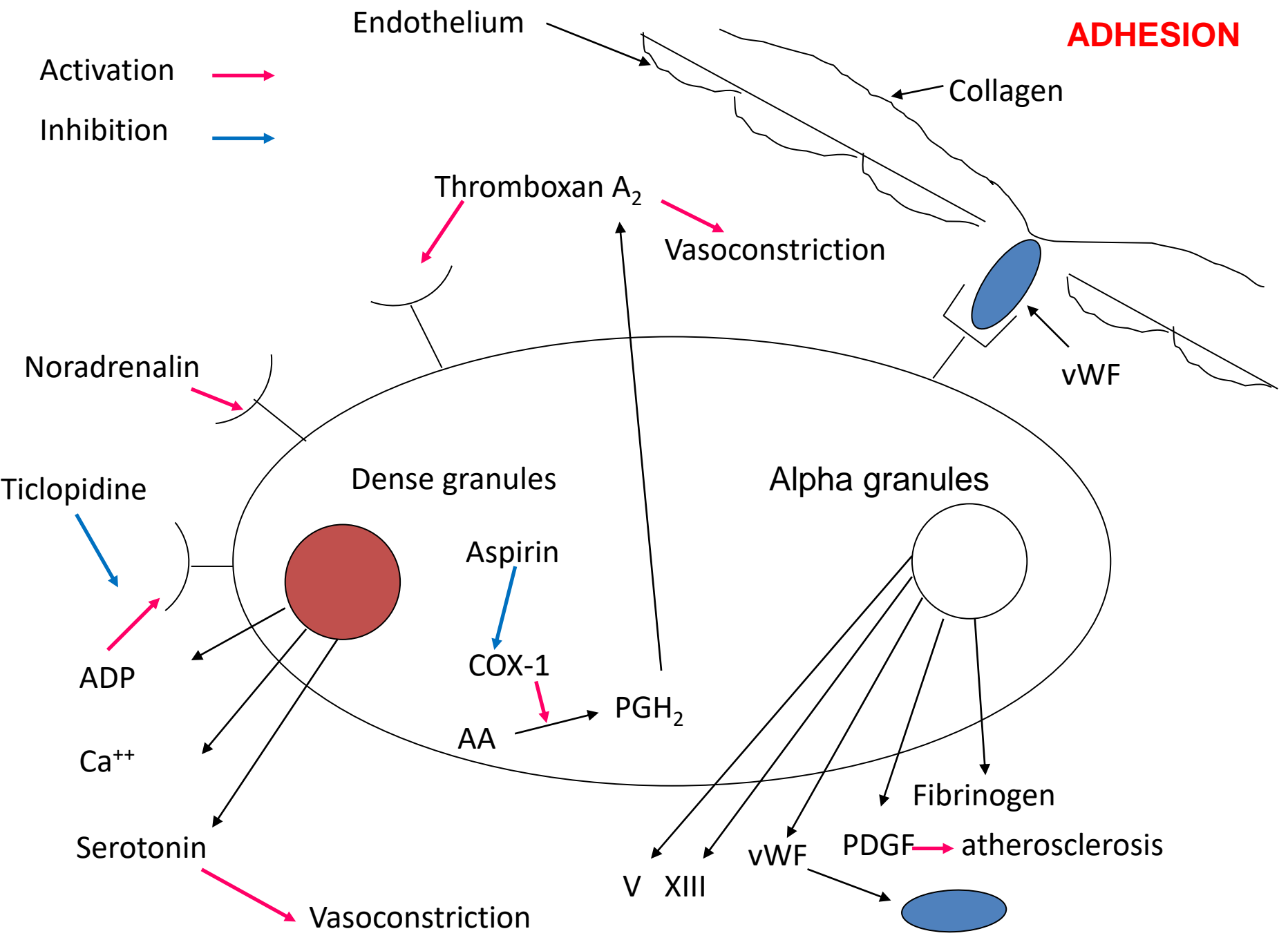
## **Aggregation.**

## **Vasoconstriction.**

## **Convolution of inner layer of vessel wall (at the place of rupture).**

# ADHESION

Activation   
Inhibition 



Inhibition →

Fibrinogen



vWF

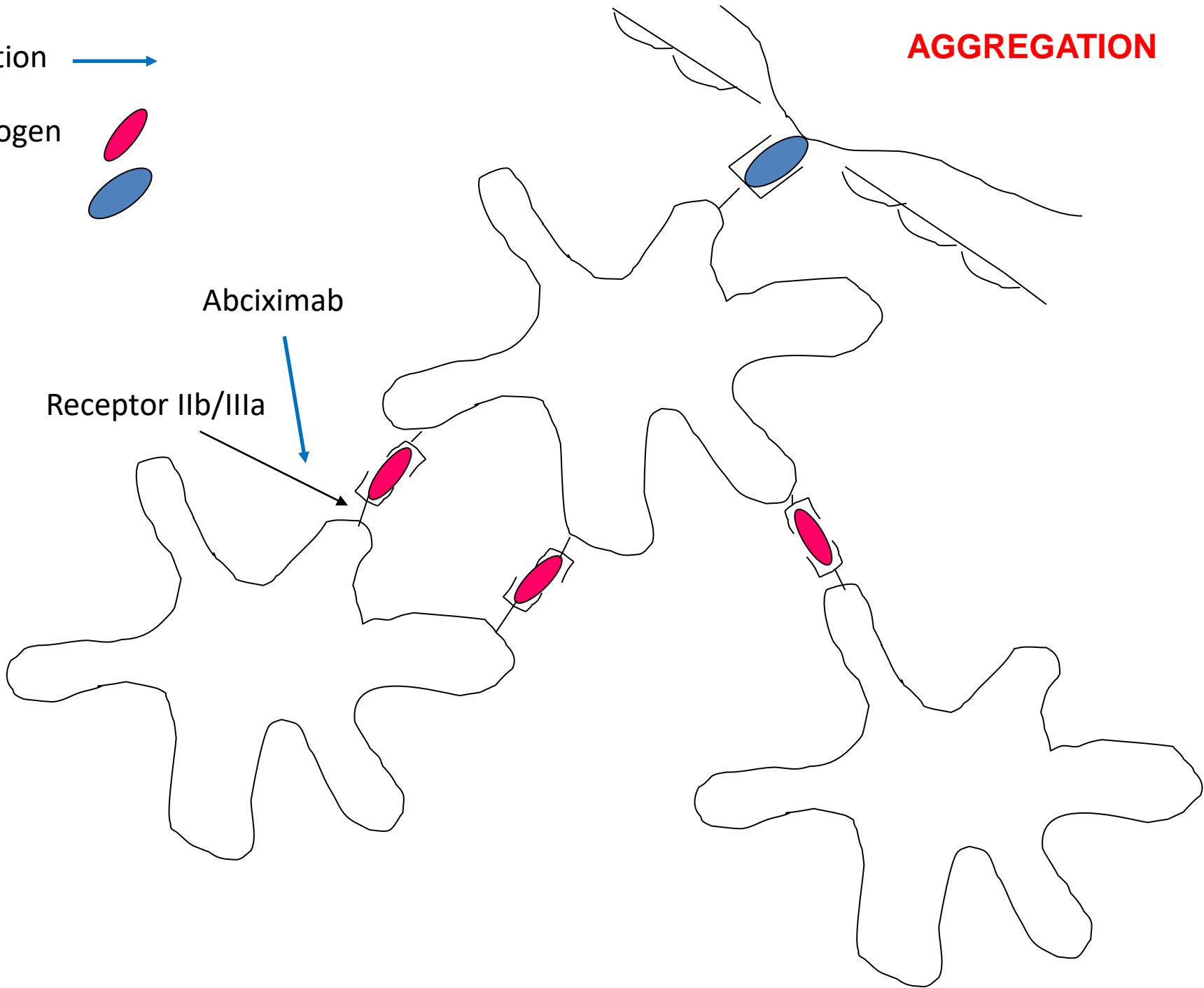
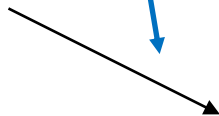


