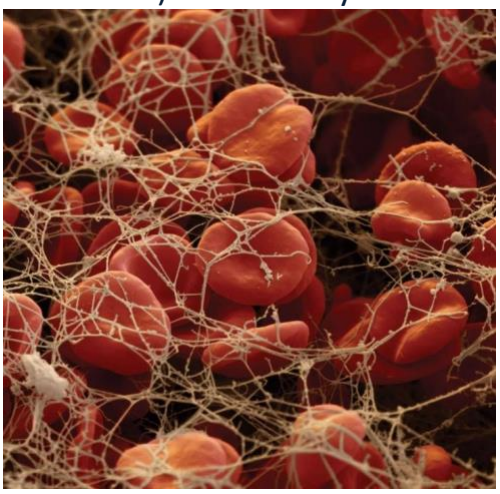
Blood coagulation

In this lecture, we'll be talking about blood coagulation, this is a beautiful lecture, I hope you will enjoy it 😁.

What is blood coagulation (clotting)?

What was mentioned in the slide:

- It is an *orchestrated*, biochemical process that is initiated as a result of vascular injury where a small area blood of surrounding injury changes from liquid to gel, forming a clot made of fibrin, which results in hemostasis (the cessation of blood loss) followed by clot dissolution and repair.



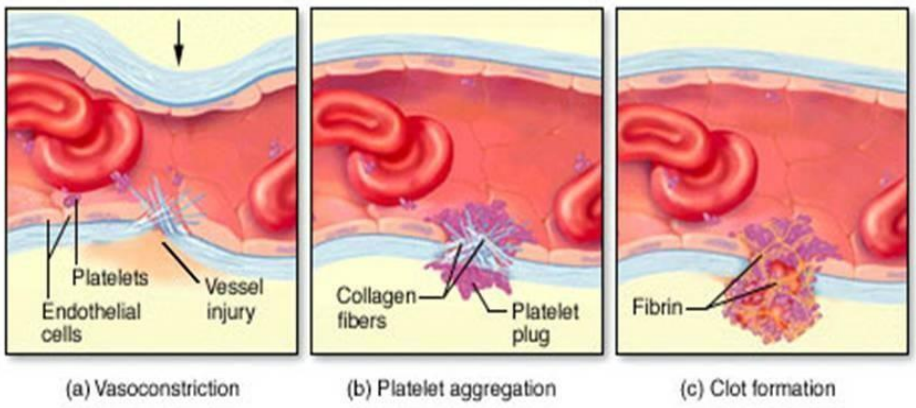
What was mentioned in the lecture:

Let's start with a definition of what blood coagulation is, blood coagulation is basically an orchestrated biochemical process, the word “orchestrated” was chosen because as you go through blood coagulation, it is almost like music, it's like a classical symphony of Beethoven or Mozart, it goes slow and then it goes up and up until it reaches a climax and then you see the music going down until you get to the end.
This biochemical process is initiated as a result of vascular injury, so you have an injury in a small area and blood in this area changes from liquid to gel, so basically you have the formation of a clot of fibers of a protein known as fibrin, that results in hemostasis so there is no more blood loss, and then this is followed by clot dissolution and repair, that is the end of the symphony.

Steps of hemostasis

What was mentioned in the slide:

- Vascular constriction limiting blood flow to the area of injury
- Activation then aggregation of platelets at the site of injury, forming a loose platelet plug
- Formation of a fibrin mesh to entrap the plug
- Dissolution of the clot in order for normal blood flow to resume following tissue repair
-



What was mentioned in the lecture:

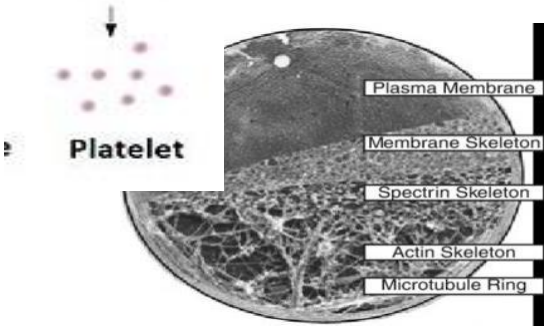
these are the basic steps of hemostasis, which is the thing that happens normally at the site of vascular injury, firstly, you have a physiological effect through vascular construction, this constriction is important initially as it limits blood flow to the area of injury, this is followed by cellular and biochemical processes, you have first activation of platelets and their aggregation at the site of injury forming what is known as a platelet plug, this platelet plug is loose, but then it becomes solidified and hard, forming what we call a hard clot, this hardening occurs via the formation of a fibrin mesh, so you have a network of fibers surrounding and entrapping the platelet cells, then, this is followed by dissolution of the clot enzymatically.

