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.

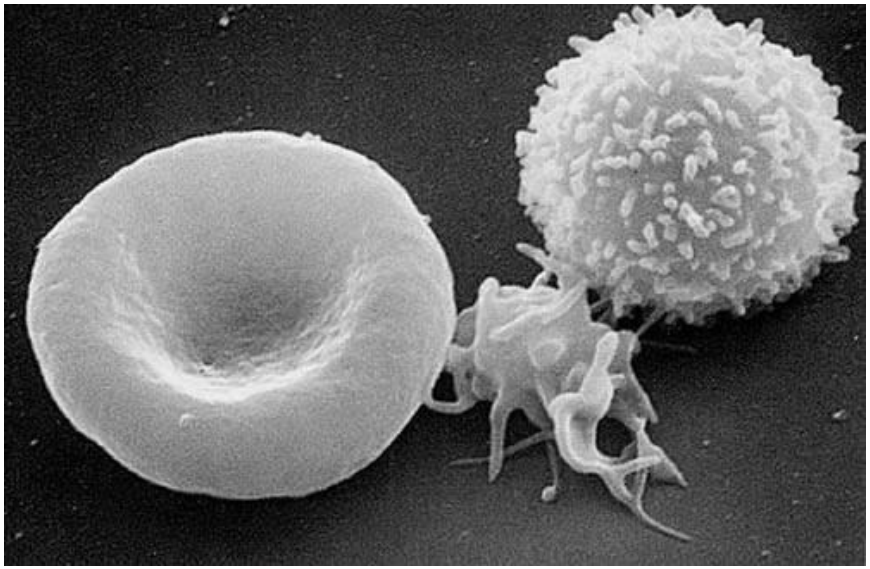
Origin: megakaryocytes of bone marrow under the effect of colony stimulating factors – interleukins (*IL-1, IL-3, IL-6*) and granulocytes and macrophages stimulating factor (*GM-CSF*)

Number: 200 000 – 500 000 in μl, one third in lien and two thirds in peripheral blood

No age and gender differences in platelet count.

Trombocytosis – after splenectomy.

Size: 2 – 4 μm in diameter, 0,5 – 1 μm 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

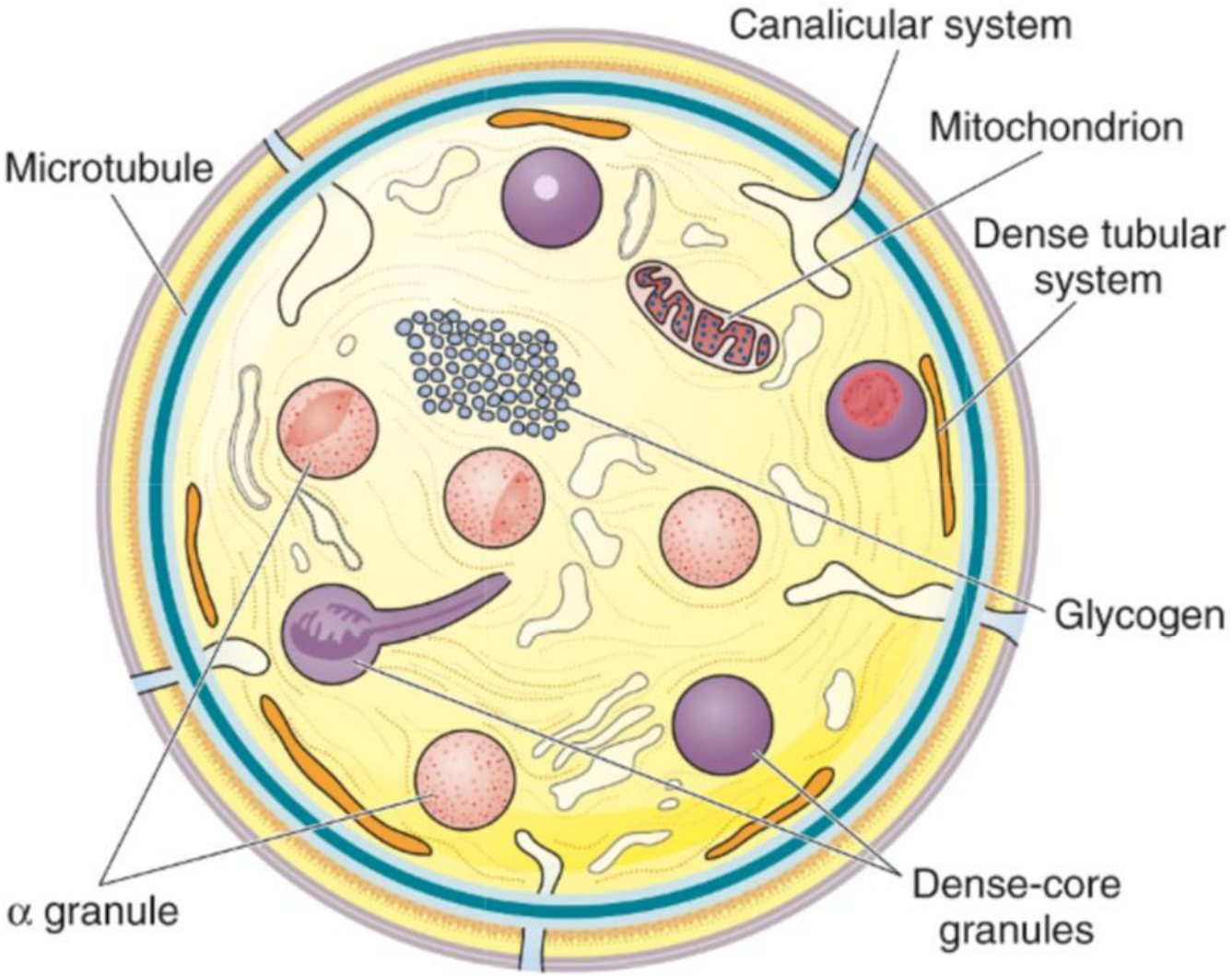
Cytoplasm: contains actin, myosin, glycogen, lysozomes and

Granules: ***dense granules*** (non-protein substances – serotonin, ADP, adenonucleotides) and ***α 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 trombocyte



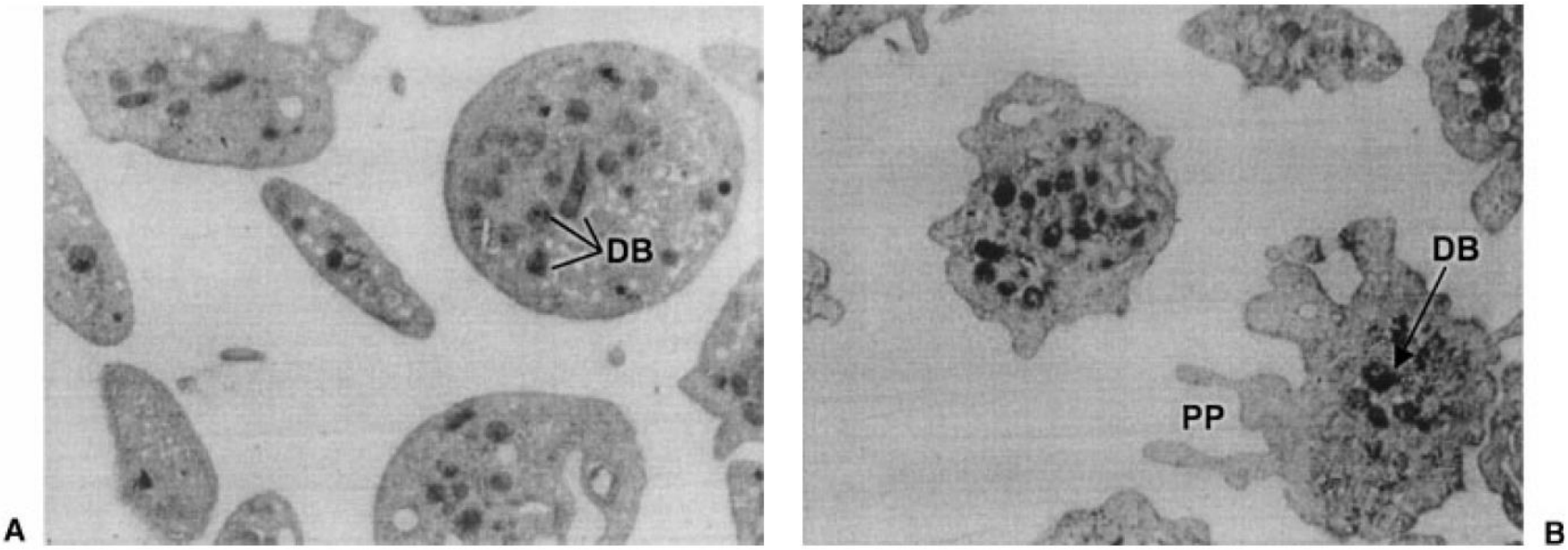


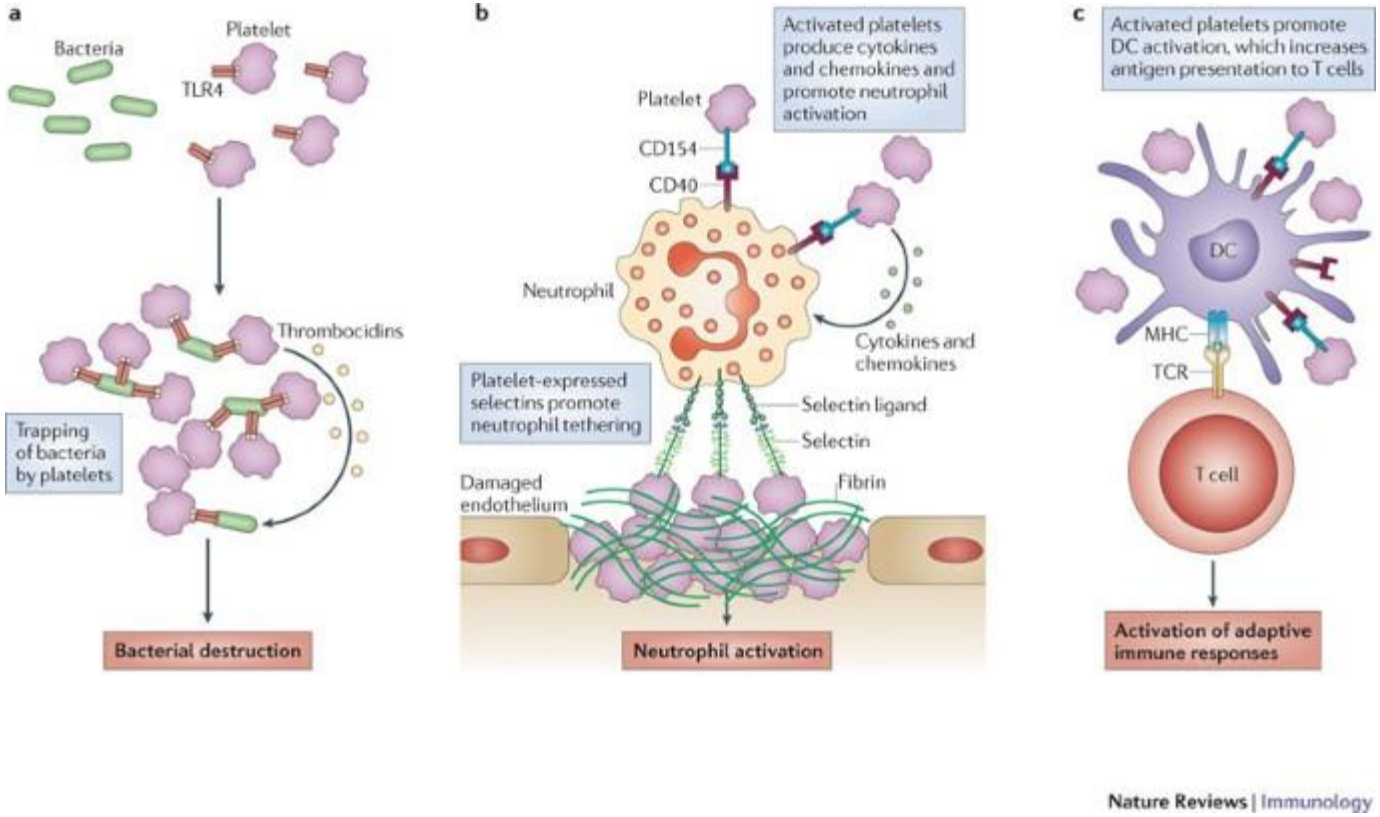
Figure 1 Morphology of human platelets. (A) Thin section of discoid resting platelets with evenly distributed granules. (B) Thin section of stimulated platelets, showing formation of pseudopodia and centralization of granules. DB, dense body; PP, pseudopodium. Magnification × 21,000.

