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

HEMOSTASIS (blood clotting, stop of bleeding)

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.

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.

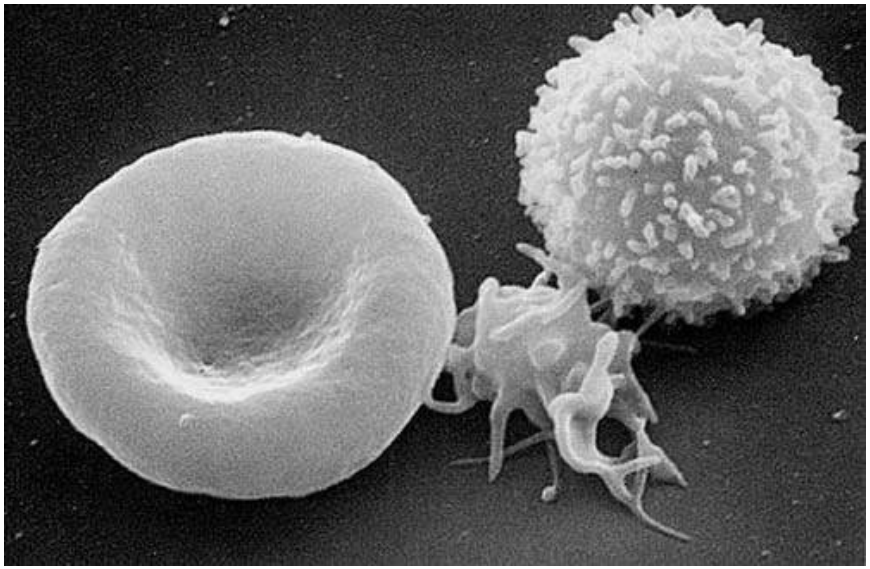
Origin: megakaryocytes of bone marrow under the effect of colony stimulating factors – interleukins (*IL-1, IL-3, IL-6*) and granulocytes and macrophages stimulating factor (*GM-CSF*)

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

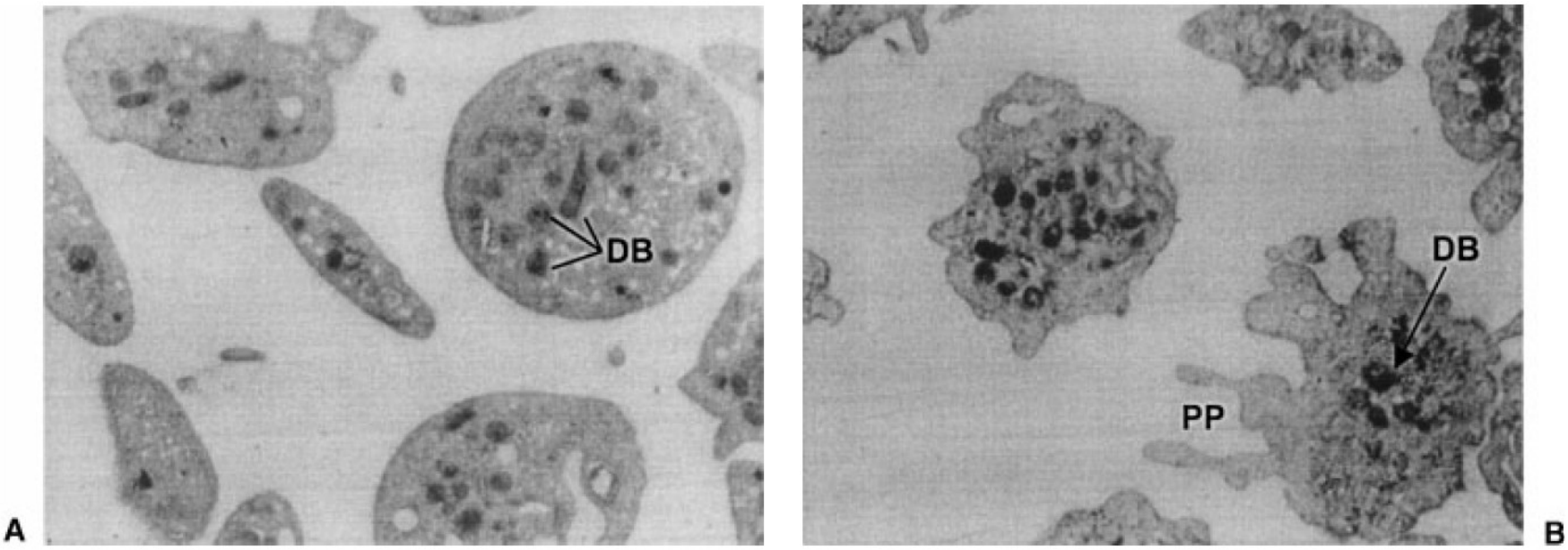


Figure 1 Morphology of human platelets. (A) Thin section of discoid resting platelets with evenly distributed granules. (B) Thin section of stimulated platelets, showing formation of pseudopodia and centralization of granules. DB, dense body; PP, pseudopodium. Magnification × 21,000.

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

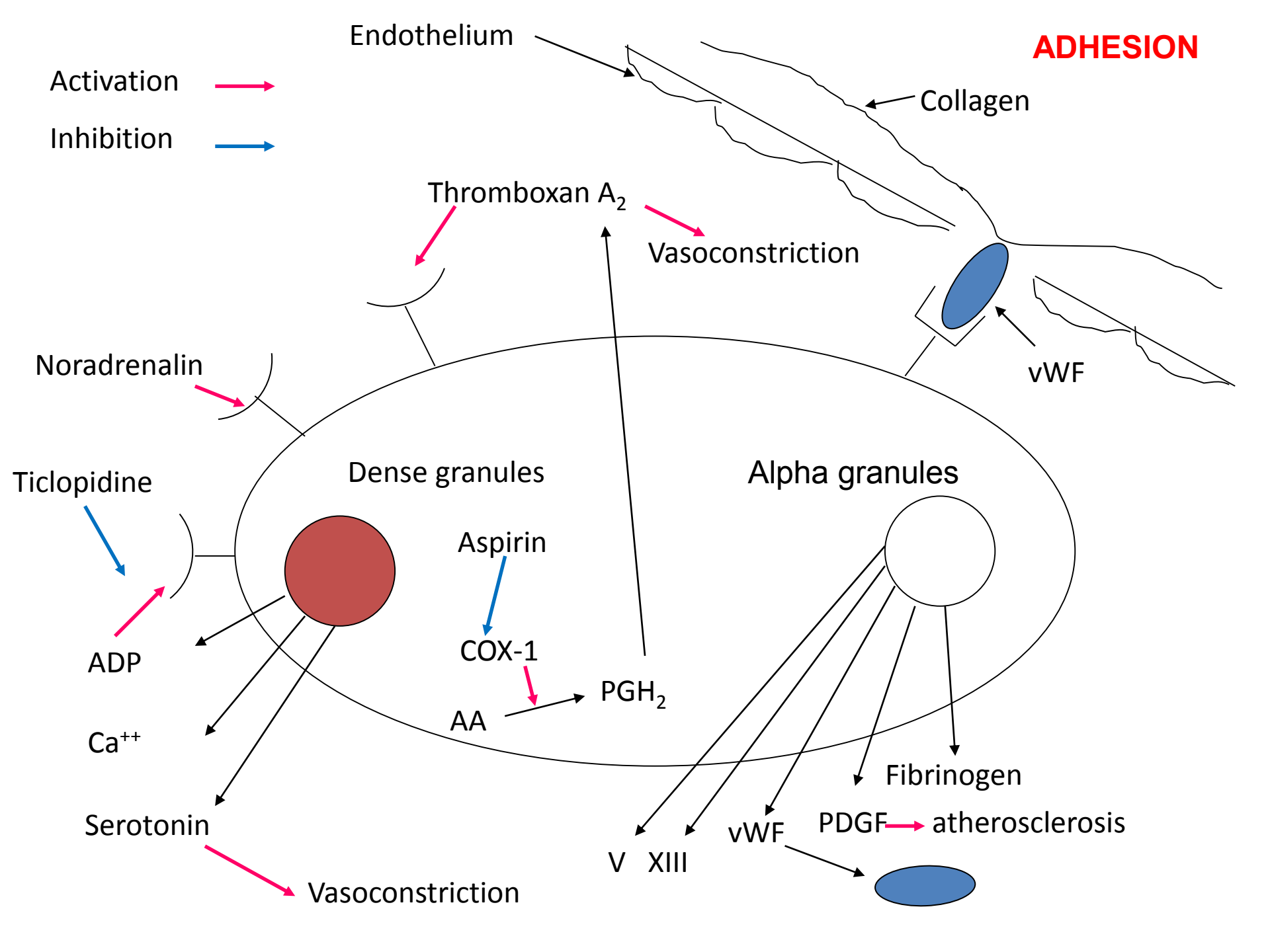
mitogenic effects – growth factor (**PDGF**)

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
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

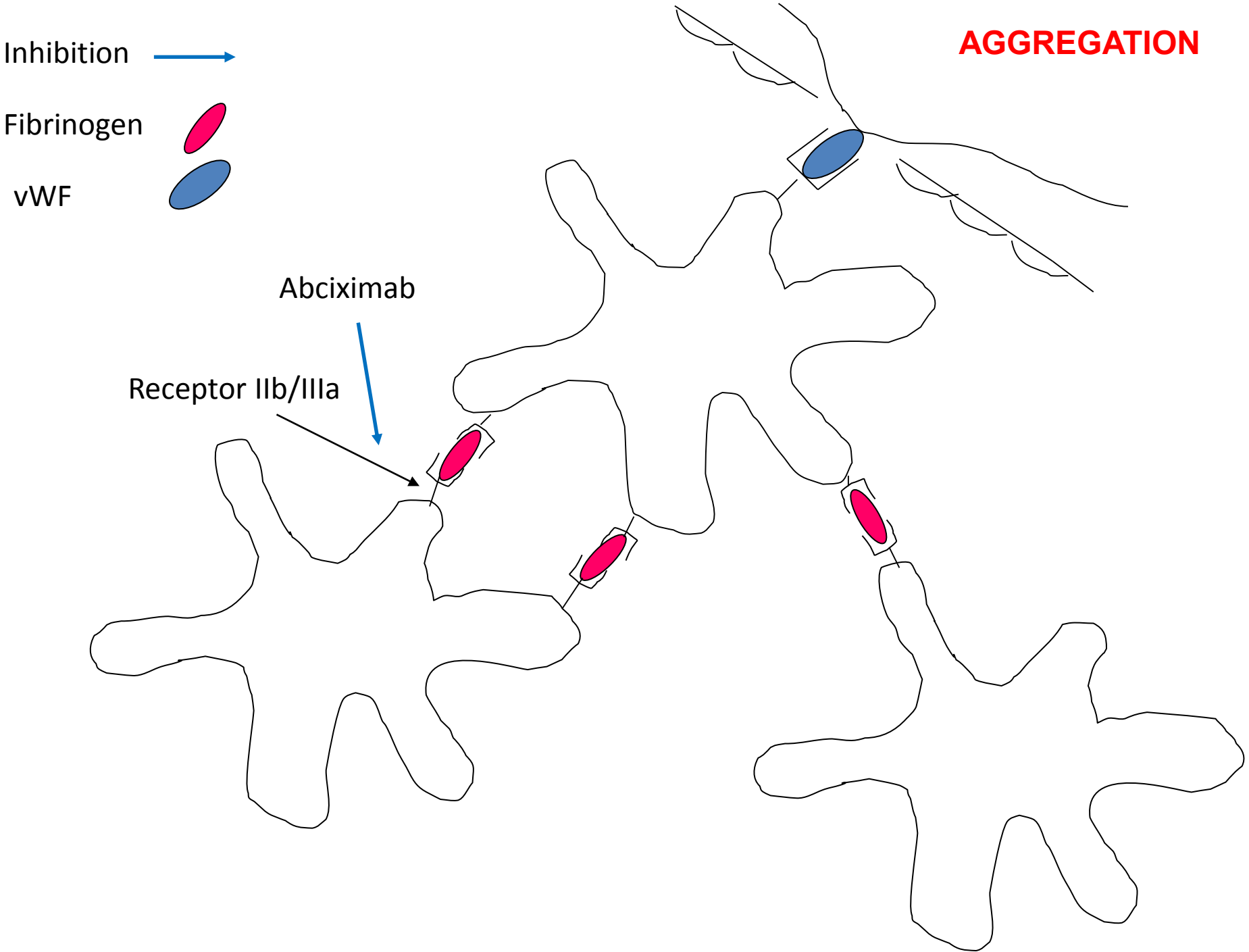
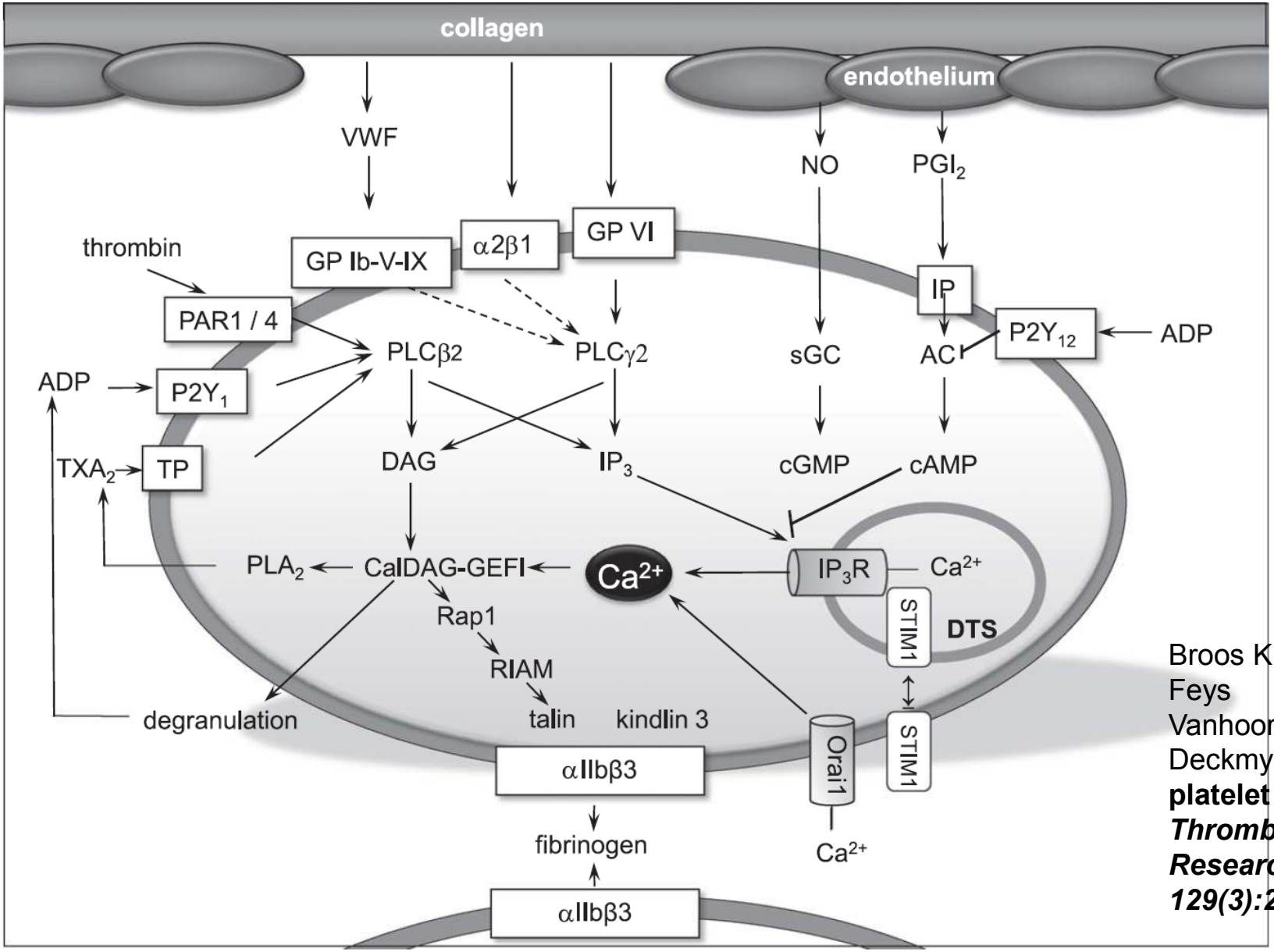

Inhibition
Fibrinogen
vWF
AGGREGATION
Abciximab
Receptor IIb/IIIa

Table 1 Agonists, Ligands, and Receptors Important for Platelet Function

Platelet Function	Agonists, Ligands	Receptors
Initial and firm adhesion	vWF	GPIb/V/IX
	TSP1	GPIb/V/IX, CD36
	Collagen	$\alpha_2\beta_1$, GPVI, CD36
	Fibrinogen	$\alpha_{IIb}\beta_3$
	Fibronectin	$\alpha_5\beta_1$[73]
	Vitronectin	$\alpha_v\beta_3$[77]
	Laminin	$\alpha_6\beta_1$[74]
	High shear stress	GPIb/V/IX
Activation and amplification	Thrombin	PAR1, PAR4, GPIb/V/IX
	ADP	$P2Y_1$, $P2Y_{12}$
	TxA_2	TPα, TPβ
	Epinephrine	α_{2A}
	Serotonin	5-HT2A
	MMP-2, MMP-1[75,76]	?
	Immune complexes	FcγIIa
	Complement factors	C1q, C3a, C5a receptors
	Plasmin	?
	Streptokinase	?
Aggregation/amplification and stabilization	Fibrin	Activated $\alpha_{IIb}\beta_3$
	vWF	Activated $\alpha_{IIb}\beta_3$, GPIb/V/IX
	TSP-1[77]	Activated $\alpha_{IIb}\beta_3$, CD36,IAP
	Fibronectin	Activated $\alpha_{IIb}\beta_3$
	sCD40L	Activated $\alpha_{IIb}\beta_3$
	Gas6	Axl[78,79]
	SDF-1, TARC, MDC	CXCR4, CCR4[80–82]

vWF, von Willebrand factor; TSP1, thrombospondin-1; ADP, adenosine diphosphate; TxA_2, thromboxane A_2; MMP, matrix metalloproteinase; IAP, integrin associated protein; SDF, stromal cell-derived factor; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine.