**AGGREGATION**

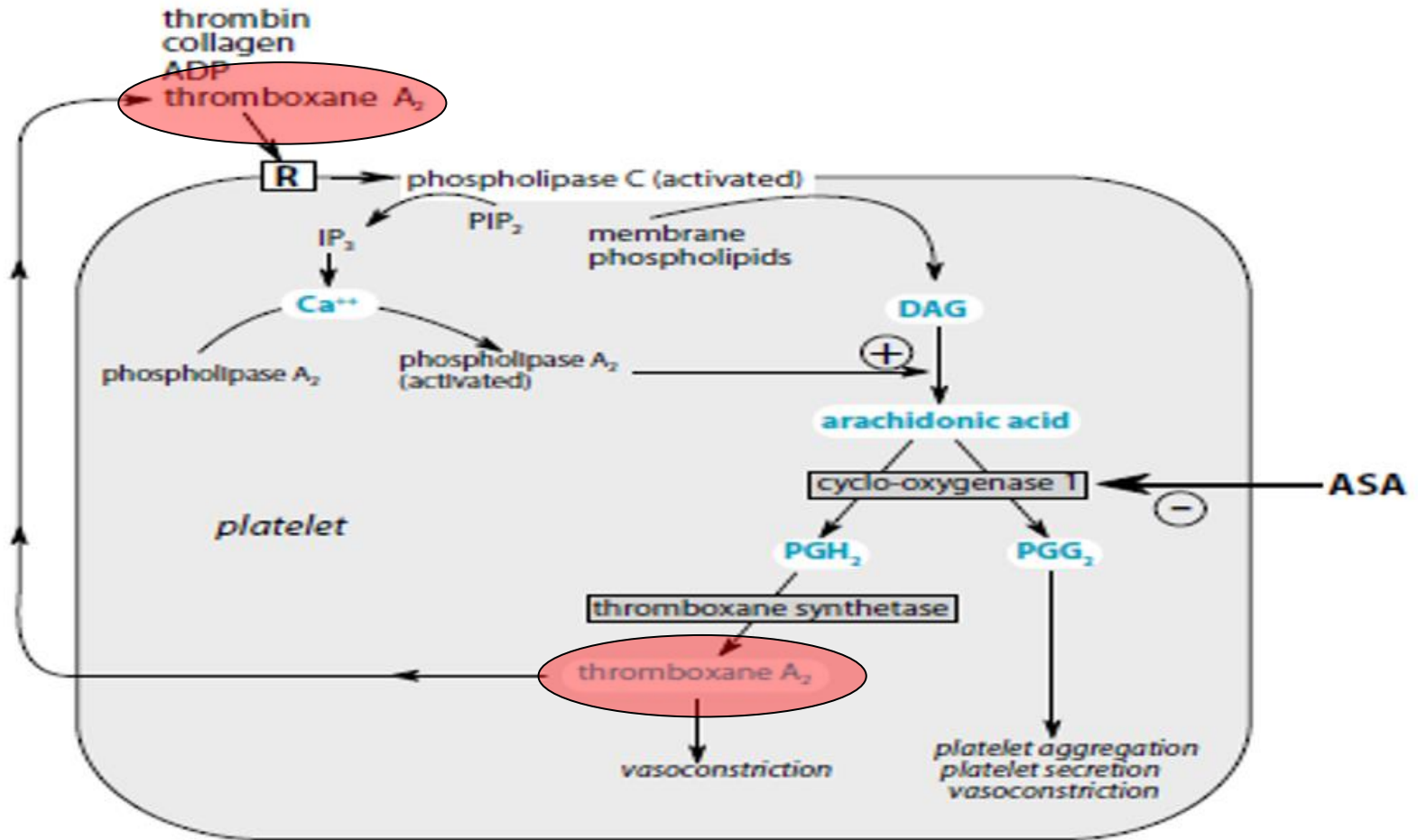
Abciximab



Receptor IIb/IIIa



# Aggregation – an example of positive feedback



# HEMOSTASIS II. – red clot

Prothrombin (factor X) – thrombin.

Fibrinogen – fibrin monomer – fibrin polymer (factor III,  $\text{Ca}^{2+}$ ).

*Intrinsic* pathway – *extrinsic* pathway of factor X activation.



# The three pathways that makeup the classical blood coagulation pathway

## Intrinsic

surface contact

XII → XII<sub>a</sub>

XI → XI<sub>a</sub>

IX → IX<sub>a</sub>

(VIII, PL, Ca<sup>++</sup>)

X → X<sub>a</sub>

(V, PL, Ca<sup>++</sup>)

prothrombin → thrombin (serine protease)

fibrinogen → fibrin → XIII<sub>a</sub> → stable fibrin clot

XII – Hageman factor, a serine protease  
 XI – Plasma thromboplastin, antecedent serine protease  
 IX – Christmas factor, serine protease  
 VII – Stable factor, serine protease  
 XIII – Fibrin stabilising factor, a transglutaminase  
 PL – Platelet membrane phospholipid  
 Ca<sup>++</sup> – Calcium ions  
 TF – Tissue Factor ( <sub>a</sub> =active form)

## Extrinsic

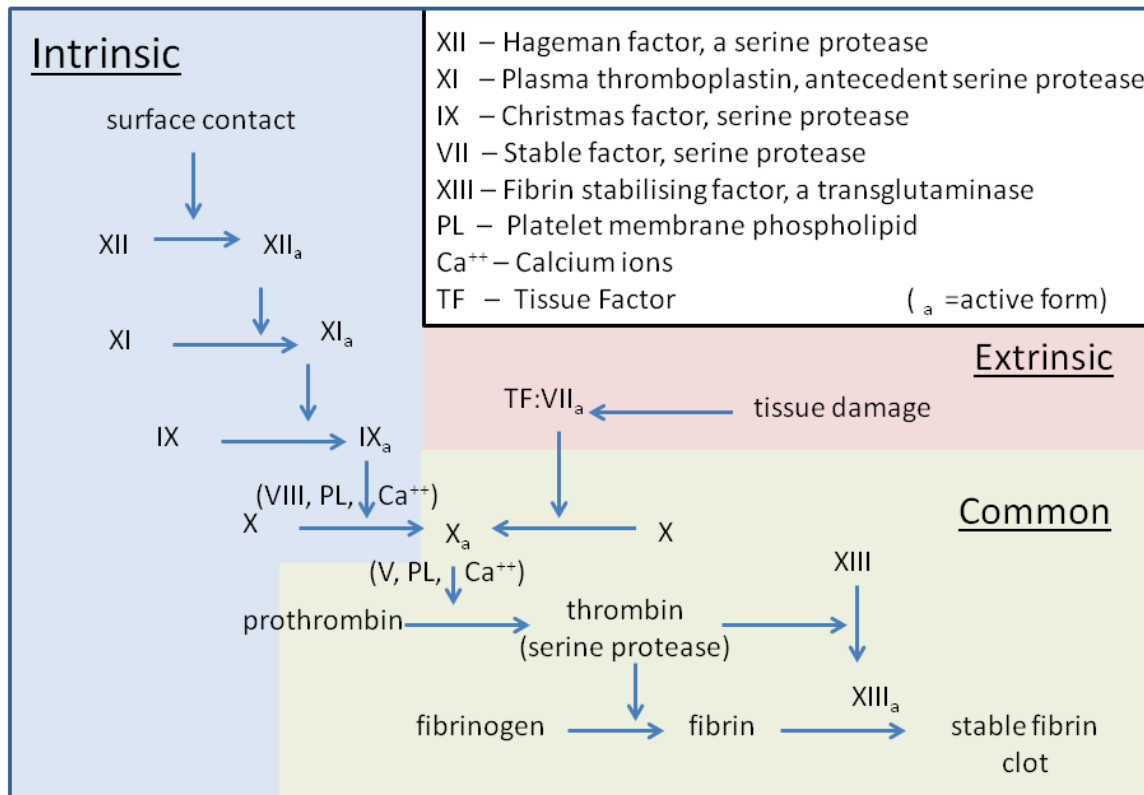
TF:VII<sub>a</sub>

tissue damage

## Common

# Intrinsic pathway

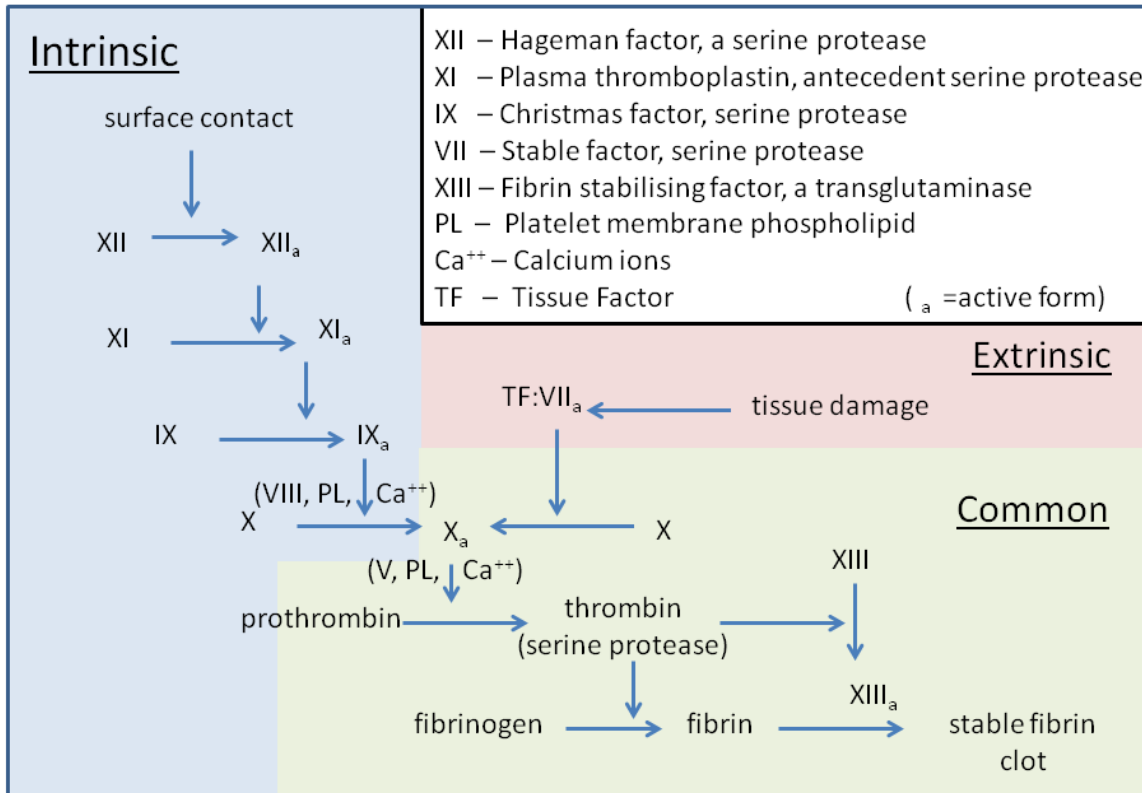
The three pathways that make up the classical blood coagulation pathway



- Factors IX<sub>a</sub>, X<sub>a</sub>, and thrombin proteolytically cleave Factor VIII to form VIII<sub>a</sub>, which is the co-factor of the next reaction.
- VIII<sub>a</sub>, together with IX<sub>a</sub>, calcium ions (from the platelets) and negatively charged phospholipids, form the trimolecular complex of tenase
- Tenase converts factor X to X<sub>a</sub>.