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

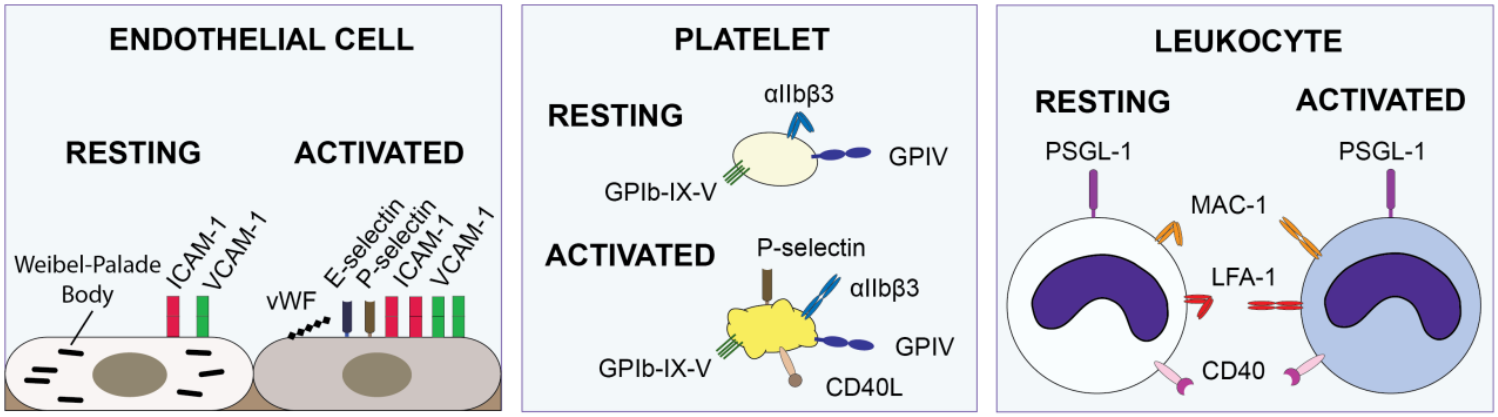
Function of platelets

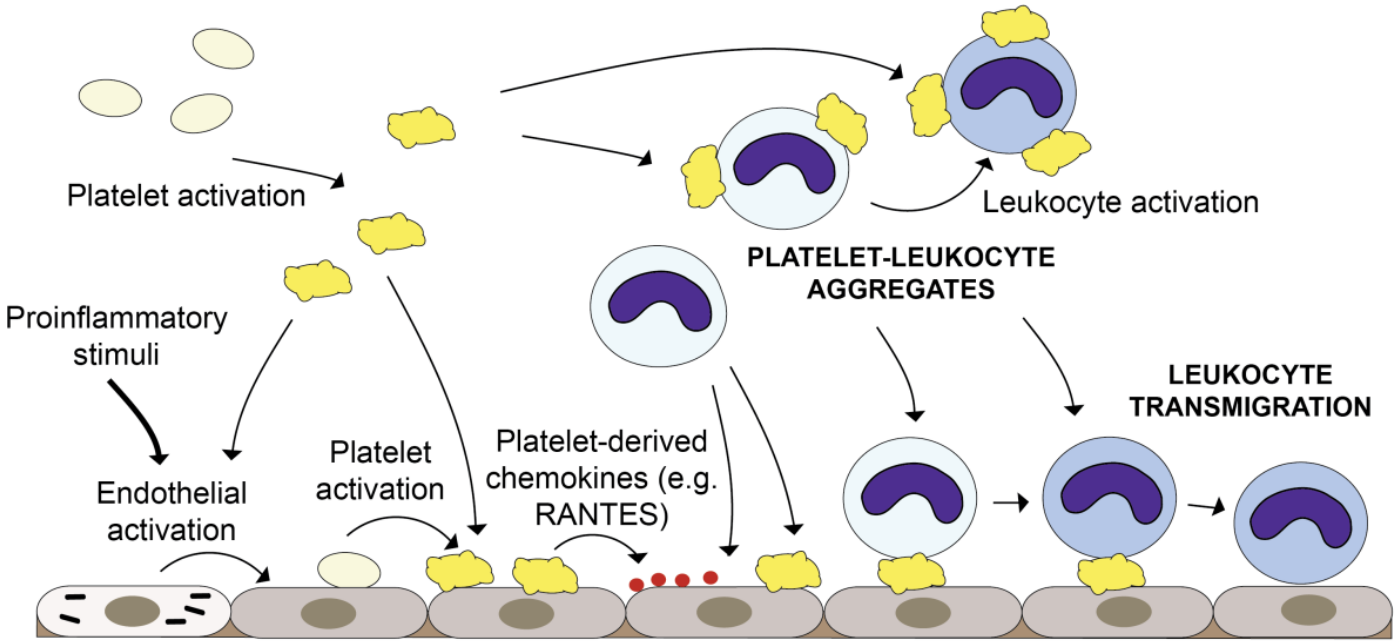
- Protection of organism from blood loss
- Keeping the integrity of vessel wall and healing of the ruptured vessel (PDGF from α-granules)
- Inflammatory reactions, changes in permeability of capillaries, removing of xenogenous substances, viruses, bacteria, graft rejection …
- Carrier for many substances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

Immune function of platelets



Platelets and inflammation





Trombocytes in hemostasis

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, tromboxane A_2

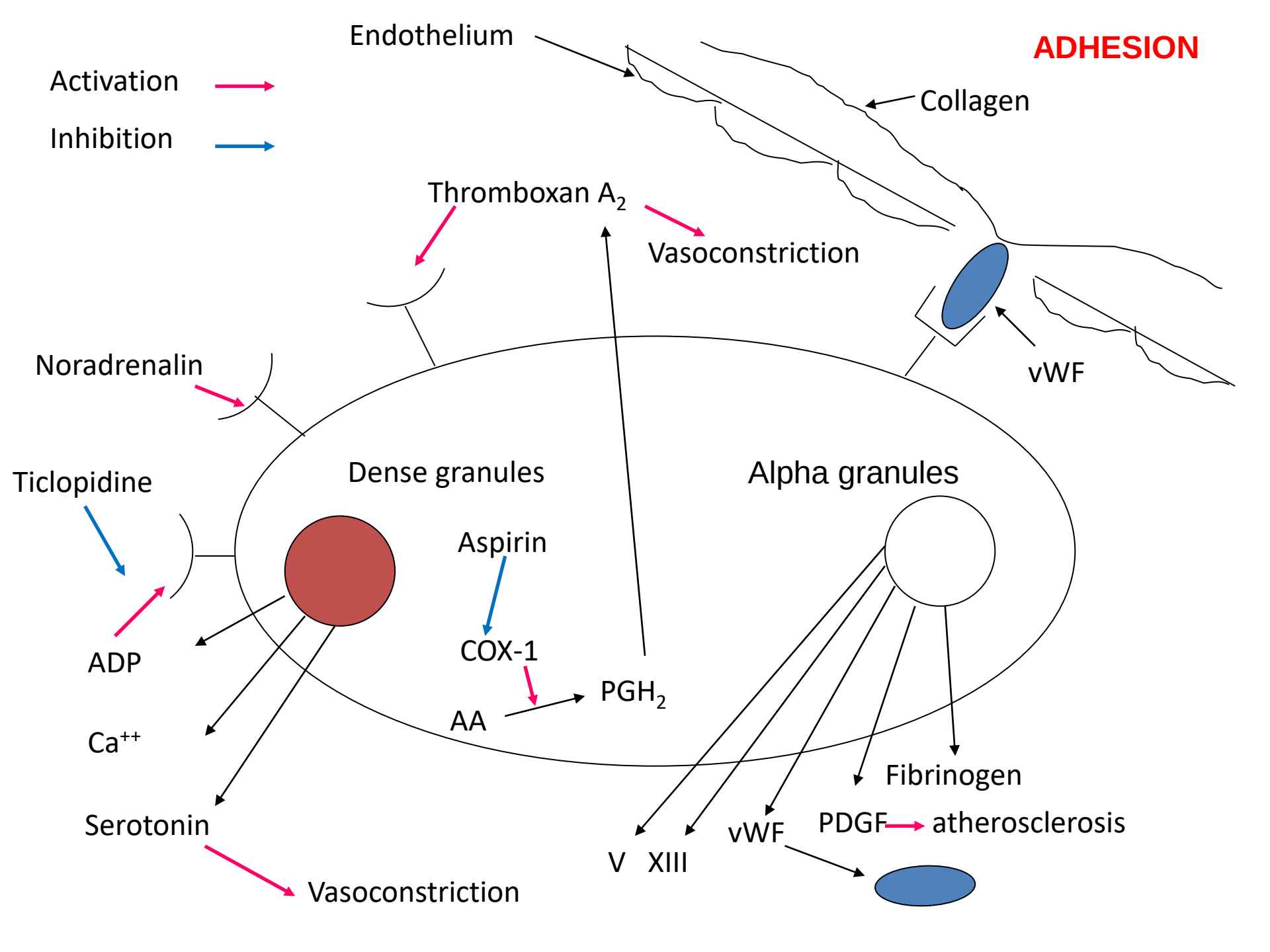
mitogenic effects – growth factor (**PDGF**)

