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.

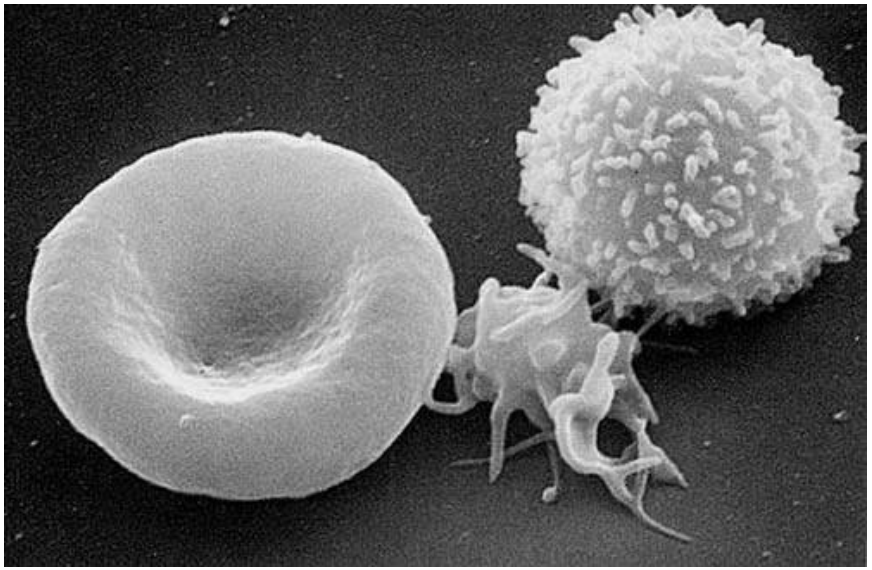
Origin: megakaryocytes of bone marrow under the effect of colony stimulating factors – interleukins (*IL-1, IL-3, IL-6*) and granulocytes and macrophages stimulating factor (*GM-CSF*)

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

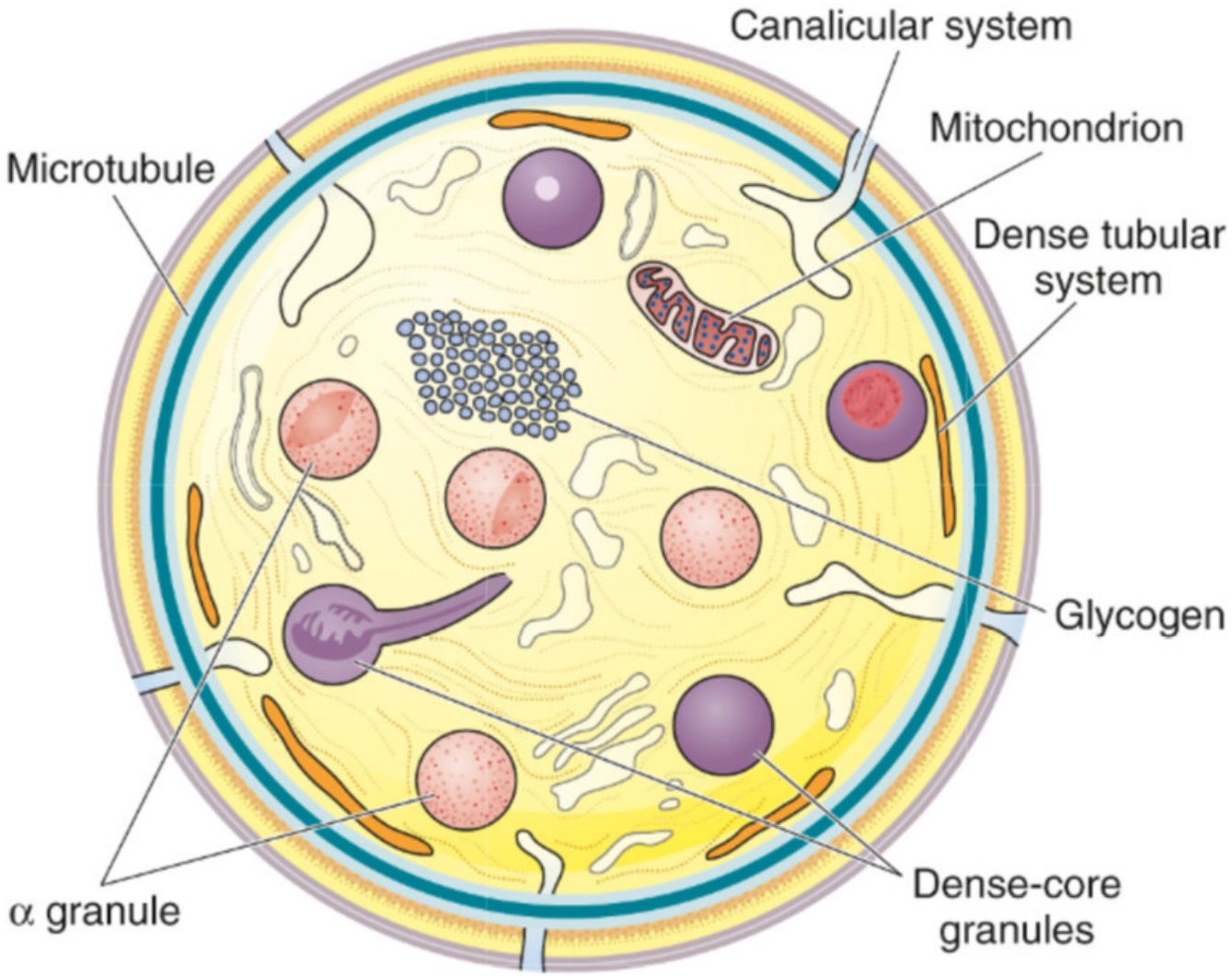
Cytoplasm: contains actin, myosin, glycogen, lysozomes and

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



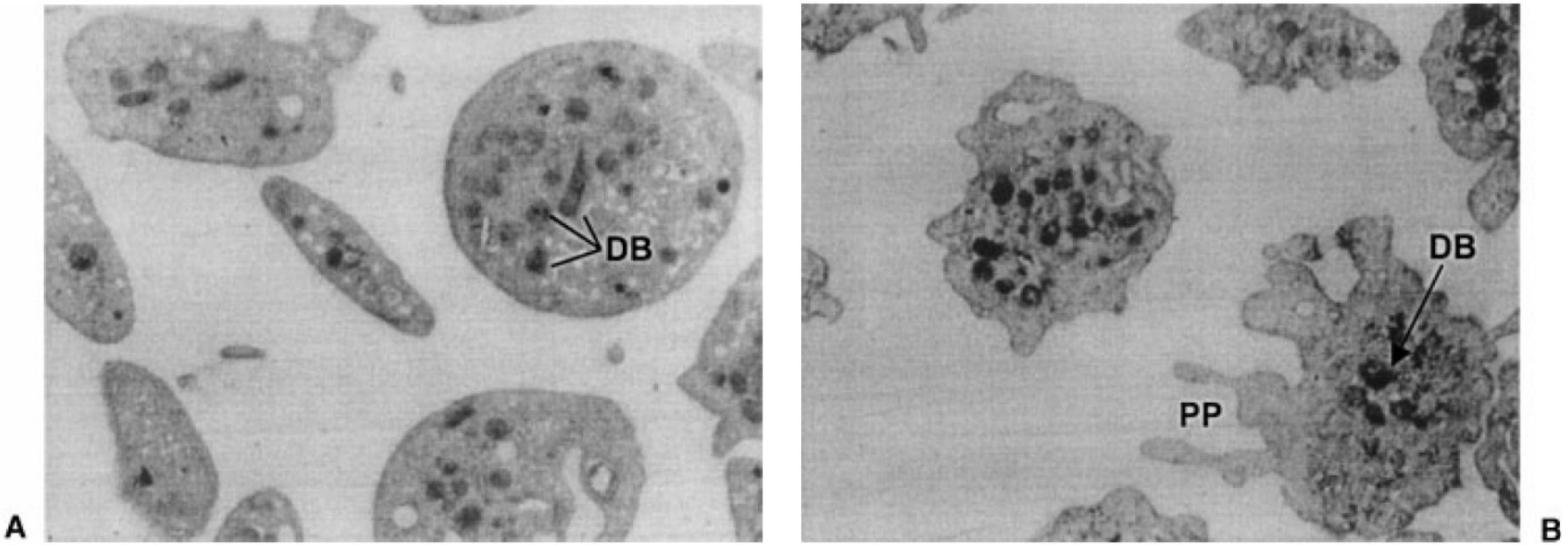


Figure 1 Morphology of human platelets. (A) Thin section of discoid resting platelets with evenly distributed granules. (B) Thin section of stimulated platelets, showing formation of pseudopodia and centralization of granules. DB, dense body; PP, pseudopodium. Magnification × 21,000.

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

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, tromboxane A_2

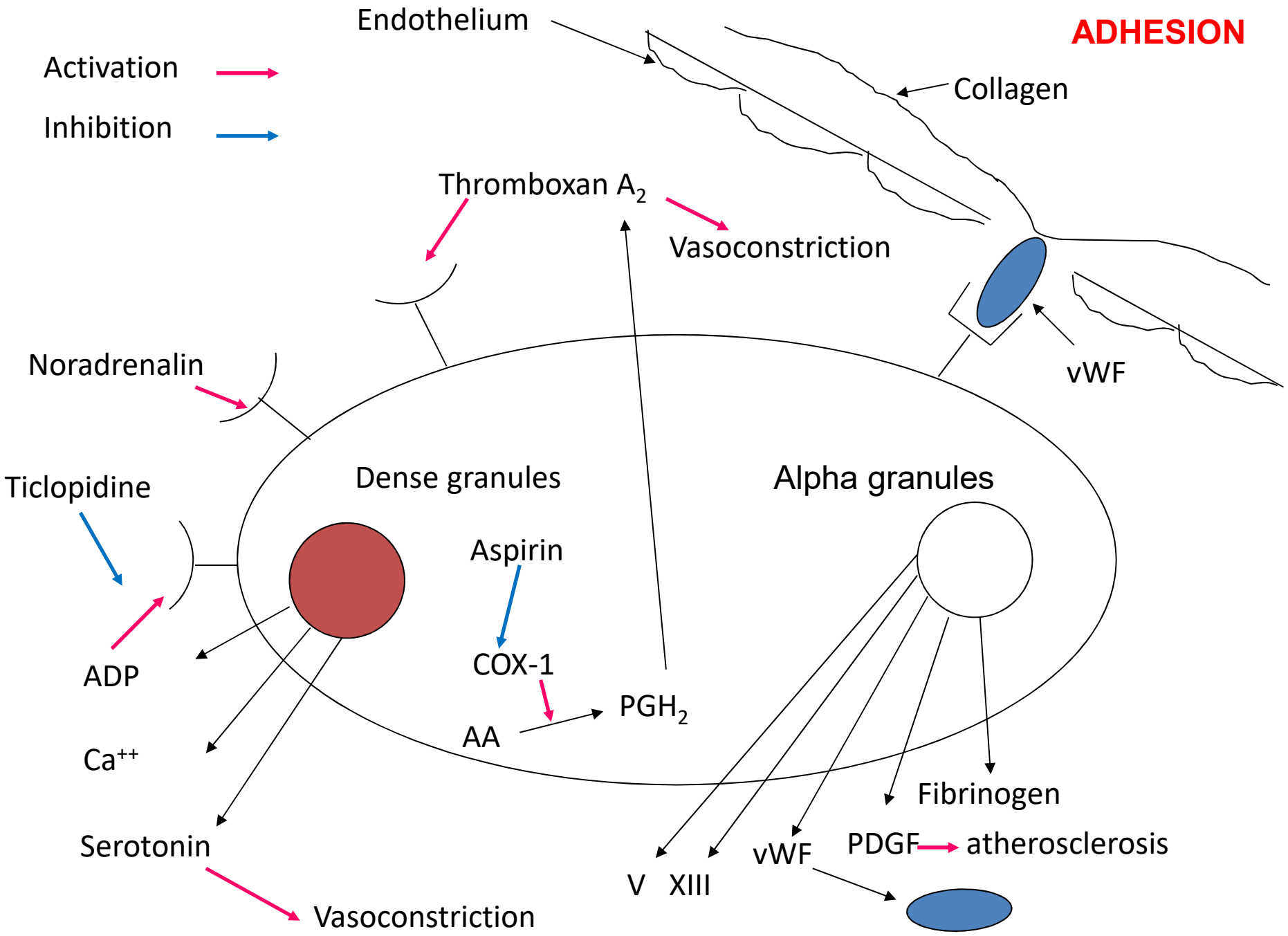
mitogenic effects – growth factor (**PDGF**)