# Extrinsic pathway

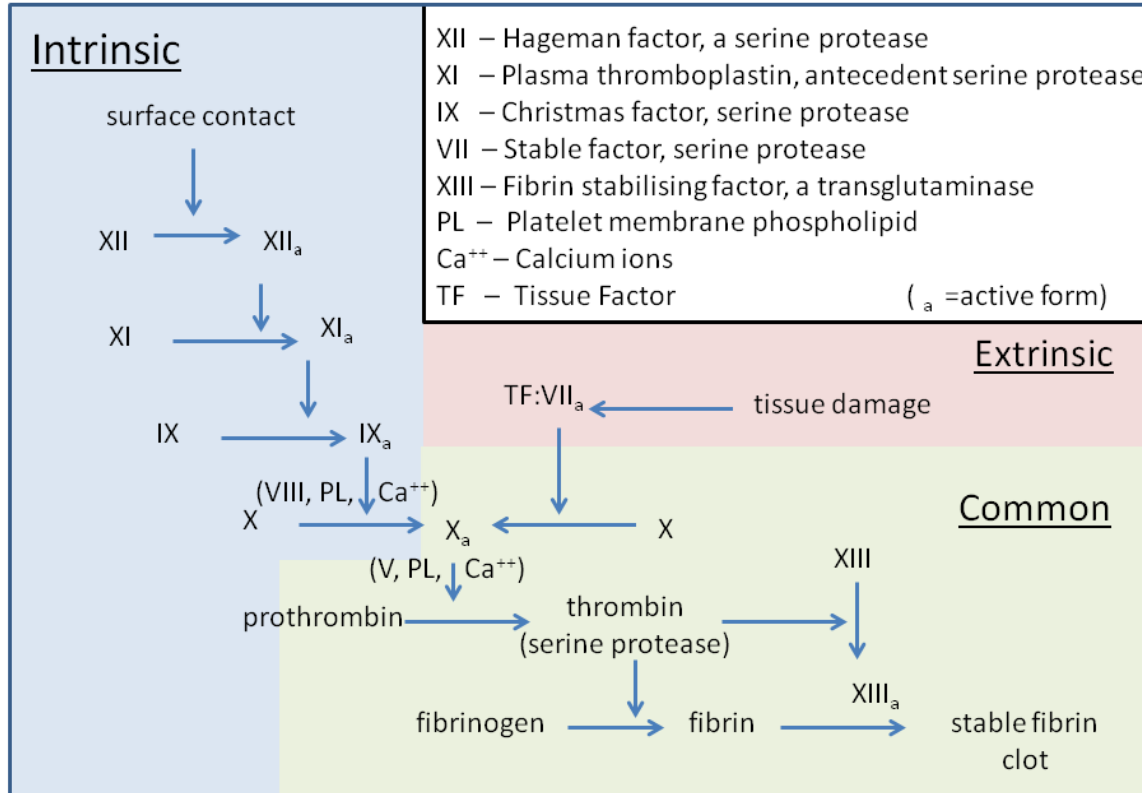
The three pathways that make up the classical blood coagulation pathway



- Initiated by factors outside of the vascular system
- Expression of tissue factor outside the vessel
- It is a receptor for plasma protein - factor VII
- Activation - VII<sub>a</sub>
- Together with calcium ions, the formation of a trimolecular complex, which resembles tenase
- Proteolytic activation of factor X

# Common pathway

The three pathways that make up the classical blood coagulation pathway



- Initiated by factor X<sub>a</sub>
- Subsequent activation of Factor V<sub>a</sub>
- Creation of the trimolecular complex (X<sub>a</sub>, V<sub>a</sub>, calcium ions together with PL) = prothrombinase
- Conversion of prothrombin to thrombin
- Conversion of fibrinogen to fibrin

## Thrombin

- Thrombin catalyses the conversion of proteolysis of fibrinogen
- Fibrin monomers spontaneously polymerize and form gel - capture of blood elements
- Activation of factor XIII and formation of polymer network
- Thrombin catalyses the formation of further thrombin, and V<sub>a</sub> and VIII<sub>a</sub> - positive feedback
- Paracrine action of thrombin - endothelial cells release NO, prostaglandin I<sub>2</sub>, ADP, vWF, TPA - thrombocytes (PAR-1) - thrombocyte association with coagulation cascade

# Modern concept - phases of coagulation

## 1. Initiation phase

- = extrinsic pathway, exposure of TF and subsequent cascade

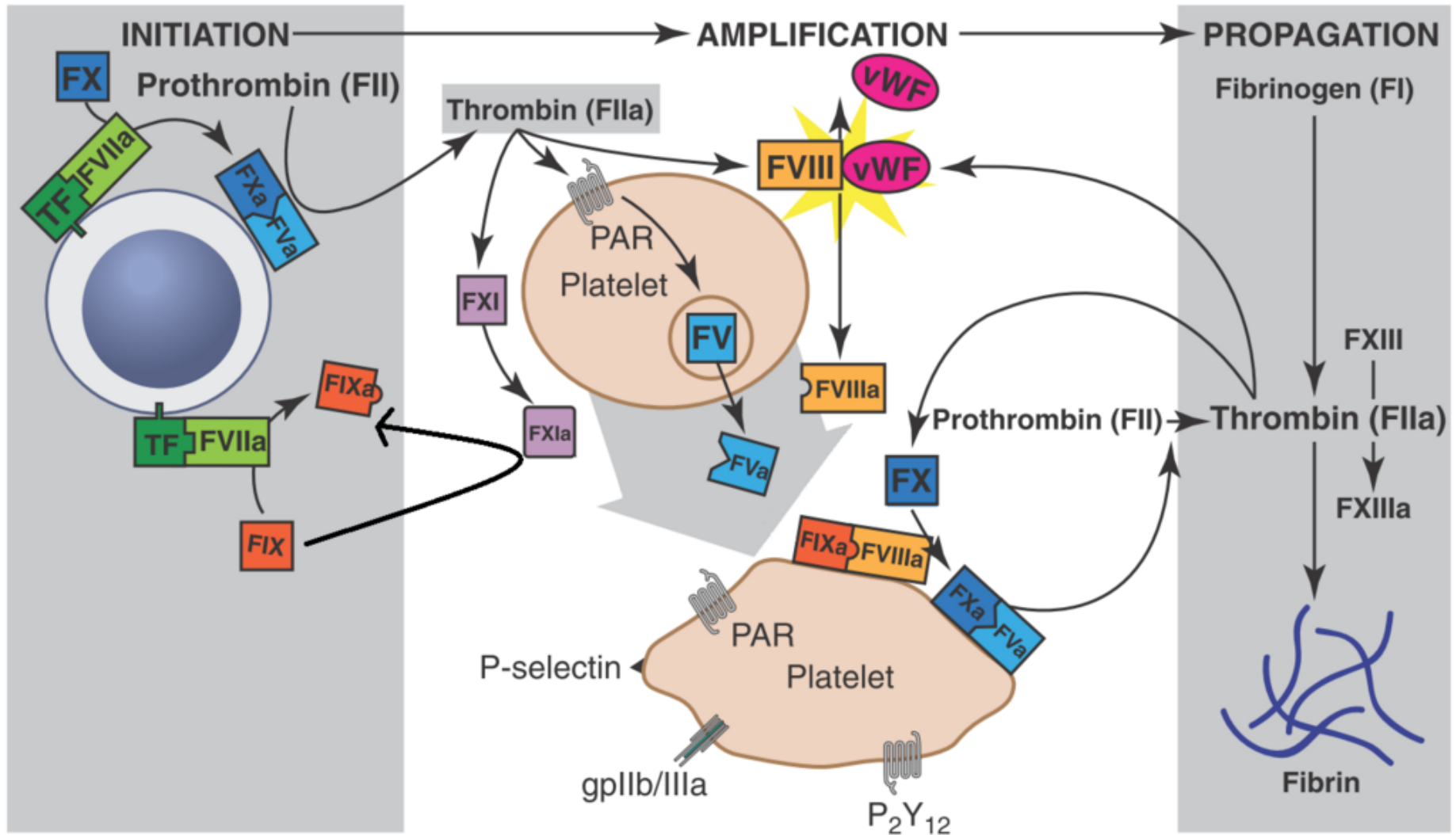
## 2. Amplification phase

- Slow accumulation of thrombin
- Recruitment of other thrombocytes at the site of the vessel injury
- Creation of Va and amplification of prothrombinase activity

## 3. Promotion phase

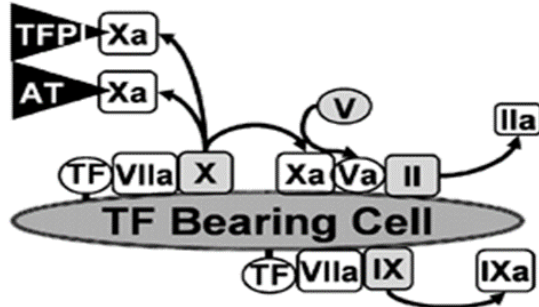
- On the surface of procoagulant phospholipids - platelets
- Cascade with the formation of thrombin, fibrin and its polymerization - crosslinking