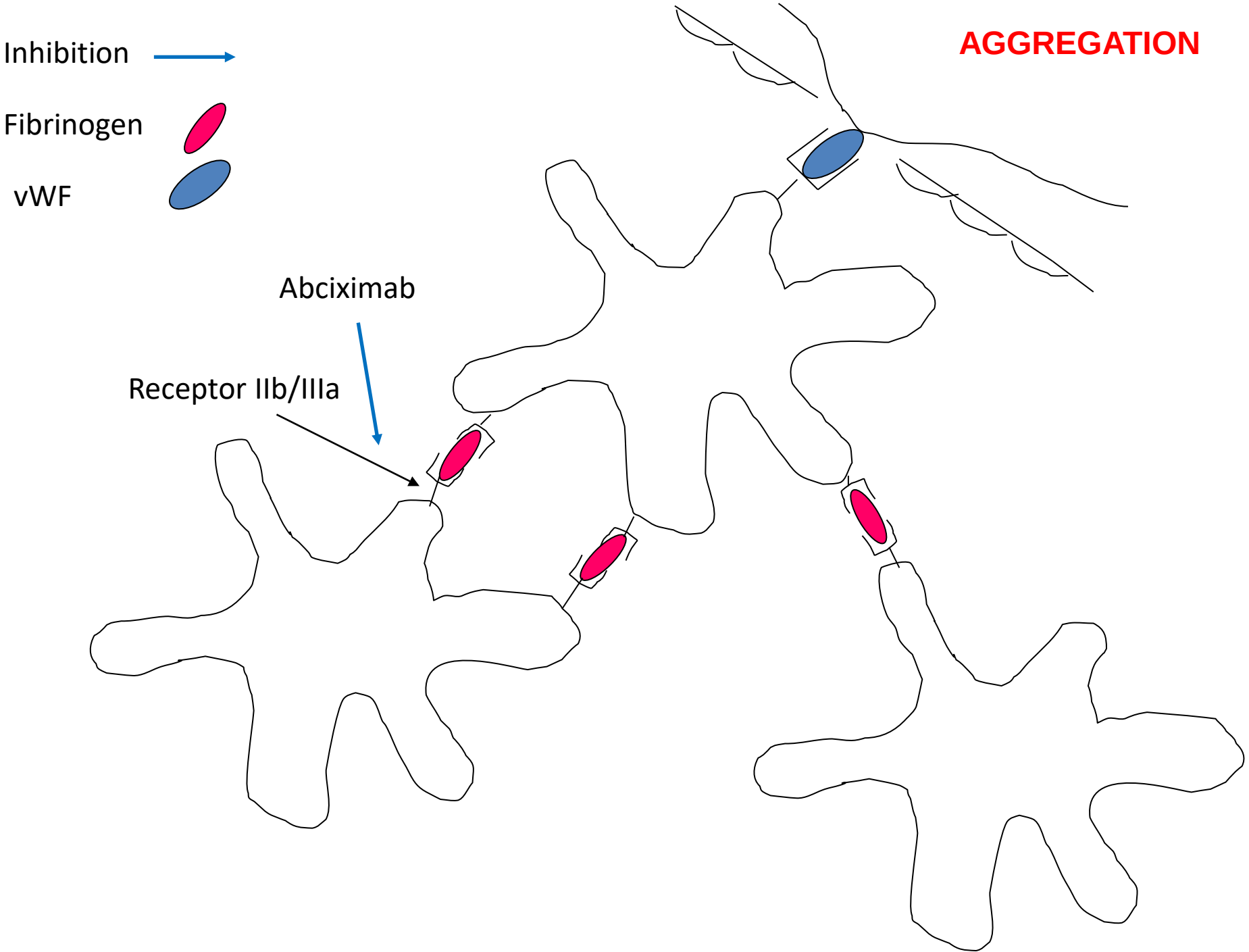
activation of platelets and phagocytes – **PAF** (cytokine, G-coupled receptor, phospholipase C, DAG, increase of intracellular Ca^{2+}concentration, phospholipase A_2 – arachidonic acid – thromboxane A_2)!!! Therapeutic use of acetylsalicylic acid!!!

Aggregation.

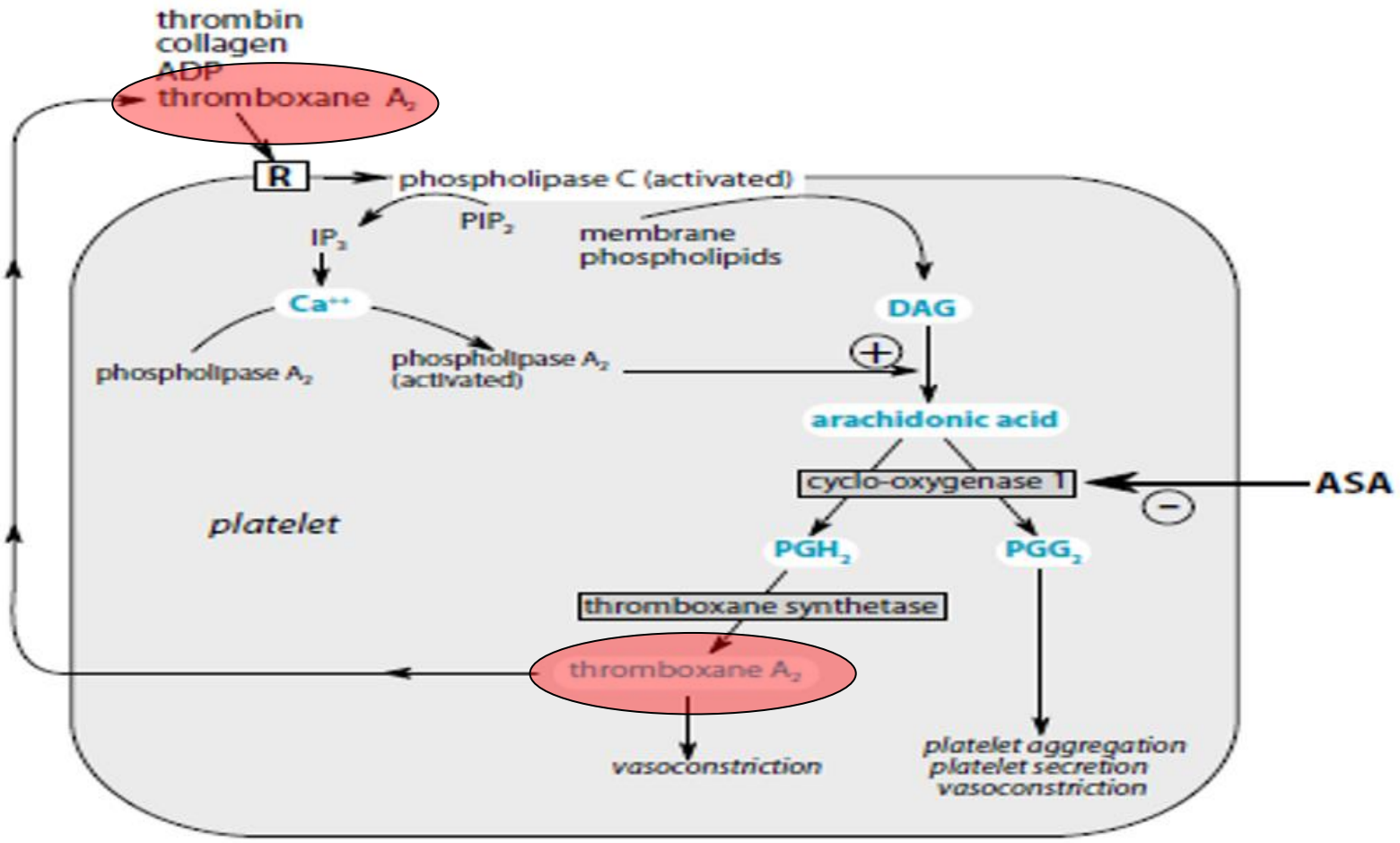
Vasoconstriction.

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


ADHESION
Activation
Inhibition
Endothelium
Collagen
Thromboxan A_2
Vasoconstriction
vWF
Noradrenalin
Ticlopidine
Dense granules
Alpha granules
Aspirin
ADP
COX-1
PGH_2
AA
Ca^{++}
Fibrinogen
Serotonin
V XIII
vWF
PDGF
atherosclerosis
Vasoconstriction


Inhibition
Fibrinogen
vWF
AGGREGATION
Abciximab
Receptor IIb/IIIa

Aggregation – an example of positive feedback



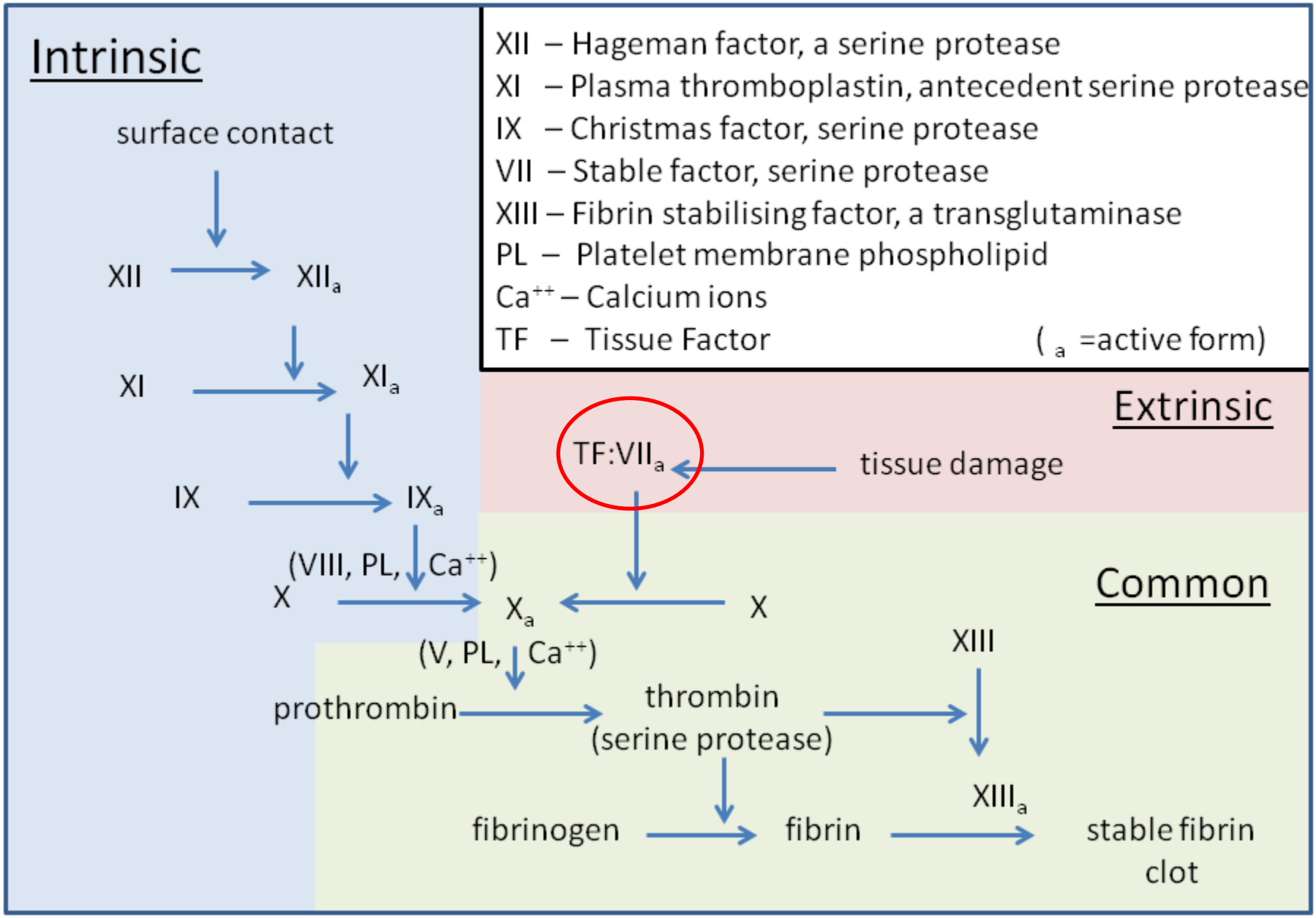
Hemocoagulation as a part of hemostasis

Prothrombin (factor X) – thrombin.