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



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.

Processes in platelets after „activation“ of receptors

- ✓ Activation of PLC ⇒ PIP_2 cleavage to IP_3 and DAG
- ✓ IP3 mobilizes Ca^{2+} from ER
- ✓ ↑Ca^{2+} ⇒
  - ✓ 1. activation of PLA_2 ⇒ activation of arachidonic acid pathway ⇒ ↑ TXA_2 = ↑ degranulation
  - ✓ 2. activation of MLCK (Myosin light-chain kinase)
    - ✓ ⇒ contraction of contractile elements
    - ✓ ⇒ platelet shape change
- ✓ ↑ degranulation
- ✓ DAG activates PKC ⇒ ↑ degranulation

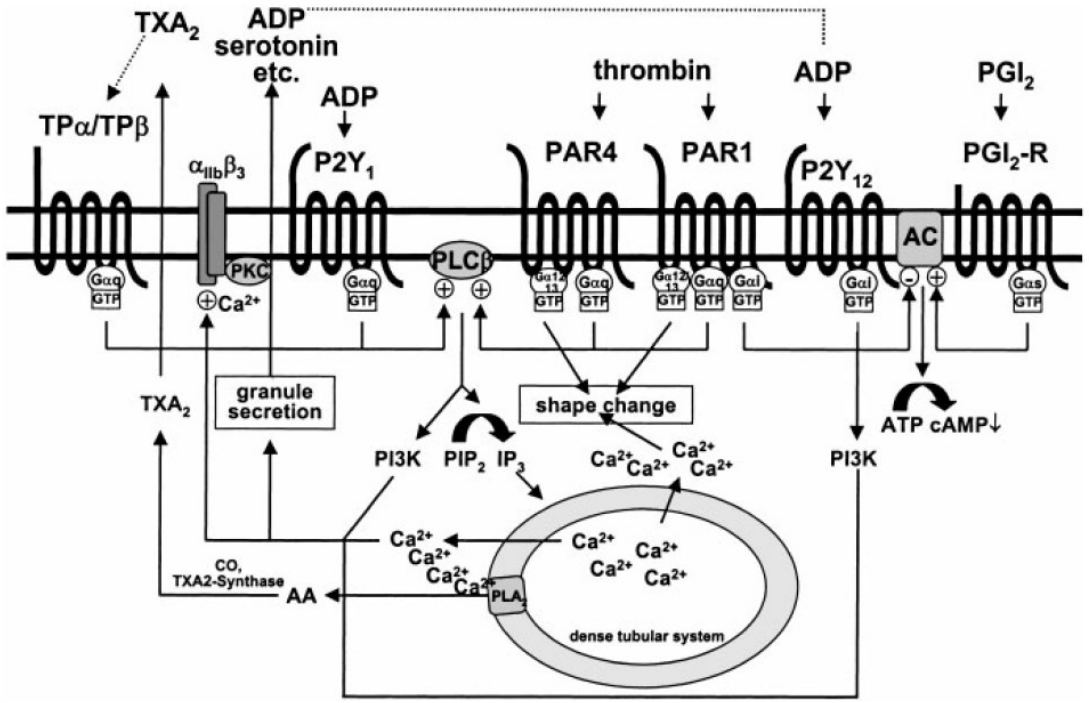
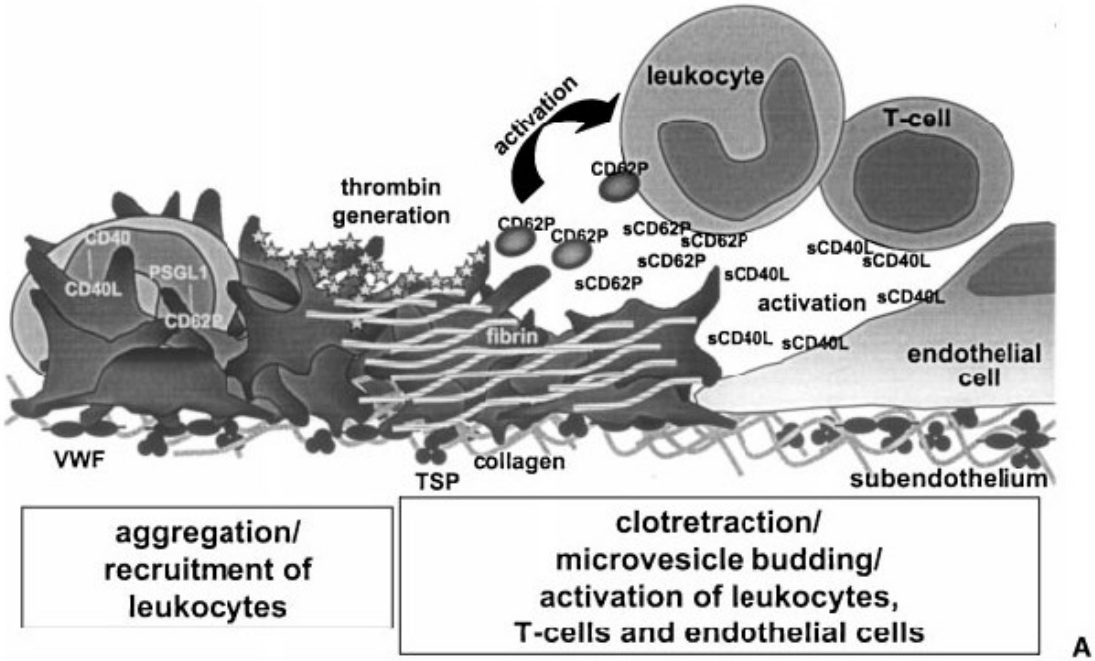


Figure 5 G-protein coupled seven-transmembrane receptor signaling in platelets. GTP, guanidine triphosphate; PKC, protein kinase C; TxA_2, thromboxane A_2, CO, cyclooxygenase; PI3K, phosphatidylinositol-3 kinase; PIP2, phosphatidylinositolbiphosphate; IP3, inositol-1,4,5-triphosphate; PLA, phospholipase A; AC, adenylatecyclase; cAMP, cyclic adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; PGI_2, prostaglandin I_2, R, receptor; PAR, protease activated receptor.

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



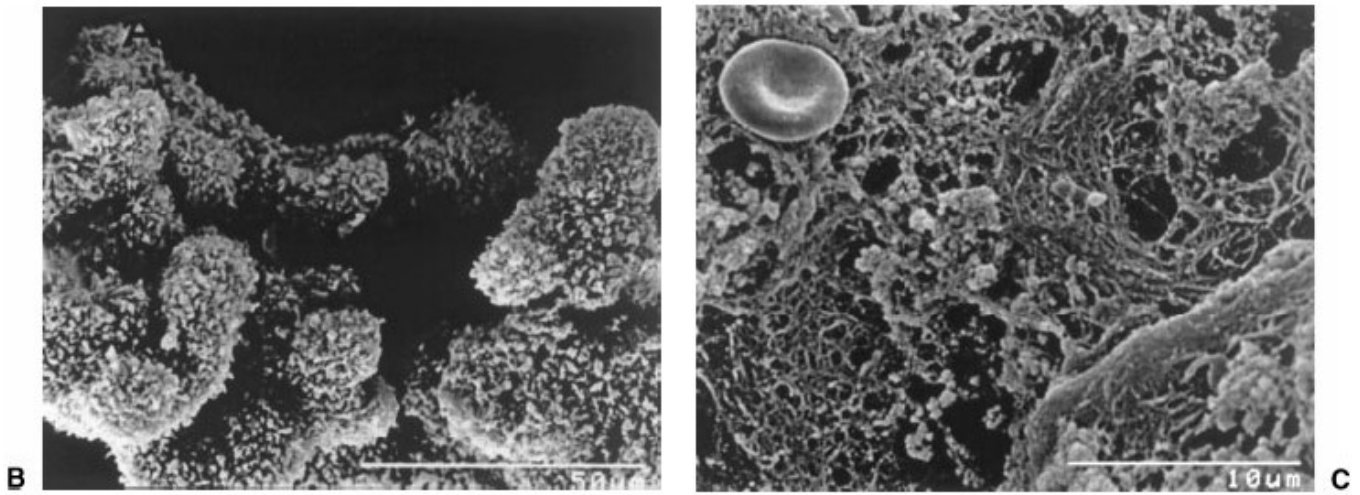


Figure 3 Aggregation and secondary hemostasis. (A) Fibrinogen or in high shear environments vWF bridges platelets through activated GPIIb/IIIa, leading to formation of an unstable platelet plug. Leukocytes are recruited to aggregated platelets via CD40/CD40L and PSGL1/CD62P interactions. Increased amounts of thrombin are generated on the platelet plug, which converts bound fibrinogen to fibrin, leading to plug stabilization and clot retraction. Microparticles as well as adhesion molecules (sCD62P, sCD40L) are shed from the platelet surface into the circulation as stimuli for leukocytes, T cells, and endothelial cells. (B) Scanning electron microscope preparation of a nonretracted platelet plug, induced by collagen. (C) REM-preparation of a platelet-fibrin clot with recruited red cell(s). vWF, von Willebrand factor; TSP, thrombospondin.

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

Factors Involved in Platelet Function

Table 16.4

Chemical Factor	Source	Activated by or Released in Response to	Role in Platelet Plug Formation	Other Roles and Comments
Collagen	Subendothelial extracellular matrix	Injury exposes platelets to collagen	Binds platelets to begin platelet plug	N/A
von Willebrand factor (vWF)	Endothelium, megakaryocytes	Exposure to collagen	Links platelets to collagen	Deficiency or defect causes prolonged bleeding
Serotonin	Secretory vesicles of platelets	Platelet activation	Platelet aggregation	Vasoconstrictor
Adenosine diphosphate (ADP)	Platelet mitochondria	Platelet activation, thrombin	Platelet aggregation	N/A
Platelet-activating factor (PAF)	Platelets, neutrophils, monocytes	Platelet activation	Platelet aggregation	Plays role in inflammation; increases capillary permeability
Thromboxane A2	Phospholipids in platelet membranes	Platelet-activating factor	Platelet aggregation	Vasoconstrictor; eicosanoid
Platelet-derived growth factor (PDGF)	Platelets	Platelet activation	N/A	Promotes wound healing by attracting fibroblasts and smooth muscle cells

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

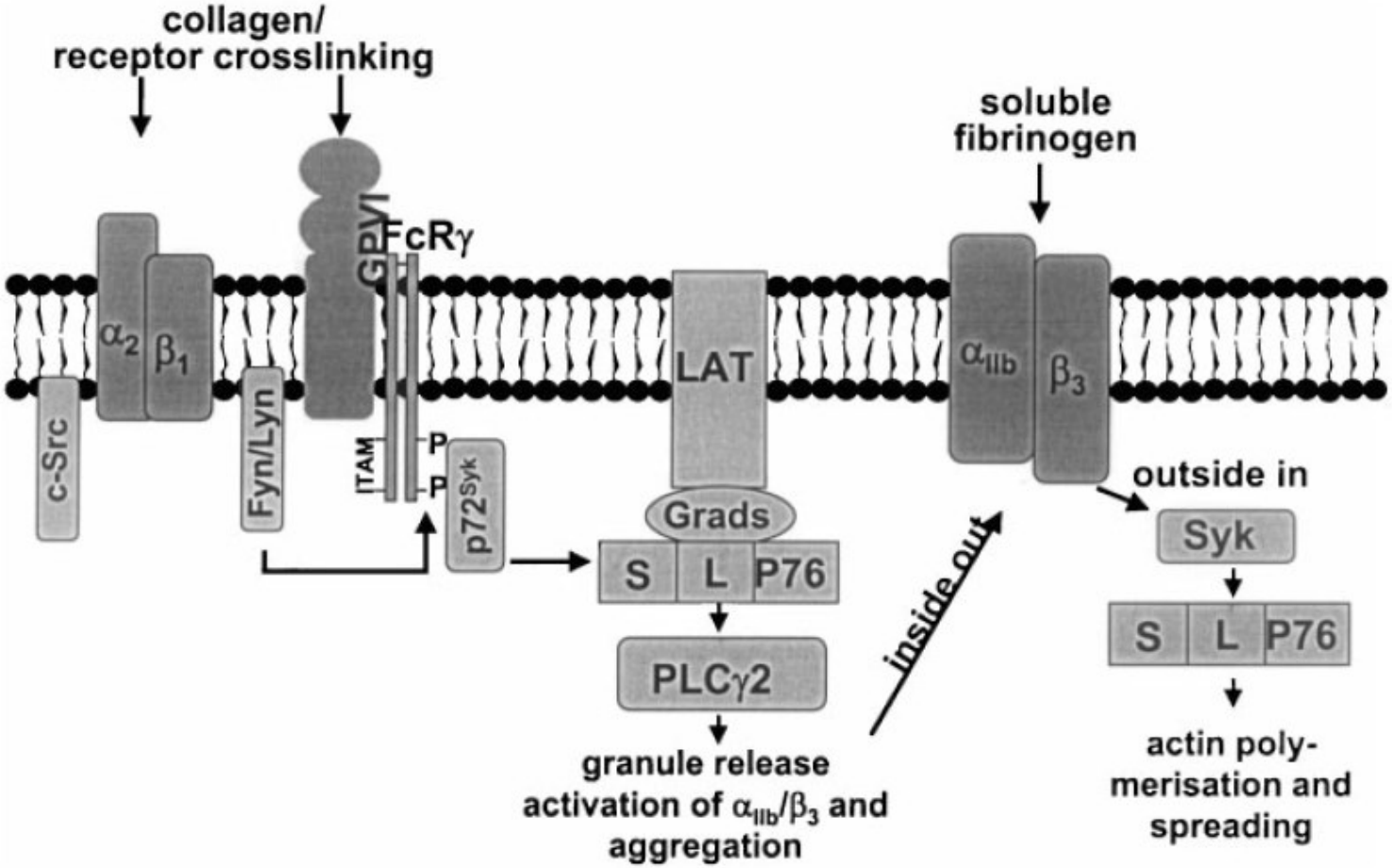
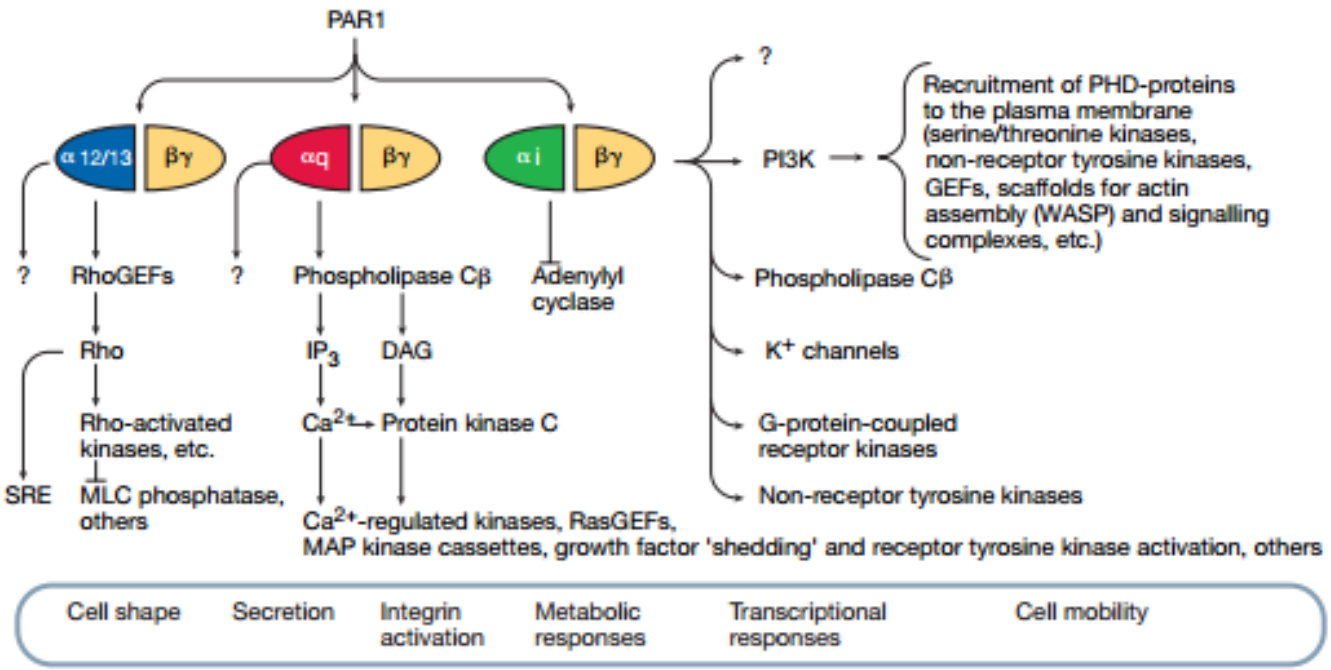


Figure 6 Nonreceptor tyrosine kinase mediated collagen signaling in platelets. Tyrosine-kinases: c-Src, Fyn/Lyn; non receptor tyrosine kinases; $p72^{Syk}$, Syk, adapter molecules: LAT, Grads, SLP76; PLC: phospholipase C; ITAM: immunoreceptor tyrosine based activation motif.

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

Box 1

Thrombin receptor signalling



PAR1 can couple to members of the $G_{12/13}$, G_q, and G_i families[45–47] to impact on a substantial network of signalling pathways, as shown in the figure. The α-subunits of G_{12} and G_{13} bind RhoGEFs (guanine-nucleotide exchange factors, which activate small G proteins such as Rho)[48–50], providing a pathway to Rho-dependent cytoskeletal responses that are likely to be involved in shape changes in platelets[51] and permeability and migration in endothelial cells[52,53]. $G\alpha_q$ activates phospholipase Cβ[54], triggering phosphoinositide hydrolysis which results in calcium mobilization and activation of protein kinase C. This provides a pathway to calcium-regulated kinases and phosphatases, GEFs, mitogen-activated protein (MAP) kinase cassettes, and other proteins that mediate cellular responses ranging from granule secretion, integrin activation and aggregation in platelets[55], to transcriptional responses in endothelial and mesenchymal cells. $G\alpha_i$ inhibits adenylate cyclase, an action known to promote platelet responses. Gβγ subunits can activate phosphoinositide 3-kinase (PI(3)K)[56] and other lipid-modifying enzymes, protein kinases and ion channels[57]. PI(3)K modifies the inner leaflet of the plasma membrane to provide attachment sites for a host of signalling proteins[58]. PAR1 activation can also activate cell-surface 'sheddases' which liberate ligands for receptor tyrosine kinases, providing a link between thrombin and receptors involved in cell growth and differentiation[59]. The pleiotropic effects of PAR1 activation are consistent with many of thrombin's diverse actions on cells. IP_3, inositol trisphosphate; DAG, diacylglycerol; SRE, serum response element; PHD, pleckstrin homology domain.