# Cell model

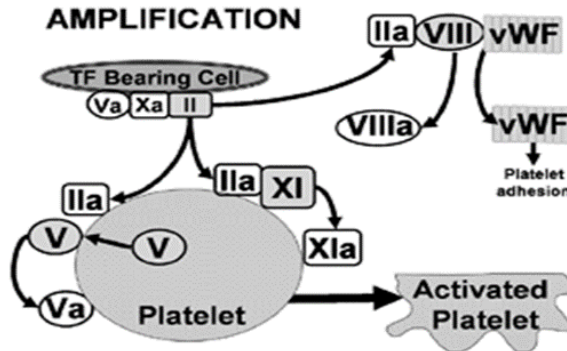


# Cell model

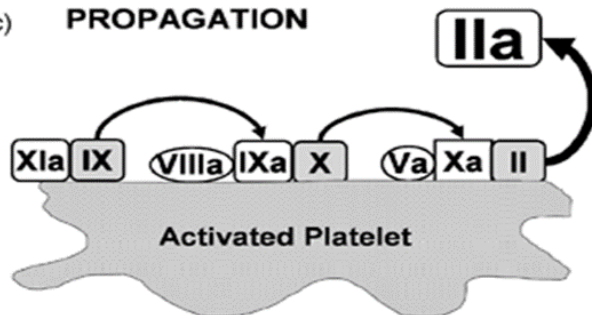
## (a) INITIATION



## (b) AMPLIFICATION



## (c) PROPAGATION

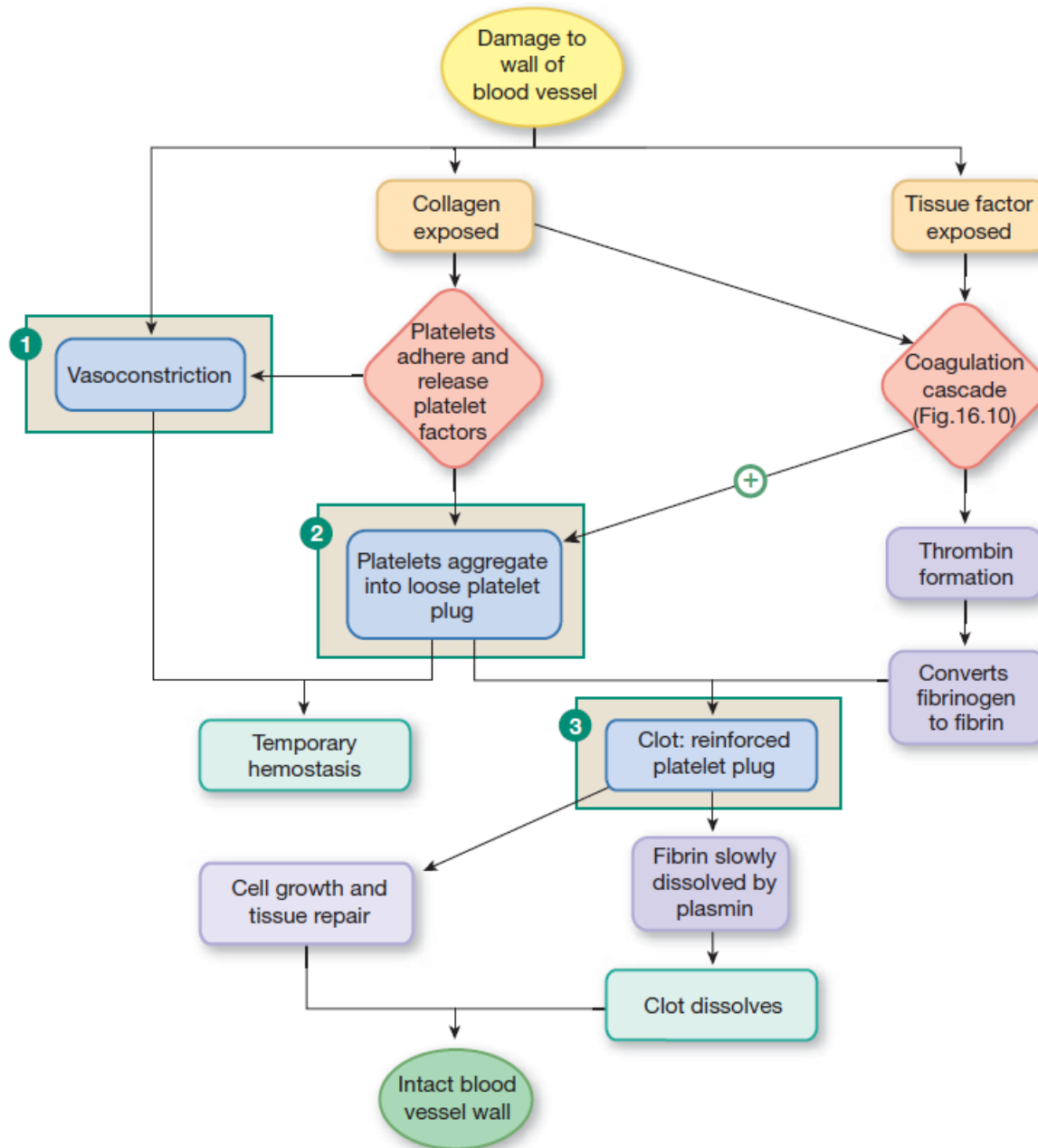


No.	Name	Role
I	Fibrinogen	Clot formation
II	Prothrombin	Activation of factors I, V, VII, VIII, XI, XIII, protein C and platelets
III	Tissue factor	Cofactor VIIa
IV	Calcium	Role in binding of phospholipid coagulation factors
V	Proaccelerin	Cofactor of X – prothrombinase complex
VI		Activated form of V
VII	Proconvertin	Enables factors IX and X
VIII	Antihemophilic factor A	Cofactor of IX complex
IX	Antihemophilic factor B or Christmas factor	Enables factor X, forms the complex tenase with factor VIII
X	Stuart–Prower factor	Forms the prothrombinase complex together with factor V, which will activate factor II
XI	Antecedent of plasma thromboplastin	Activates factor IX
XII	Hageman factor	Enables factors XI, VII and prekallikrein
XIII	Fibrin stabilizing factor	Creating cross-links between fibrin monomers
XIV	Prekallikrein – Fletcher factor	Precursor of kallikrein
XV	HMWK – Fitzgerald factor	Cofactor
XVI	von Willebrand factor	Role in platelet adhesion; it is linked to factor VIII
XVII	Antithrombin III	Inhibits IIa, Xa and other proteases
XVIII	Heparin cofactor II	Inhibits IIa
XIX	Protein C	Inactivates factors Va and VIIIa
XX	Protein S	Cofactor for activated C protein

HMWK: High-molecular-weight kinogen.

prothrombinase complex

**SUMMARY**



Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.

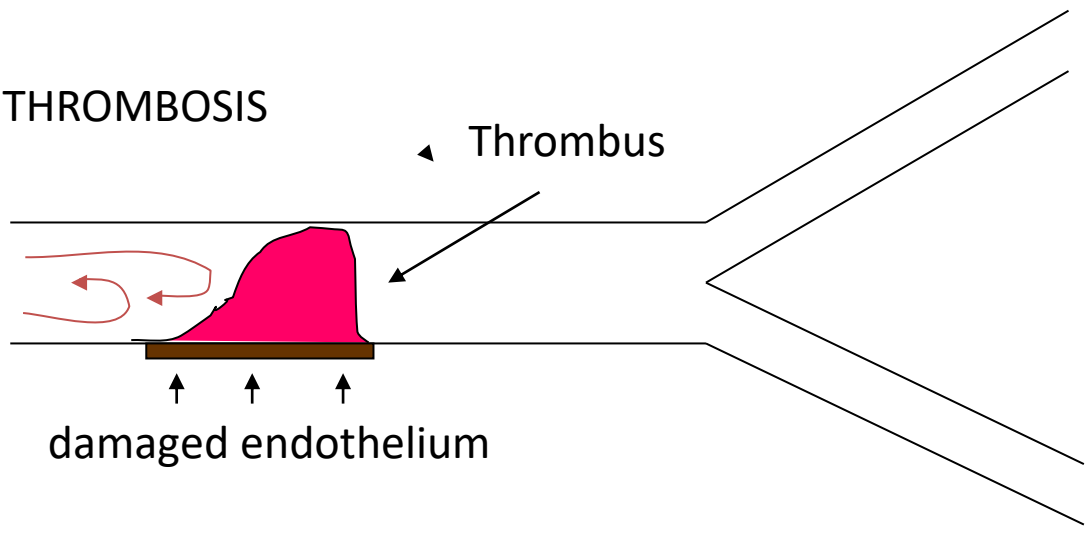


# INTRAVASCULAR COAGULATION

Damage of epithelium caused by:

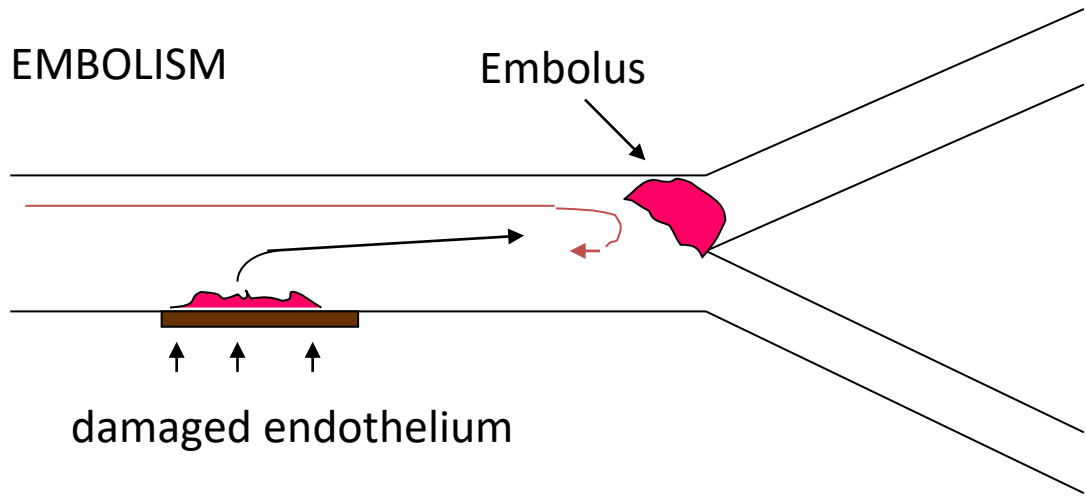
- 1) Atherosclerosis (myocardial infarction, stroke)
- 2) Inflammation (venous thrombosis, pulmonary embolism)

THROMBOSIS



Example:  
MI  
Stroke

EMBOLISM



Example:  
Pulmonary embolism

## Antithrombotic Drugs

Antiplatelet drugs

Anticoagulants

Fibrinolytic agents

Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: <http://www.accessmedicine.com>

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# Antithrombotic drugs?

- We influence function of thrombocytes, not number of thrombocytes!
- Primary and secondary prevention of atherothrombosis
  - Acute Coronary Syndromes (ACS)
  - Cerebrovascular Ischemic Attack
  - Peripheral arterial disease (PAD)
- antiplatelet agents?
- Inhibitors of cyclooxygenase/inhibitors of thromboxane A<sub>2</sub> synthesis or antagonists of the receptors
- Inhibitors of ADP receptors (P2Y<sub>12</sub>)
- Antagonists of protease-activated receptors (PAR-1)
- Antagonists of surface glycoproteins (GP IIb/IIIa)
- Blockage of serotonin pathway
- Other mechanisms

# CONTROL OF HAEMOCOAGULATION

Clotting is counteracted by anti-coagulating mechanisms:

## Non-humoral control:

Endothelial surface factors.