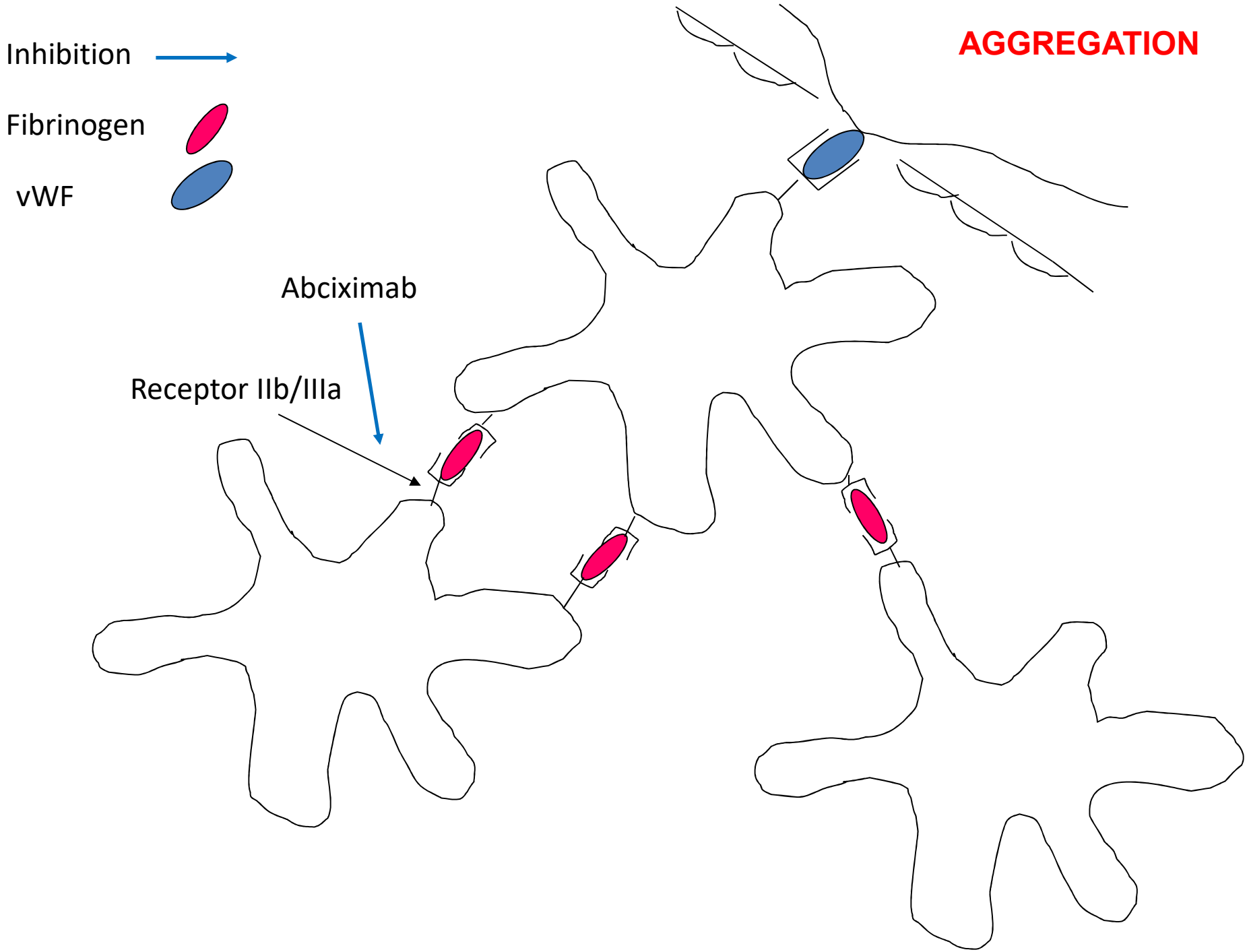
activation of platelets and phagocytes – **PAF** (cytokine, G-coupled receptor, phospholipase C, DAG, increase of intracellular Ca^{2+}concentration, phospholipase A_2 – arachidonic acid – thromboxane A_2)!!! Therapeutic use of acetylsalicylic acid!!!

Aggregation.

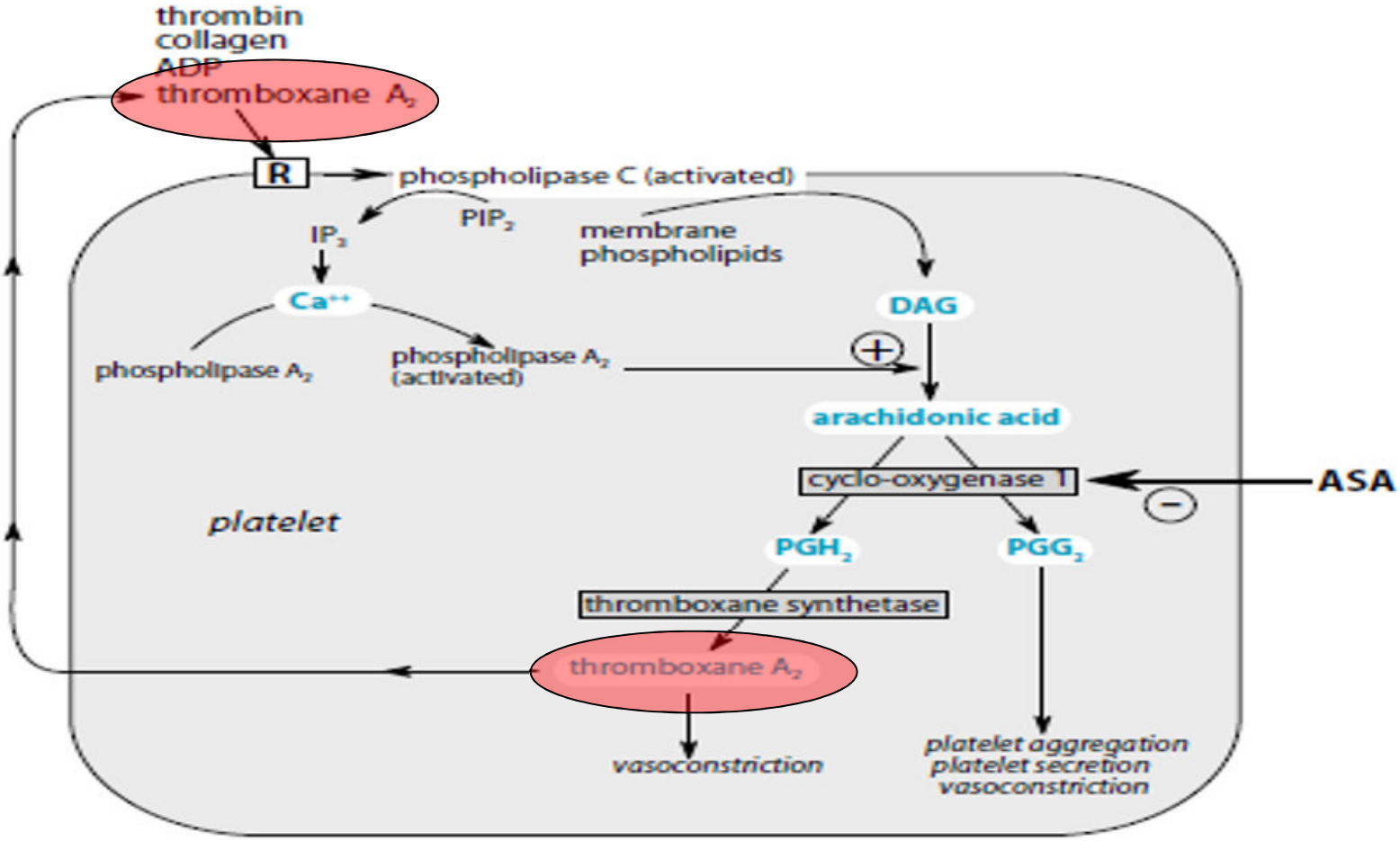
Vasoconstriction.

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

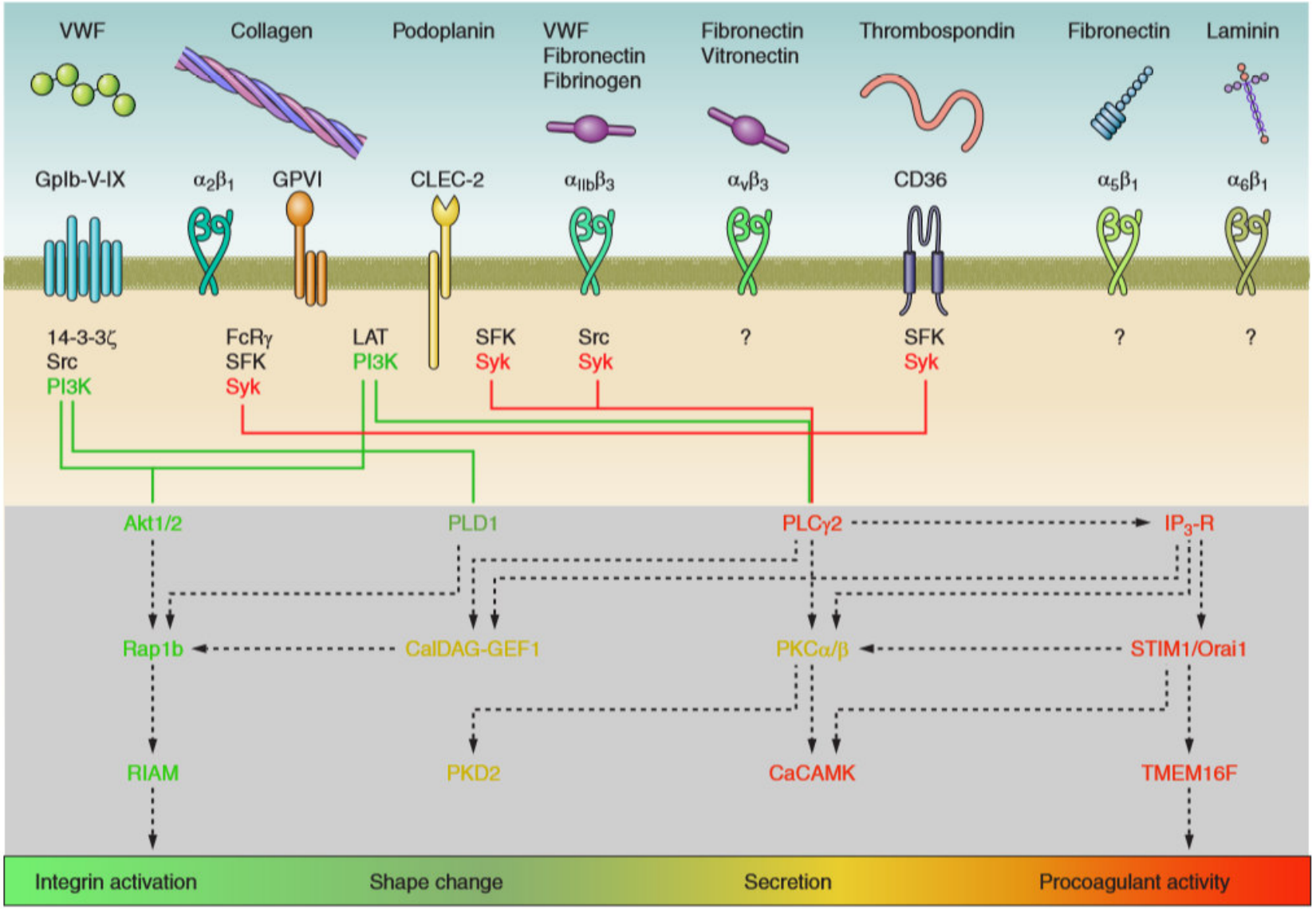

ADHESION
Endothelium
Activation
Inhibition
Collagen
Thromboxan A2
Vasoconstriction
vWF
Noradrenalin
Ticlopidine
Dense granules
Alpha granules
Aspirin
ADP
COX-1
PGH2
AA
Ca++
Fibrinogen
Serotonin
vWF
PDGF
atherosclerosis
V XIII
Vasoconstriction