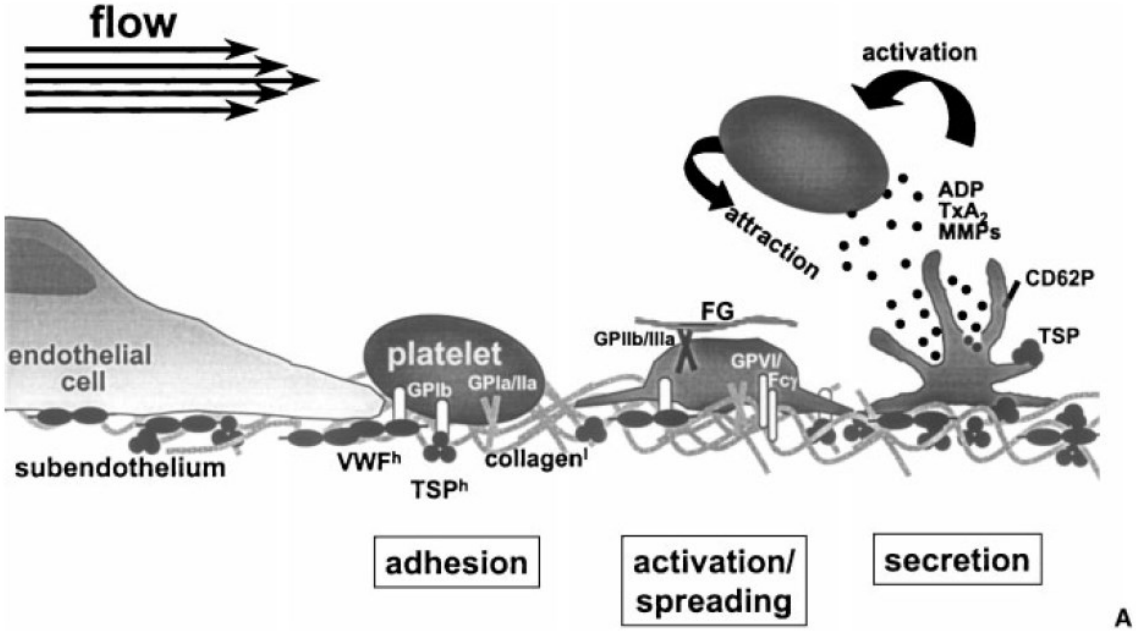
Coughlin SR: **Thrombin signalling and protease-activated receptors.** ***Nature 2000, 407(6801):258-264.***

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.



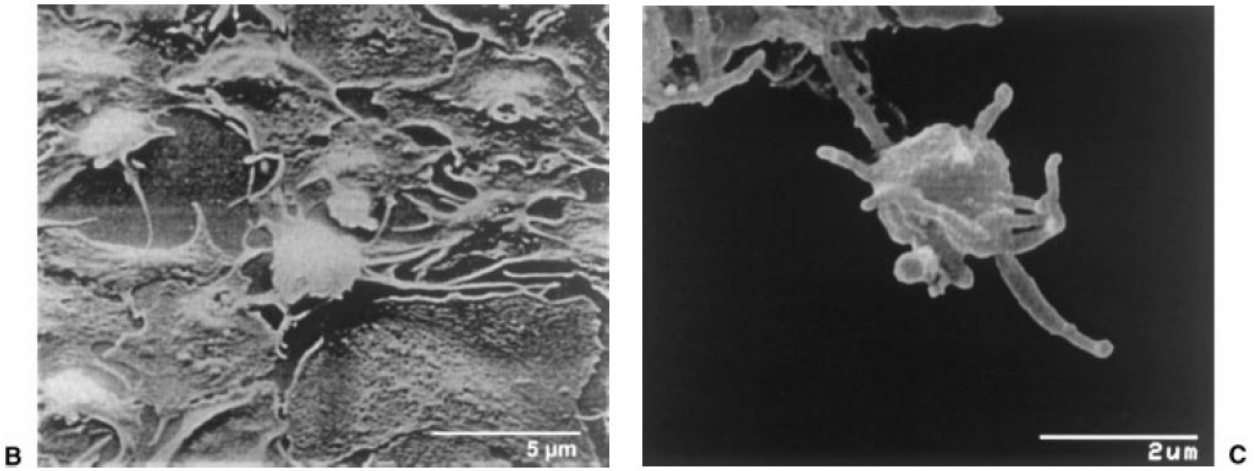


Figure 2 Primary hemostasis. (A) Platelets recruited from the circulation to the subendothelium of a damaged vessel wall adhere to vWF and TSP via GPIb/V/IX under high shear conditions (h). Collagen serves as adhesive substrate for platelets (via GPIa/IIa and GPVI/Fcγ) under low shear conditions (l). Receptor clustering through multiple binding sites of matrix proteins induce activation of GPIIb/IIIa with subsequent binding of fibrinogen (FG) and platelet spreading. Activated platelets secrete several adhesive proteins, including TSP, which binds back to the platelet surface. Secreted secondary agonists (ADP, TxA_2 and MMPs) amplify activation and attraction of additional circulating platelets. (B) REM-preparation of human platelets adhered and spread on immobilized collagen. (C) Scanning electron microscope preparation of a thrombin-stimulated human platelet with marked pseudopodia. vWF, von Willebrand factor; TSP, thrombospondin, ADP, adenosine diphosphate; TxA_2, thromboxane A_2; MMPs, matrix metalloproteinases.

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

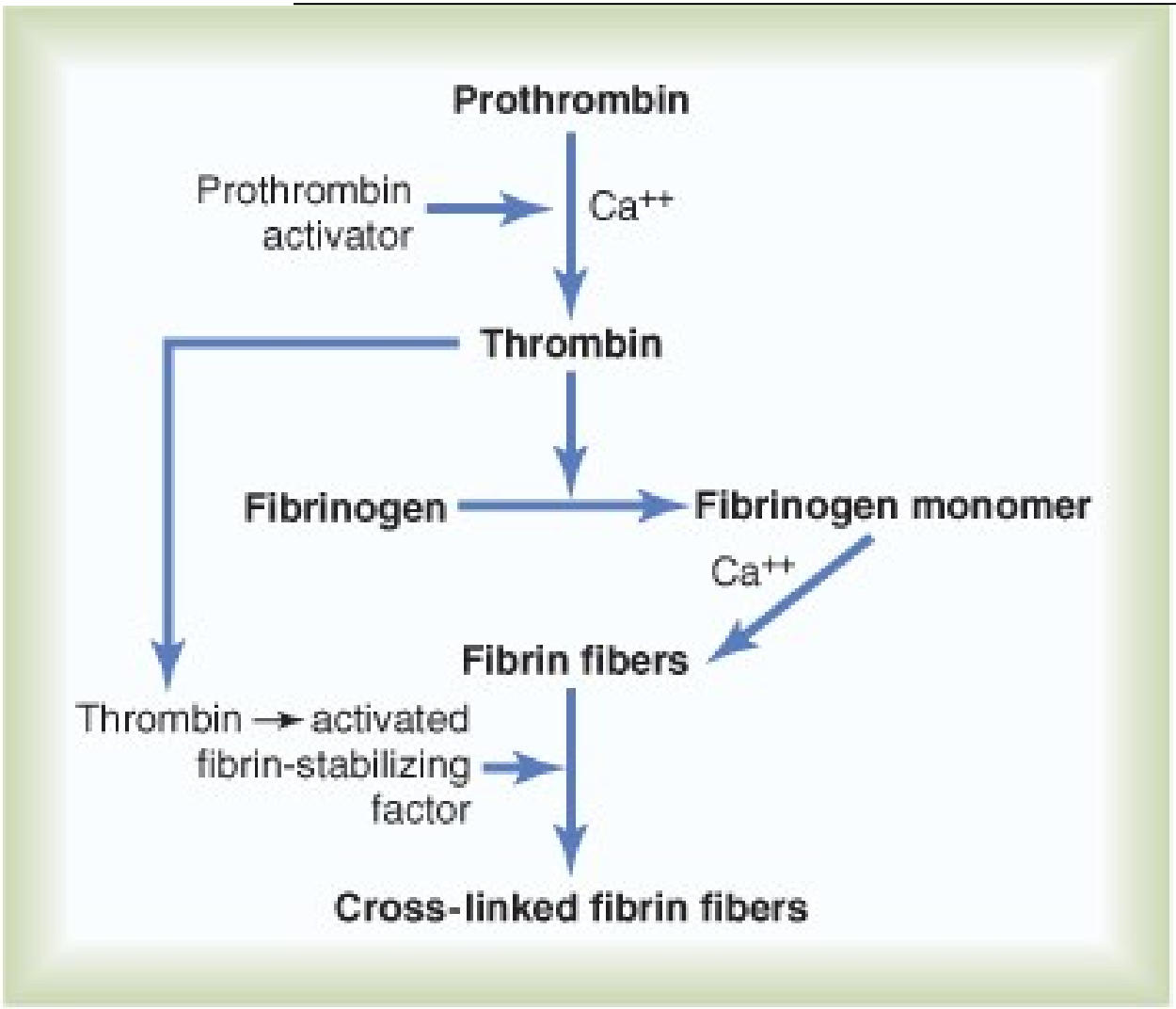
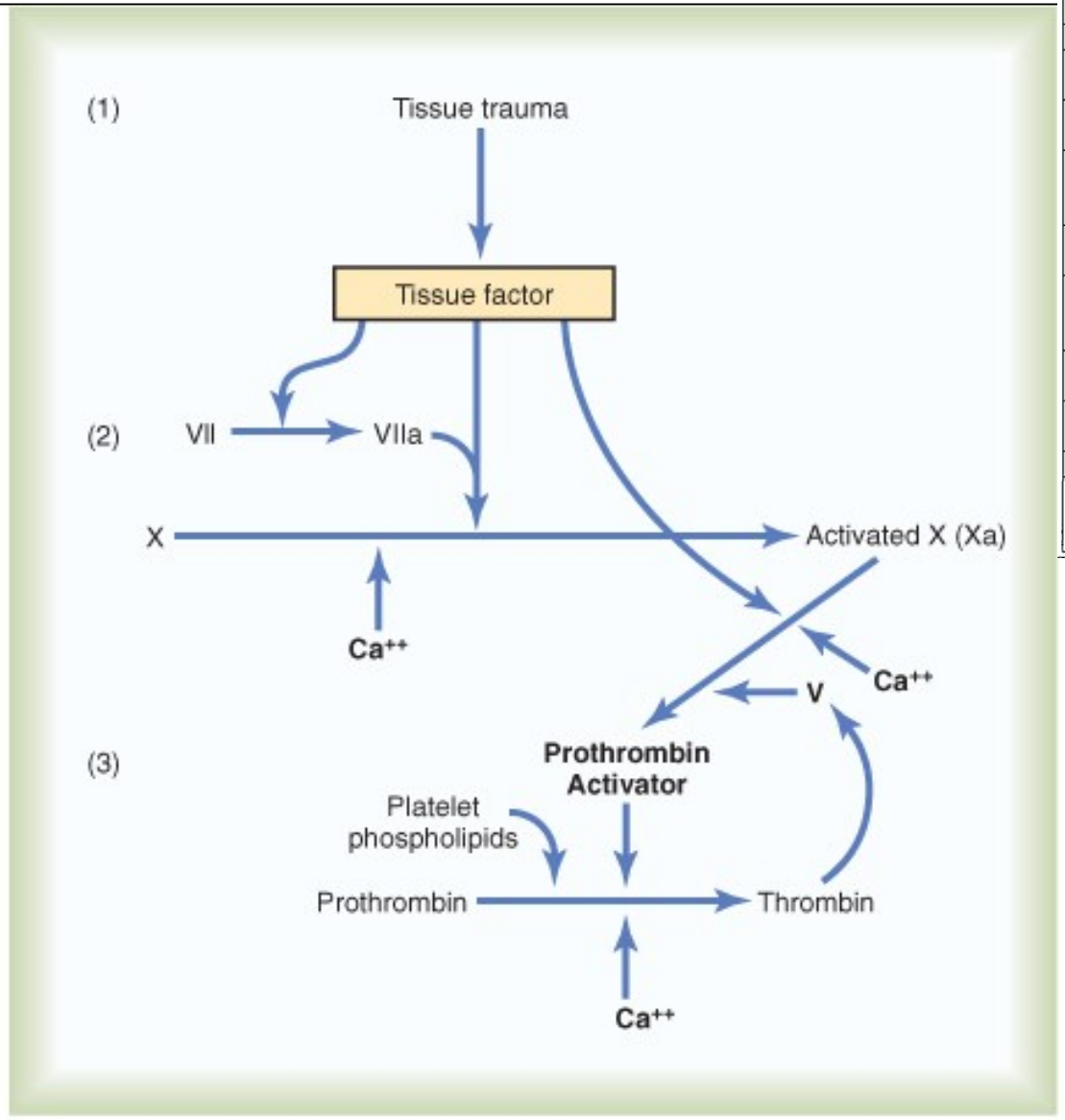
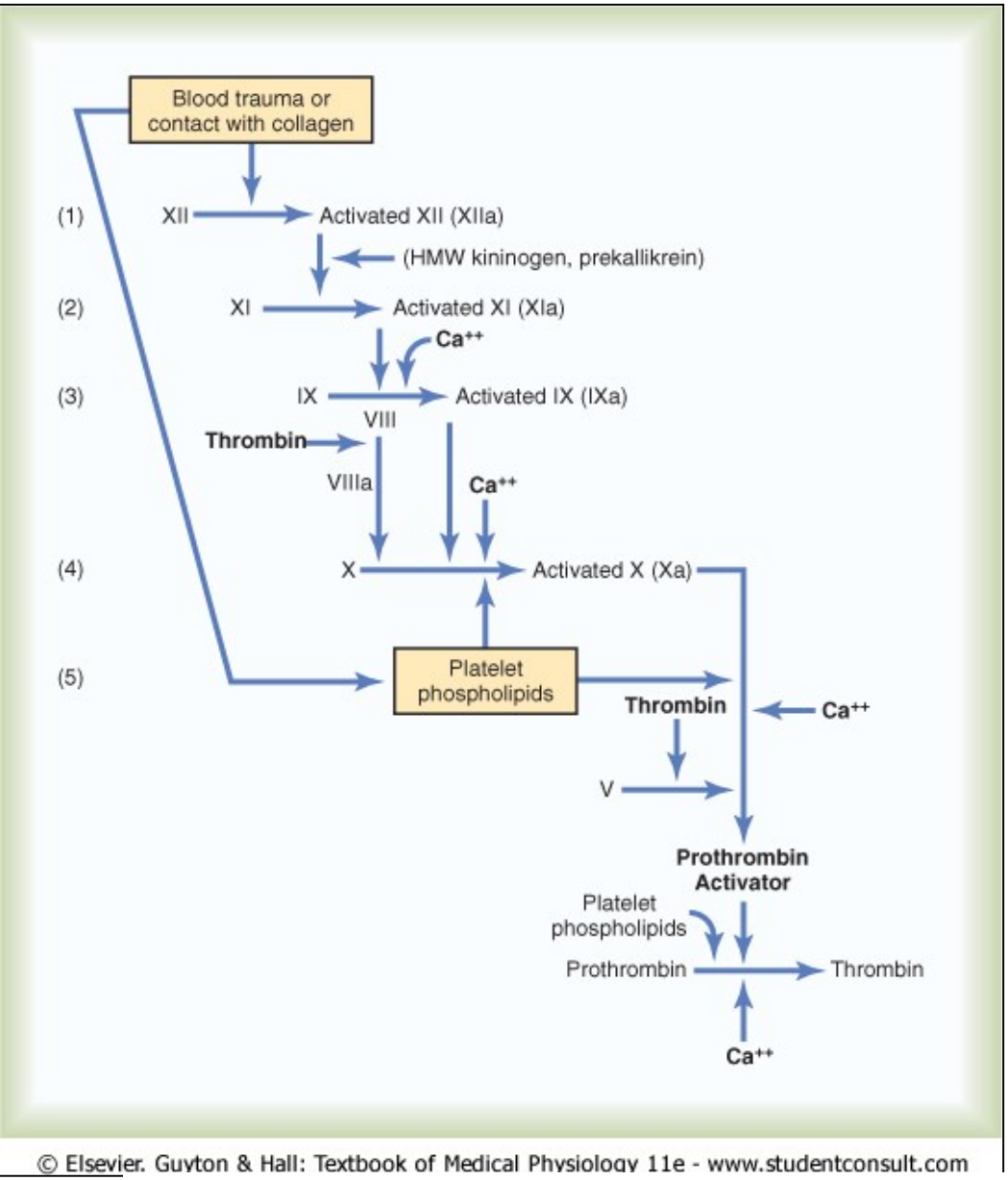


Figure 36-2 Schema for conversion of prothrombin to thrombin and polymerization of fibrinogen to form fibrin fibers.



Factor	Common Name	Pathway	Characteristic
I	Fibrinogen	Both	-
II	Prothrombin	Both	Contains N-terminal Gla domain
III	Tissue Factor	Extrinsic	-
IV	Calcium	Both	-
V	Proaccelerin, labile factor, Accelerator globulin	Both	Protein cofactor
VI (Va)	Accelerin	-	(Redundant to factor V)
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA) cothromboplastin	Extrinsic	Endopeptidase with Gla domain
VIII	Antihemophiliac factor A, antihemophiliac globulin (AHG)	Intrinsic	Protein cofactor
IX	Christmas factor, antihemophiliac factor B, plasma thromboplastin component (PTC)	Intrinsic	Endopeptidase with Gla domain
X	Stuart-prower factor	Both	Endopeptidase with Gla domain
XI	Plasma thromboplastin antecedent (PTA)	Intrinsic	Endopeptidase
XII	Hageman factor	Intrinsic	Endopeptidase
XIII	Protransglutamidase, fibrin stabilizing factor (FSF), fibrinoligase	Both	Transpeptidase

Figure 36-3 Extrinsic pathway for initiating blood clotting.