Blood stream: restriction of increase of clot, dilution and removal of clotting factors.

Interaction between thromboxane  $A_2$  and prostacycline.

## Humoral control:

**Fibrin:** binds thrombin strongly – „antithrombin“

**Antithrombin III:** circulating inhibitor of proteases (active forms of factors IX, X, XI, XII), binding of proteases of clotting system is facilitated by heparin from mast cells (co-factor of heparin)

**Thrombomodulin:** thrombin binding protein, produced by endothelial cells.

Thrombin + Thrombomodulin = activator of protein C

**Protein C:** inactivation of factors V and VIII

Inhibition of the inhibitor of activator of tissue

plasminogen (= more plasmin – degradation of fibrin)

**Plasmin (fibrinolysin):** active part of fibrinolytic system.

Precursor: plasminogen, catalyzed by thrombin and **tissue**

**activator of plasmin (TPA)** – use in therapy of myocardial

infarction!!! Streptokinase.

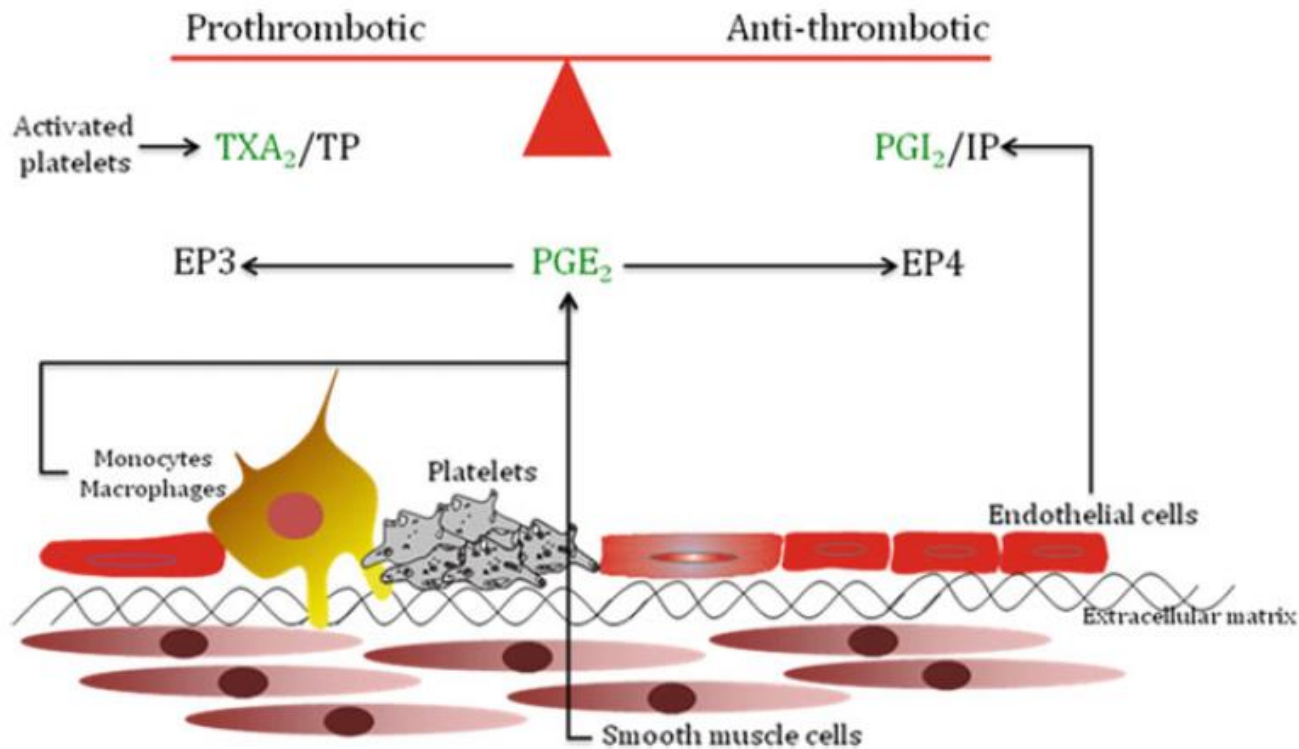
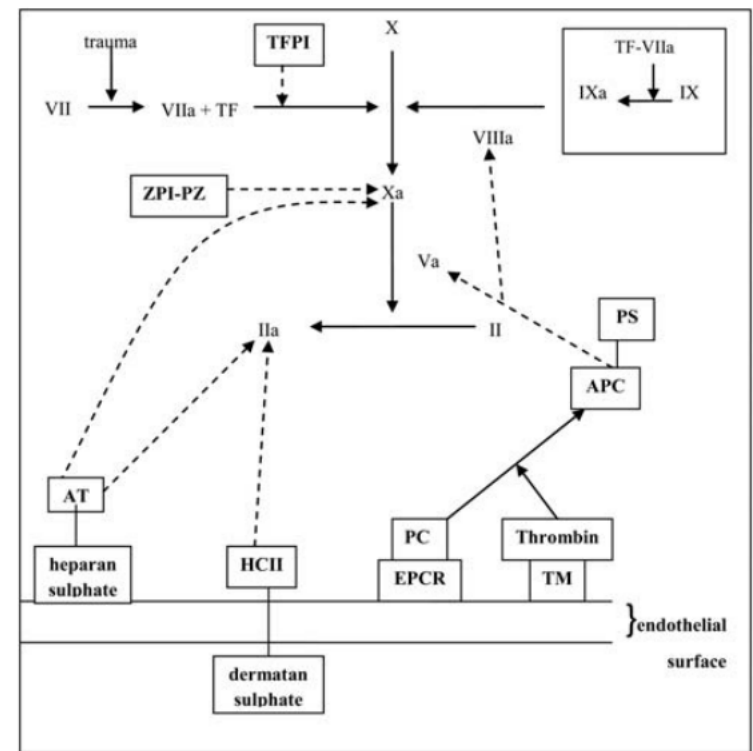
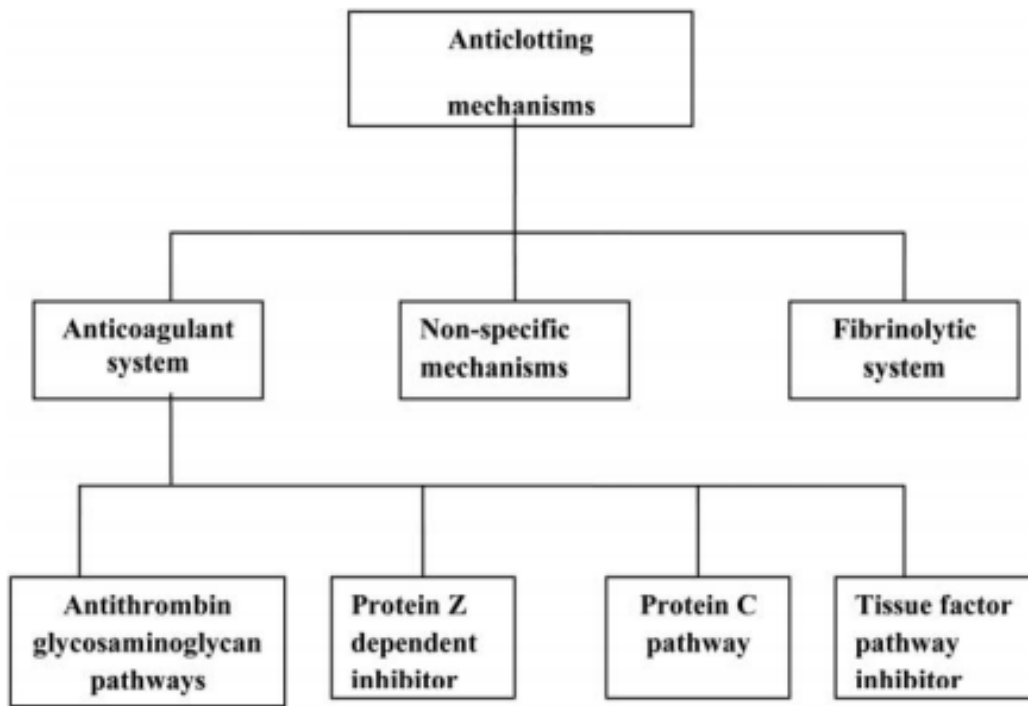


Fig. 4 The balance between thrombotic and antithrombotic effects of prostanoids. In response to vascular injury, PGI<sub>2</sub> produced by endothelial cells opposes the enhanced prothrombotic effect of TXA<sub>2</sub> produced by platelets. Smooth muscle cells, monocytes, and macrophages (accumulate in atherosclerotic plaques) release prostanoids such as PGE<sub>2</sub> during inflammation. PGE<sub>2</sub> shows a biphasic, dose-dependent effect on platelet aggregation

Kauskot A, Hoylaerts MF: Platelet receptors. *Handbook of experimental pharmacology* 2012(210):23-57.