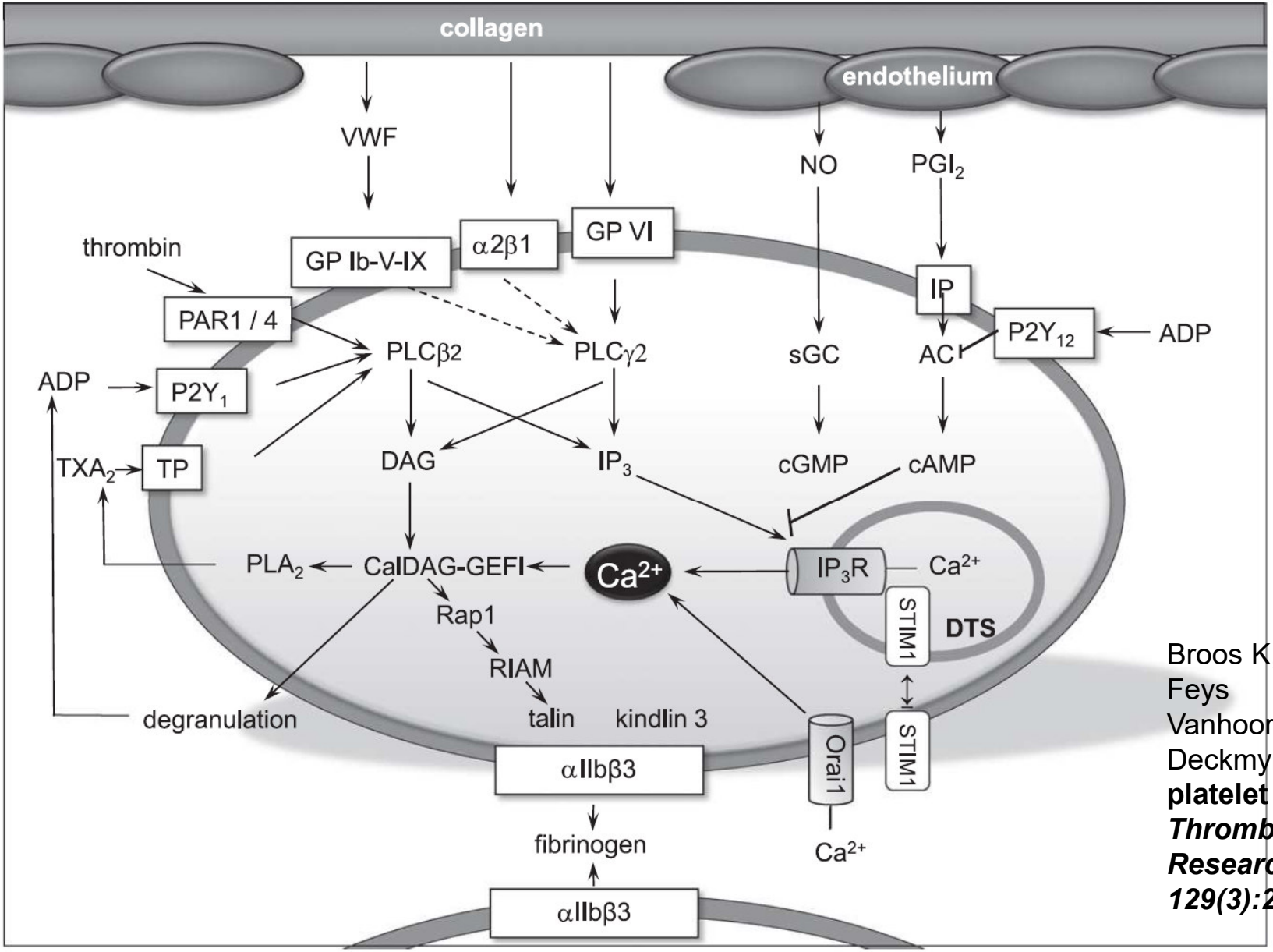

Inhibition
Fibrinogen
vWF
AGGREGATION
Abciximab
Receptor IIb/IIIa

Aggregation – an example of positive feedback



Ligands and receptors involved in adhesion of platelets

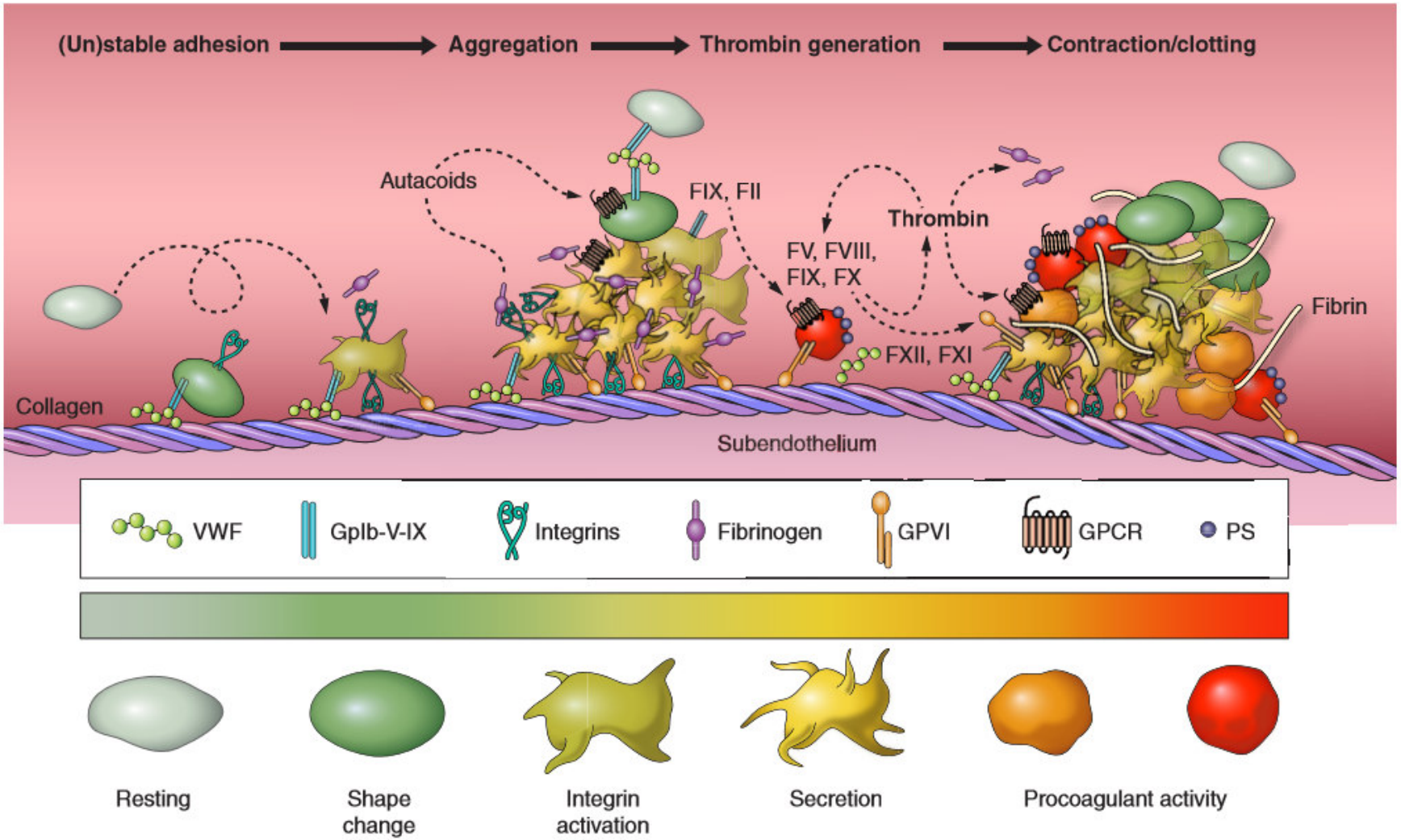




Broos K, De Meyer SF, Feys HB, Vanhoorelbeke K, Deckmyn H: **Blood platelet biochemistry.** ***Thrombosis Research 2012, 129(3):245-249.***

Fig. 1. Schematic overview of the main platelet receptors and effectors involved in platelet activation, amplification, aggregation and inhibition.

Hemostasis - white thrombus – overview and connections



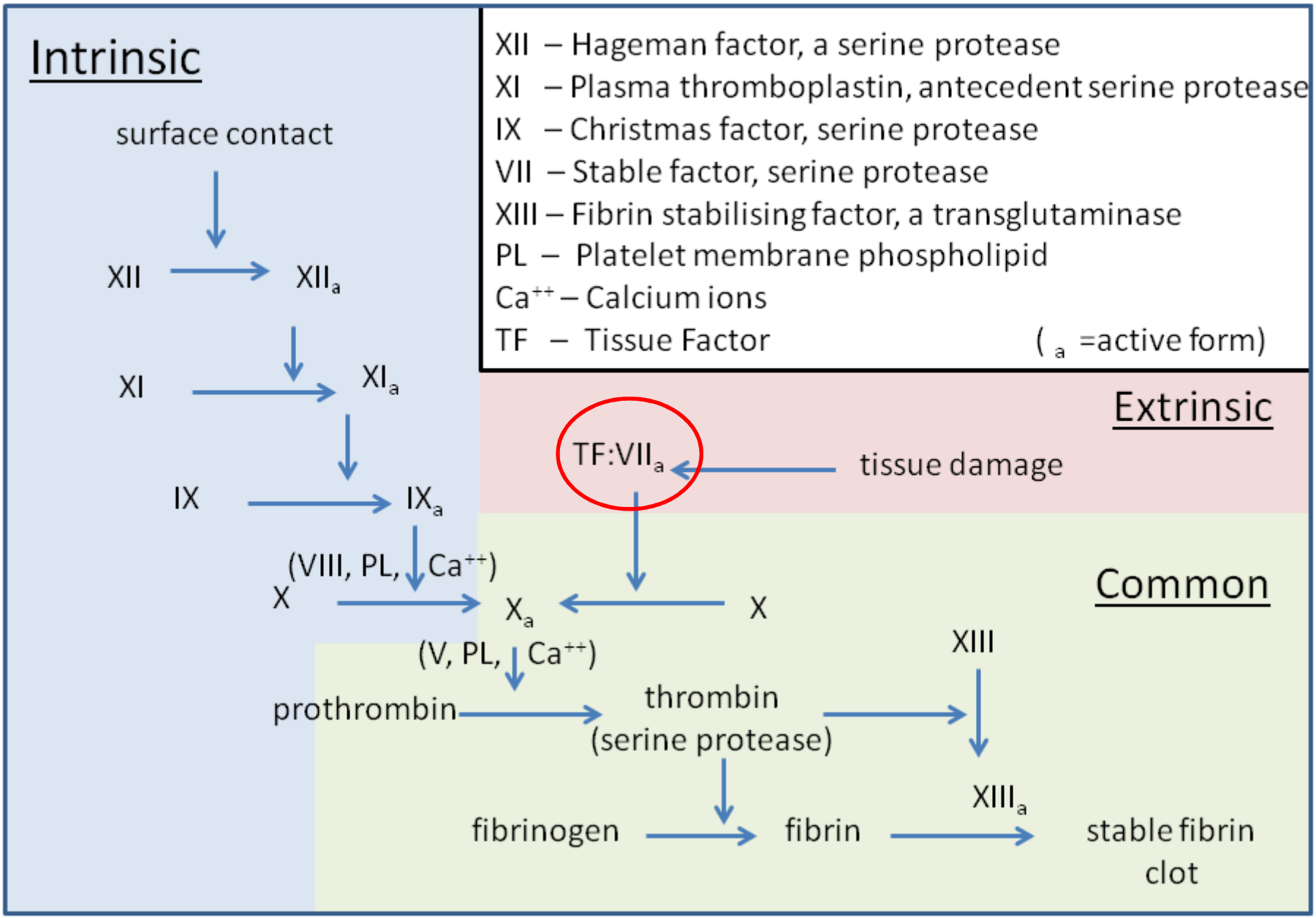
HEMOSTASIS II. – red clot

Prothrombin (factor X) – thrombin.

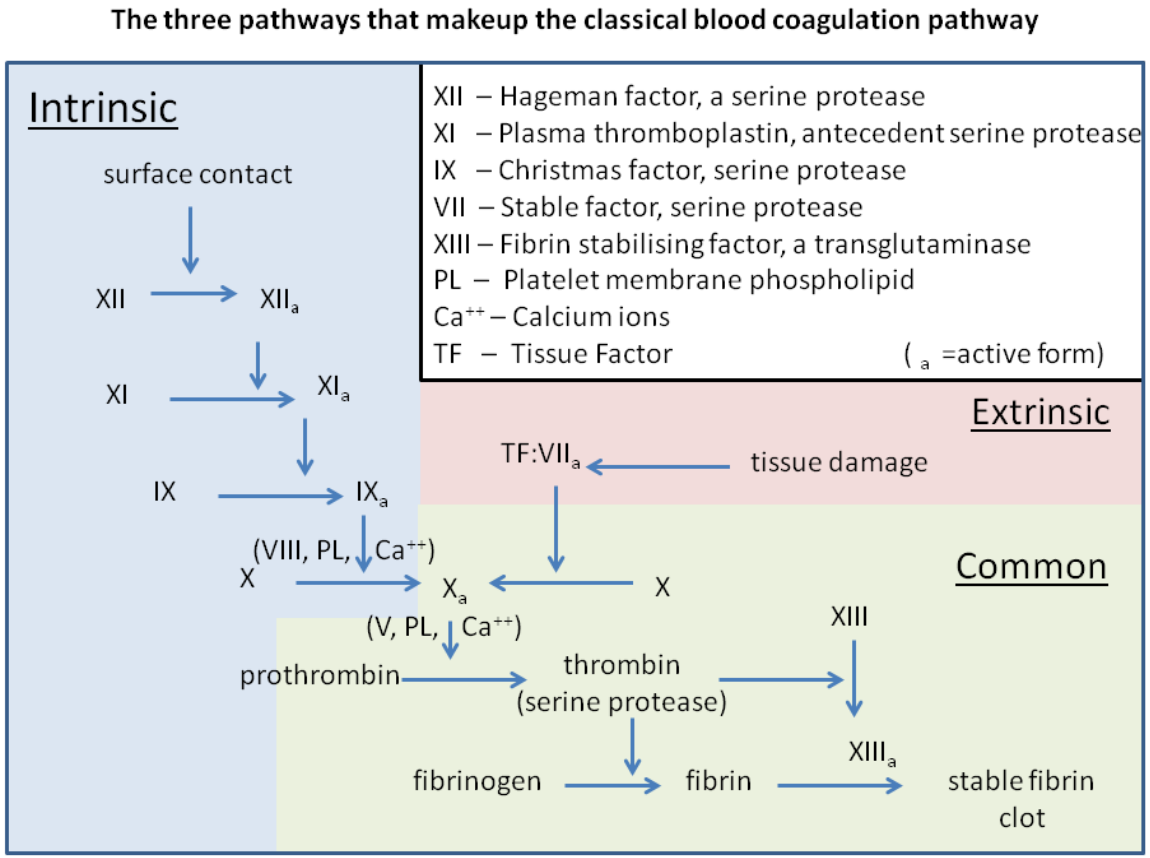
Fibrinogen – fibrin monomer – fibrin polymer (factor III, Ca^{2+}).