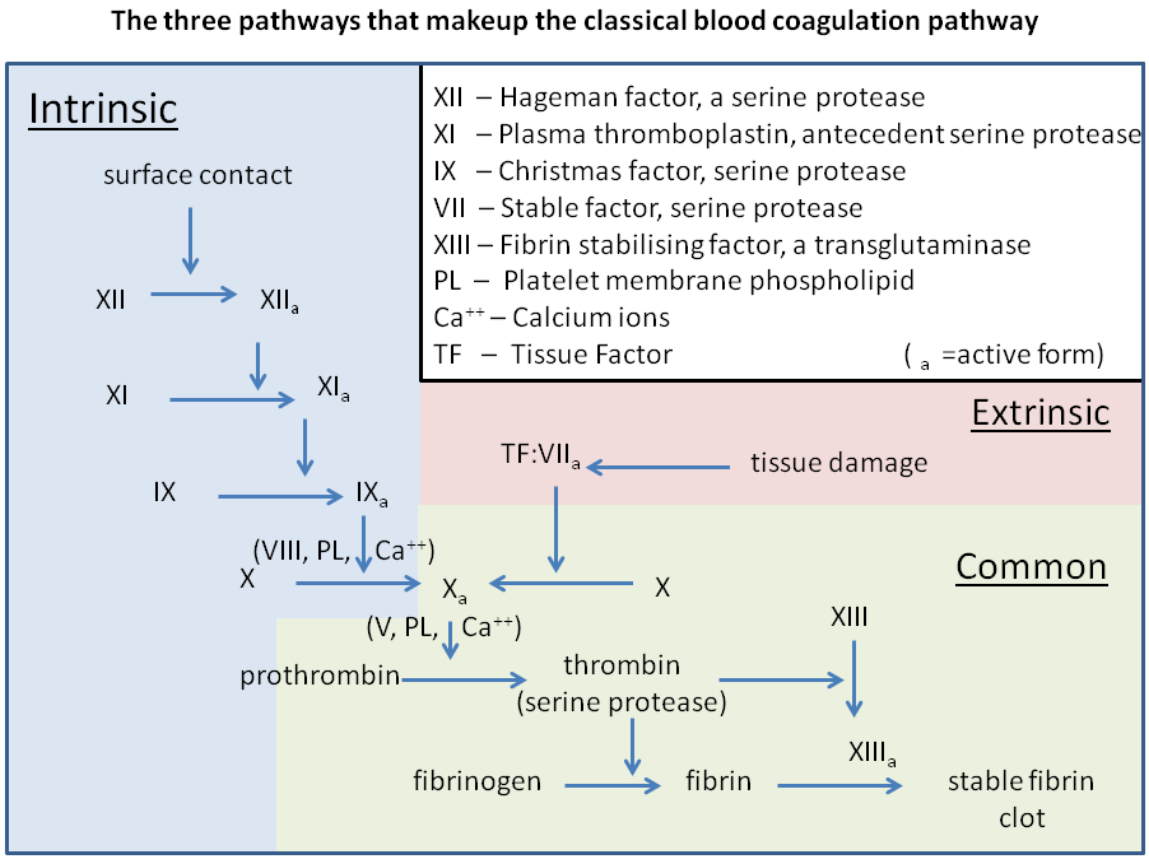
Fibrinogen – fibrin monomer – fibrin polymer (factor III, Ca^{2+}).

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

The three pathways that makeup the classical blood coagulation pathway

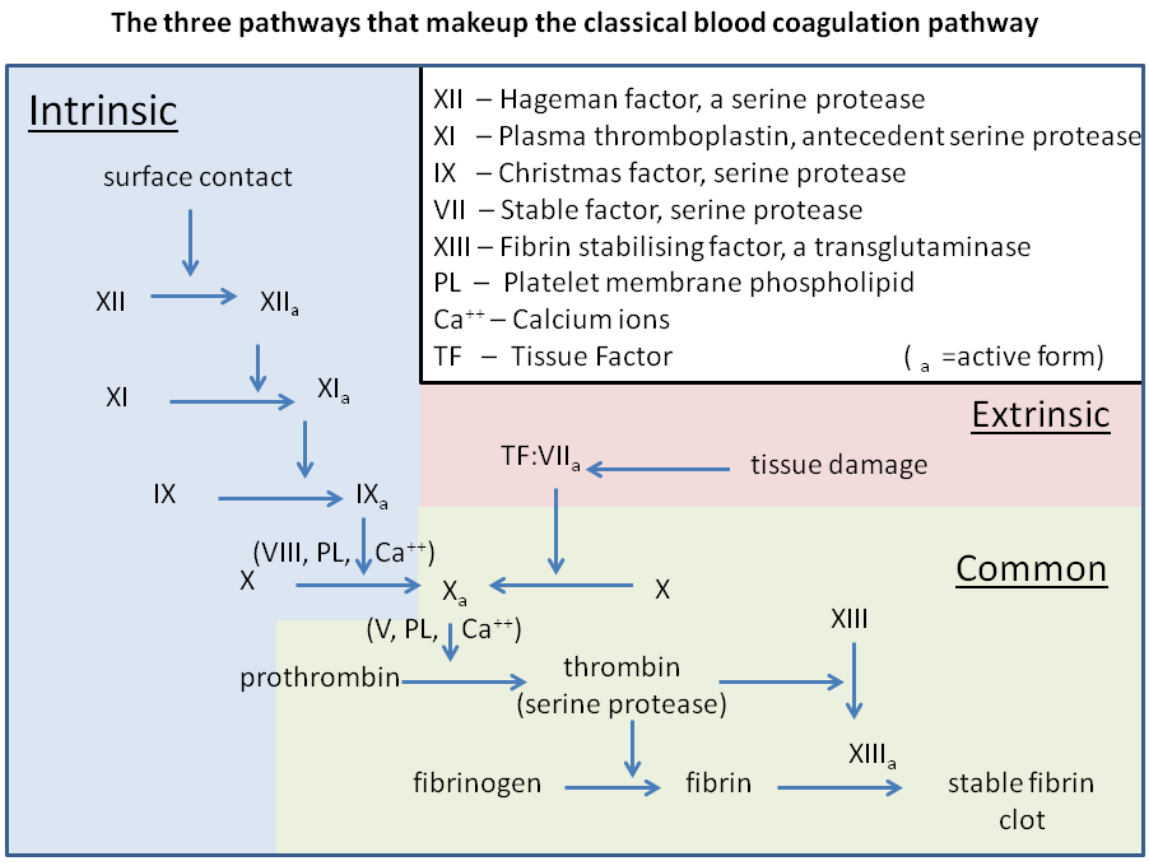


Intrinsic pathway



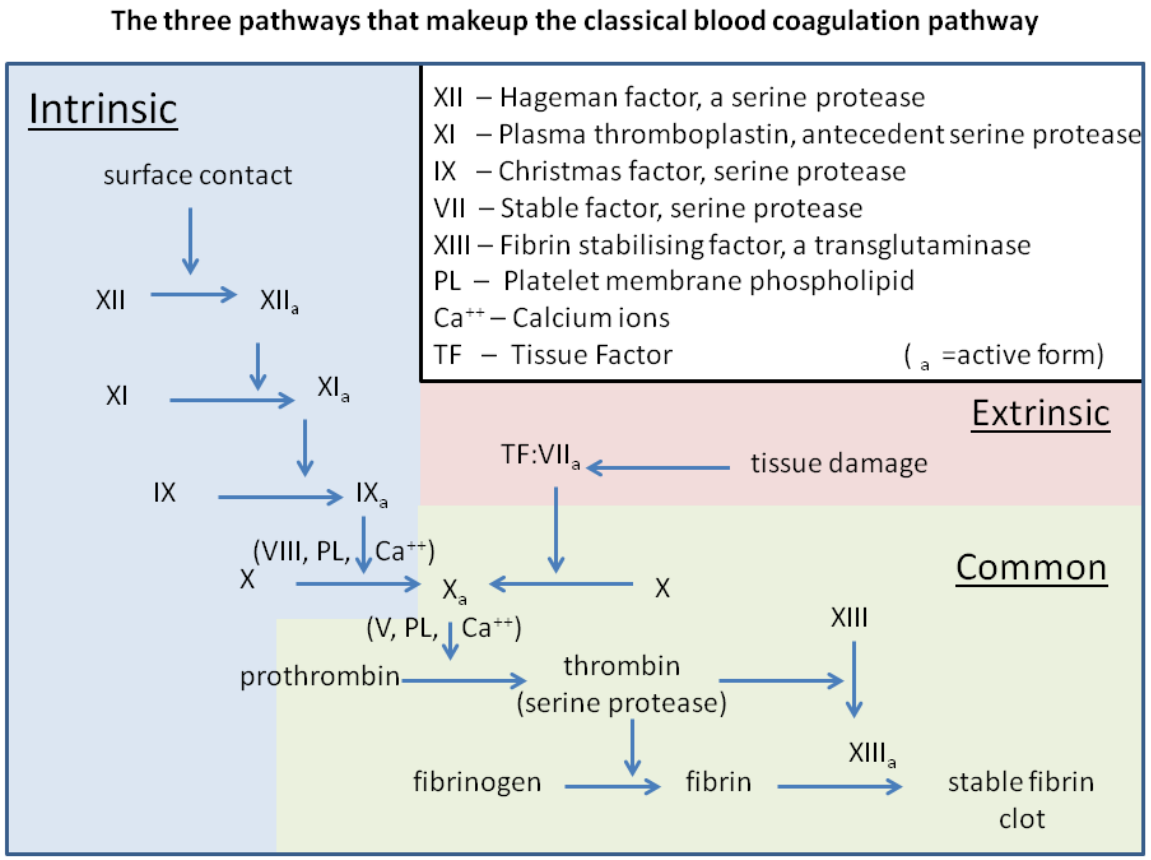
- Factors IXa, Xa, and thrombin proteolytically cleave Factor VIII to form VIIIa, which is the co-factor of the next reaction.
- VIIIa, together with IXa, calcium ions (from the platelets) and negatively charged phospholipids, form the trimolecular complex of tenase
- Tenase converts factor X to Xa.

Extrinsic 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 - VIIa
- Together with calcium ions, the formation of a trimolecular complex, which resembles tenase
- Proteolytic activation of factor X

Common pathway



- Initiated by factor Xa
- Subsequent activation of Factor Va
- Creation of the trimolecular complex (Xa, Va, 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 Va and VIIIa - positive feedback
- Paracrine action of thrombin - endothelial cells release NO, prostaglandin I2, 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