Platelets are a major player

What was mentioned in the slide:

- Small anuclear cell fragments produced from the megakaryocytes.
- Platelets have numerous kinds of surface receptors.

- Platelets also have actin filaments and myosin, which change the shape of the platelet upon activation.
- They also have three types of granules that store substances that are released upon platelet activation.



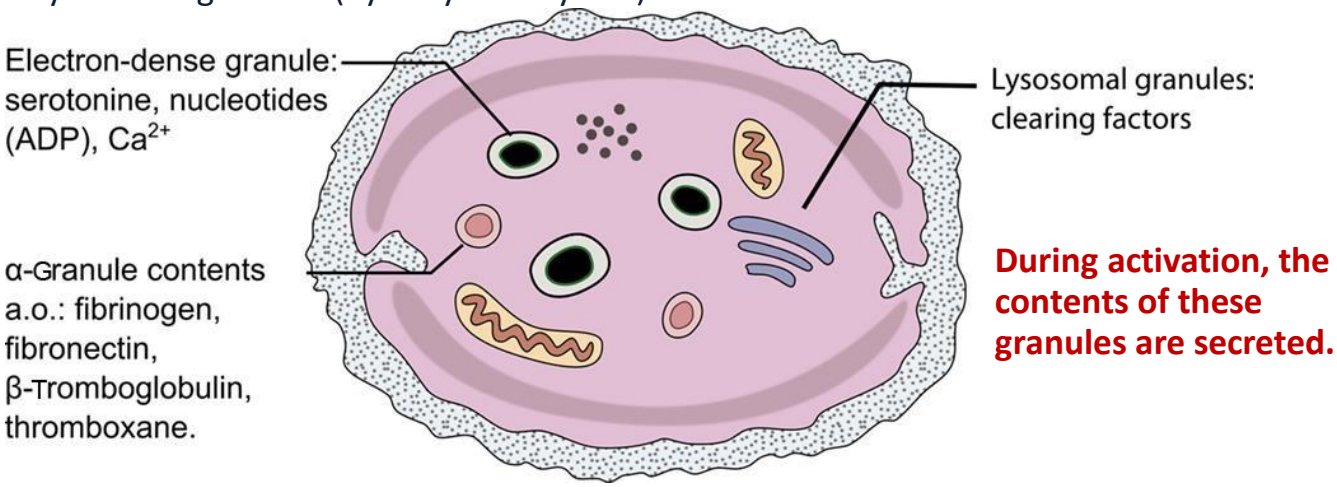
What was mentioned in the lecture:

what are platelets platelets are small anuclear cell fragments, they are produced from large precursor cells known as megakaryocytes, inside platelets, you have cellular components, you have of course the plasma membrane, actin cytoskeleton, vesicles (granules), proteins, signaling factors, etc.

The granules

What was mentioned in the slide:

- Electron-dense granules (calcium ions, ADP, ATP, serotonin)
- α-granule (a heparin antagonist, platelet-derived growth factor, fibrinogen, von Willebrand factor (vWF), clotting factors)
- Lysosomal granules (hydrolytic enzymes)