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

The three pathways that makeup the classical blood coagulation pathway

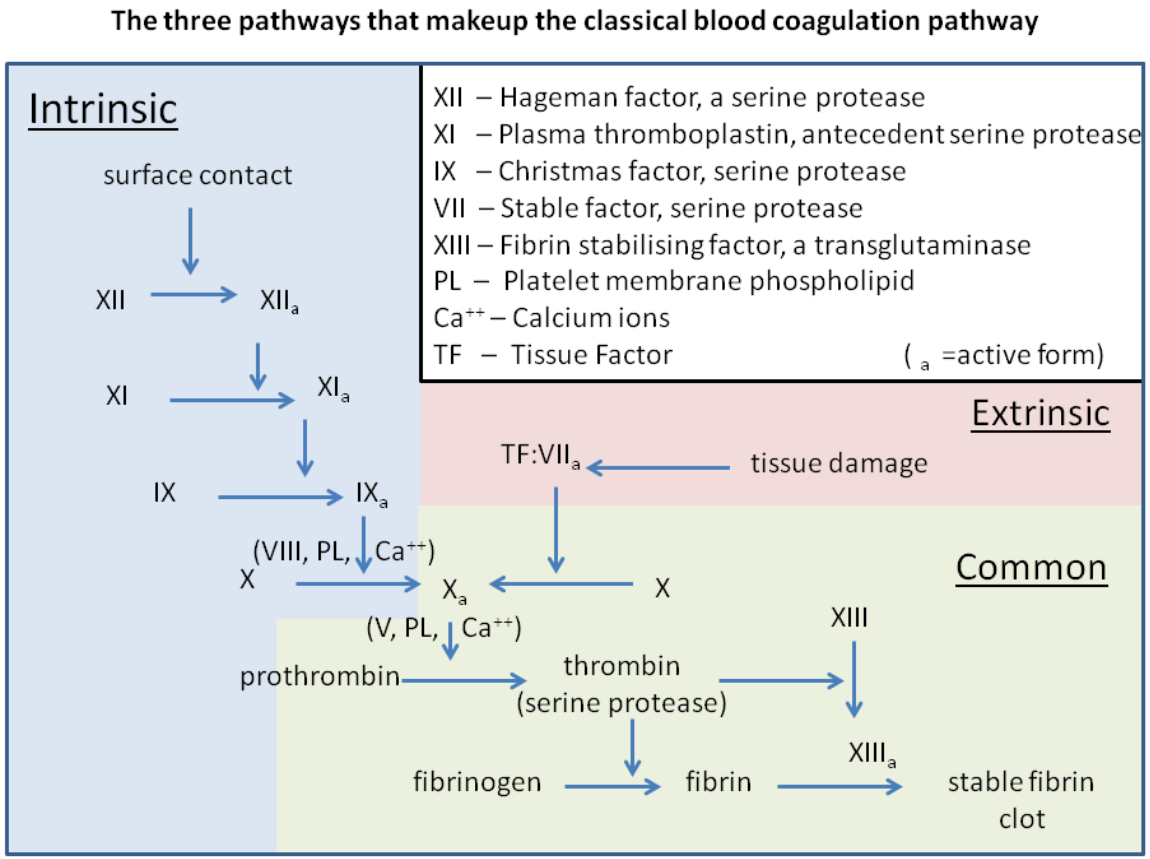


Intrinsic pathway



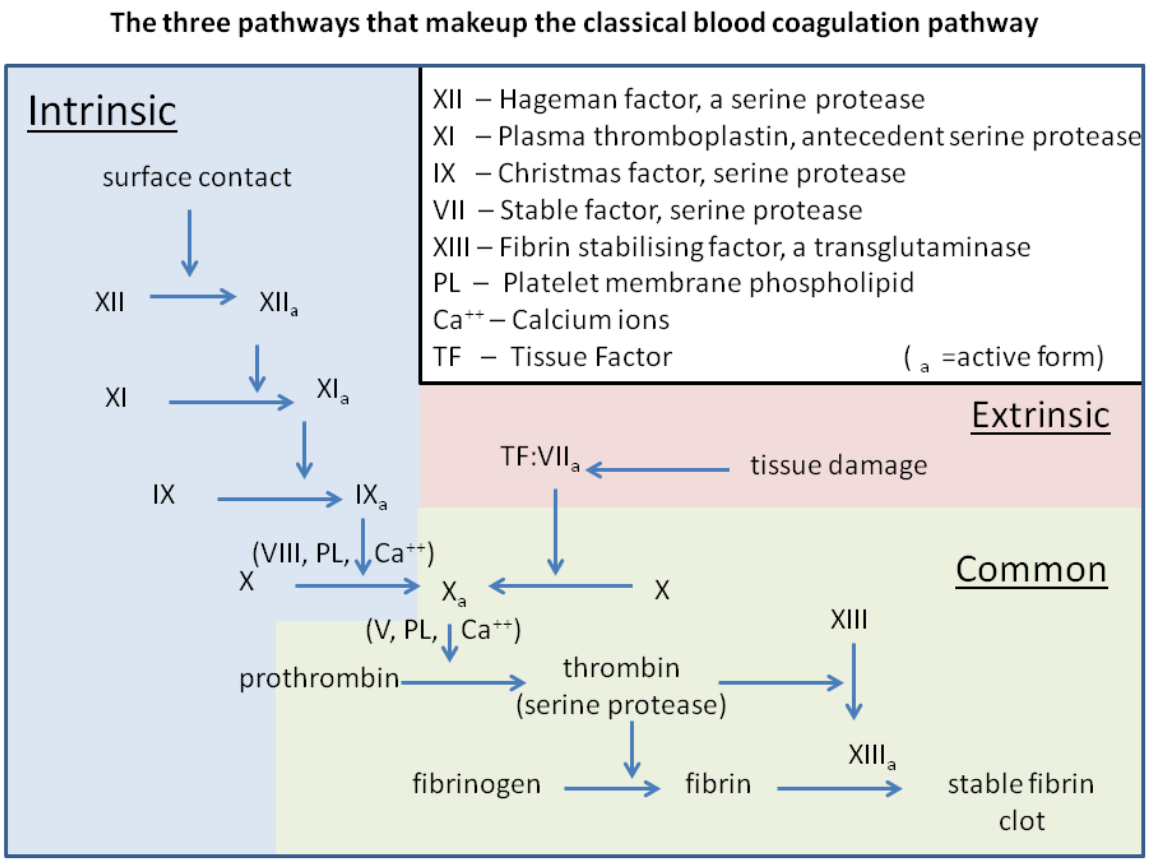
- Factors IXa, Xa, and thrombin proteolytically cleave Factor VIII to form VIIIa, which is the co-factor of the next reaction.
- VIIIa, together with IXa, calcium ions (from the platelets) and negatively charged phospholipids, form the trimolecular complex of tenase
- Tenase converts factor X to Xa.