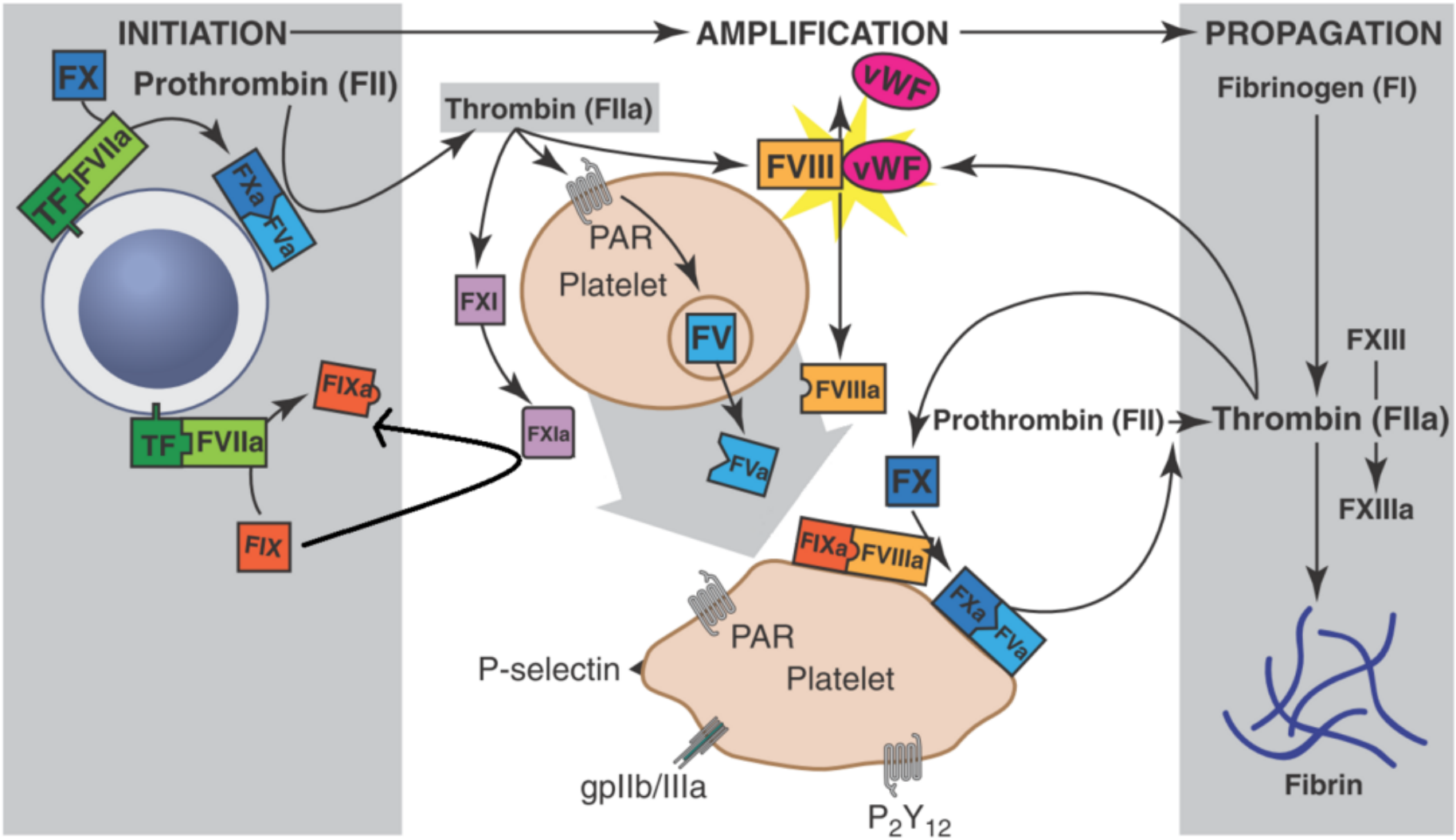
2. Amplification phase

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

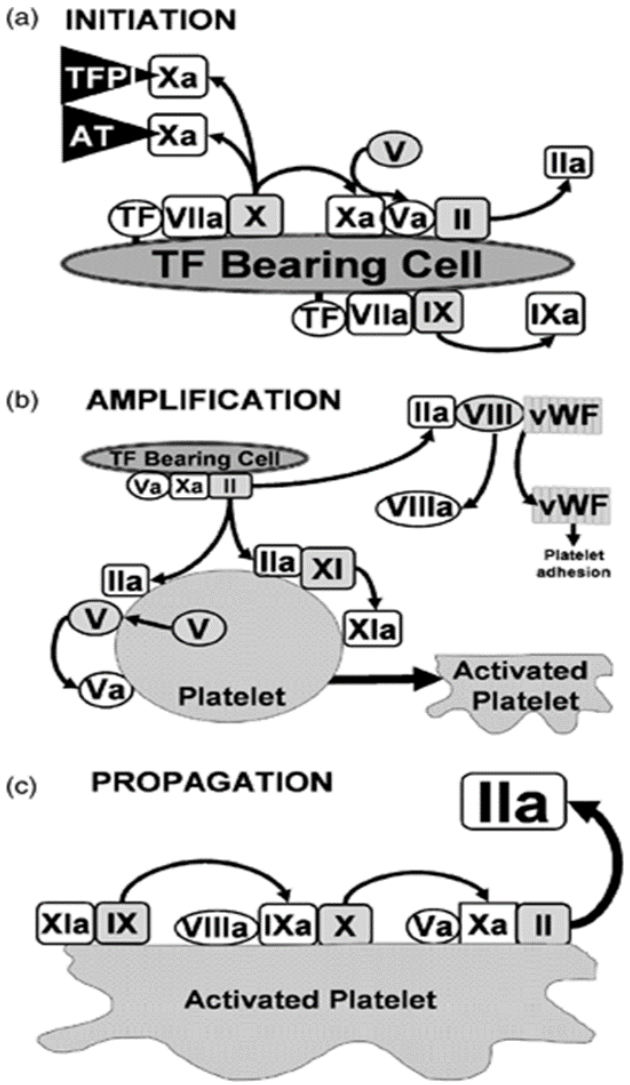
3. Promotion phase

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

Cell model


INITIATION
AMPLIFICATION
PROPAGATION
FX
Prothrombin (FII)
TF
FVIIa
FXa
FVa
Thrombin (FIIa)
vWF
FVIII
vWF
PAR
Platelet
FXI
FV
FVIIIa
FIXa
TF
FVIIa
FXIa
FVa
FIX
Prothrombin (FII)
Thrombin (FIIa)
FX
FIXa
FVIIIa
FXa
FVa
PAR
P-selectin
Platelet
gpIIb/IIIa
P_2Y_{12}
Fibrinogen (FI)
FXIII
FXIIIa
Fibrin

Cell model



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 tensor 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 – Fitzgerland 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 kininogen.

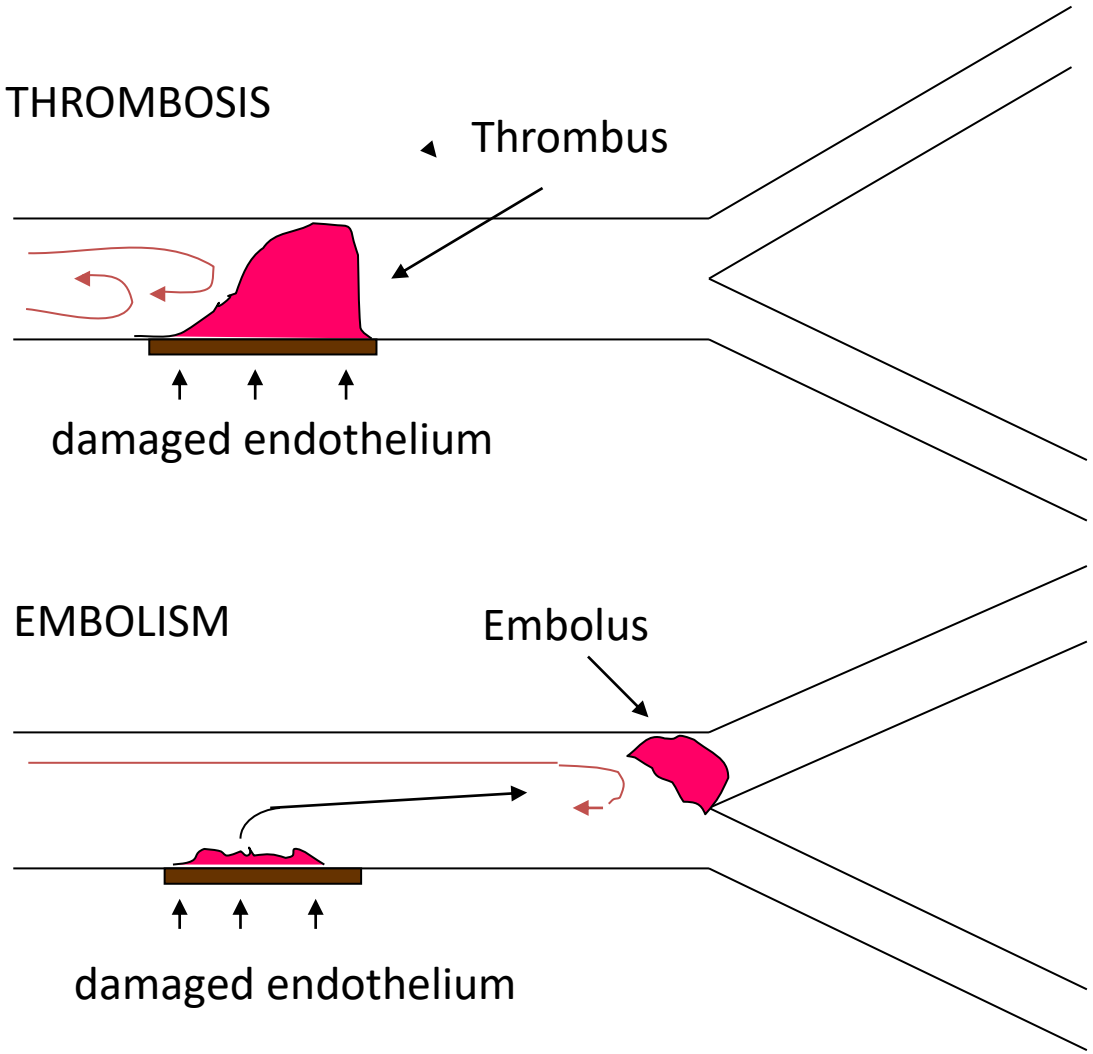
prothrombinase compl

INTRAVASCULAR COAGULATION

Damage of epithelium caused by:

1) Atherosclerosis (myocardial infarction, stroke)

2) Inflammation (venous thrombosis, pulmonary embolism)



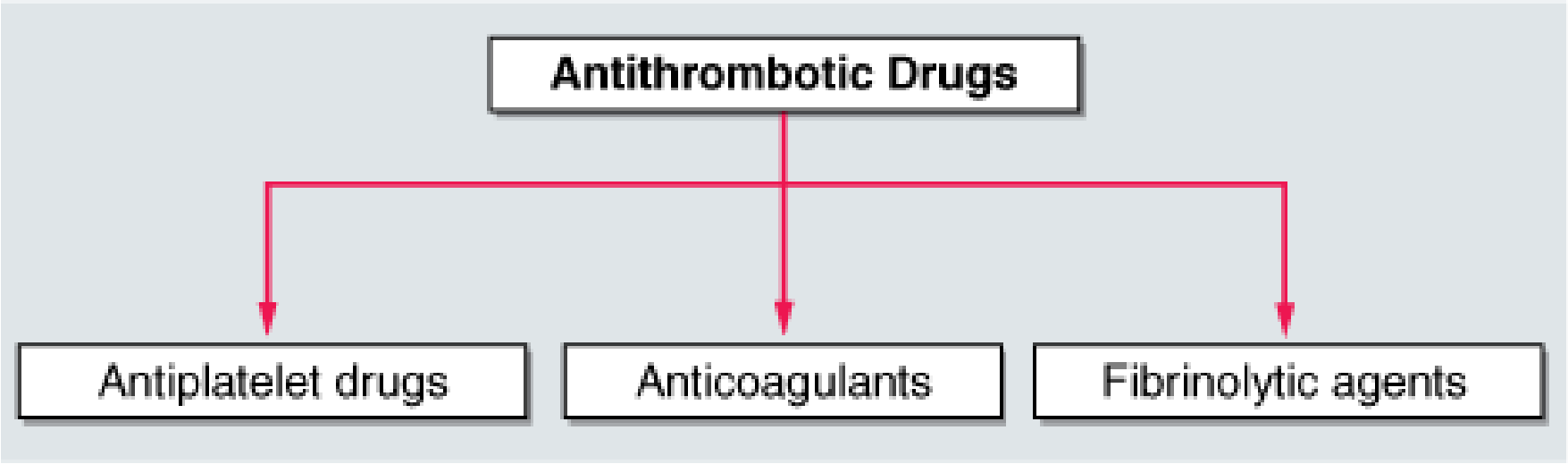
Example:

MI

Stroke

Example:

Pulmonary embolism



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

Antithrombotic drugs?

- We influence function of thrombocytes, not number of throbocytes!
- Primary and secondary prevention of atherothrombosis
  - Acute Coronary Syndromes (ACS)
  - Cerebrovascular Ischemic Attack
  - Peripheral arterial disease (PAD)
- antiplatelet agents?
- Inhibitors of cyklooxygenase/inhibitors of thromboxane A_2 synthesis or antagonists of the receptors
- Inhibitors of ADP receptors ($P2Y_{12}$)
- Antagonists of protease-activated receptors (PAR-1)
- Antagonists of surface glycoproteins (GP IIb/IIa)
- 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)

Thrombmodulin: thrombin binding protein, produced by endothelial cells.