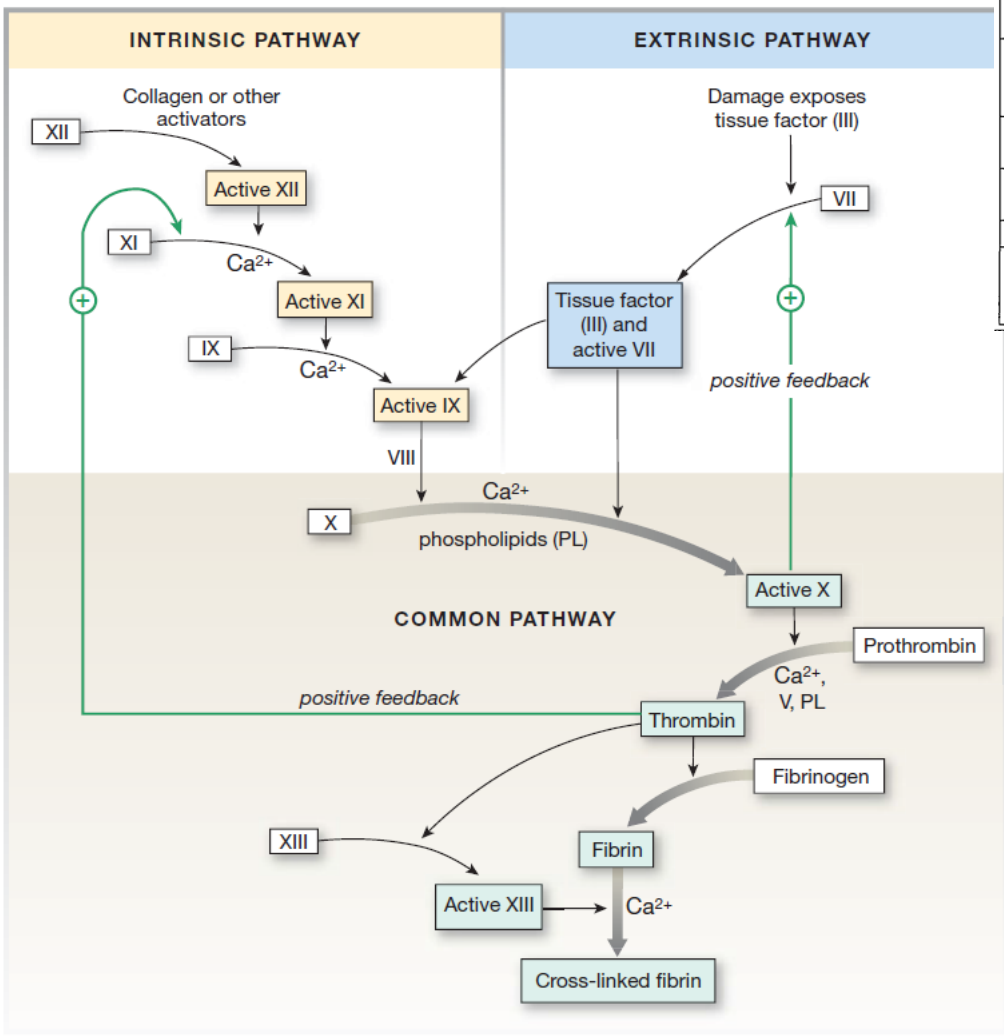
Factor	Common Name	Pathway	Characteristic
I	Fibrinogen	Both	-
II	Prothrombin	Both	Contains N-terminal Gla domain
III	Tissue Factor	Extrinsic	-
IV	Calcium	Both	-
V	Proaccelerin, labile factor, Accelerator globulin	Both	Protein cofactor
VI (Va)	Accelerin	-	(Redundant to factor V)
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA) cothromboplastin	Extrinsic	Endopeptidase with Gla domain
VIII	Antihemophiliac factor A, antihemophiliac globulin (AHG)	Intrinsic	Protein cofactor
IX	Christmas factor, antihemophiliac factor B, plasma thromboplastin component (PTC)	Intrinsic	Endopeptidase with Gla domain
X	Stuart-prower factor	Both	Endopeptidase with Gla domain
XI	Plasma thromboplastin antecedent (PTA)	Intrinsic	Endopeptidase
XII	Hageman factor	Intrinsic	Endopeptidase
XIII	Protransglutamidase, fibrin stabilizing factor (FSF), fibrinoligase	Both	Transpeptidase

Figure 36-4 Intrinsic pathway for initiating blood clotting.

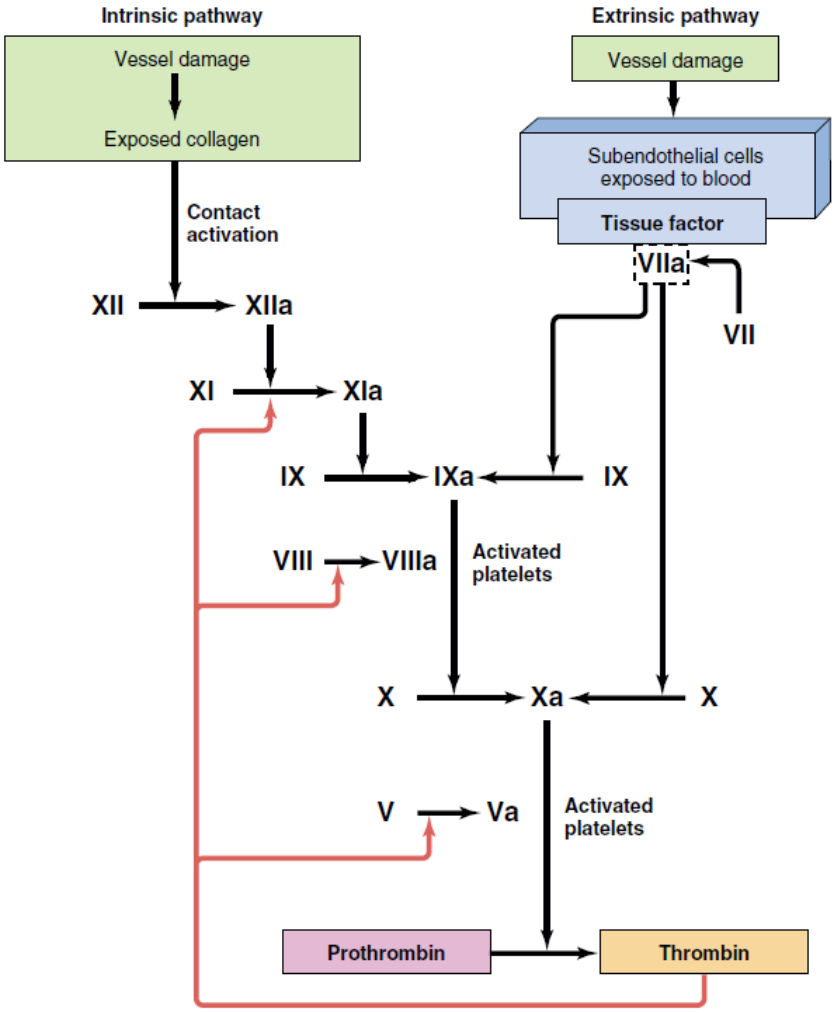
Factor	Common Name	Pathway	Characteristic
I	Fibrinogen	Both	-
II	Prothrombin	Both	Contains N-terminal Gla domain
III	Tissue Factor	Extrinsic	-
IV	Calcium	Both	-
V	Proaccelerin, labile factor, Accelerator globulin	Both	Protein cofactor
VI (Va)	Accelerin	-	(Redundant to factor V)
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA) cothromboplastin	Extrinsic	Endopeptidase with Gla domain
VIII	Antihemophiliac factor A, antihemophiliac globulin (AHG)	Intrinsic	Protein cofactor
IX	Christmas factor, antihemophiliac factor B, plasma thromboplastin component (PTC)	Intrinsic	Endopeptidase with Gla domain
X	Stuart-prower factor	Both	Endopeptidase with Gla domain
XI	Plasma thromboplastin antecedent (PTA)	Intrinsic	Endopeptidase
XII	Hageman factor	Intrinsic	Endopeptidase
XIII	Protransglutamidase, fibrin stabilizing factor (FSF), fibrinoligase	Both	Transpeptidase

THE COAGULATION CASCADE

Inactive plasma proteins (white boxes) are converted into active enzymes in each step of the pathway.



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



Factor	Common Name	Pathway	Characteristic
I	Fibrinogen	Both	-
II	Prothrombin	Both	Contains N-terminal Gla domain
III	Tissue Factor	Extrinsic	-
IV	Calcium	Both	-
V	Proaccelerin, labile factor, Accelerator globulin	Both	Protein cofactor
VI (Va)	Accelerin	-	(Redundant to factor V)
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA) cothromboplastin	Extrinsic	Endopeptidase with Gla domain
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IX	Christmas factor, antihemophiliac factor B, plasma thromboplastin component (PTC)	Intrinsic	Endopeptidase with Gla domain
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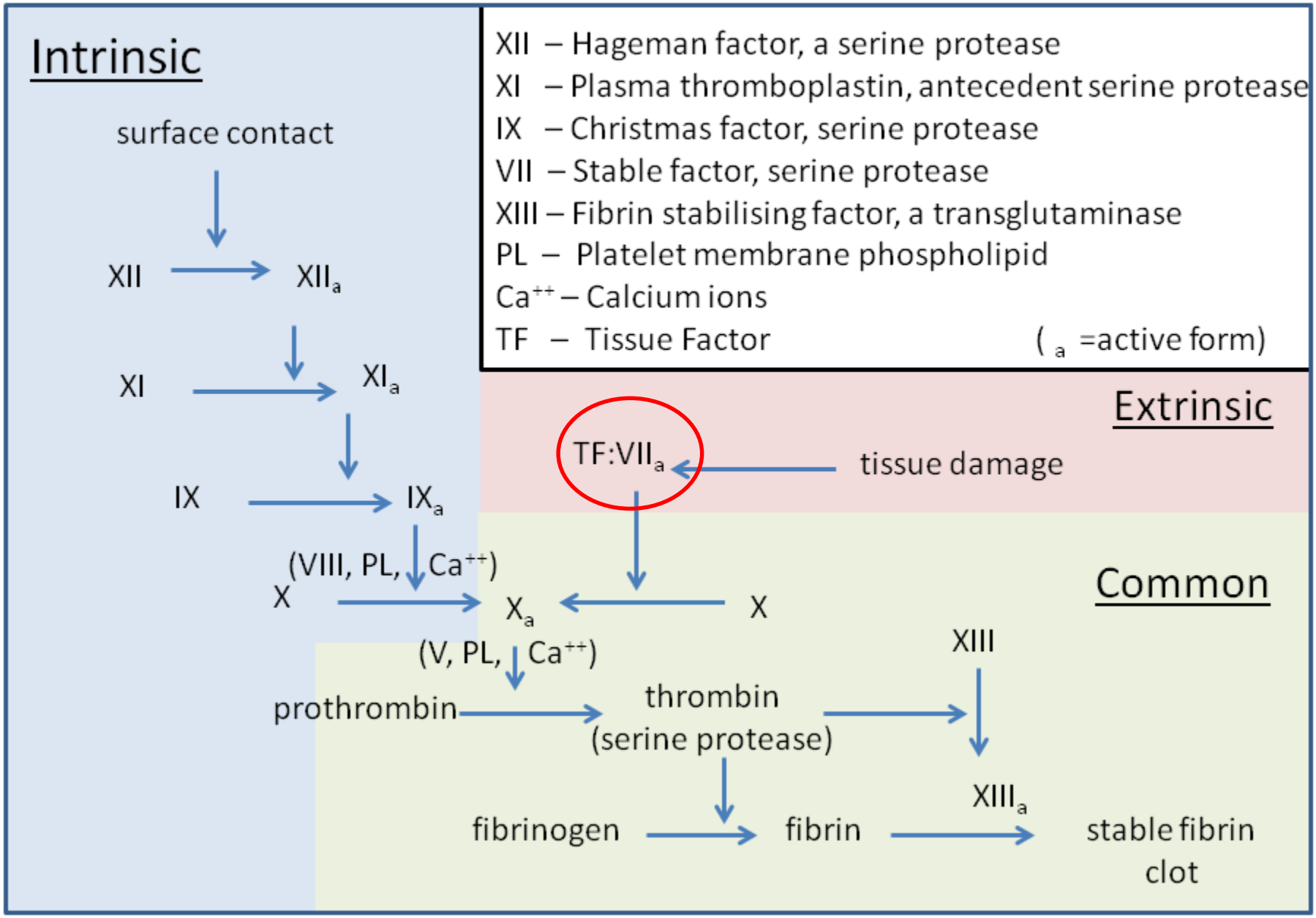
XI	Plasma thromboplastin antecedent (PTA)	Intrinsic	Endopeptidase
XII	Hageman factor	Intrinsic	Endopeptidase
XIII	Protransglutamidase, fibrin stabilizing factor (FSF), fibrinoligase	Both	Transpeptidase

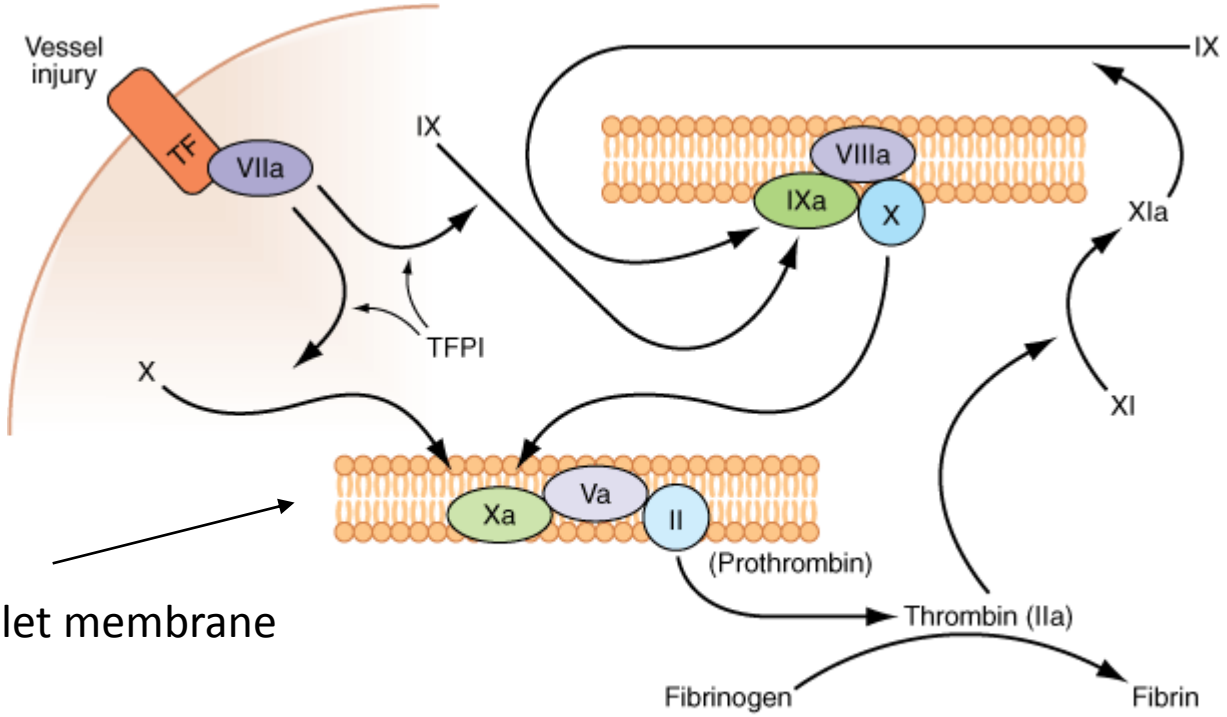
FIGURE 14–73

Two clotting pathways—called intrinsic and extrinsic—merge and can lead to the generation of thrombin. Under most physiological conditions, however, factor XII and the contact activation step that begin the intrinsic pathway probably play little, if any, roles in clotting. Rather, clotting is initiated solely by the extrinsic pathway, as described in the text. You might think that factors IX and X were accidentally transposed in the intrinsic pathway, but such is not the case; the order of activation really is XI, IX, and X. For the sake of clarity, the roles of calcium in clotting are not shown.