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

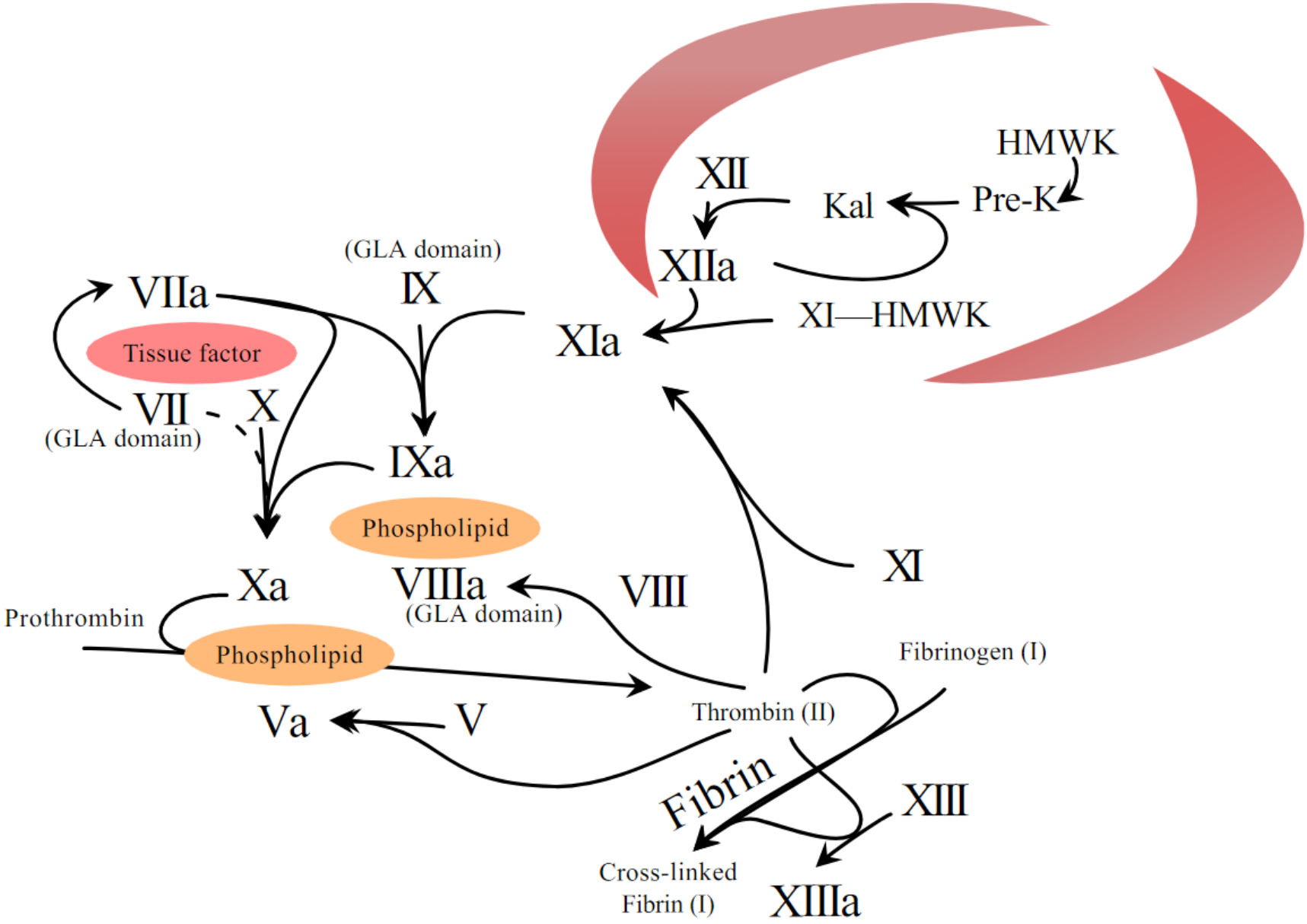
2. Amplification phase

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

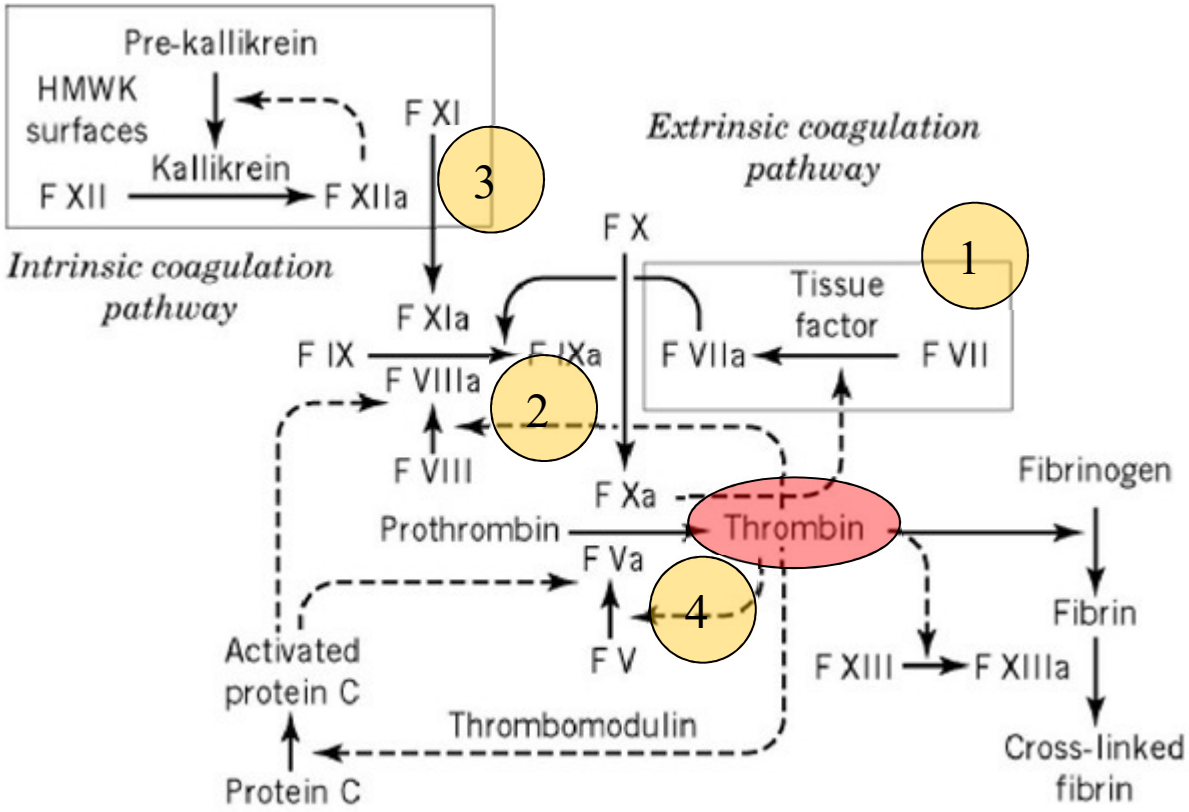
3. Promotion phase

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

Modern concept

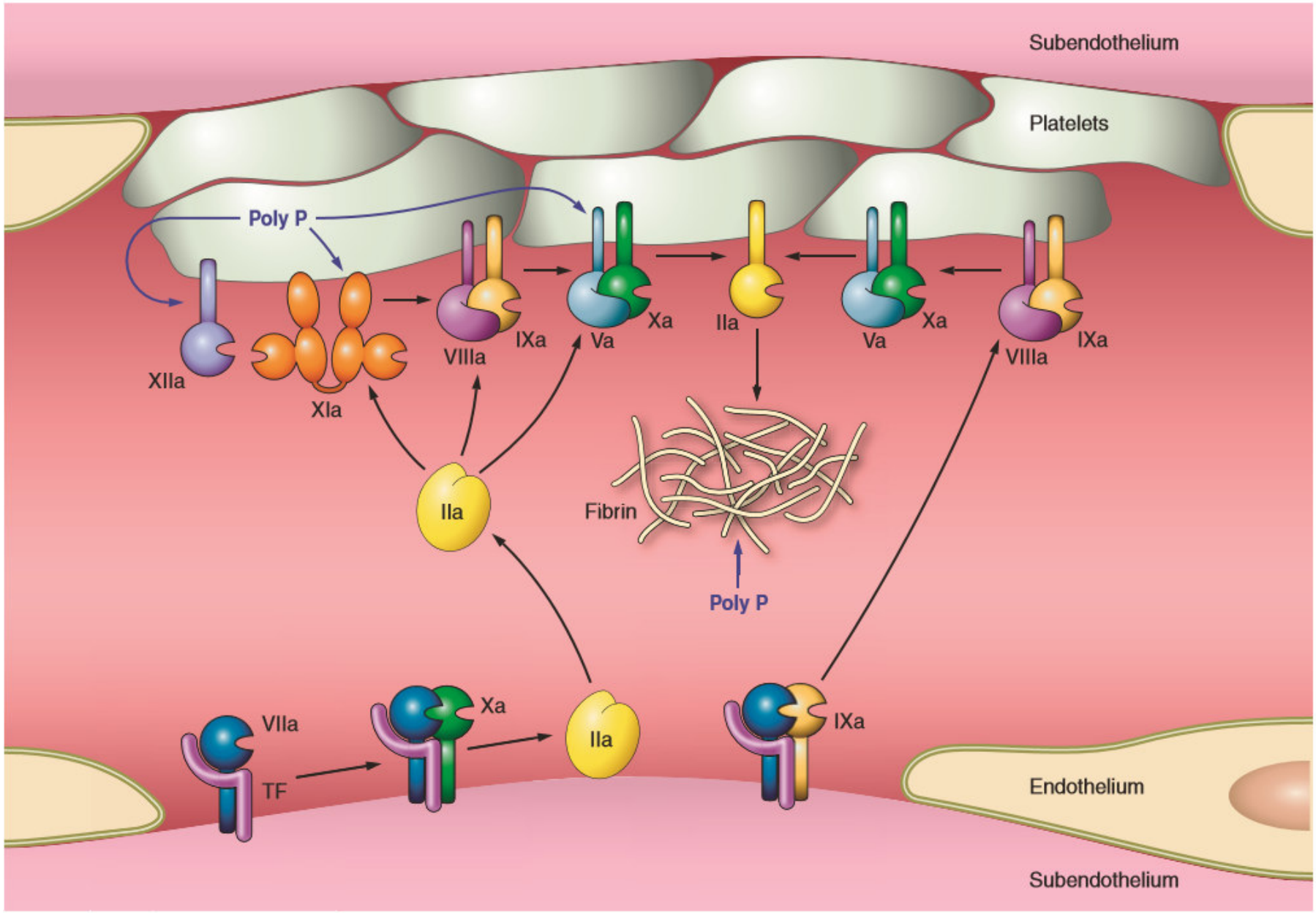


Thrombin – an example of positive feedback

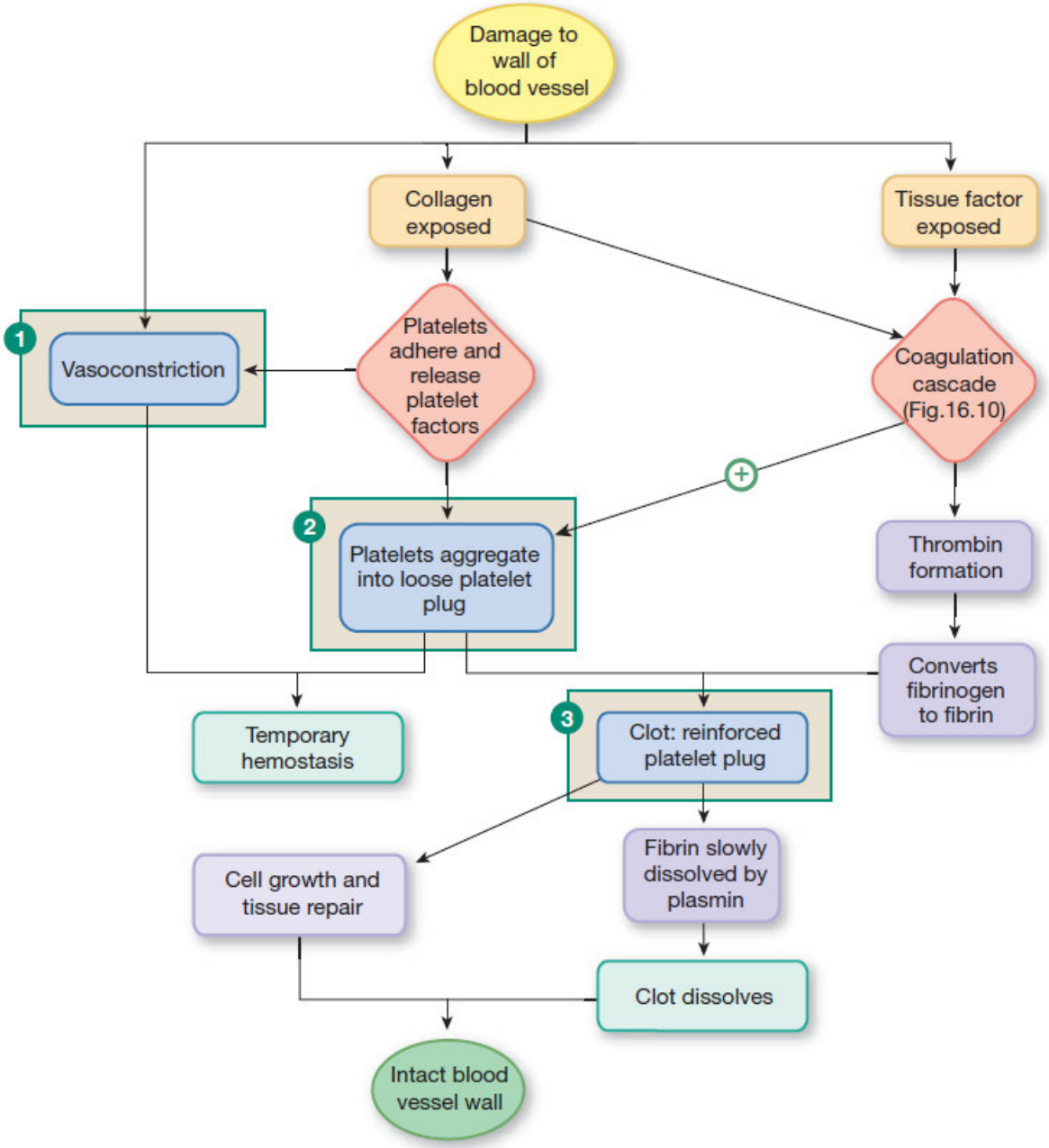


Thrombin

- very small amount of thrombin is insufficient to activate fibrinogen
- four major feedback mechanisms


Subendothelium
Platelets
Poly P
XIIa
XIa
VIIIa
IXa
Va
Xa
IIa
Va
Xa
VIIIa
IXa
IIa
Fibrin
Poly P
VIIa
TF
Xa
IIa
IXa
Endothelium
Subendothelium

SUMMARY



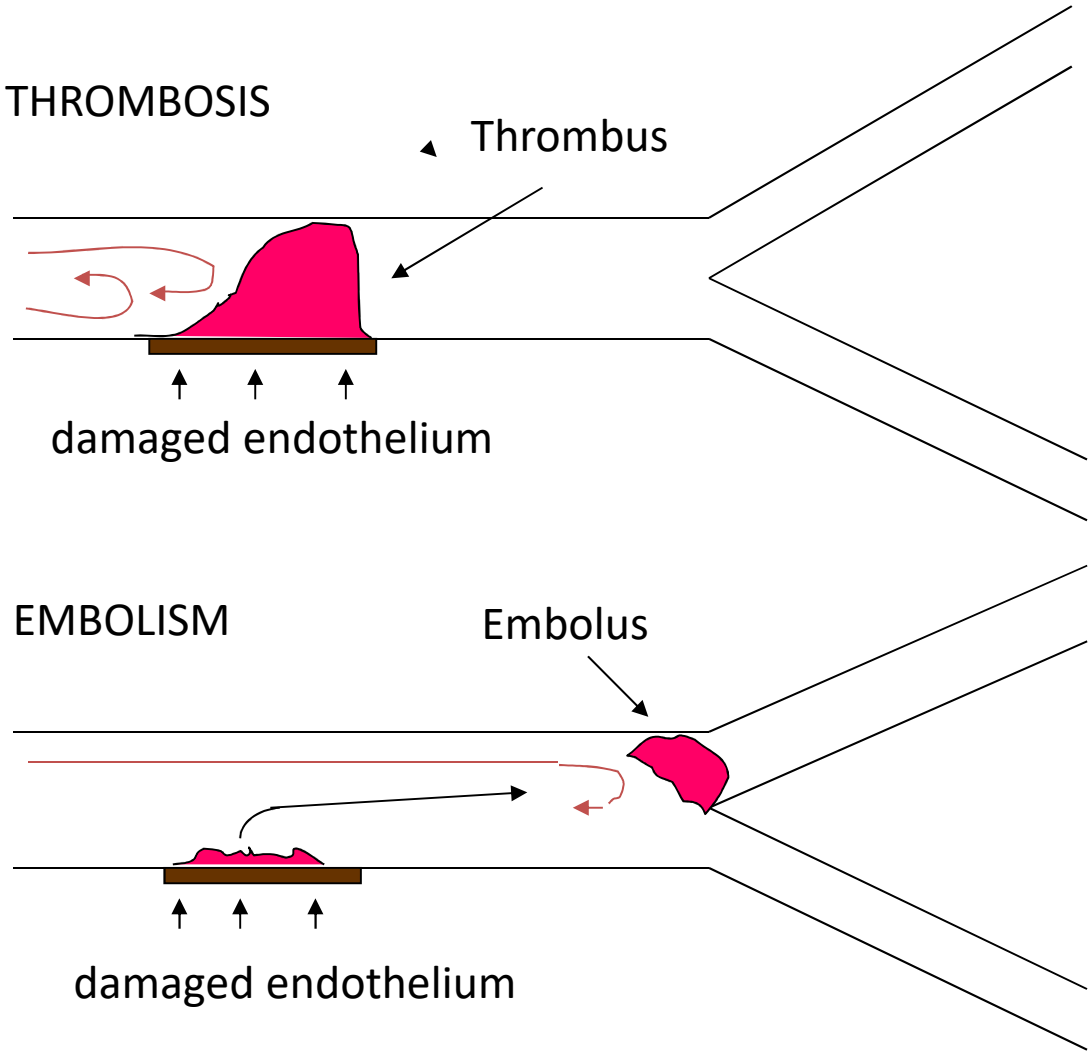
Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.

INTRAVASCULAR COAGULATION

Damage of epithelium caused by:

1) Atherosclerosis (myocardial infarction, stroke)

2) Inflammation (venous thrombosis, pulmonary embolism)



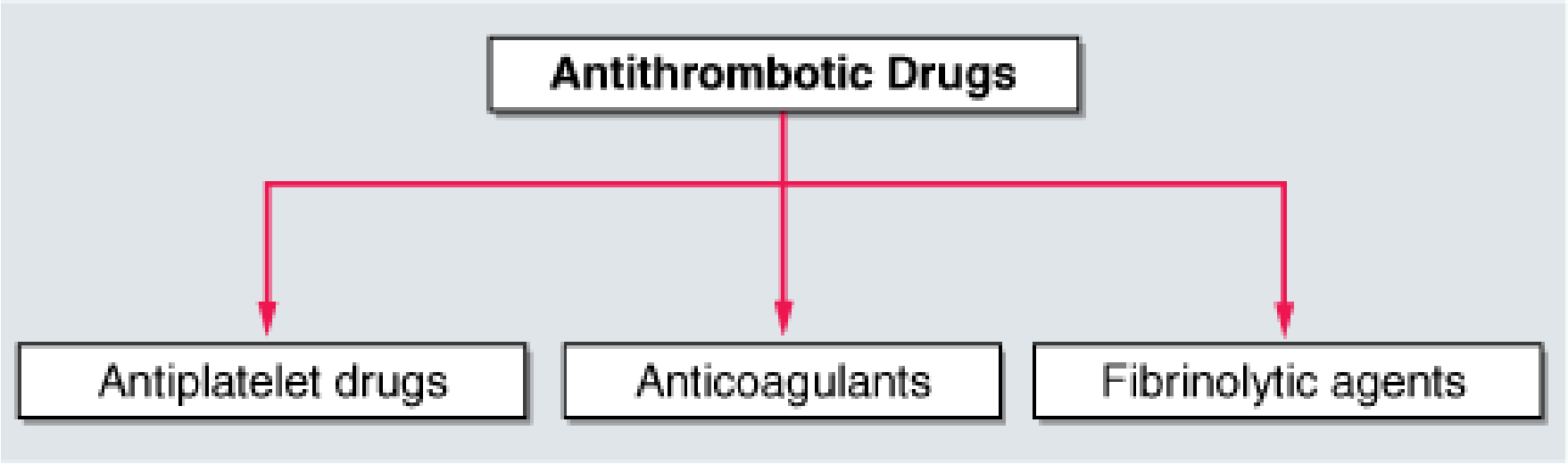
Example:

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.