Thrombin + Thrombmodulin = activator of protein C

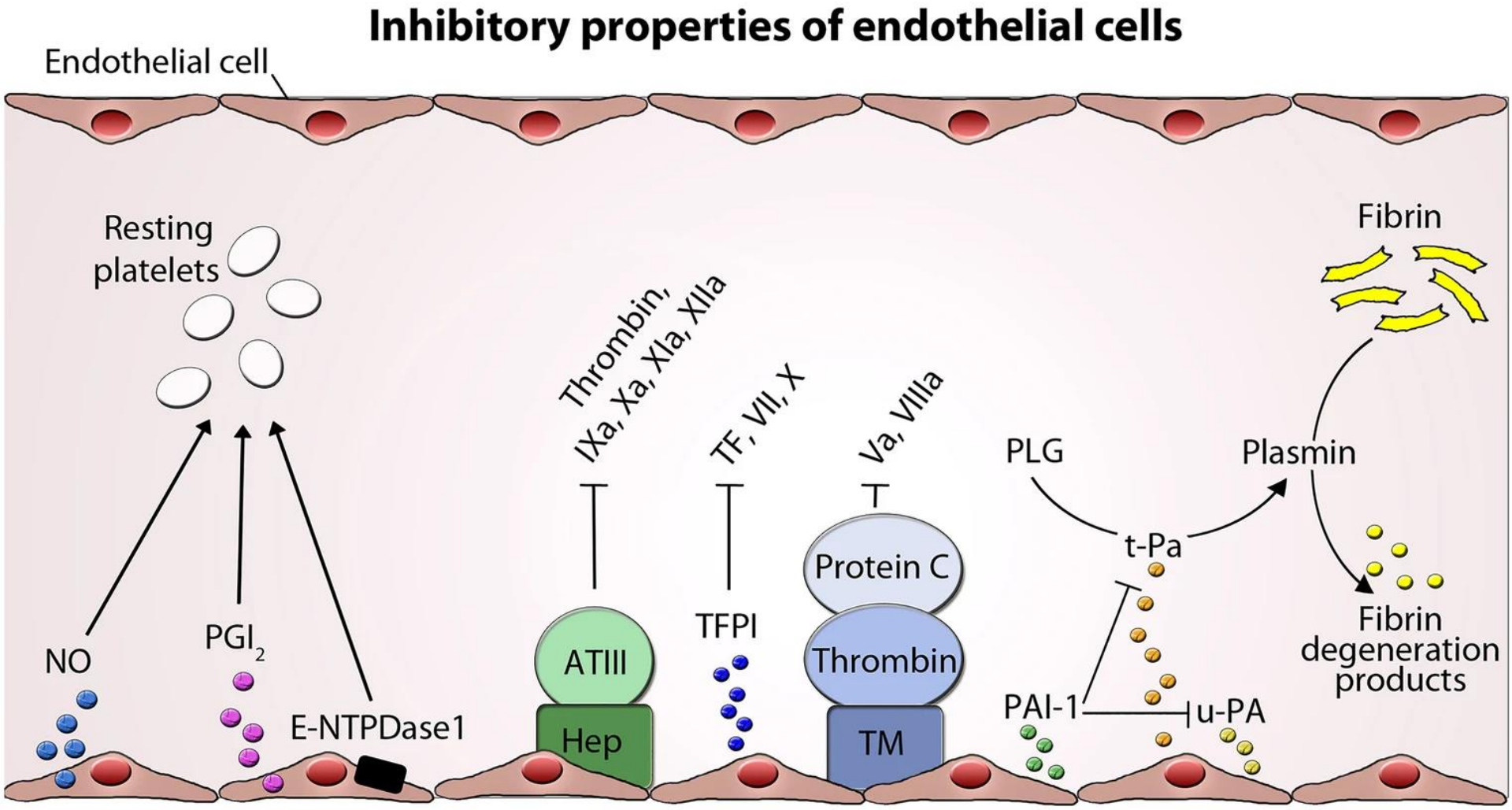
Protein C: inactivation of factors V and VIII

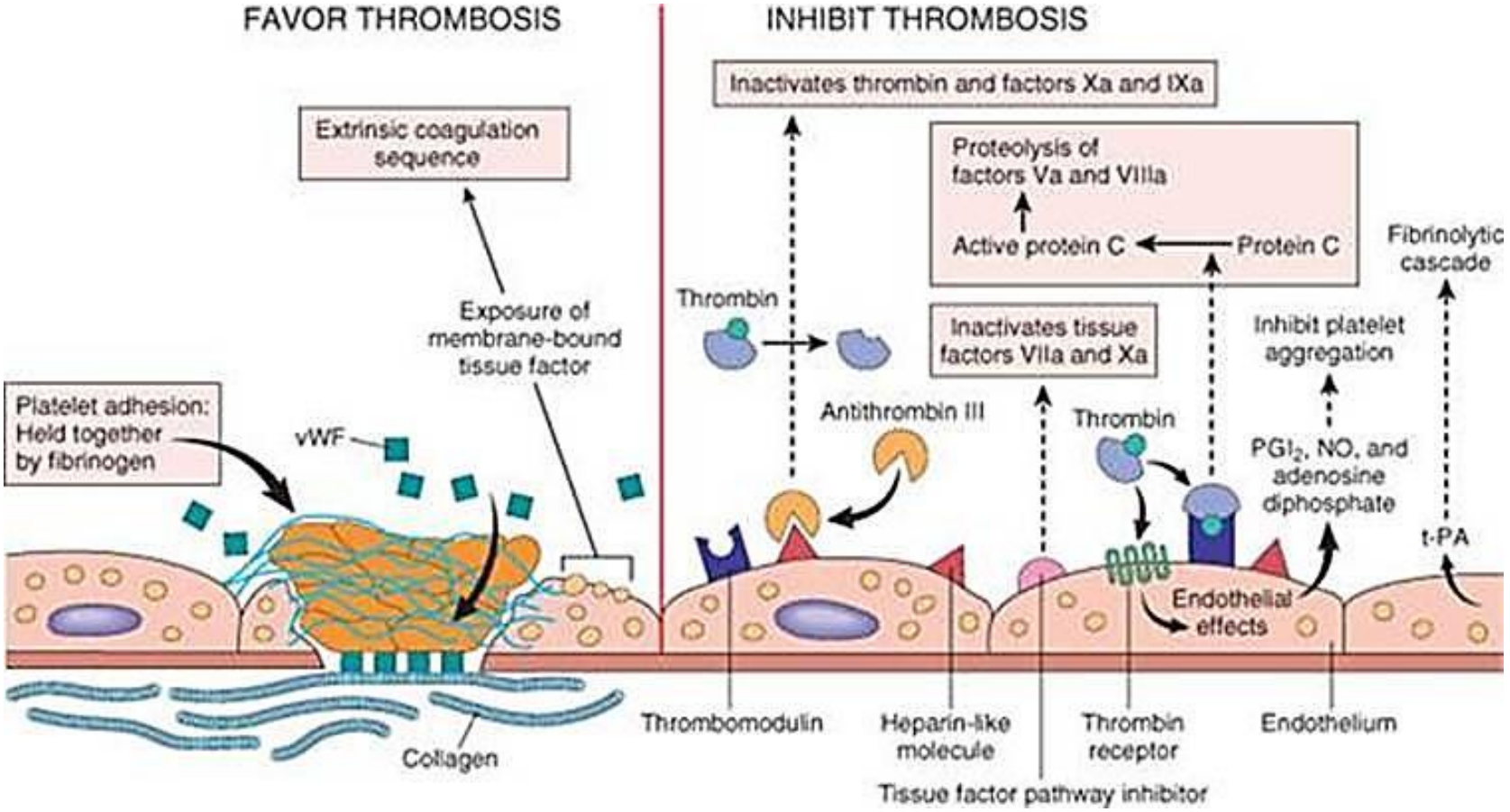
Inhibition of the inhibitor of activator of tissue plasminogen (= more plasmin – degradation of fibrin)

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.

Endothelium nad hemostasis




FAVOR THROMBOSIS
INHIBIT THROMBOSIS
Extrinsic coagulation sequence
Exposure of membrane-bound tissue factor
Platelet adhesion: Held together by fibrinogen
vWF
Collagen
Inactivates thrombin and factors Xa and IXa
Proteolysis of factors Va and VIIIa
Active protein C
Protein C
Fibrinolytic cascade
Thrombin
Inactivates tissue factors VIIa and Xa
Inhibit platelet aggregation
Antithrombin III
Thrombin
PGI2, NO, and adenosine diphosphate
t-PA
Endothelial effects
Thrombomodulin
Heparin-like molecule
Thrombin receptor
Endothelium
Tissue factor pathway inhibitor

FIBRINOLYSIS AND TROMBOLYSIS

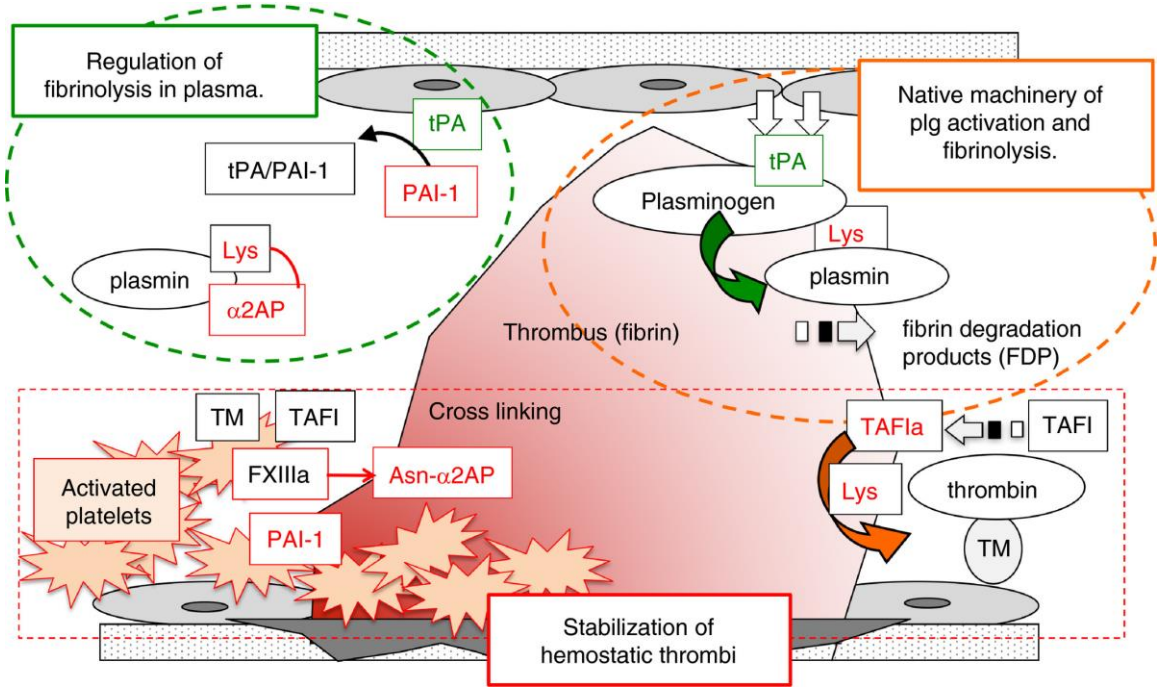
Inactive plasminogen.

Active plasmin (fibrinolysin).

Activators of plasminogen.

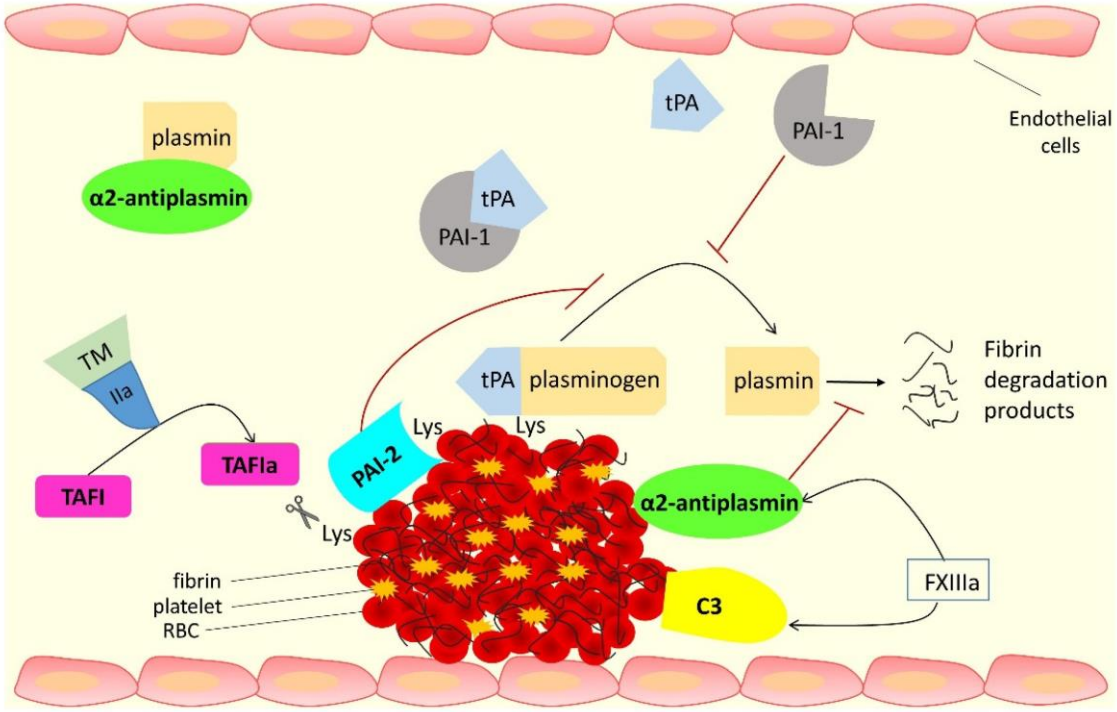
Inhibitors of plasminogen.