The three pathways that makeup the classical blood coagulation pathway

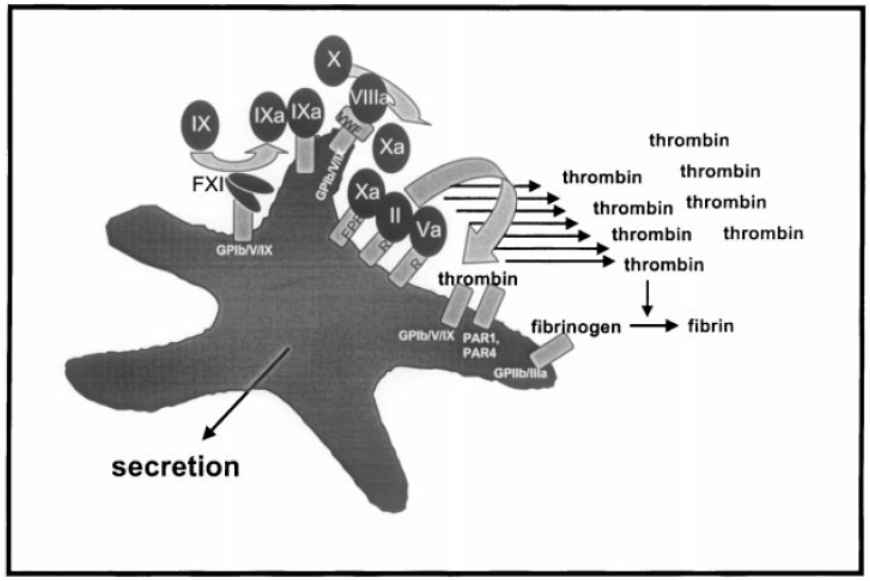


CLOTTING MECHANISM



Activated platelet membrane

TF, tissue factor TFPI, tissue factor pathway inhibitor



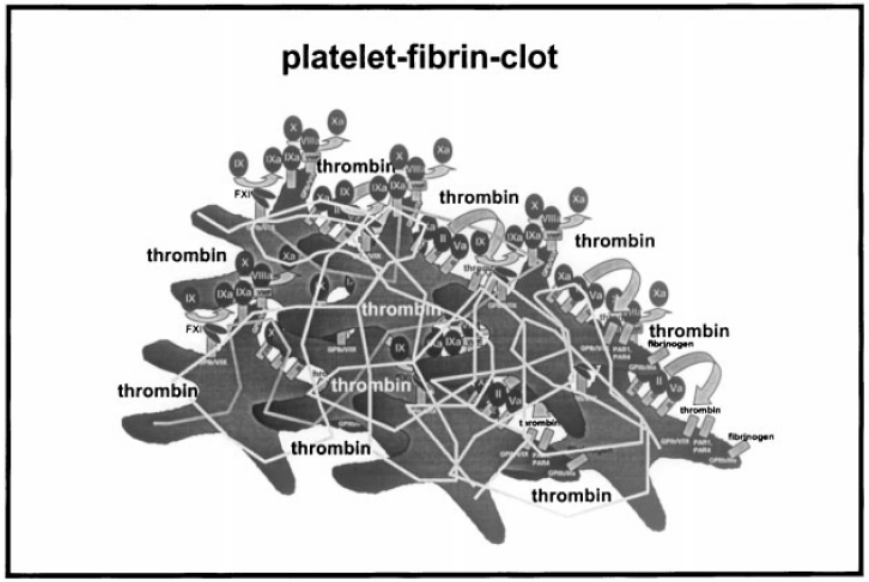


Figure 4 Model of receptor-mediated thrombin generation. (A) Small amounts of thrombin formed on the surface of a tissue factor (TF) presenting cell (fibroblast or activated monocyte or activated endothelial cell, respectively). These amounts of thrombin are not able to produce a stable fibrin clot, but are enough to activate platelets. (B) Activated platelets can then bind coagulation factors and cofactors via Ca^{2+} and by specific receptors. (C) Platelet-bound cofactors FV and FVIII are protected against cleavage by activated protein C. (D) On the surface of the platelet FXIa binds to its receptor GPIb and activates FIX. In contrast to FXa that is readily inhibited by tissue factor pathway inhibitor (TFPI) as soon as it enters the plasma, FIXa, built on TF/FVIIa presenting cells can in addition diffuse to the activated platelets. On the platelet surface the Xase-complex and the prothrombinase-complex have optimal conditions. (E) The concerted actions of coagulation factors on the platelet surface lead to a burst of thrombin formation, so that a stable fibrin clot can be formed. aPC, activated protein C; R, receptor; EPR1, effector cell protease receptor 1, PAR, protease activated receptor.

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

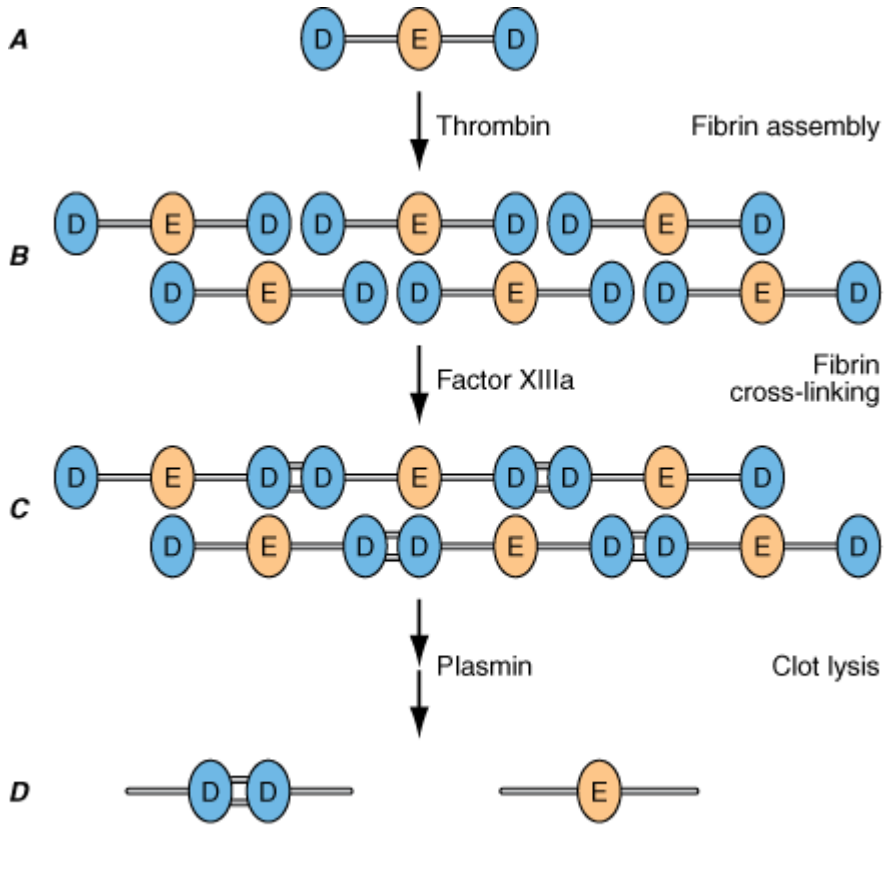
Factors Involved in Coagulation

Table 16.5

Chemical Factor	Source	Activated by or Released in Response to	Role in Coagulation	Other Roles and Comments
Collagen	Subendothelial extracellular matrix	Injury that exposes collagen to plasma clotting factors	Starts intrinsic pathway	N/A
von Willebrand factor (vWF)	Endothelium, megakaryocytes	Exposure to collagen	Regulates level of factor VIII	Deficiency or defect causes prolonged bleeding
Kininogen and kallikrein	Liver and plasma	Cofactors normally present in plasma pathway	Cofactors for contact activation of intrinsic pathway	Mediate inflammatory response; enhance fibrinolysis
Tissue factor (tissue thromboplastin or factor III)	Most cells except platelets	Damage to tissue	Starts extrinsic pathway	N/A
Prothrombin and thrombin (factor II)	Liver and plasma	Platelet lipids, Ca^{2+} and factor V	Fibrin production	N/A
Fibrinogen and fibrin (factor I)	Liver and plasma	Thrombin	Form insoluble fibers that stabilize platelet plug	N/A
Fibrin-stabilizing factor (XIII)	Liver, megakaryocytes	Platelets	Cross-links fibrin polymers to make stable mesh	N/A
Ca^{2+} (factor IV)	Plasma ions	N/A	Required for several steps of coagulation cascade	Never a limiting factor
Vitamin K	Diet	N/A	Needed for synthesis of factors II, VII, IX, X	N/A

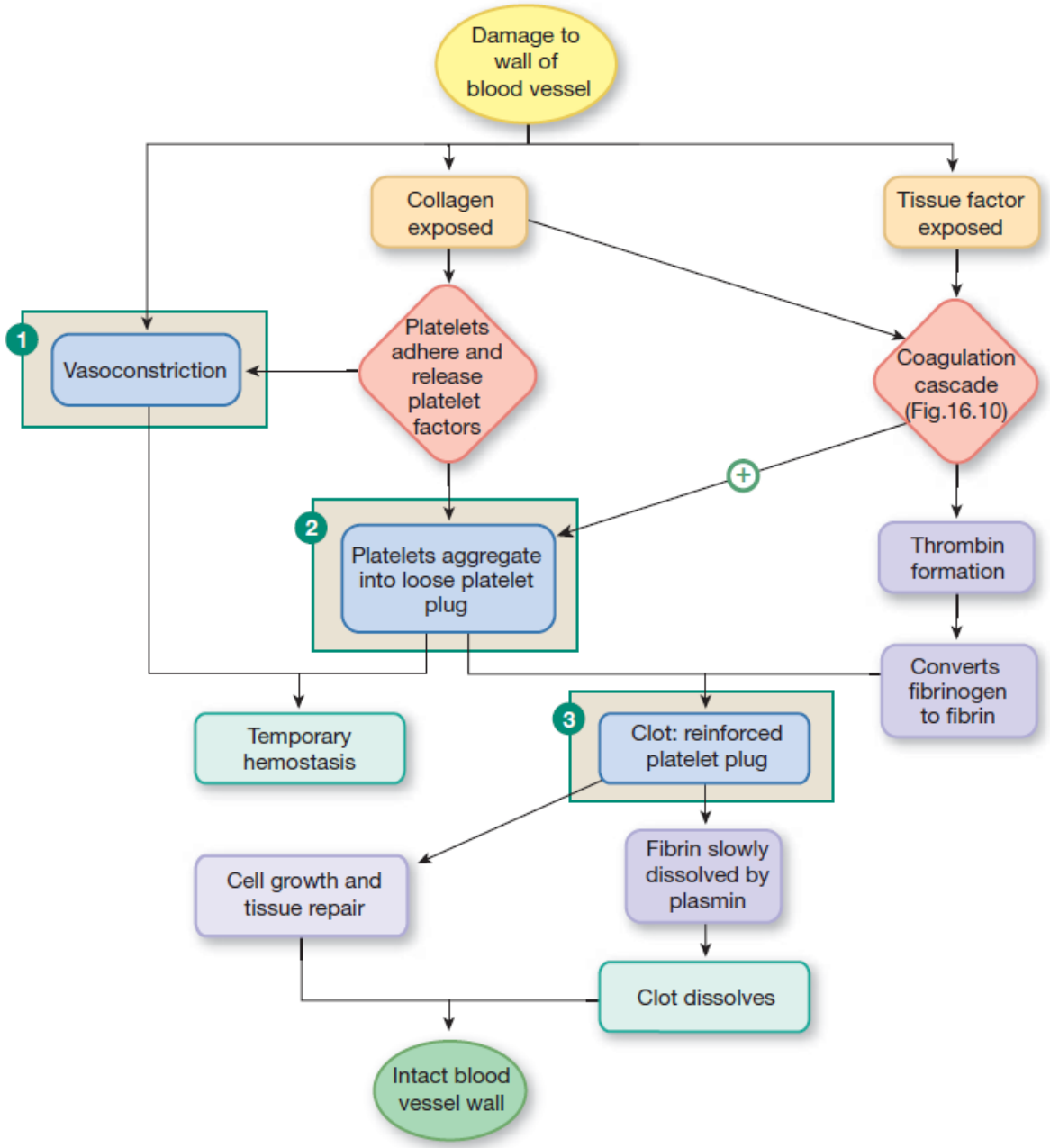
Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.

FIBRIN FORMATION AND DEGRADATION



FDPs, fibrin degradation products

SUMMARY

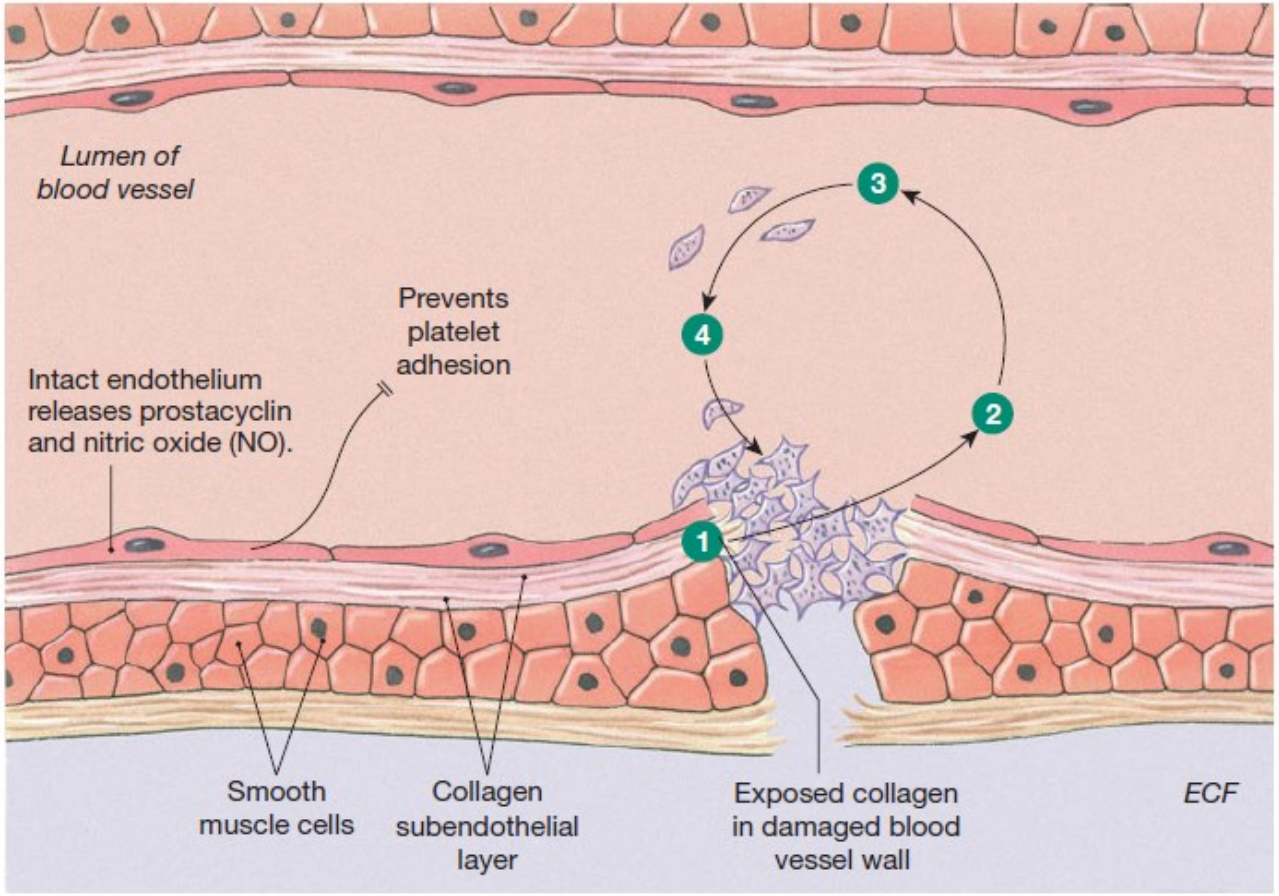


Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.

PLATELET PLUG FORMATION

SUMMARY

Platelets will not adhere to intact endothelium. Damage triggers platelet plug formation where collagen has been exposed.



1. Exposed collagen binds and activates platelets.
2. Release of platelet factors
3. Factors attract more platelets.
4. Platelets aggregate into platelet plug.

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

Factor	Name of factor	Biological half-time (h)
I	fibrinogen	120-144
II	prothrombin	48
III	thromboplastin, thrombokinase	very short
IV	calcium ions	
V	proaccelerin	12-15
VII	(AHF) proconvertin, stabile factor	2-5
VIII	antihaemofilic factor A, a. globulin	5-12
IX	Christmas factor, antihem. f. B	12-30
X	Stuart-Prower factor	32
XI	antihaemofilic factor C, PTA	less than 12
XII	Hageman factor	less than 12
XIII	factor stabilising fibrin	48-72
HMW-K	Fitzgerald f. (high-molecular-weight kininogen)	
Pre-K	prekallikrein	
Ka	kallikrein	
PL	Platelet phospholipids	

Endogenous Factors Involved in Fibrinolysis and Anticoagulation

Table 16.6

Chemical Factor	Source	Activated by or Released in Response to	Role in Anticoagulation or Fibrinolysis	Other Roles and Comments
Plasminogen and plasmin	Liver and plasma	tPA and thrombin	Dissolves fibrin and fibrinogen	N/A
Tissue plasminogen activator (tPA)	Many tissues	Normally present; levels increase with stress, protein C	Activates plasminogen	Recombinant tPA used clinically to dissolve clots
Antithrombin III	Liver and plasma	N/A	Anticoagulant; blocks factors IX, X, XI, XII, thrombin, kallikrein	Facilitated by heparin; no effect on thrombin despite name
Prostacyclin (prostaglandin I, or PGI_2)	Endothelial cells	N/A	Blocks platelet aggregation	Vasodilator