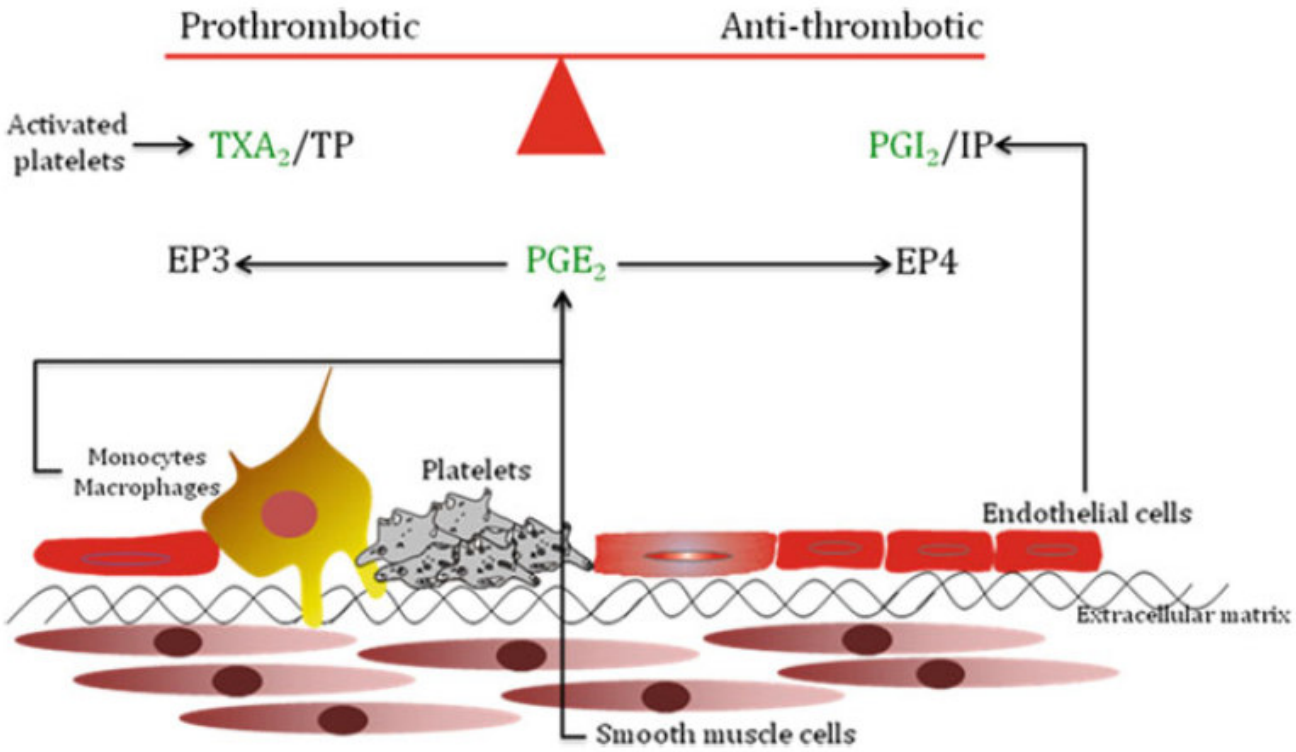
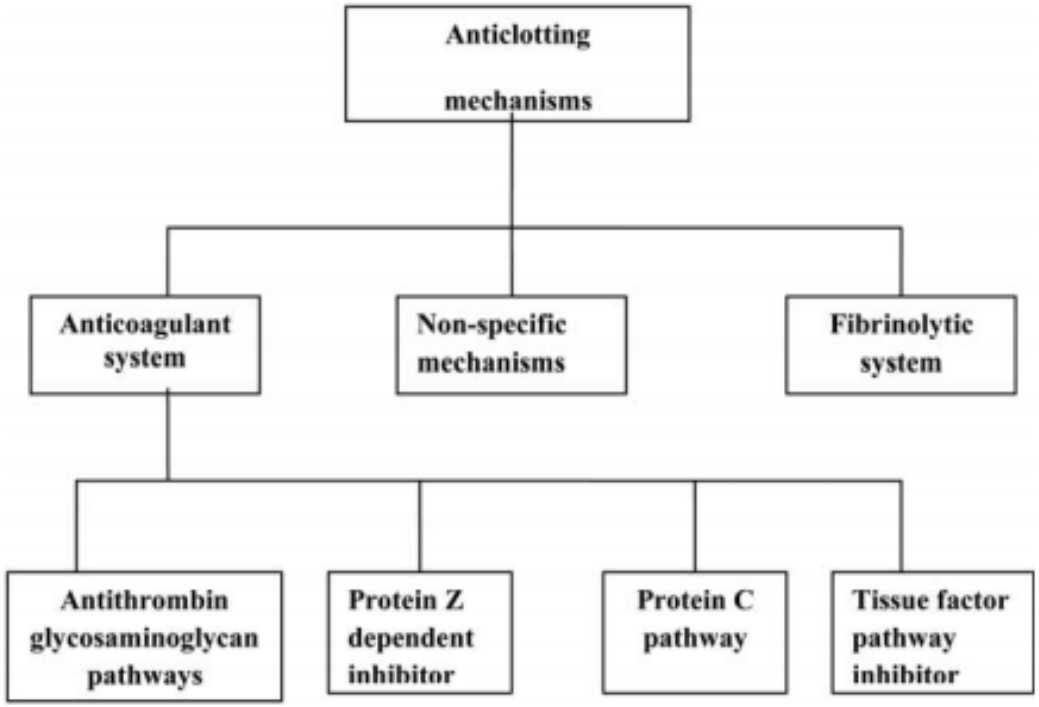


Fig. 4 The balance between thrombotic and antithrombotic effects of prostanoids. In response to vascular injury, PGI_2 produced by endothelial cells opposes the enhanced prothrombotic effect of TXA_2 produced by platelets. Smooth muscle cells, monocytes, and macrophages (accumulate in atherosclerotic plaques) release prostanoids such as PGE_2 during inflammation. PGE_2 shows a biphasic, dose-dependent effect on platelet aggregation

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



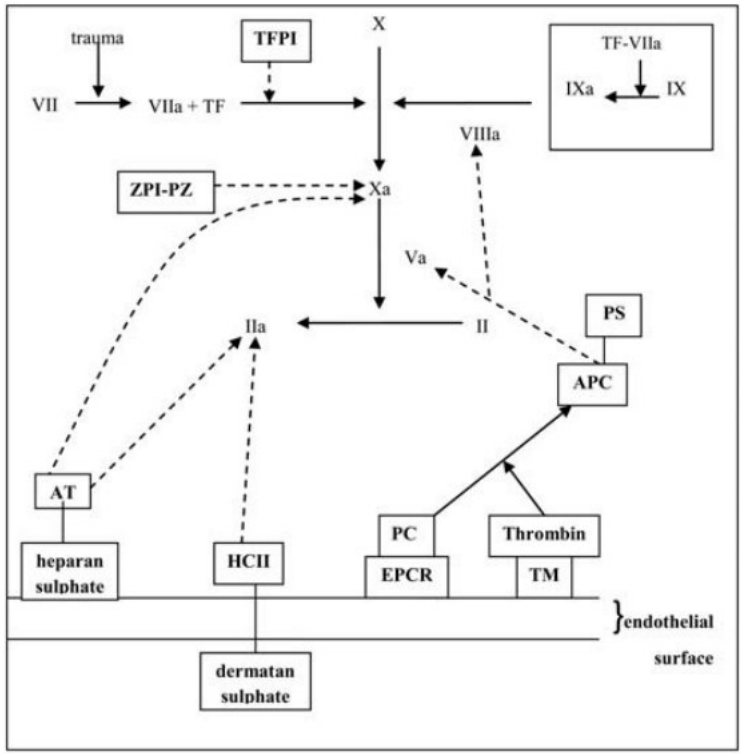
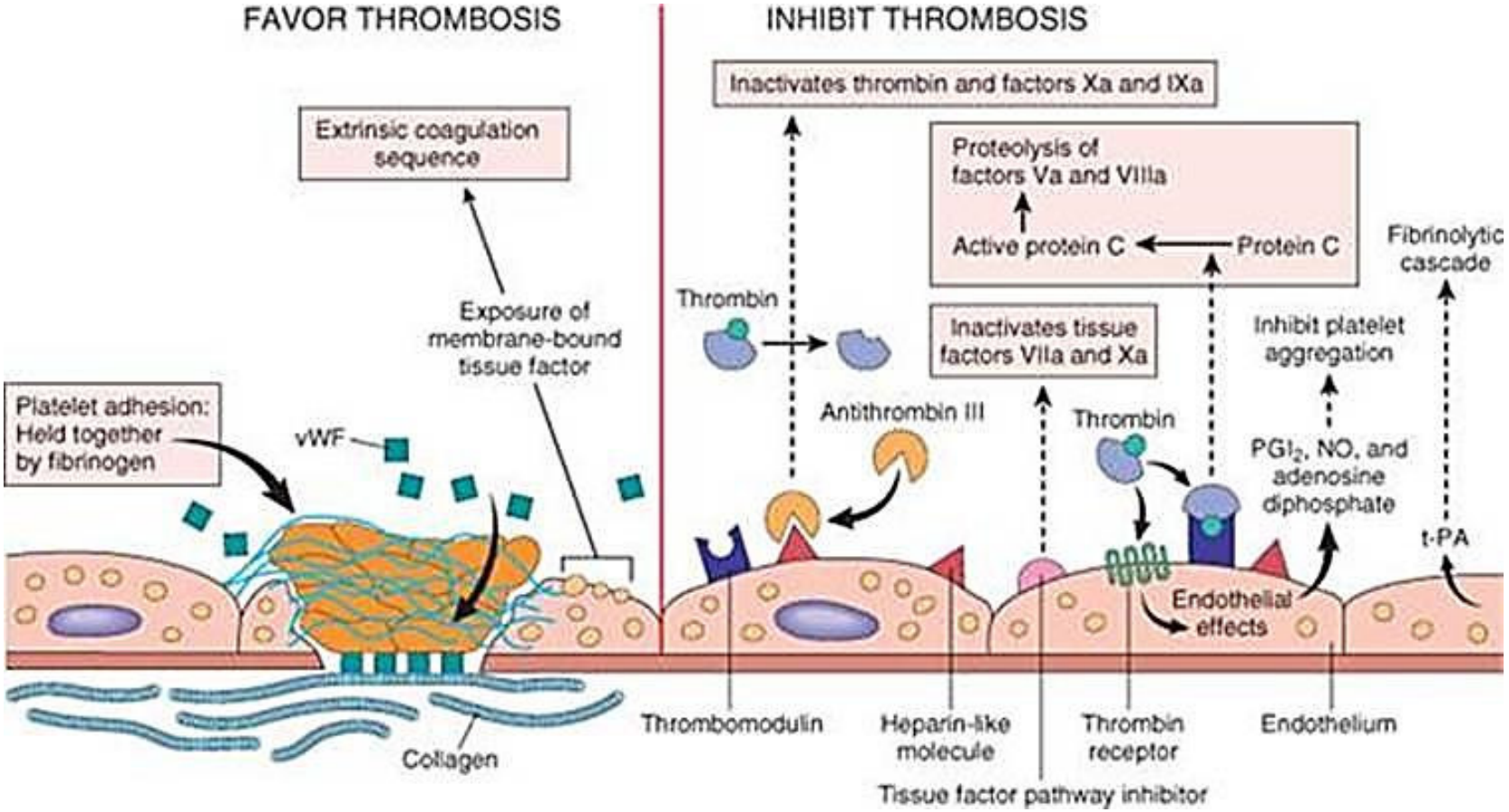


Fig 2 The anticoagulant system. AT, antithrombin; HCII, heparin cofactor II; TFPI, tissue factor pathway inhibitor; ZPI, protein Z-dependent protease inhibitor; PZ, protein Z; PC, protein C; APC, activated protein C; PS, protein S; EPCR, endothelial protein C receptor; TM, thrombomodulin. Solid arrows 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
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
PGI_2, NO, and adenosine diphosphate
t-PA
Endothelial effects
Thrombomodulin
Heparin-like molecule
Thrombin receptor
Endothelium
Tissue factor pathway inhibitor