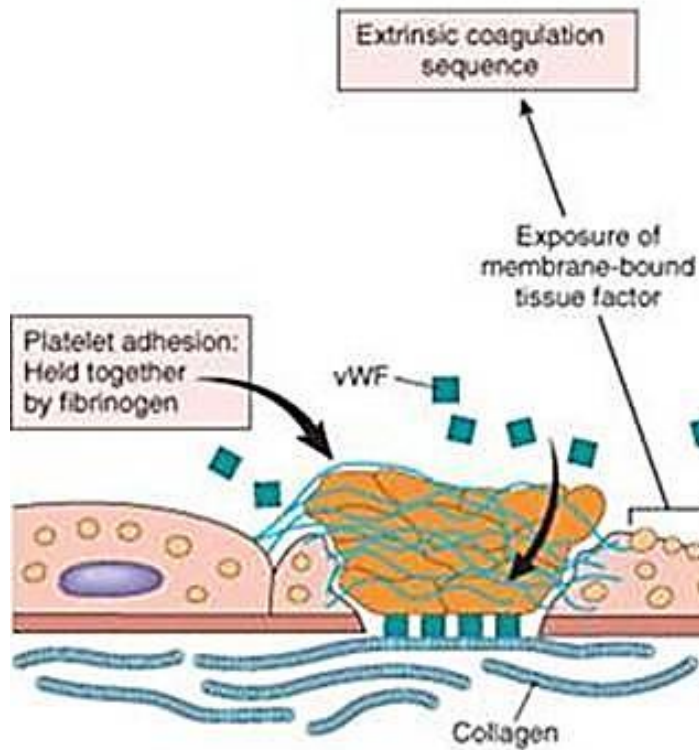




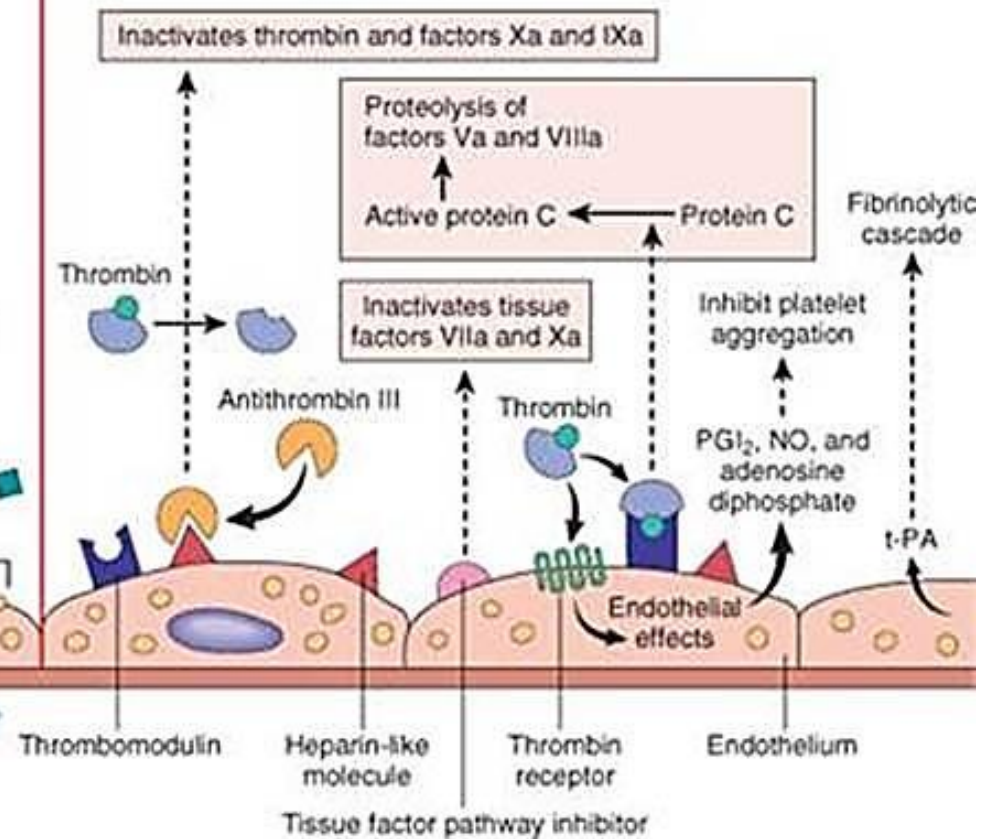
**Fig 2** The anticoagulant system. AT, antithrombin; HCII, heparin cofactor II; TFPI, tissue factor pathway inhibitor; ZPI, protein Z-dependent protease inhibitor; PZ, protein Z; PC, protein C; APC, activated protein C; PS, protein S; EPCR, endothelial protein C receptor; TM, thrombomodulin. Solid arrows indicate activation and dashed arrows indicate inhibition.

Ezihe-Ejiofor JA, Hutchinson N: **Anticlotting mechanisms 1: physiology and pathology. *Continuing Education in Anaesthesia, Critical Care & Pain Advance Access 2013***

## FAVOR THROMBOSIS



## INHIBIT THROMBOSIS



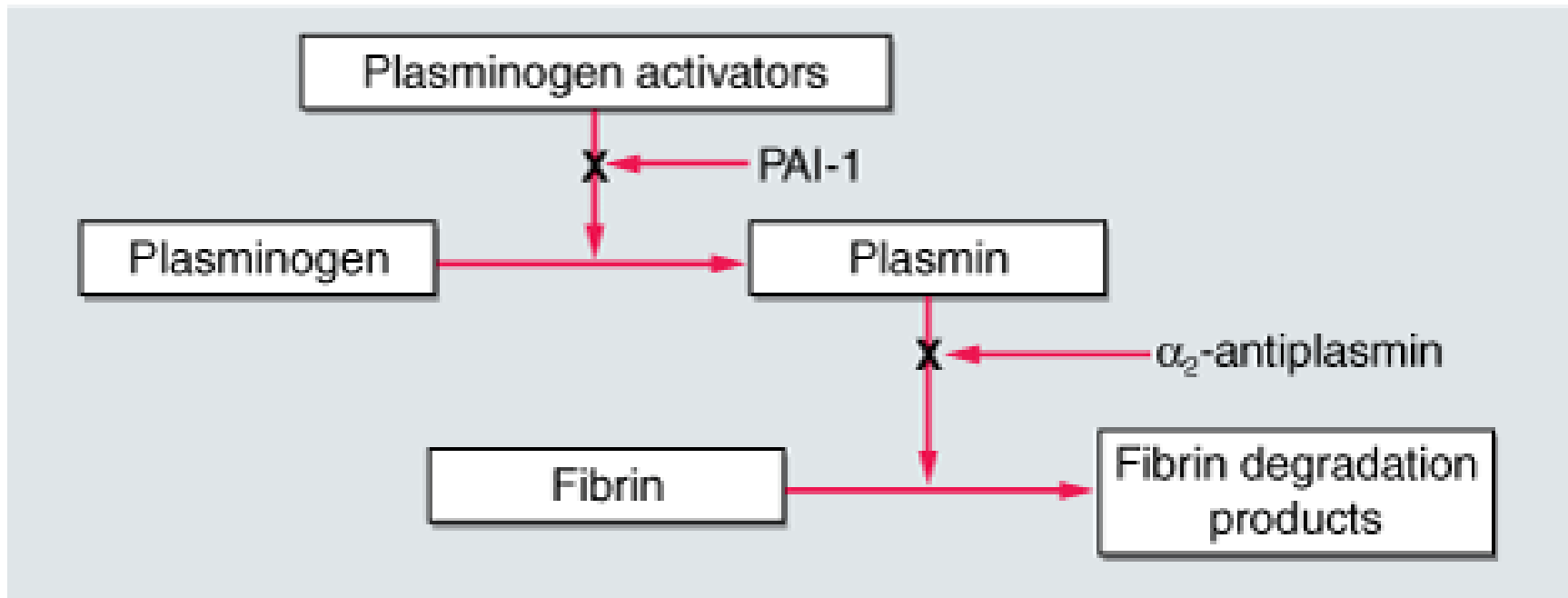
# FIBRINOLYSIS

Inactive plasminogen.

Active plasmin (fibrinolysin).

Activators of plasminogen.

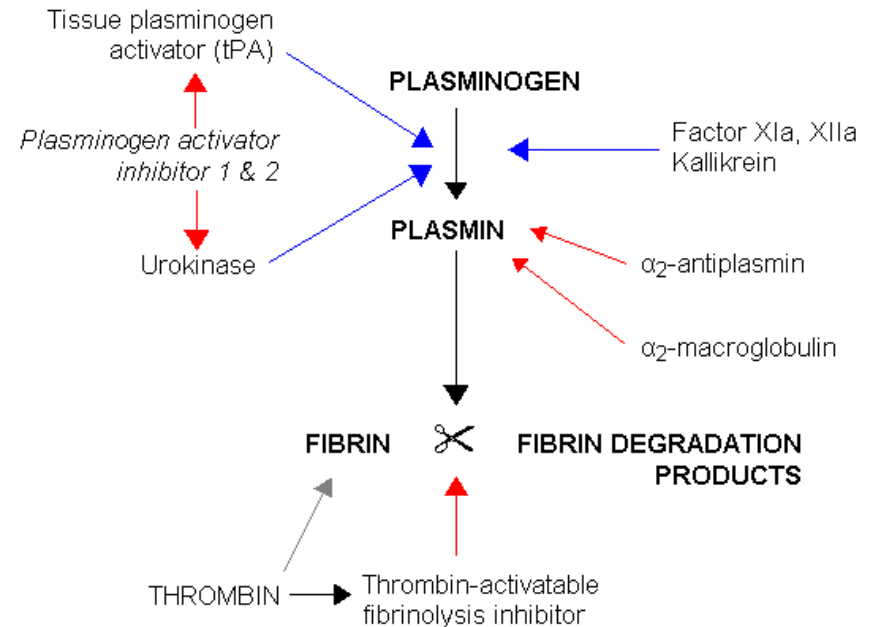
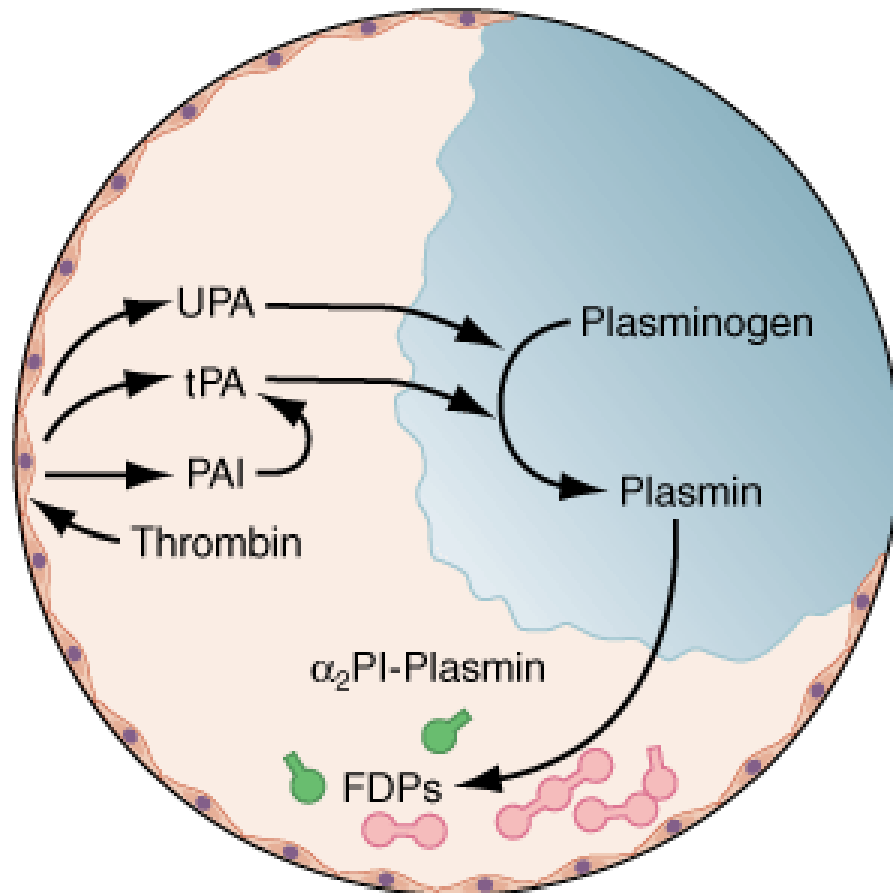
Inhibitors of plasminogen.