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

TABLE 14–16 Anticlotting Roles of Endothelial Cells

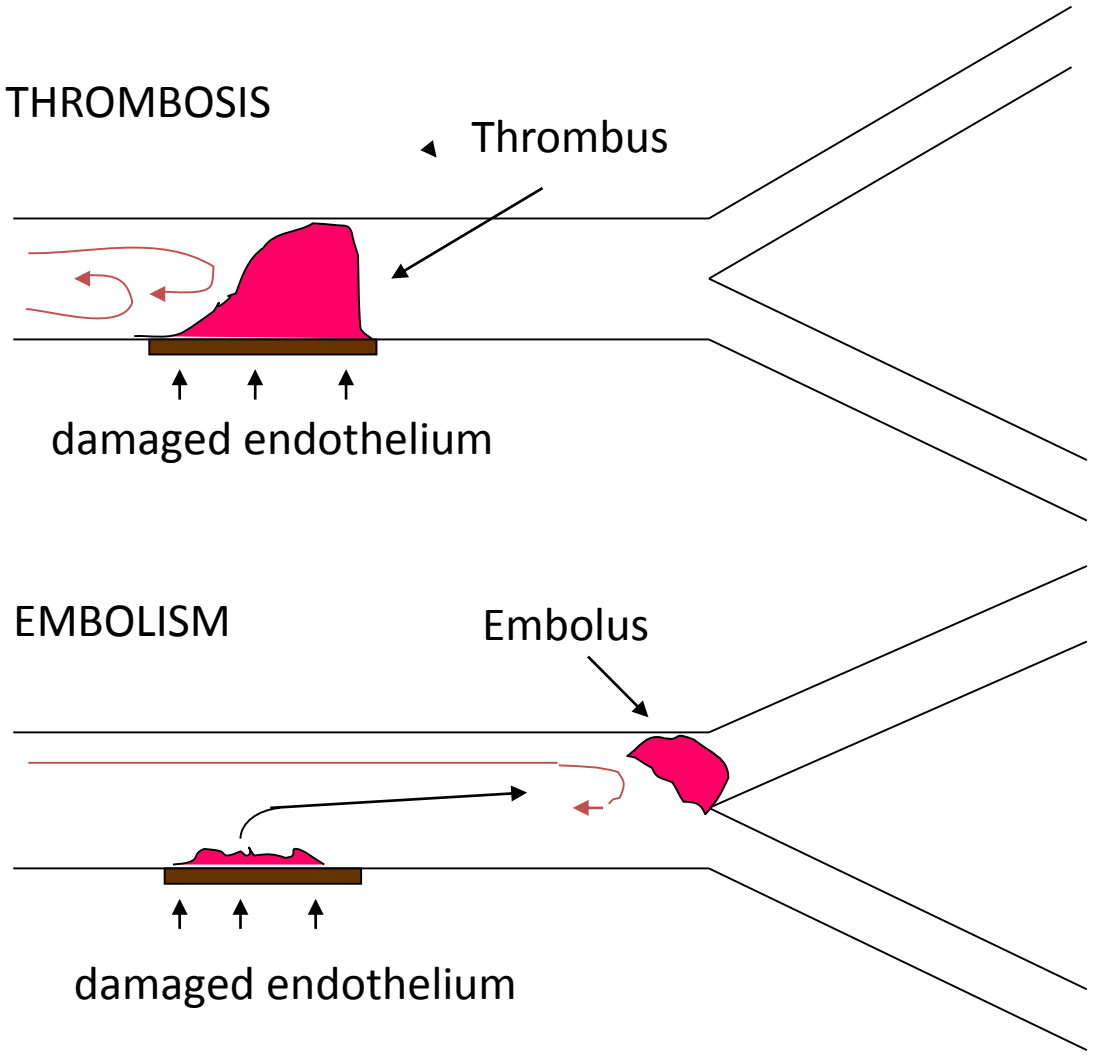
Action	Result
Normally provide an intact barrier between the blood and subendothelial connective tissue	Platelet aggregation and the formation of tissue factor–factor VIIa complexes are not triggered
Synthesize and release PGI_2 and nitric oxide	These inhibit platelet activation and aggregation
Secrete tissue factor pathway inhibitor	Inhibits the ability of tissue factor–factor VIIa complexes to generate factor Xa
Bind thrombin (via thrombomodulin), which then activates protein C	Active protein C inactivates clotting factors VIIIa and Va
Display heparin molecules on the surfaces of their plasma membranes	Heparin binds antithrombin III, and this molecule then inactivates thrombin and several other clotting factors
Secrete tissue plasminogen activator	Tissue plasminogen activator catalyzes the formation of plasmin, which dissolves clots

INTRAVASCULAR COAGULATION

Damage of epithelium caused by:

1) Atherosclerosis (myocardial infarction, stroke)

2) Inflammation (venous thrombosis, pulmonary embolism)



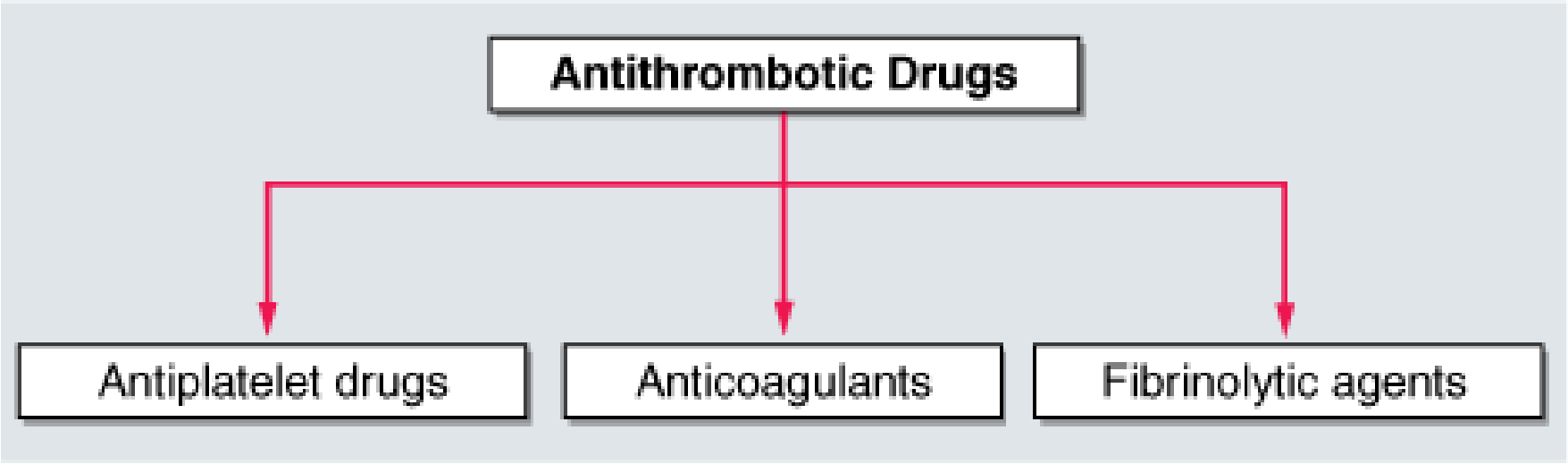
Example:

MI

Stroke

Example:

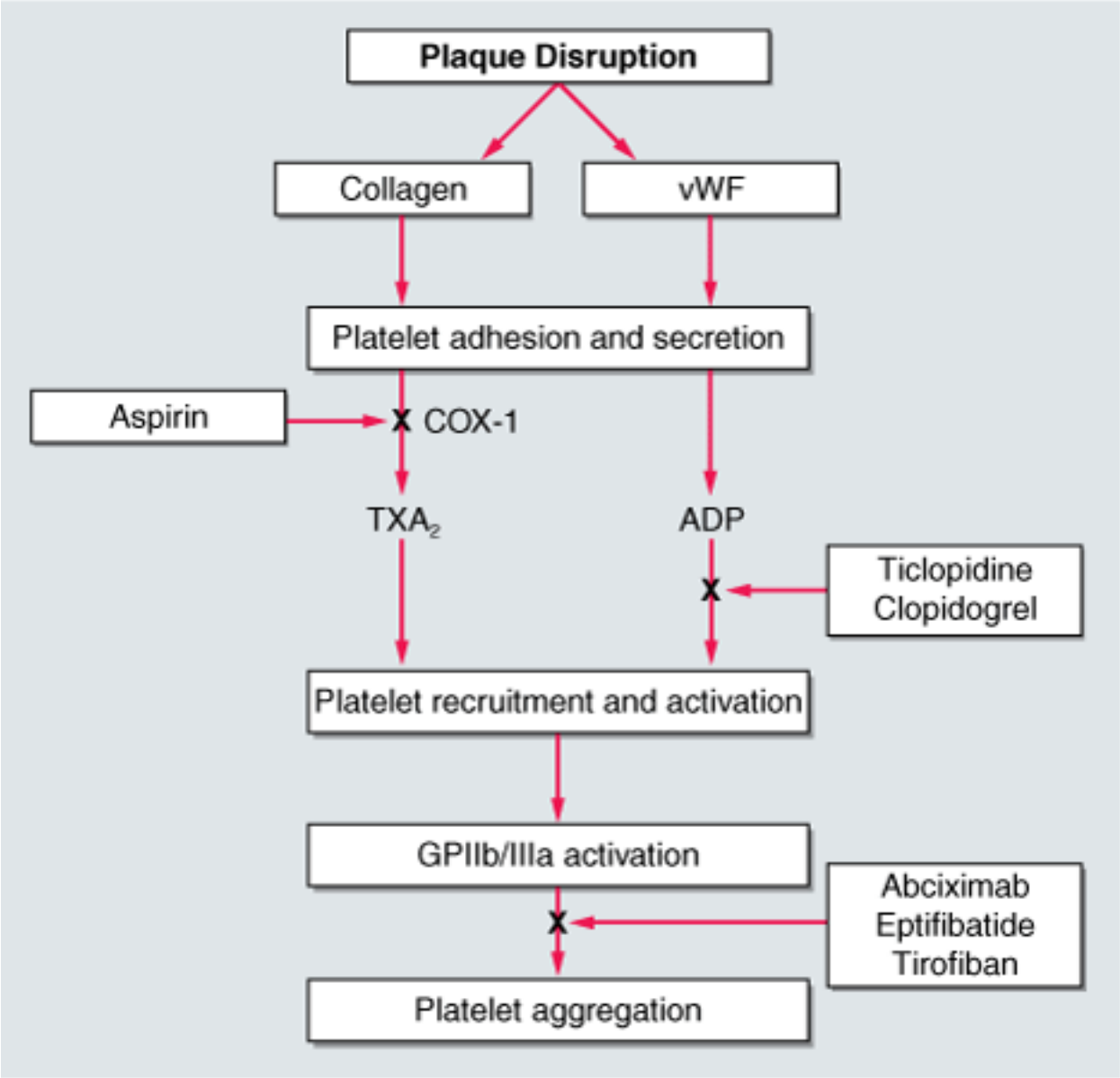
Pulmonary embolism



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



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

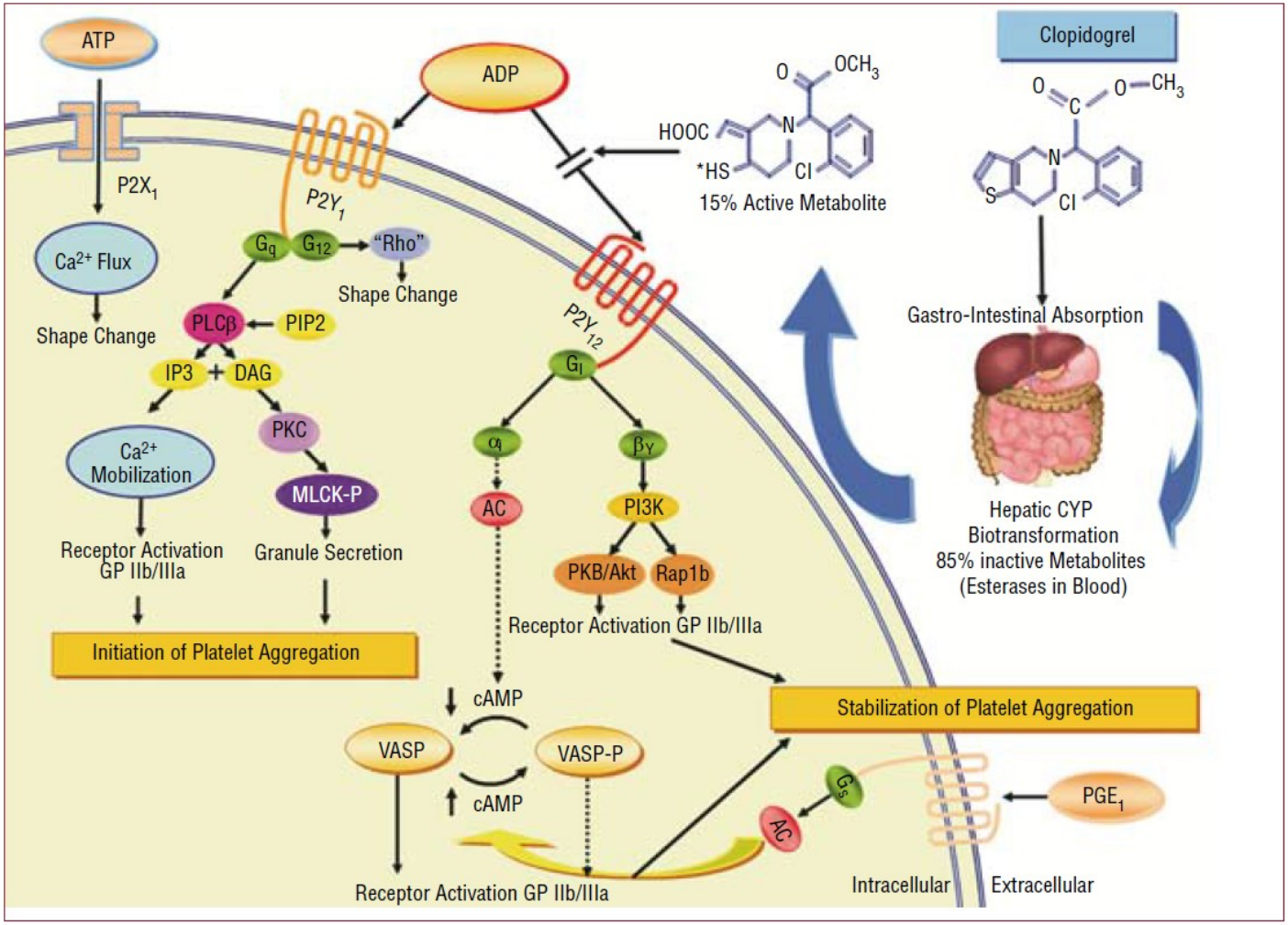


Figure 1. Purinergic receptors and mechanism of action of clopidogrel. Clopidogrel is a pro-drug of which approximately 85% is hydrolyzed by esterases in the blood to inactive metabolites and only 15% is metabolized by the cytochrome P450 (CYP) system in the liver into an active metabolite. The active metabolite irreversibly inhibits the adenosine diphosphate (ADP) $P2Y_{12}$ receptor. The $P2X_1$ receptor, which uses adenosine triphosphate (ATP) as an agonist, is involved in platelet shape change through extracellular calcium influx and helps to amplify platelet responses mediated by other agonists. Activation of the $P2Y_1$ receptor leads to alteration in shape and initiates a weak and transient phase of platelet aggregation. The binding of ADP to the G_q-coupled $P2Y_1$ receptor activates phospholipase C (PLC), which generates diacylglycerol (DAG) and inositol triphosphate (IP3) from phosphatidylinositol biphosphate (PIP2). Diacylglycerol activates protein kinase C (PKC) leading to phosphorylation of myosin light chain kinase (MLCK-P) and IP3 leads to mobilization of intracellular calcium. The $P2Y_1$ receptor is coupled to another G-protein, G_{12}, which activates the "Rho" protein and leads to the change in platelet shape. The binding of ADP to the G_i-coupled$P2Y_{12}$ receptor liberates the G_i protein subunits a_i and β_γ, resulting in stabilization of platelet aggregation. The α_i subunit inhibits adenylyl cyclase (AC) and, thus, reduces cyclic adenosine monophosphate (cAMP) levels, which diminishes cAMP-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP-P). The status of VASP-P modulates glycoprotein (GP) IIb/IIIa receptor activation. The subunit β_γ activates the phosphatidylinositol 3-kinase (PI3K), which leads to GP IIb/IIIa receptor activation through activation of a serine-threonine protein kinase B (PKB/Akt) and of Rap1b GTP binding proteins. Prostaglandin E_1 (PGE_1) activates AC, which increases cAMP levels and status of VASP-P. Solid arrows indicate activation; dotted arrows indicate inhibition. With permission from Angiolillo DJ et al.[6]

Angiolillo DJ, Ferreiro JL: **Platelet Adenosine Diphosphate P2Y(12) Receptor Antagonism: Benefits and Limitations of Current Treatment Strategies and Future Directions.** ***Revista Espanola De Cardiologia 2010, 63(1):60-76.***

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.

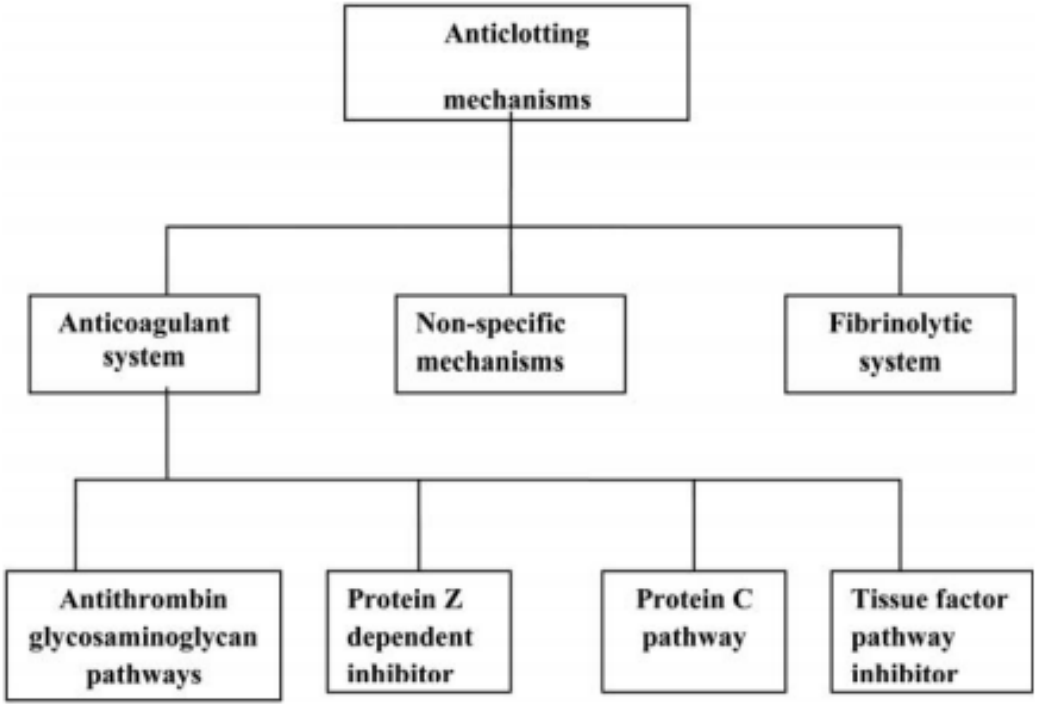
Thrombin + Thrombmodulin = activator of protein C

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.