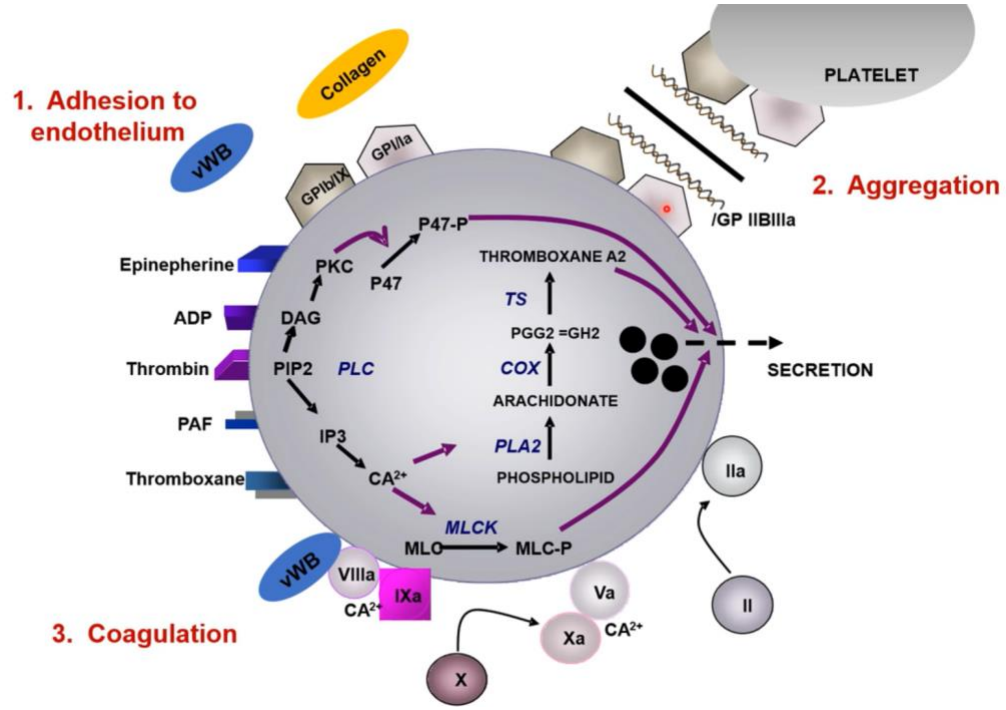


During activation, the contents of these granules are secreted.

What was mentioned in the lecture:

Platelets contain three types of vesicles (granules), 1-you have electron dense vesicles (also known as δ-granules), these contain calcium ions (Ca^{+2}), ADP, ATP and serotonin, the reason why you have ADP and ATP inside these vesicles is that they're not really used as sources of energy, rather, they are used as signaling molecules 2- you have also α-granules inside platelets and these granules contain a heparin antagonist, the signaling molecule platelet-derived growth factor, the structural protein fibrinogen, a regulatory protein known as von Willebrand factor and other numerous clotting factors, 3- also you have lysosomal vesicles (also known as λ granules) and

these contain hydrolytic enzymes, these enzymes are necessary for removal of the clot as well as activation of different proteins.
so what happens with these platelets is that once they're activated in response to an injury, these granules fuse with the plasma membrane releasing their contents.



What was mentioned in the lecture:

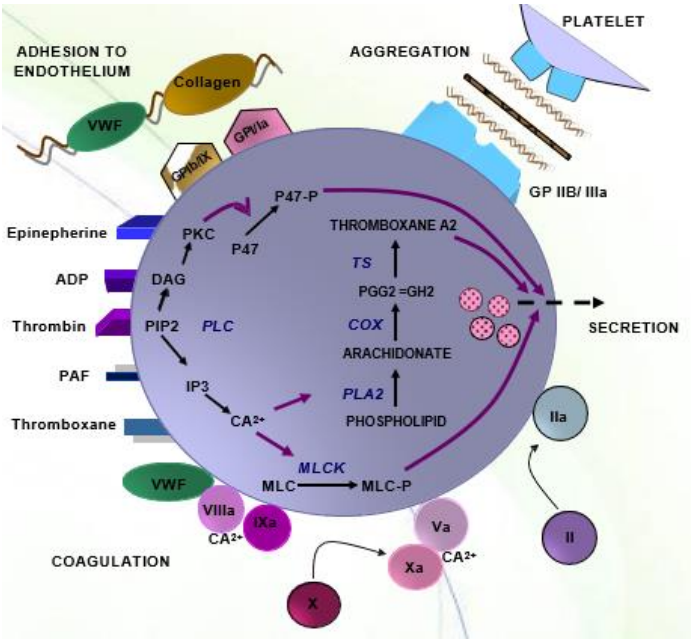
here we have a representation of a platelet and you can see there are numerous receptors on the cell surface for example, you have receptors for epinephrine, receptors for ADP, others for thrombin and so on, there are also glycoproteins (namely glycoprotein Ib and glycoprotein IIb-IIIa) on the cell surface and these are important for interacting with collagen, von Willebrand factor, and fibrinogen, these are also important for forming aggregation of platelets at the site of injury, (remember from the pathology course: deficiencies of these glycoproteins has been associated with several bleeding disorders, namely Glanzmann thrombasthenia and Bernard-Soulier disease), now also on the plasma membrane of platelets, you have the process of blood coagulation going on.