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: <http://www.accessmedicine.com>

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# Thrombolysis



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: <http://www.accessmedicine.com>

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UPA, urokinase plasmin activator    tPA, tissue plasmin activator

PAI, plasmin activator inhibitor    alpha<sub>2</sub>PI-Plasmin, complex

# ANTI-CLOTTING TREATMENT

**Defibrination:** removal of fibrin (substances from snake poisons) – *in vitro*

**Decalcification:** binding or removal of calcium ions (sodium citrate, potassium or ammonium oxalate) – *in vitro*

**Heparin:** natural anticoagulant, mast cells, active only in the presence of antithrombin III, used also *in vivo*

**Cumarin derivatives** (dicumarol, warfarin): inhibition of effects of vitamin K in liver – disorders of factors II, VII, IX, X, protein C, protein S (facilitates activation of Va and VIIIa via protein C)

**Hirudin:** obsolete, salivary glands of leech (*Hirudo medicinalis*)

# Anticoagulants

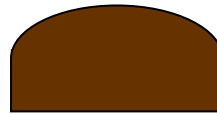
Activation →

Inhibition ←

*In vivo:* Heparin → antithrombin III  
test: aPTT,  
antifactor Xa level

Coumarin (warfarin)

Vitamin K →



Liver

→ prothrombin

test: PT

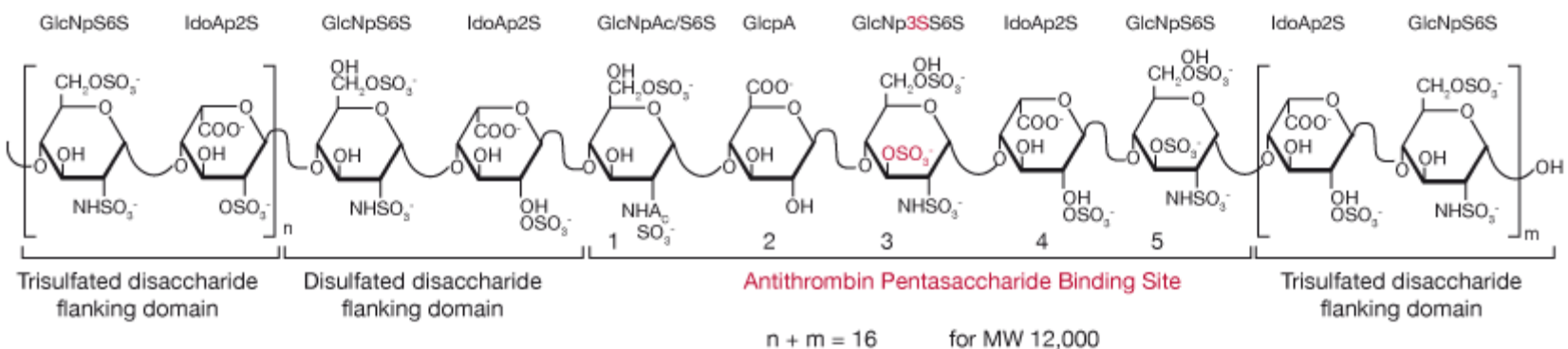
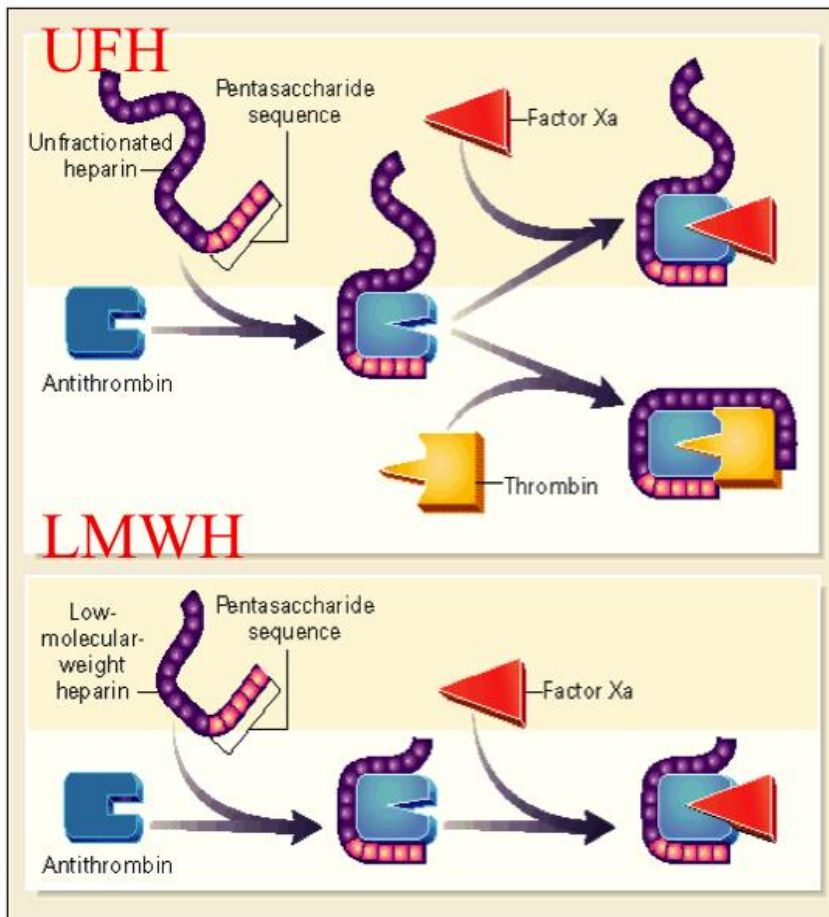
$INR = \frac{PT_{\text{patient}}}{PT_{\text{norm}}}$

warfarin → INR 2-3

*In vitro:* Heparin → antithrombin III

Sodium citrate →  $Ca^{++}$

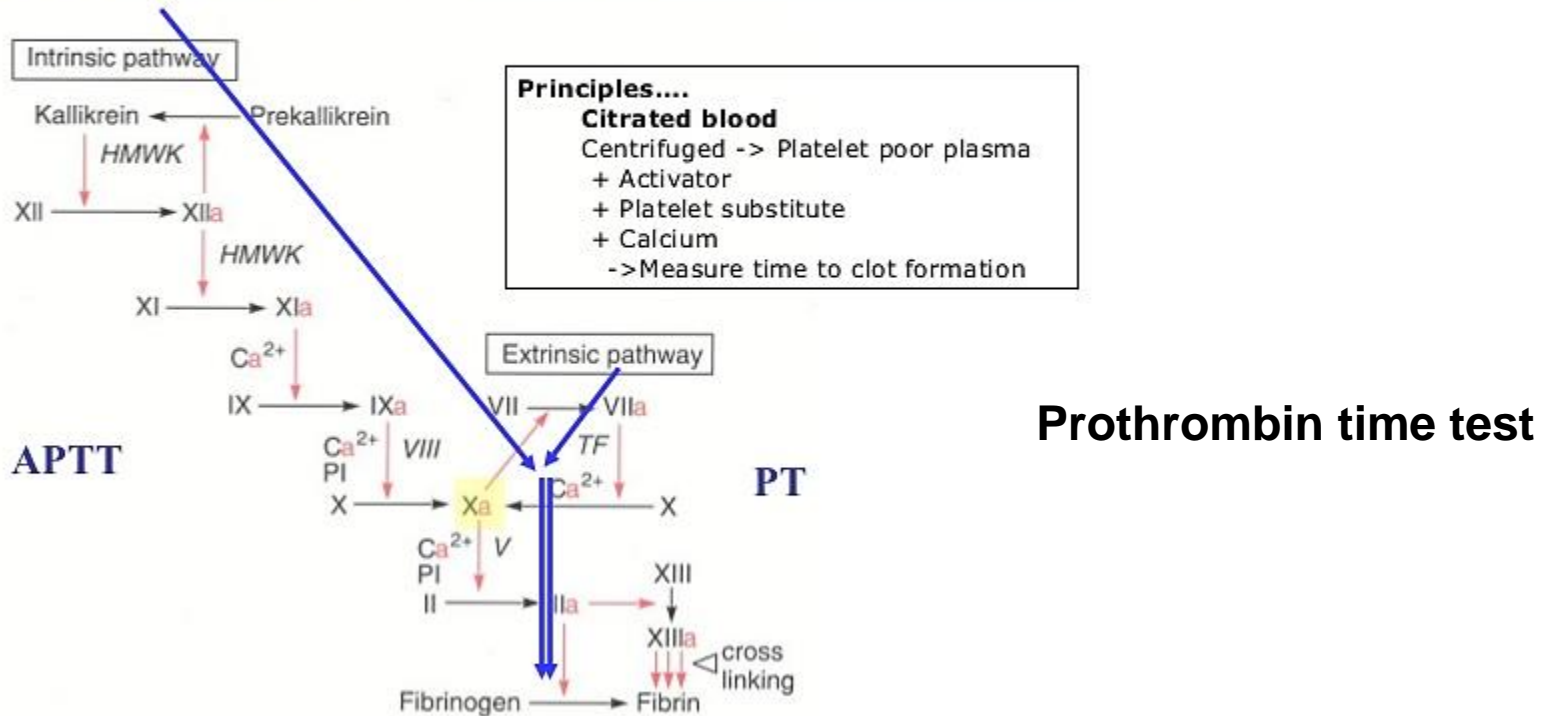
aPTT: activated partial thromboplastin time    PT: prothrombin time