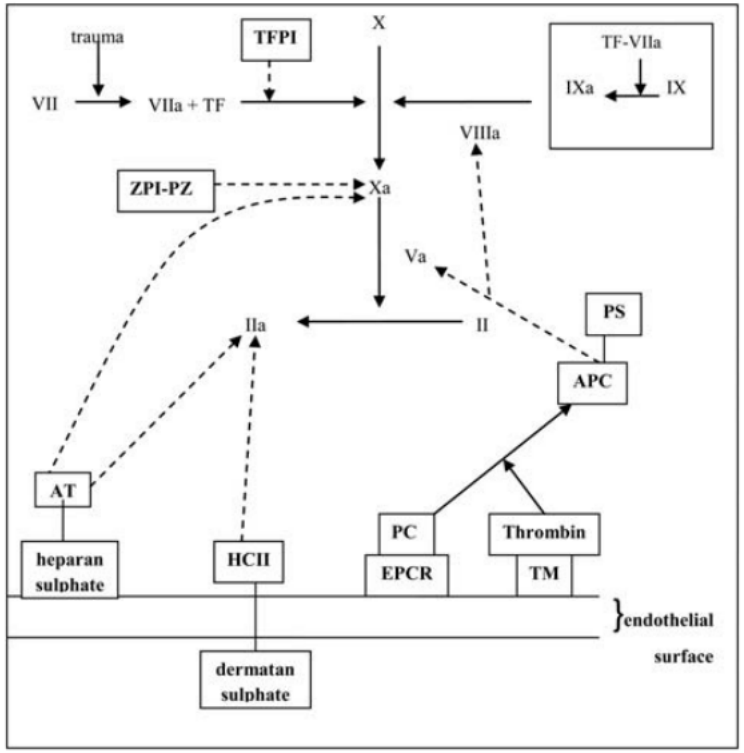


Fig 2 The anticoagulant system. AT, antithrombin; HCII, heparin cofactor II; TFPI, tissue factor pathway inhibitor; ZPI, protein Z-dependent protease inhibitor; PZ, protein Z; PC, protein C; APC, activated protein C; PS, protein S; EPCR, endothelial protein C receptor; TM, thrombomodulin. Solid arrows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

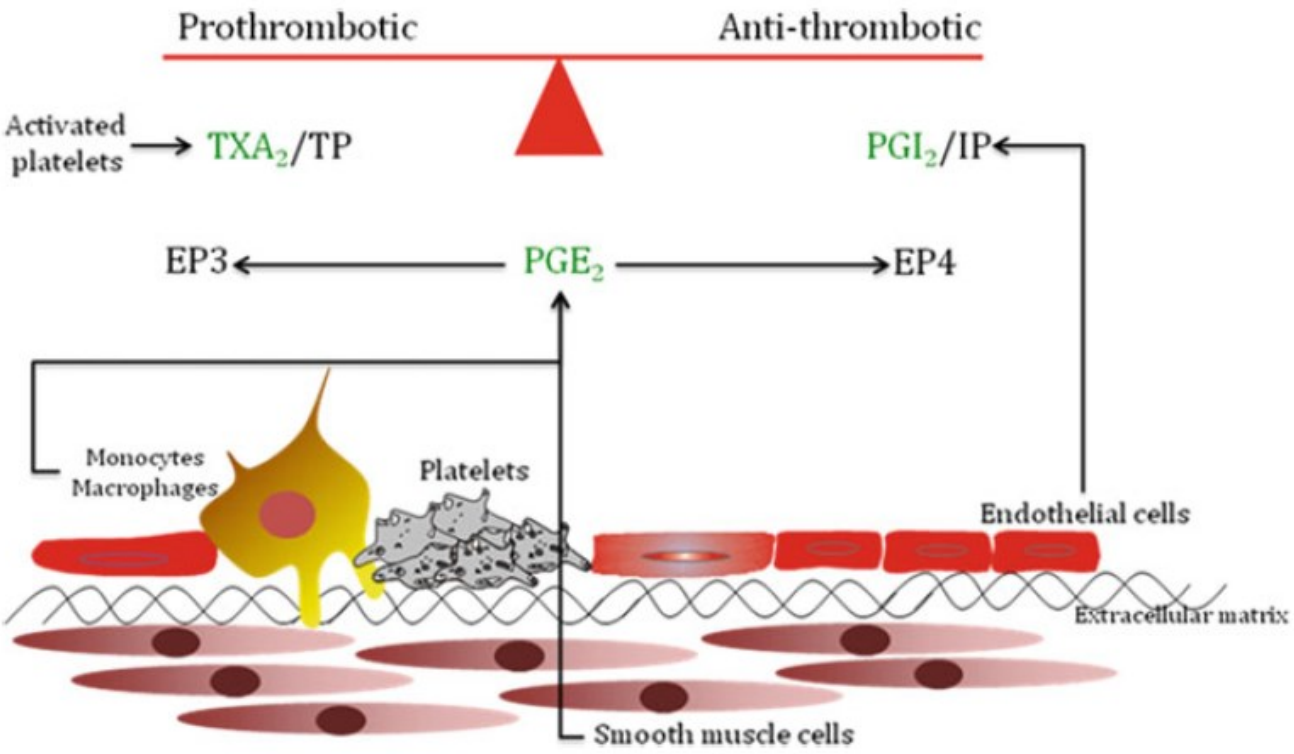
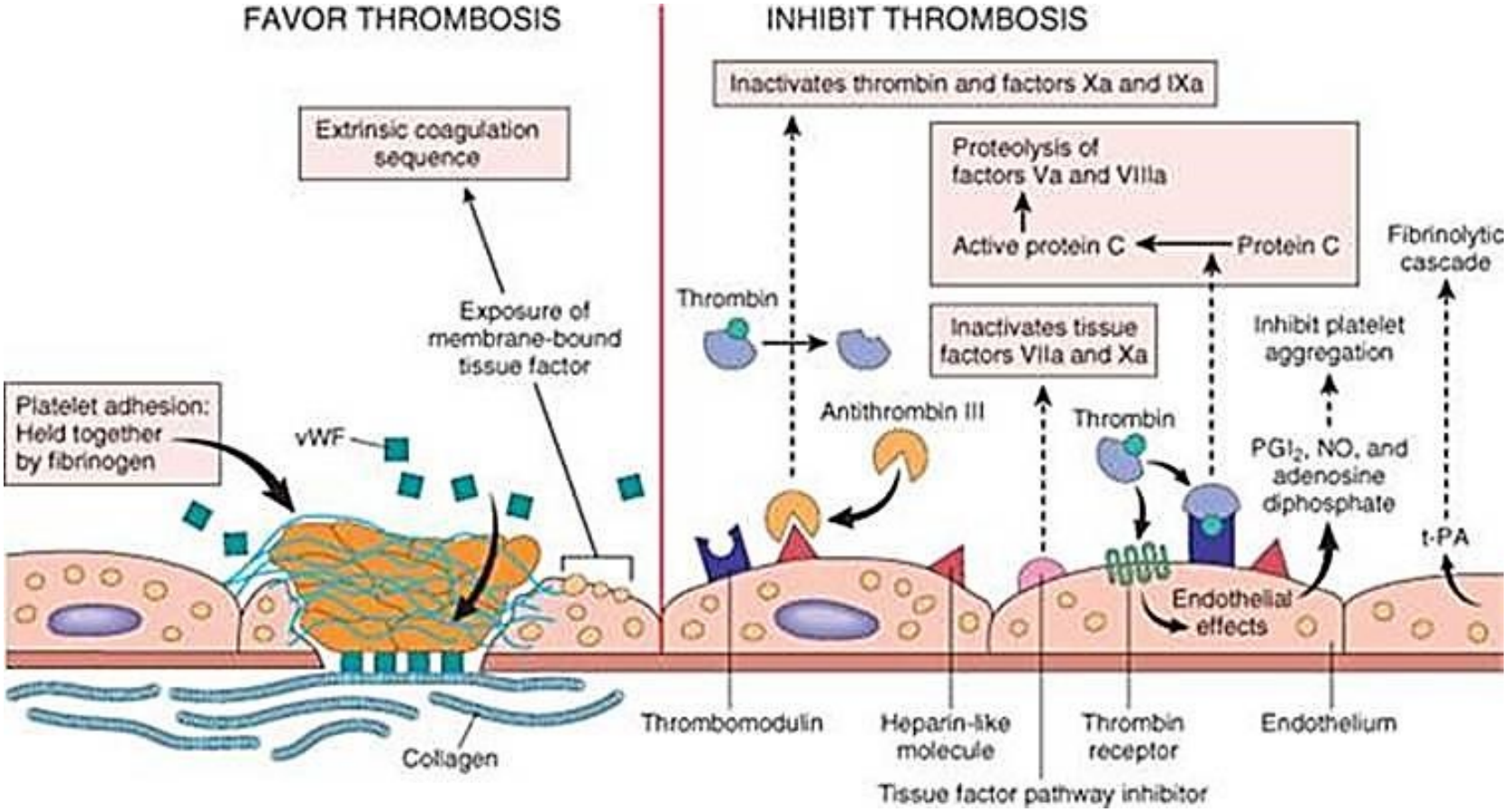


Fig. 4 The balance between thrombotic and antithrombotic effects of prostanoids. In response to vascular injury, PGI_2 produced by endothelial cells opposes the enhanced prothrombotic effect of TXA_2 produced by platelets. Smooth muscle cells, monocytes, and macrophages (accumulate in atherosclerotic 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.***


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
Thrombin
Inactivates tissue factors VIIa and Xa
Antithrombin III
Thrombin
Fibrinolytic cascade
Inhibit platelet aggregation
PGI2, NO, and adenosine diphosphate
t-PA
Endothelial effects
Thrombomodulin
Heparin-like molecule
Thrombin receptor
Endothelium
Tissue factor pathway inhibitor

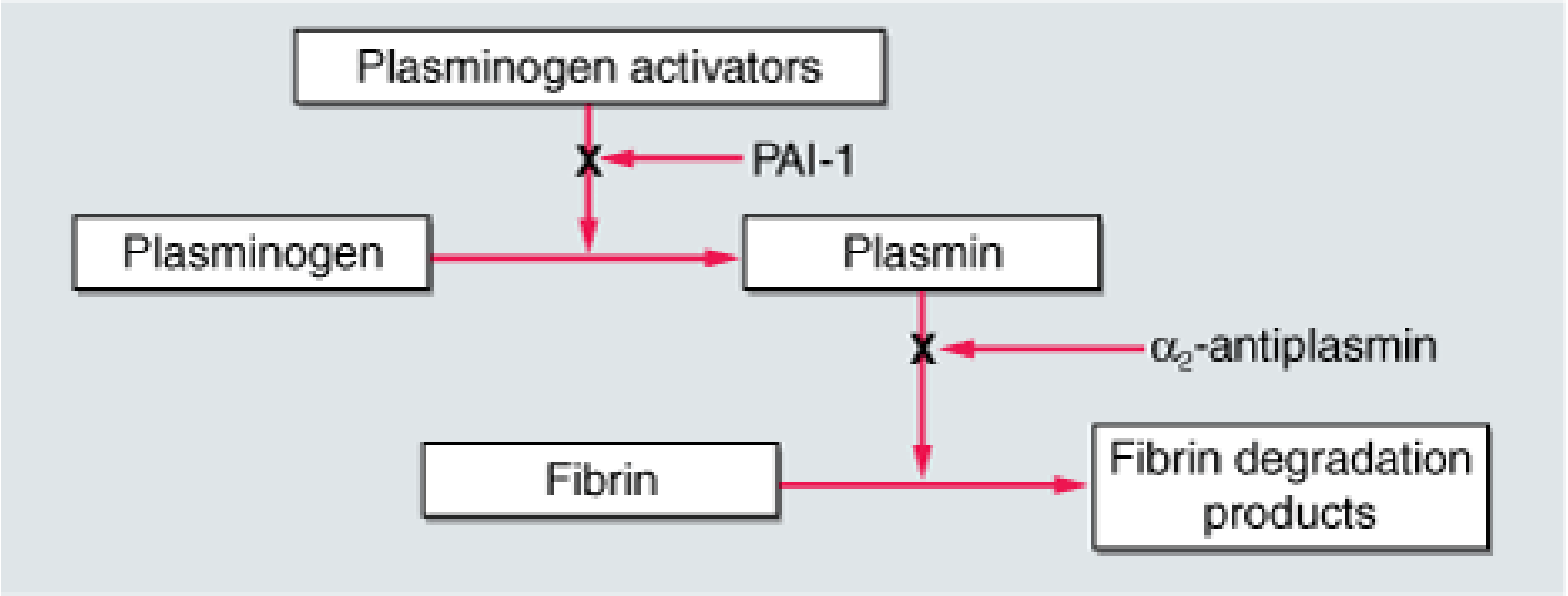
FIBRINOLYSIS

Inactive plasminogen.

Active plasmin (fibrinolysin).

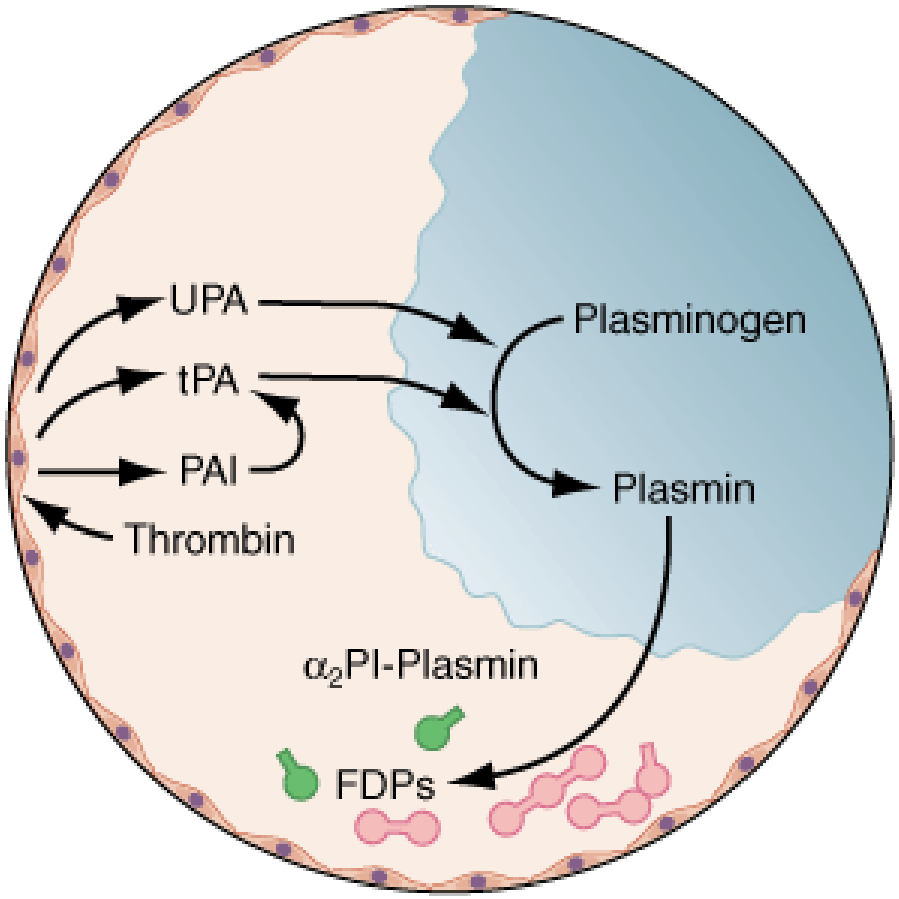
Activators of plasminogen.

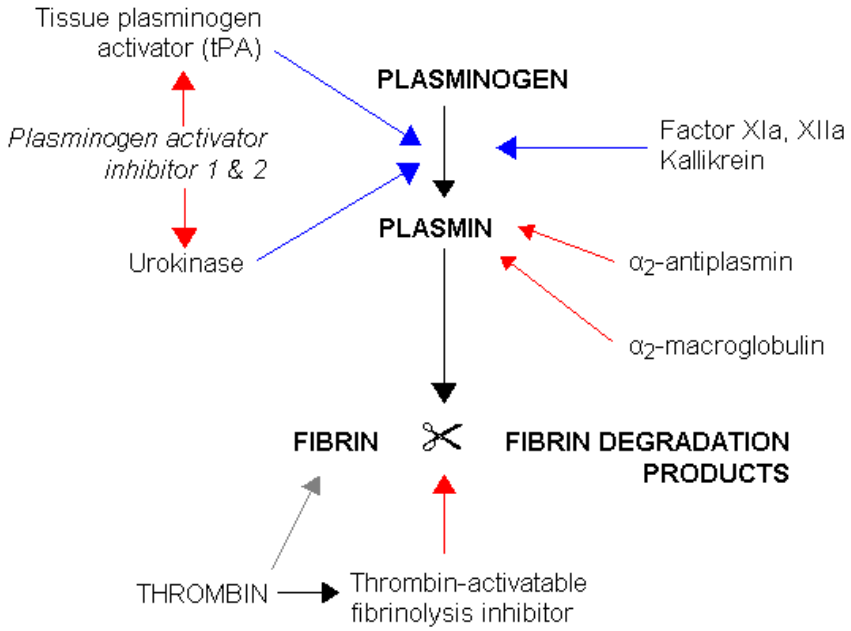
Inhibitors of plasminogen.