Fibrinolysis

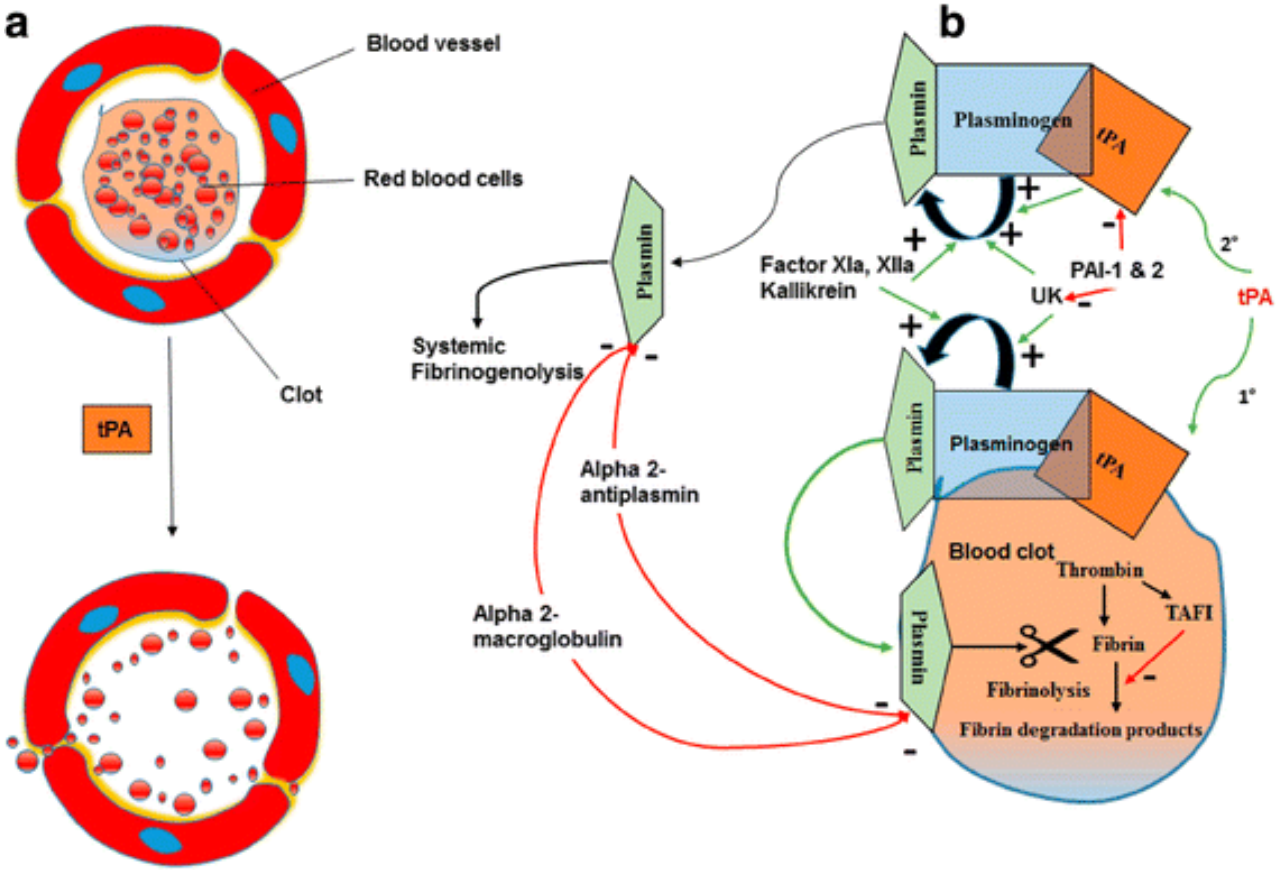


Native machinery of plasminogen (plg) activation and fibrinolysis and its modification by vascular endothelial cells (VECs) and platelets. (1) The upper right panel shows native machinery of plg activation and fibrinolysis. VECs secrete and retain tissue-type plasminogen activator (tPA) and maintain adequate fibrinolytic potential on their surfaces. When fibrin is generated, tPA and plg accumulate on fibrin surfaces through C-terminal lysine residues bound to the lysine binding sites (LBSs) of plg, which trigger plg activation and fibrinolysis. (2) The lower right panel illustrates the inhibitory function of VECs on fibrinolysis. When thrombin is generated adjacent to thrombomodulin (TM)-expressing VECs, TM-bound thrombin effectively generates the carboxypeptidase B, thrombin-activatable fibrinolysis inhibitor (TAFIa), which specifically cleaves C-terminal lysine residues and inhibits fibrinolysis. (3) The lower left panel shows the inhibitory function of platelets on fibrinolysis. Activated platelets secrete plasminogen activator inhibitor type 1 (PAI-1) and the FXIIIa catalytic A subunit, the latter of which cross-links α2AP to fibrin to stabilize thrombus. The mechanisms shown in (2) and (3) are coordinated to protect hemostatic thrombi from immature lysis. (4) In plasma, free tPA and free plasmin are effectively inhibited by PAI-1 and α2AP, respectively. PAI-1 also inhibits tPA activity on VECs by facilitating the dissociation of the membrane-bound tPA on the surface of VECs and regulates plg activation potential (upper left panel).

https://onlinelibrary.wiley.com/doi/full/10.1111/jth.14157



The role of antifibrinolytic proteins in the fibrinolytic process. The fibrin component of the thrombus is degraded by plasmin, generated by tissue plasminogen activator (tPA) activation of plasminogen. Anti-fibrinolytic protein plasminogen activator inhibitor-1 (PAI-1) binds tPA, preventing plasminogen activation. Alpha-2 antiplasmin (α2AP) forms a stable complex with plasmin in the circulation or becomes cross-linked into the fibrin clot by activated FXIII (FXIIIa), which makes the clot more resistant to fibrinolysis. Thrombin activatable fibrinolysis inhibitor (TAFI) is activated by thrombin (IIa) in complex with thrombomodulin (TM). Activated TAFI (TAFIa) cleaves off lysine residues (Lys) from the fibrin surface therefore decreasing plasminogen and tPA binding, thus reducing plasmin generation. TAFI, as well as plasminogen activator inhibitor-2 (PAI-2), can also be cross-linked into the fibrin clot by FXIIIa. Complement C3 is bound and cross-linked to the fibrin clot by FXIIIa, causing prolongation of fibrinolysis.


a
Blood vessel
Red blood cells
Clot
tPA
b
Plasmin
Plasminogen
tPA
Factor XIa, XIIa
Kallikrein
PAI-1 & 2
UK
2°
tPA
1°
Systemic
Fibrinogenolysis
Alpha 2-
antiplasmin
Alpha 2-
macroglobulin
Blood clot
Thrombin
TAFI
Fibrin
Fibrinolysis
Fibrin degradation products

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)