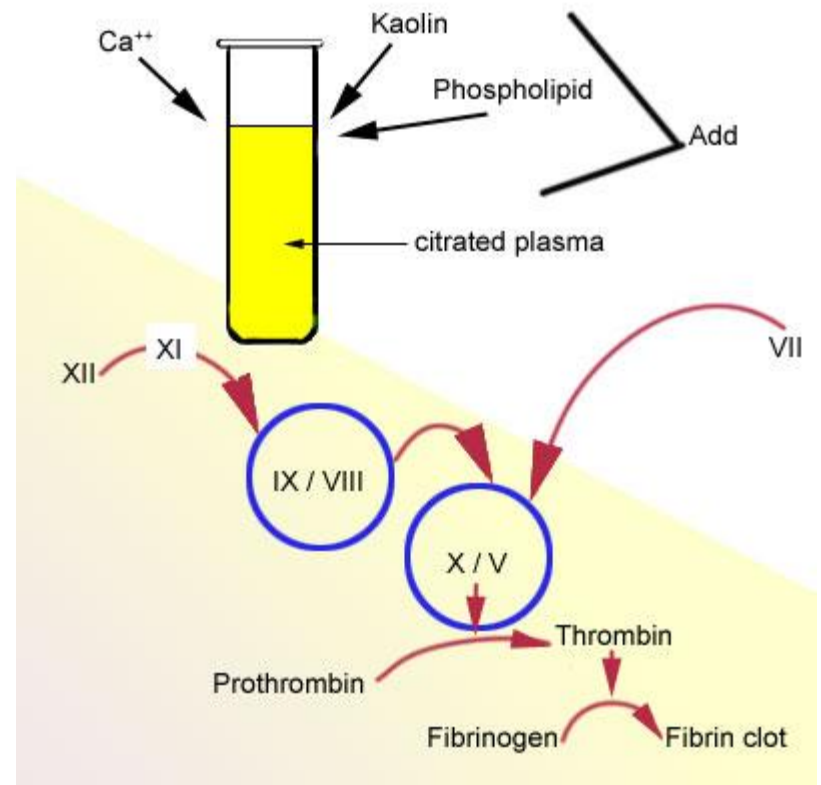
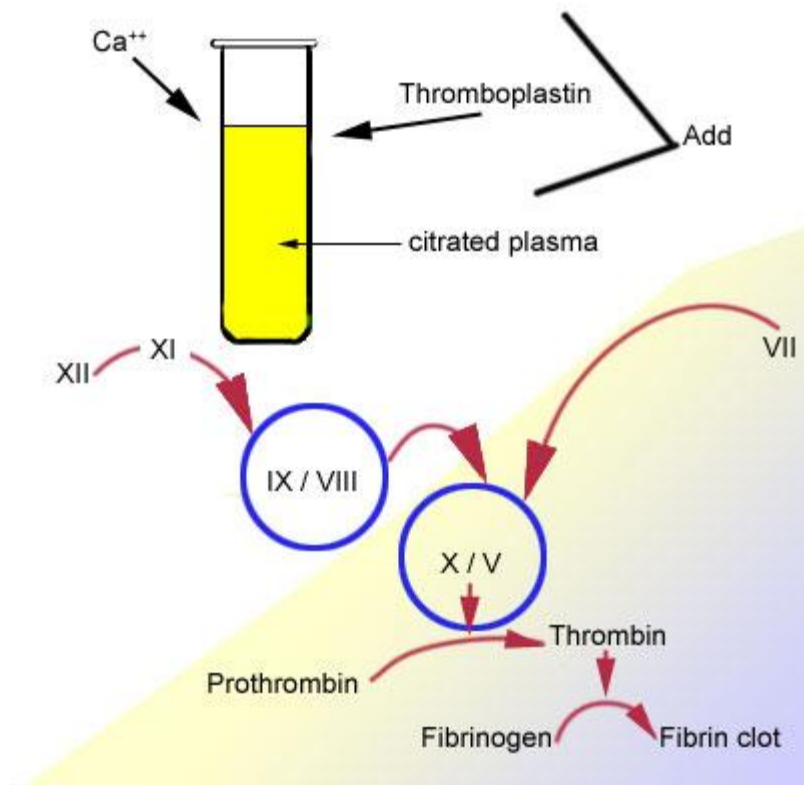
## Tests aPTT and PT

# Tests: PT and APTT

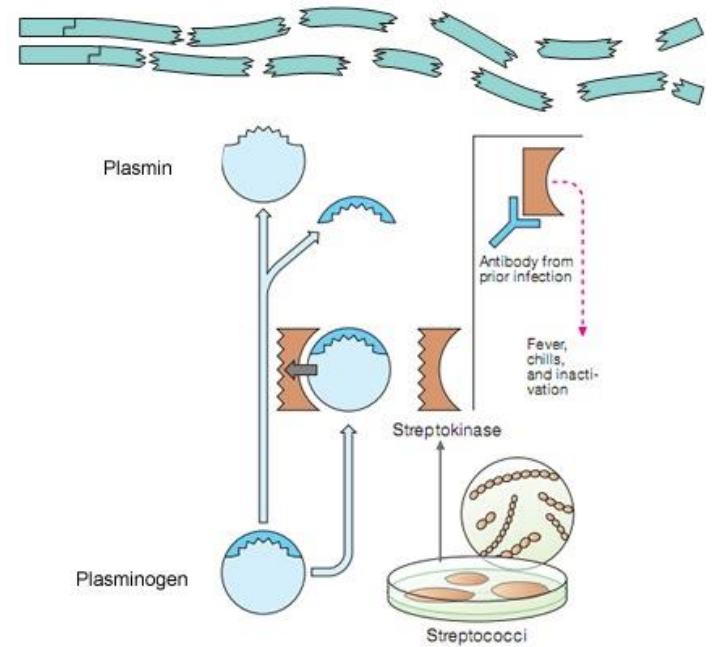
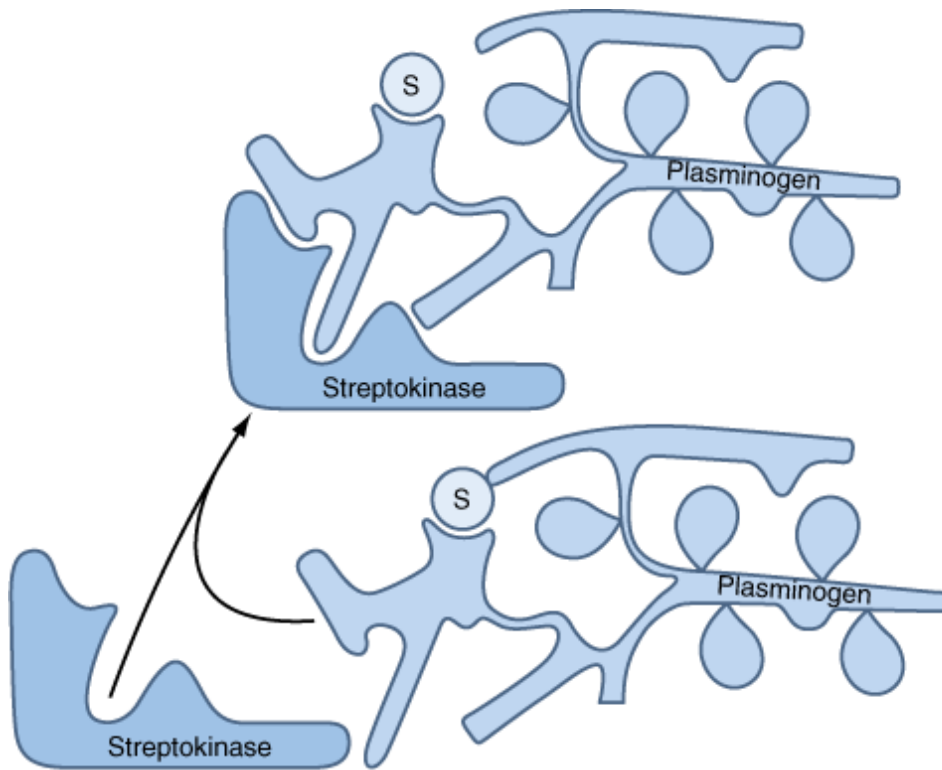


## Activated Partial Thromboplastin Time test

HMWK, high-molecular-weight-kininogen PK, prekallikrein F, factor



# STREPTOKINASE



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: <http://www.accessmedicine.com>

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# CLOTTING DISORDERS

**Clotting diseases** = disorders, in which blood clotting starts either spontaneously or after inadequately small stimulus.

**Blood clotting disorders caused by diseases of vessels**

**Disorders of platelets:**

1) *thrombocytopenia*

2) *thrombocytopathy*

**Coagulopathy** – loss or lack of plasmatic clotting factors:

1) *Disorders of synthesis*: hereditary (haemophilia), attained (hypo-vitaminosis K, therapy with derivatives of cumarin)

2) *Disorders of metabolism*:

- consumptive coagulopathy and hyperfibrinolysis
- repeated transfusions
- immunocoagulopathy
- therapy by heparin
- paraproteinemia