Adhesion

What was mentioned in the slide:

- The endothelial von Willebrand factor (vWF) protein and exposed collagen bind to the platelet glycoproteins (GP).

- Some platelets release substances from the granules:
  - ADP
  - Serotonin
  - Factor V
  - ATP
  - Calcium
  - Fibrinogen
  - vWF
  - Thrombin
  - Thromboxane
- Platelets also change shape allowing for more platelet-platelet interaction and aggregation.

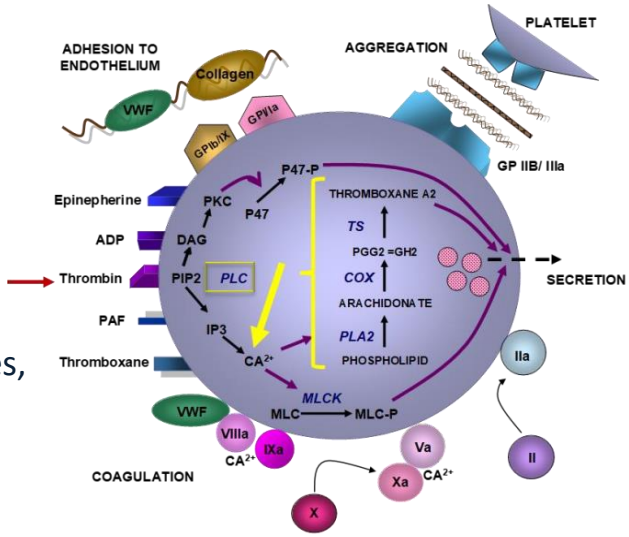


What was mentioned in the lecture:

The first thing that happens in the clotting cascade is that whenever you have a vascular injury, a protein known as von Willebrand factor is exposed, this leads to platelet binding (specifically through glycoprotein Ib) to this von Willebrand factor and activation of the platelets, that would cause a series of signaling reactions inside the cell and that leads to secretion of a number of factors, including ADP, factor V, calcium ions (Ca^{+2}), ATP, fibrinogen, more von Willebrand factor, thrombin and thromboxane, all of these bind to receptors or they have specific functions as you'll see now, what happens as well is that once the platelets are activated, their shape changes, this change in cell shape results in more platelet adhesion and aggregation.

Thrombin receptor

What was mentioned in the slide:



• Thrombin receptor activates a G-protein that activates phospholipase C-γ (PLC-γ).
• PLC-γ hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG).
• IP3 induces the release of intracellular Ca2+ stores, and DAG activates protein kinase C (PKC).

- Calcium triggers liberation of arachidonic acid from membrane phospholipids by the enzyme phospholipase A2.
- Arachidonate is converted by cyclooxygenase to prostaglandins, which are then converted by thromboxane synthetase to thromboxane A2.
- Thromboxane has vasoconstrictor activities and is
- platelet activator.
- It acts in autocrine and paracrine manners.

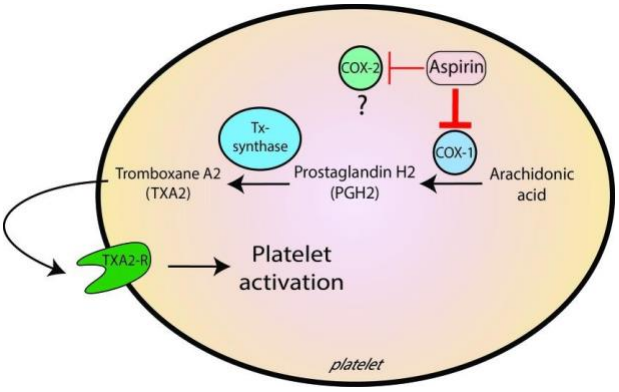
What was mentioned in the lecture:

Among the receptors that exists on the surface of platelets, which get activated when upon binding of von Willebrand factor is a thrombin receptor, thrombin receptor activates a G-protein that eventually activates the enzyme phospholipase C-γ, once phospholipase C-γ it is activated, it can act on its substrate that is phosphatidylinositol 4,5-bisphosphate (PIP_2), so it degrades (PIP_2) into two products and these are known as the diacylglycerol (DAG) and phosphatidylinositol (IP_3), IP_3 induces the release of calcium ions from its stores, namely the endoplasmic reticulum (in most cells), but from vesicles in platelets, while diacylglycerol activates protein kinase C.
let's focus now on calcium ions and what they do, calcium ions act on the plasma membrane of platelets and they activate a phospholipase enzyme known as phospholipase A_2, this phospholipase A_2 releases arachidonic acids (arachidonate) from phospholipids of the plasma membrane, then arachidonate can be converted by an enzyme known as cyclooxygenase and another enzyme known as thromboxane synthase all the way to formation of thromboxane, so thromboxane can be released, and once thromboxane is released, it has vasoconstrictor activities, limiting blood flow and helping in the aggregation of platelets, and it can also act in in autocrine and paracrine manners, so what thromboxane does is that it can bind to its receptor on the same platelet that released it (autocrine), activating a signal transduction pathway inside it, or it can also act on neighboring platelets as well (paracrine).

NSAIDs

What was mentioned in the slide:

- Non-steroidal anti-inflammatory drugs inhibit the enzyme cyclooxygenase, accounting for their anticoagulant effects.
- Serotonin is also a vasoconstrictor. PDGF stimulates proliferation of
- endothelial cells to reduce blood flow.



What was mentioned in the lecture:

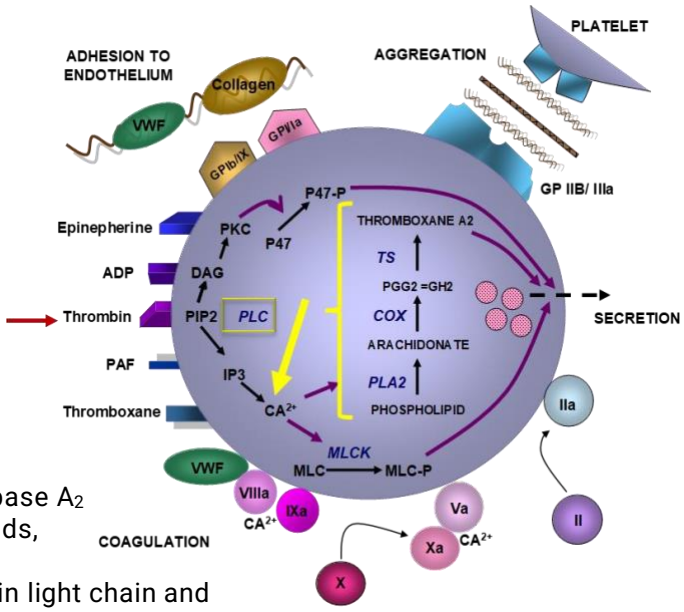
Thromboxanes, prostaglandins, leukotrienes and arachidonate are eicosanoids, that is, they are 20-carbon fatty acids, as we have said, arachidonate is converted to thromboxanes by an enzyme known as cyclooxygenase I, cyclooxygenase I is the target for aspirin, this is why it is has been always thought that aspirin is beneficial, because it prevents the aggregation of platelets, so this mechanism of action of the non-steroidal anti-inflammatory drugs, whose prototype is aspirin.

Other molecules that are secreted from platelets granules upon activation is serotonin which is also a vasoconstrictor working alongside thromboxanes and the platelet-derived growth factor which stimulates proliferation of endothelial cells and this also reduces blood flow.

More release of granular contents

What was mentioned in the slide:

- Ca2+ ions activate myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin allowing it to interact with actin resulting in altered platelet morphology, induced motility, and release of granules.
- DAG activates PKC, which phosphorylates and activates specific platelet proteins that induce the release of platelet granule contents including ADP.