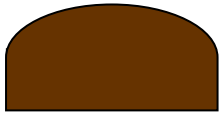
Anticoagulants

Activation →

Inhibition ⊣

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

Coumarin (warfarin) ↓

Vitamin K →  → prothrombin

Liver

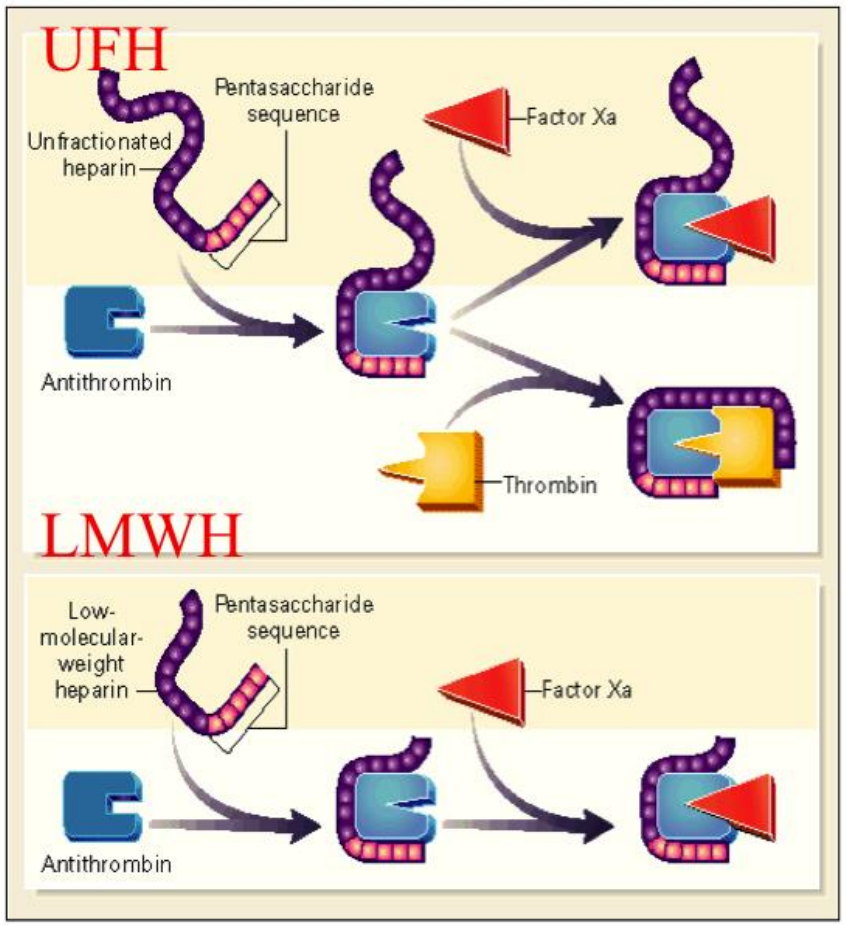
test: PT
INR=$PT_{patient}/PT_{norm}$

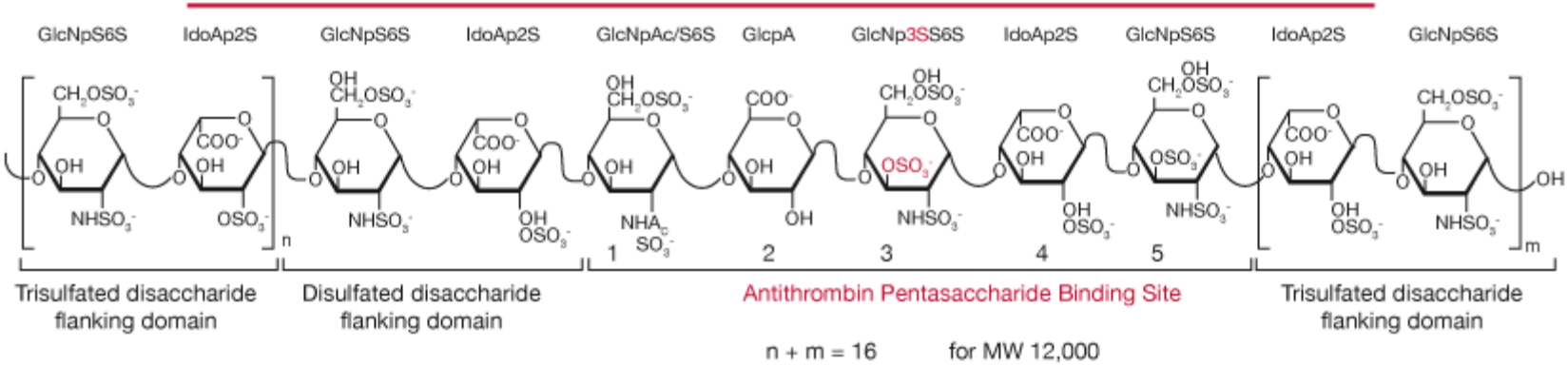
In vitro: Heparin → antithrombin III

warfarin → INR 2-3

Sodium citrate → Ca^{++}

aPTT: activated partial thromboplastin time PT: prothrombin time

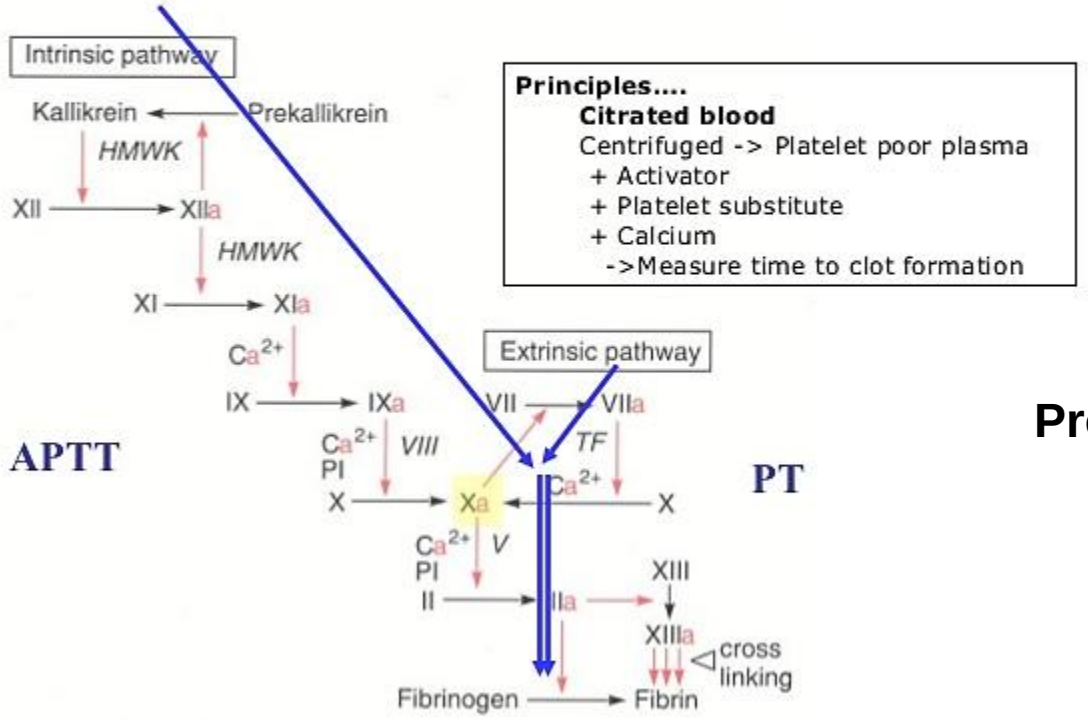




n + m = 16 for MW 12,000

Tests aPTT and PT

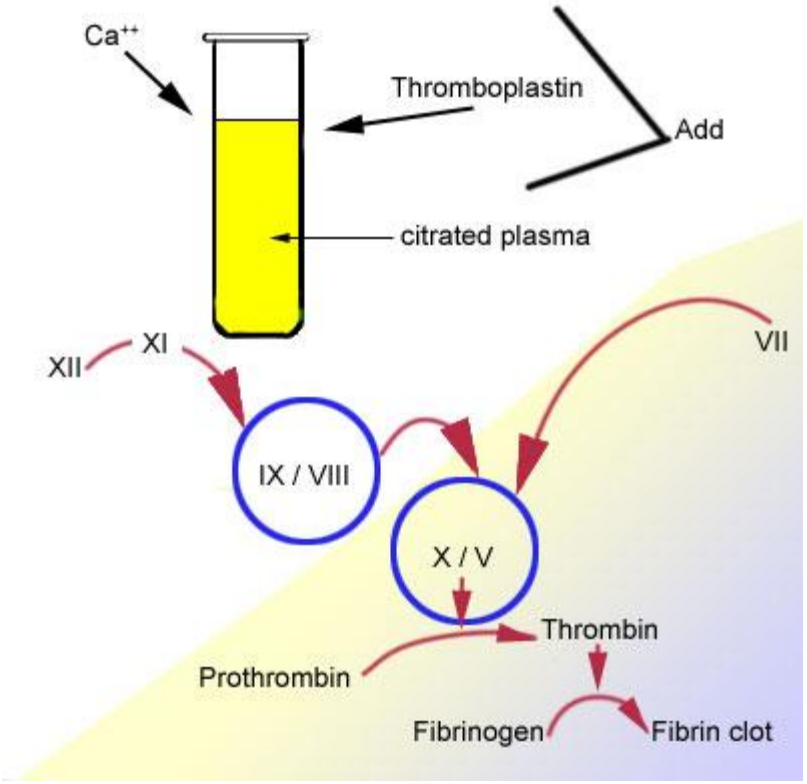
Tests: PT and APTT

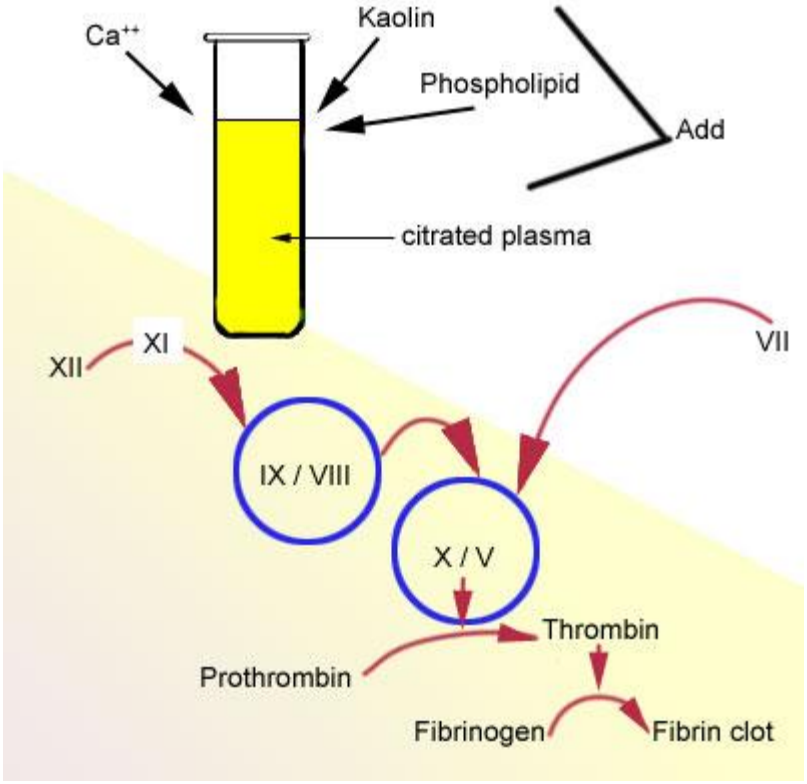


Prothrombin time test

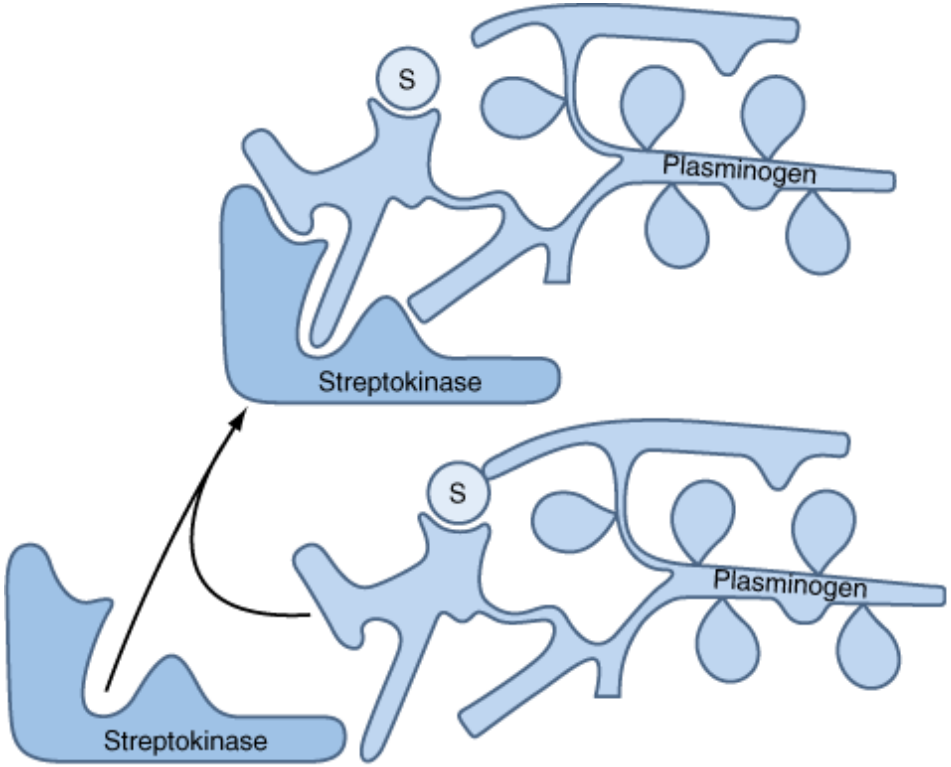
Activated Partial Thromboplastin Time test

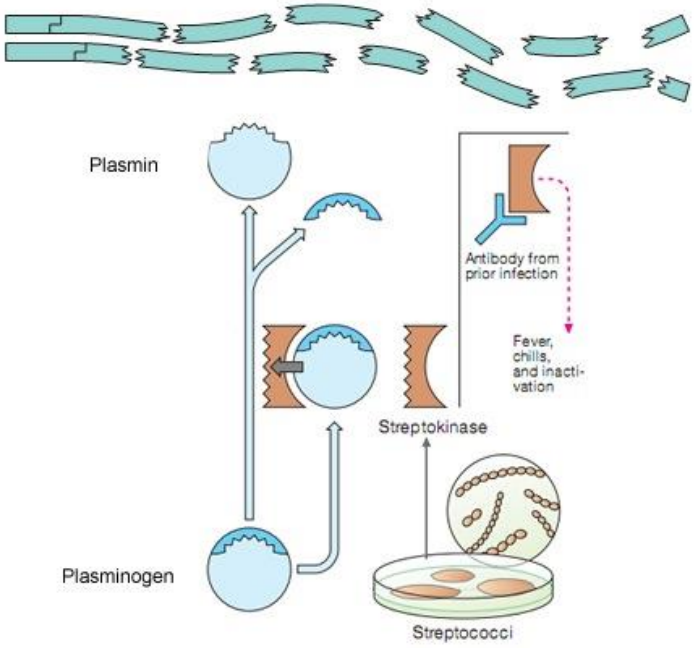
HMWK, high-molecular-weight-kininogen PK, prekallikrein F, factor


Ca++
Thromboplastin
Add
citrated plasma
XII
XI
VII
IX / VIII
X / V
Thrombin
Prothrombin
Fibrinogen
Fibrin clot


Ca++
Kaolin
Phospholipid
Add
citrated plasma
XII
XI
VII
IX / VIII
X / V
Thrombin
Prothrombin
Fibrinogen
Fibrin clot

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

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