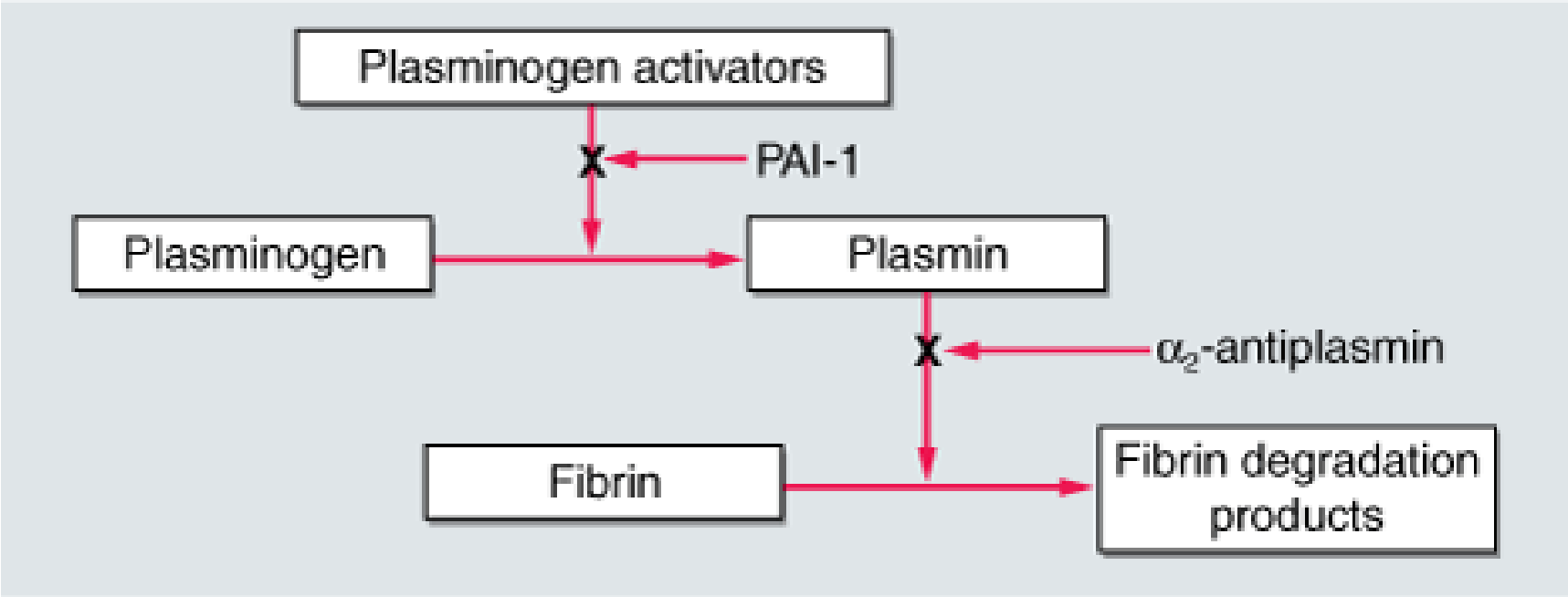
FIBRINOLYSIS

Inactive plasminogen.

Active plasmin (fibrinolysin).

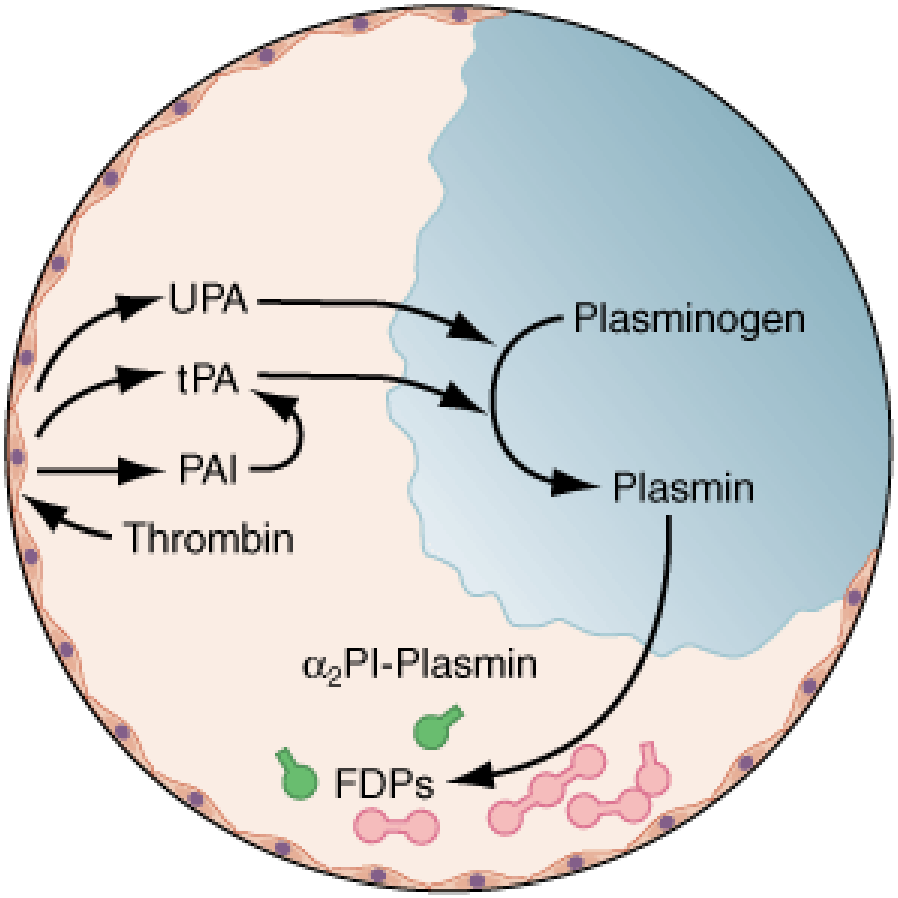
Activators of plasminogen.

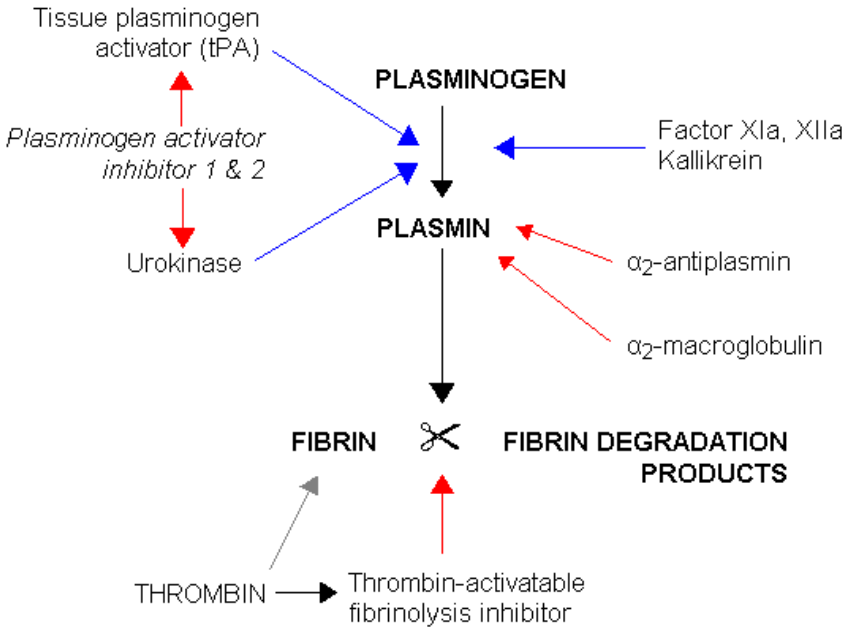
Inhibitors of plasminogen.



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

Thrombolysis





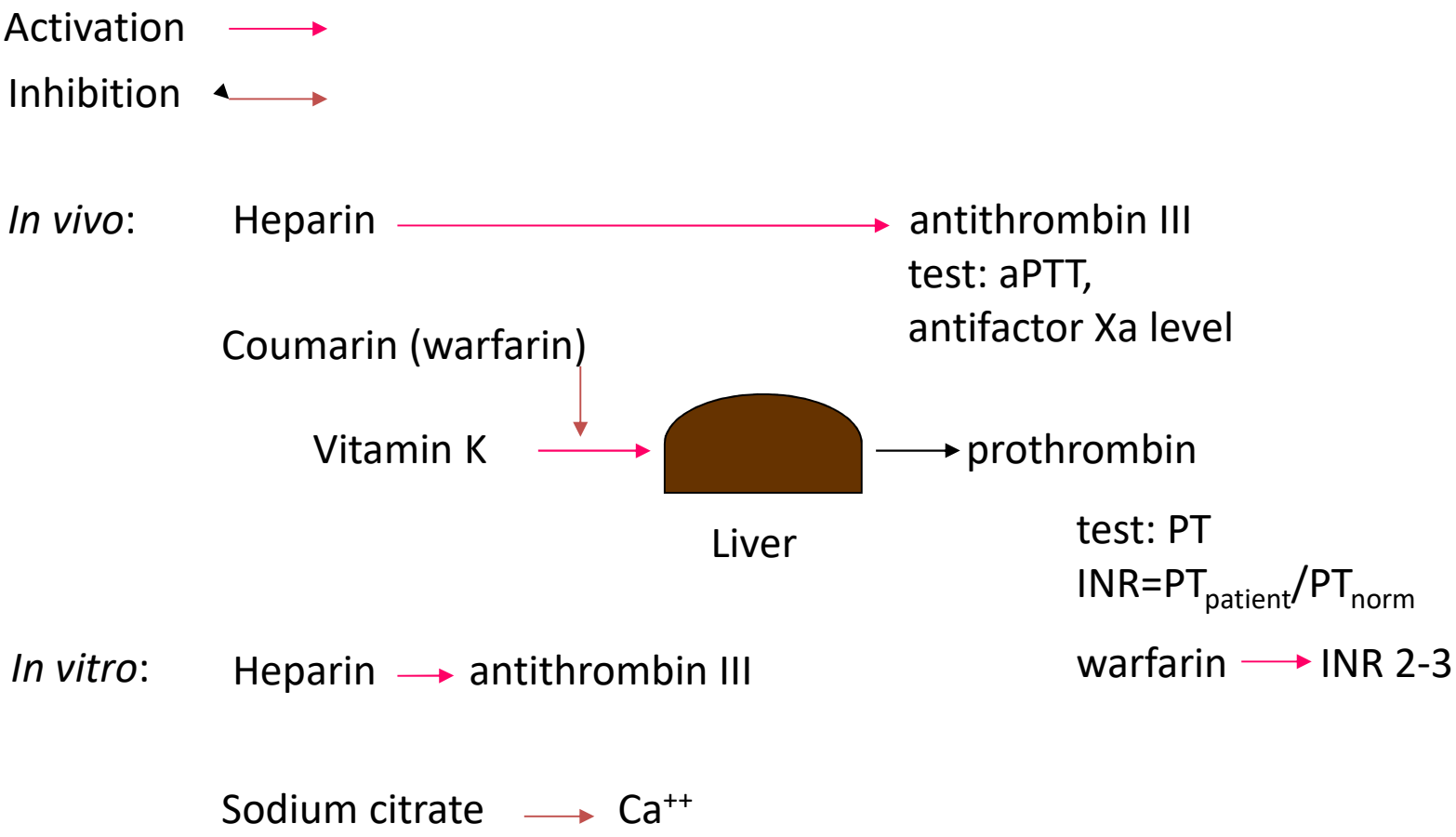
UPA, urokinase plasmin activator tPA, tissue plasmin activator

PAI, plasmin acivator inhibitor alpha2PI-Plasmin, complex

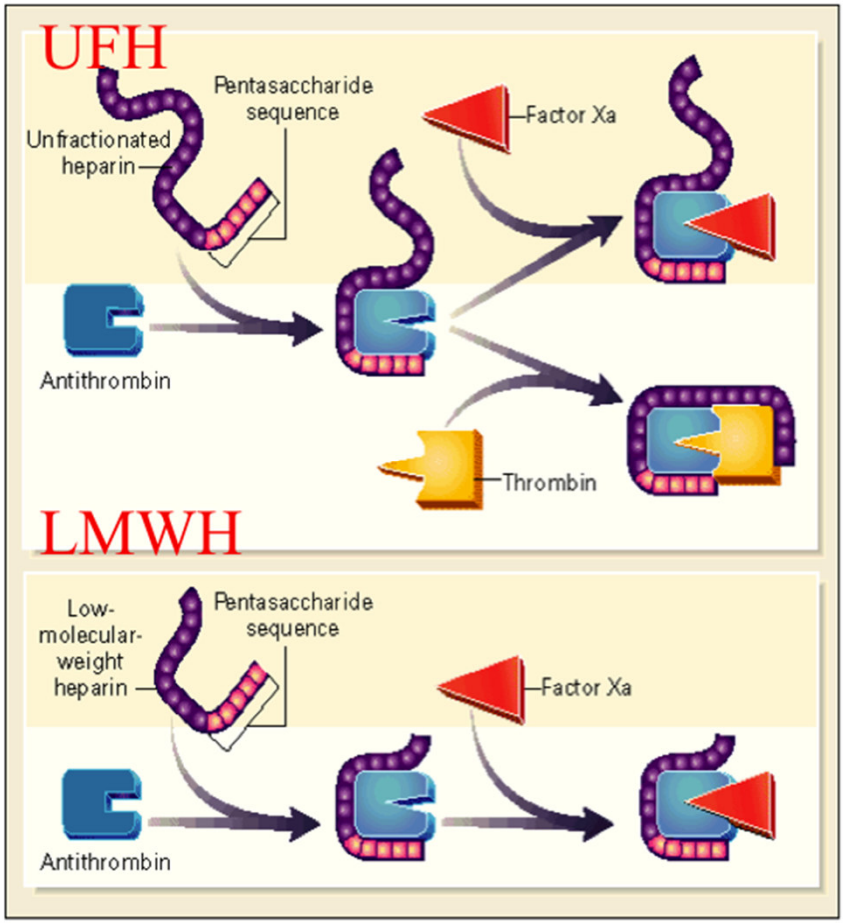
ANTI-CLOTTING TREATMENT

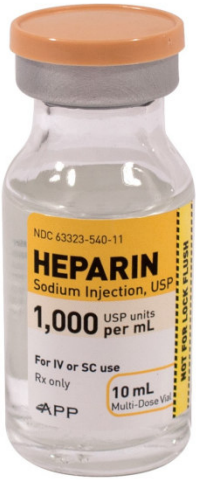
Defibrination: removal of fibrin (substances from snake poisons) – *in vitro*
Decalcification: binding or removal of calcium ions (sodium citrate, potassium or ammonium oxalate) – *in vitro*
Heparin: natural anticoagulant, mast cells, active only in the presence of antithrombin III, used also *in vivo*
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)