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

Activation →

Inhibition ⟷

In vivo: Heparin → antithrombin III

test: aPTT,

antifactor Xa level

Coumarin (warfarin) → Vitamin K → Liver → prothrombin

test: PT

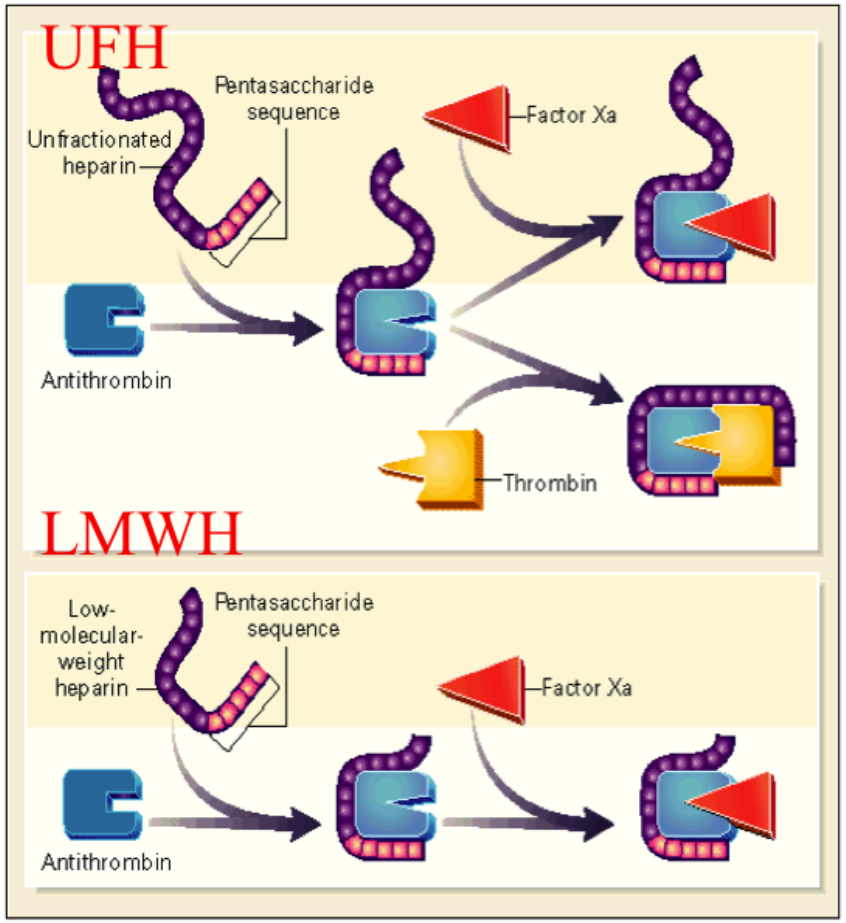
$INR = PT_{patient}/PT_{norm}$

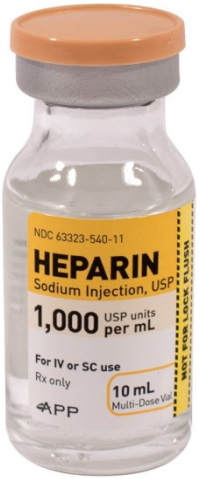
warfarin → INR 2-3

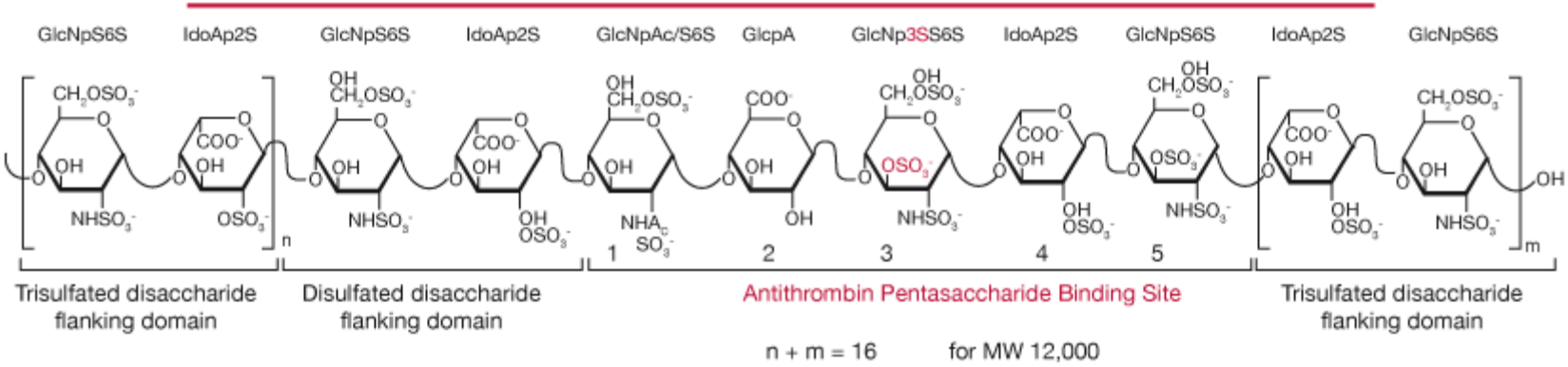
In vitro: Heparin → antithrombin III

Sodium citrate → Ca^{++}

aPTT: activated partial thromboplastin time PT: prothrombin time

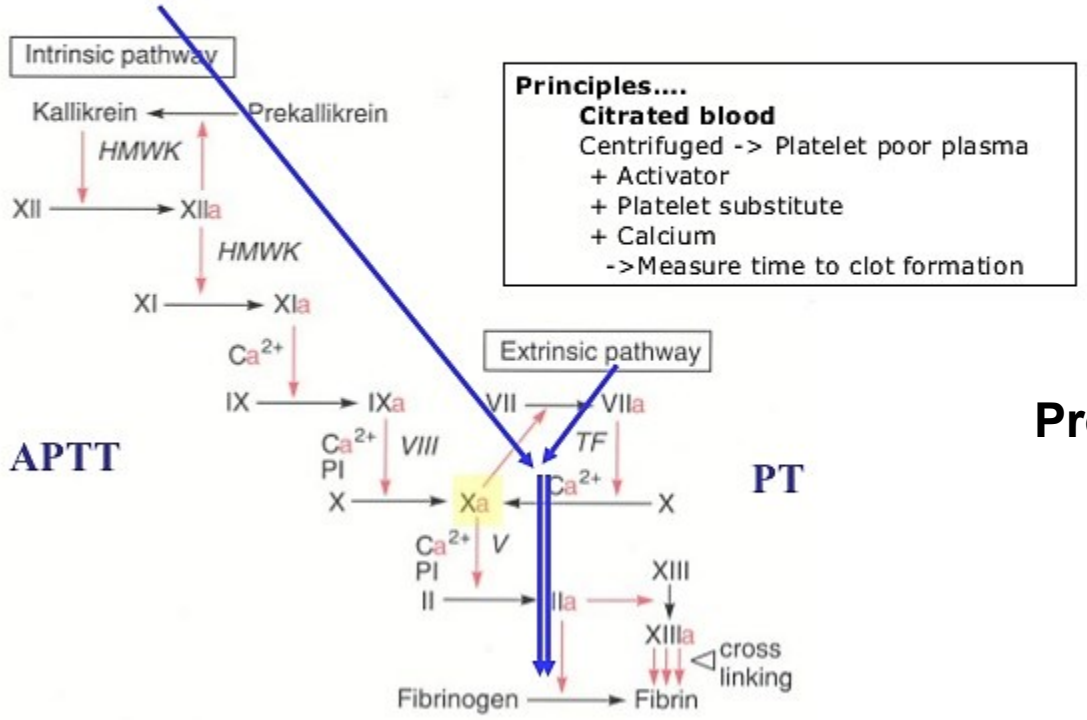

UFH
Pentasaccharide sequence
Unfractionated heparin
Factor Xa
Antithrombin
Thrombin
LMWH
Low-molecular-weight heparin
Pentasaccharide sequence
Factor Xa
Antithrombin


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
NOT FOR LOCK FLUSH


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

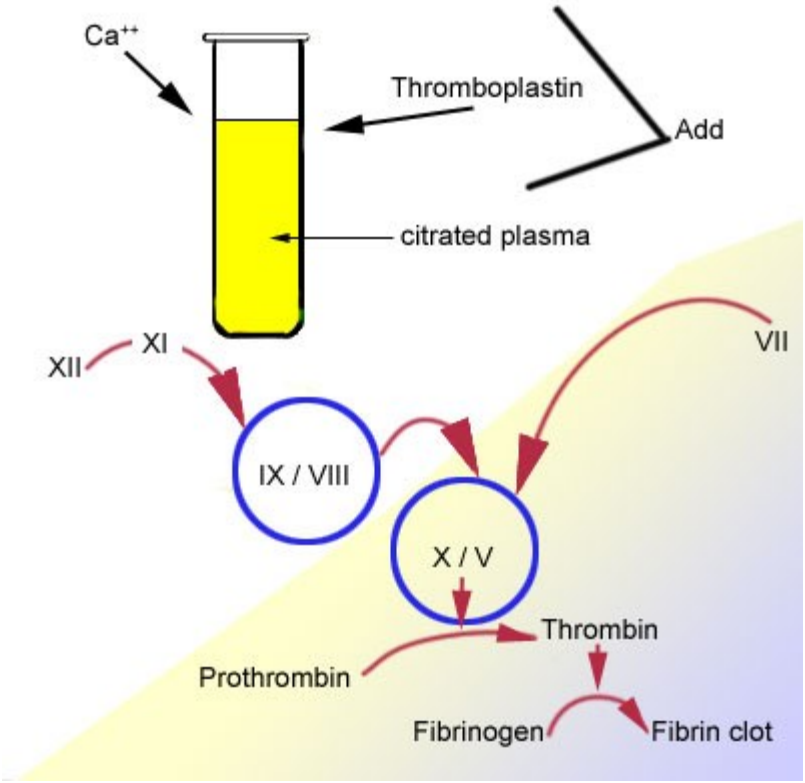
Tests: PT and APTT

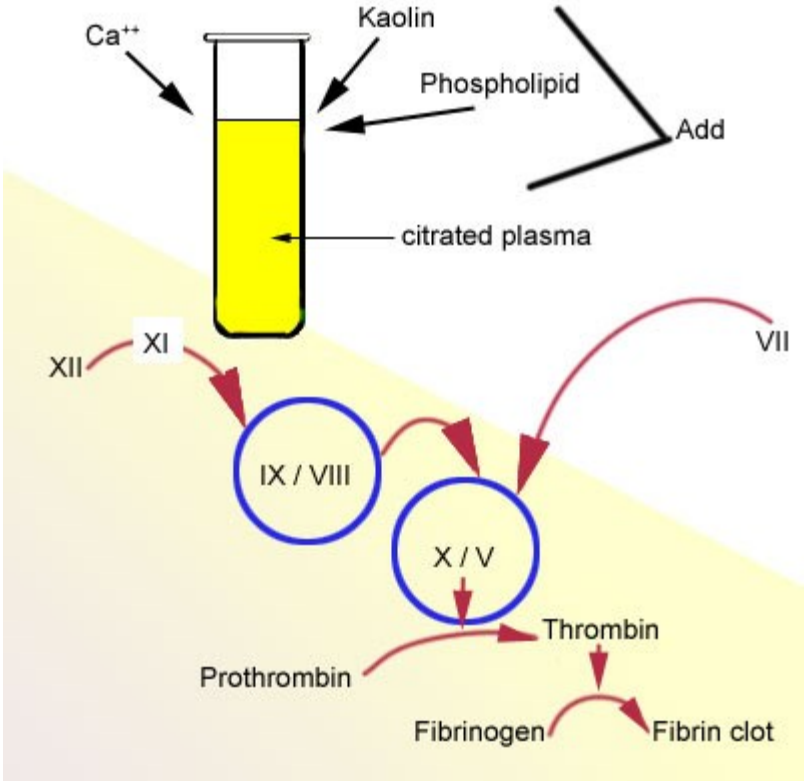


Prothrombin time test

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

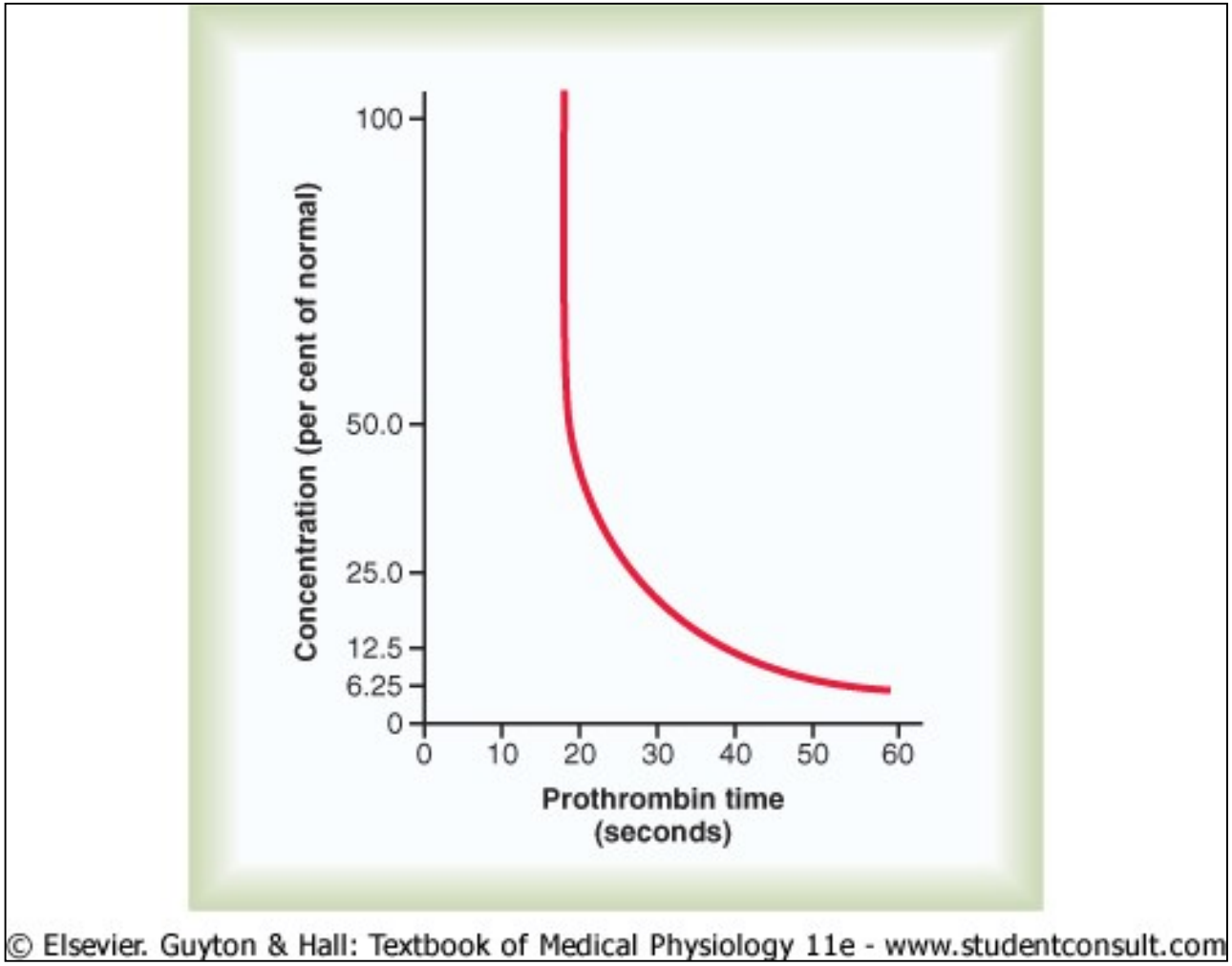
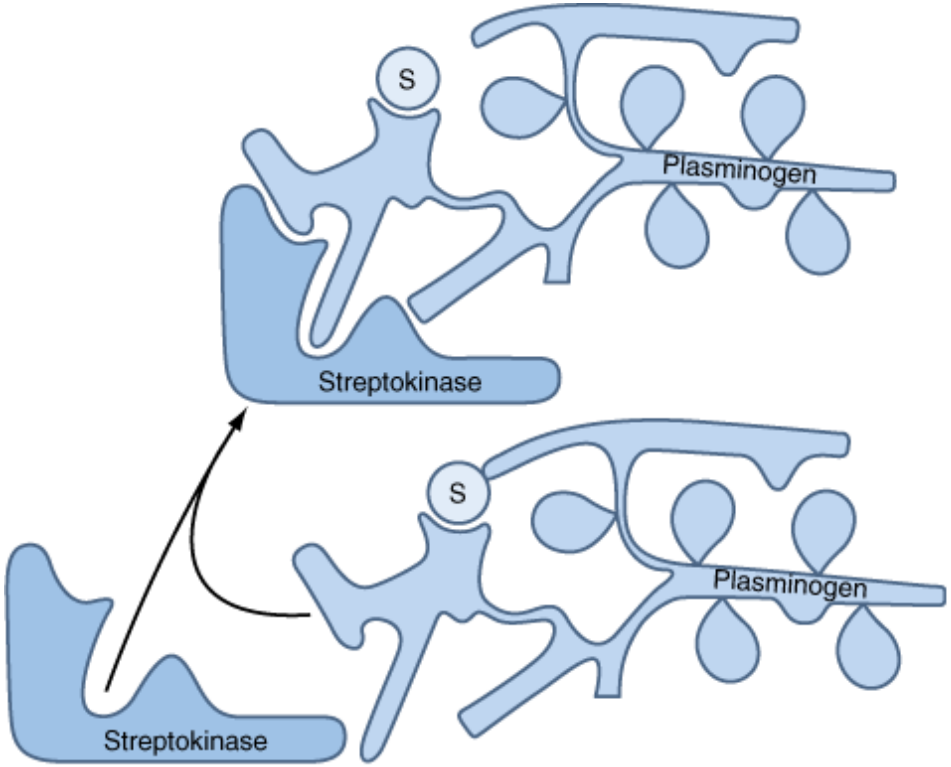
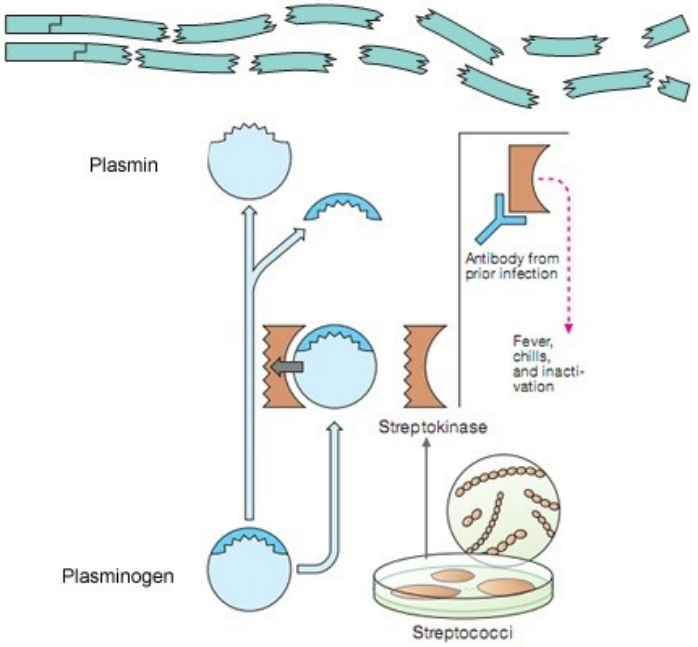


Figure 36-5 Relation of prothrombin concentration in the blood to "prothrombin time."

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