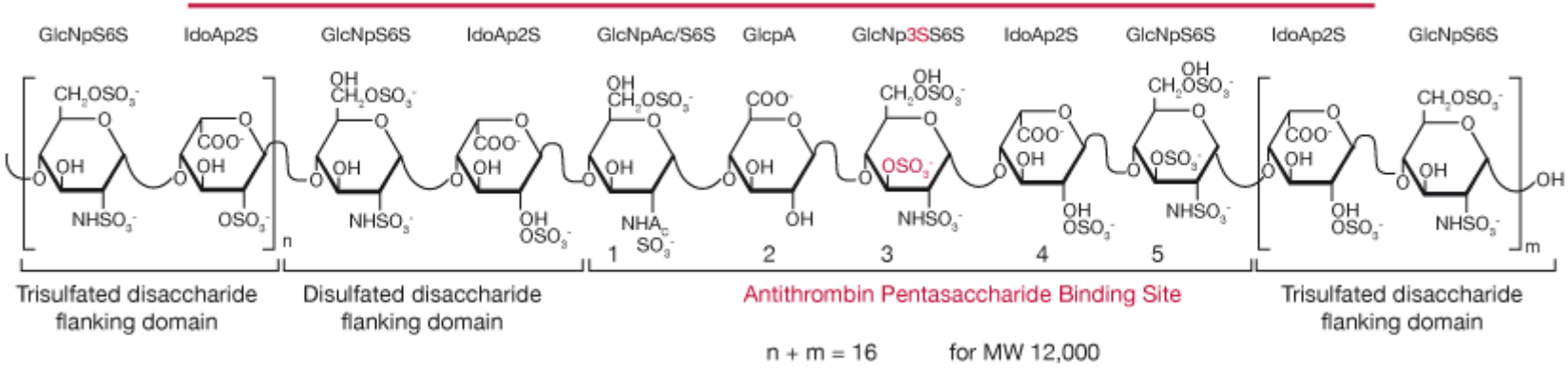
Anticoagulants



aPTT: activated partial thromboplastin time PT: prothrombin time

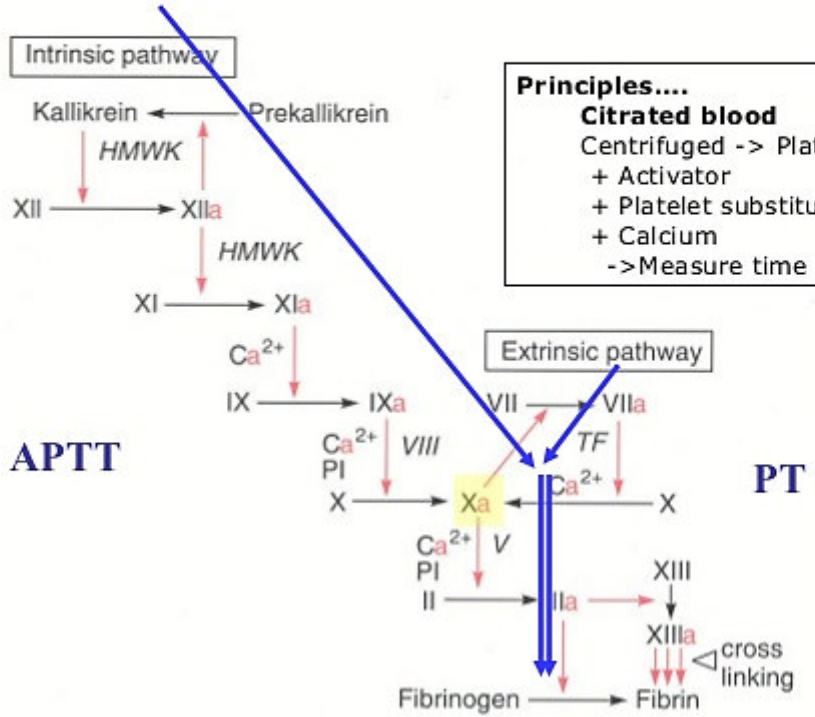

UFH
Pentasaccharide sequence
Unfractionated heparin
Factor Xa
Antithrombin
Thrombin
LMWH
Low-molecular-weight heparin
Pentasaccharide sequence
Factor Xa
Antithrombin


NDC 63323-540-11
HEPARIN
Sodium Injection, USP
1,000 USP units per mL
For IV or SC use
Rx only
10 mL
Multi-Dose Vial
APP


GlcNpS6S
IdoAp2S
GlcNpS6S
IdoAp2S
GlcNpAc/S6S
GlcpA
GlcNp3SS6S
IdoAp2S
GlcNpS6S
IdoAp2S
GlcNpS6S
1
2
3
4
5
Trisulfated disaccharide flanking domain
Disulfated disaccharide flanking domain
Antithrombin Pentasaccharide Binding Site
Trisulfated disaccharide flanking domain
n + m = 16
for MW 12,000

Tests aPTT and PT

Tests: PT and APTT

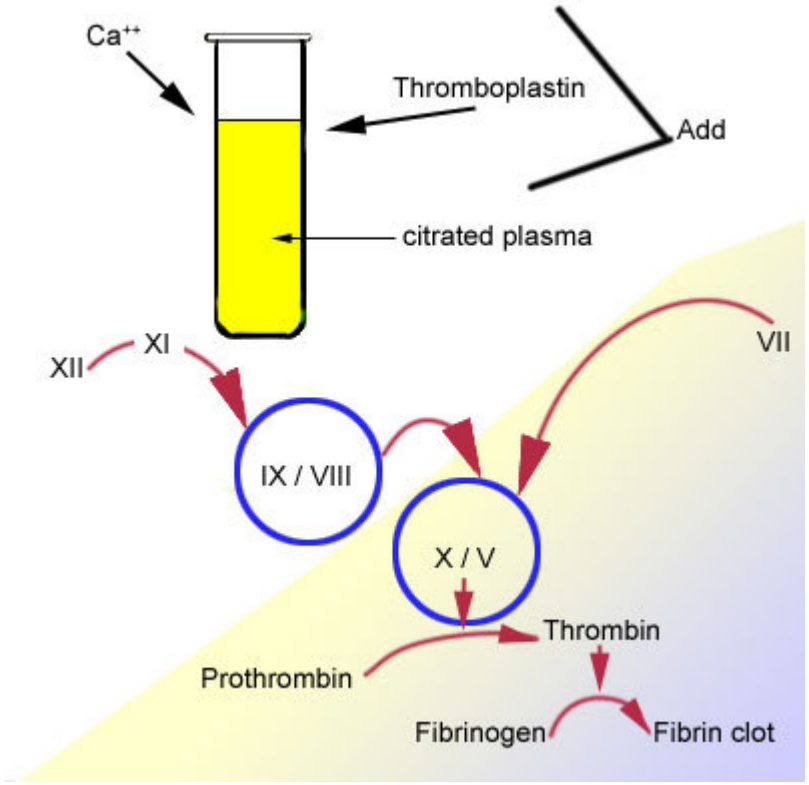


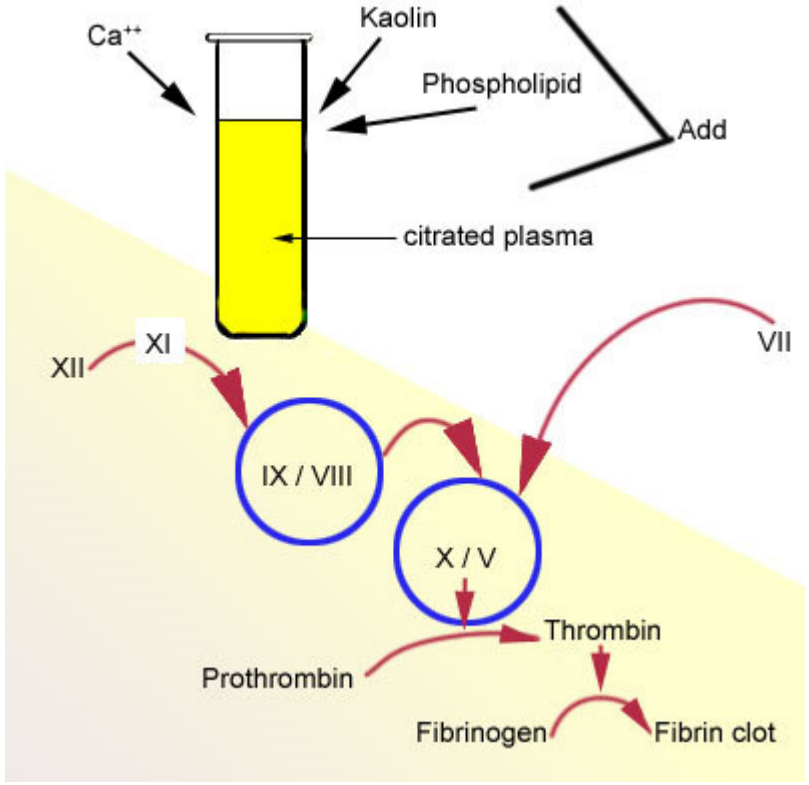
Principles....
Citrated blood
Centrifuged -> Platelet poor plasma
+ Activator
+ Platelet substitute
+ Calcium
->Measure time to clot formation

Prothrombin time test

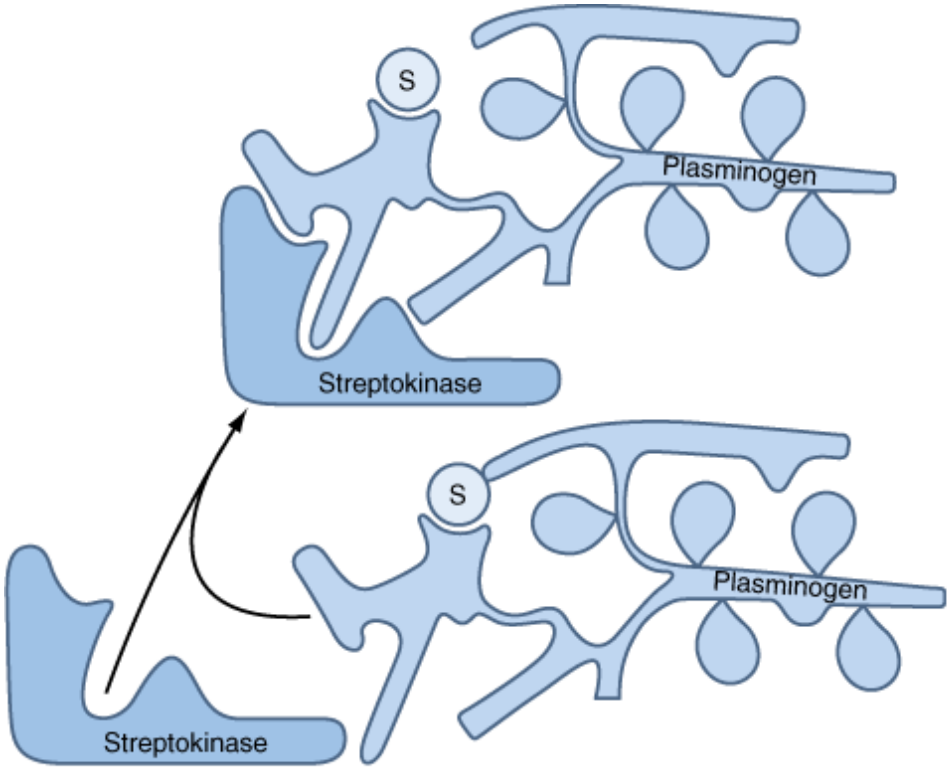
Activated Partial Thromboplastin Time test

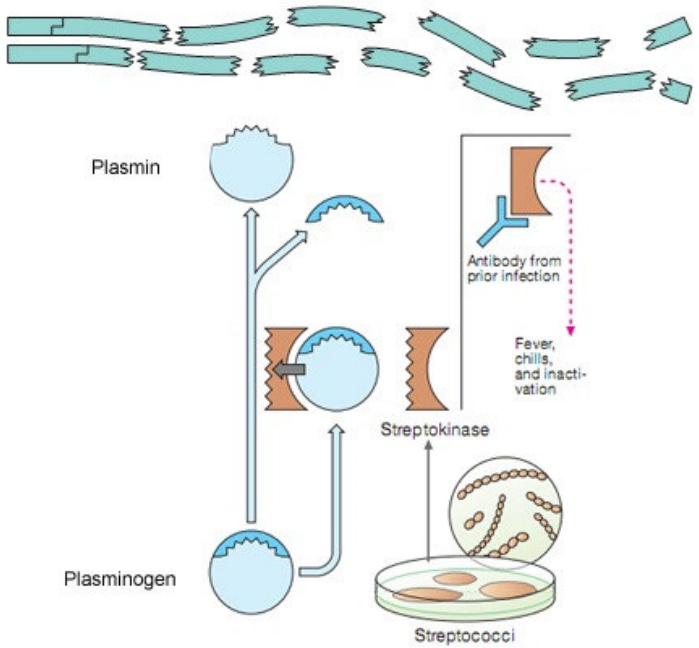
HMWK, high-molecular-weight-kininogen PK, prekallikrein F, factor


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