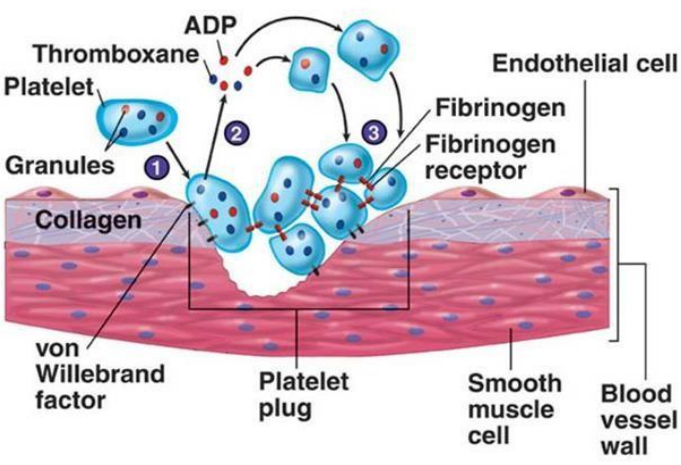
What was mentioned in the lecture:

what calcium ions do in addition to activation of phospholipase A_2 and triggering the release of arachidonate from phospholipids, calcium ions also can bind to a kinase known as myosin light chain kinase, this kinase phosphorylates myosin light chain and this phosphorylated myosin light chain has a number of effects, one of them is that it stimulates further fusion of platelets granules with the plasma membrane and releasing of their contents, myosin light chain kinase also modifies the actin cytoskeleton so it can modulate it can induce motility of platelets, it also changes platelet morphology leading to more aggregation of platelets.
On the other side of the equation is diacylglycerol which is the second product of hydrolysis of PIP_2, what diacylglycerol does is that it binds to protein kinase C, which phosphorylates several proteins including protein 47, which leads to further release of granular contents including ADP.

Role of ADP

What was mentioned in the slide:

- ADP is a platelet activator that binds to its receptor and modifies the platelet membrane allowing fibrinogen to adhere to platelet surface glycoproteins resulting in fibrinogen-induced platelet aggregation, called platelet plug.



What was mentioned in the lecture:

so what does ADP do? ADP is a platelet activator, what it does is that it binds to its receptor, modifying platelet membrane and allowing for fibrinogen to bind to the glycoproteins on platelets, resulting in further platelet aggregation, this is known as a platelet plug.

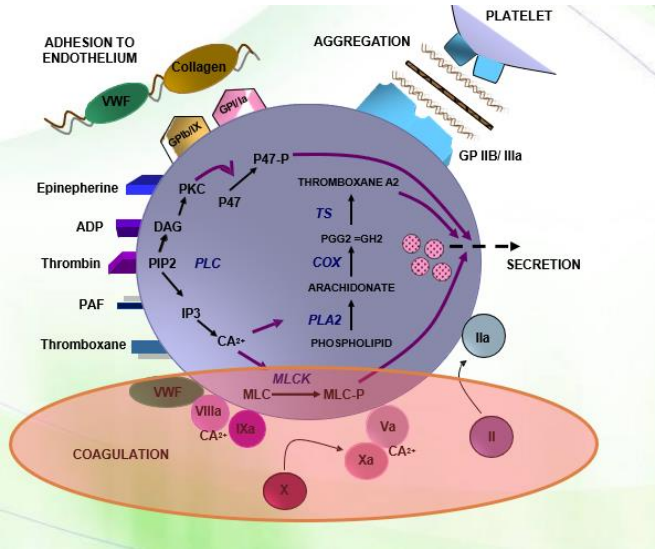
Role of platelet cell surface

What was mentioned in the slide:

- The accumulated platelet plug provides an important surface on whichcoagulation reactions occur.

What was mentioned in the lecture:

something else is that the surface of platelets is the theater for the blood coagulation process, which is the biochemical reactions that lead to blood coagulation, and this is the next topic of this lecture.



Biochemistry of coagulation

Components of coagulation

What was mentioned in the slide:

- An organizing surface (platelets)
- Proteolytic zymogens (prekallikrein, prothrombin, and factors VII, IX, X, XI, XII, and XIII)
- These are mainly serine proproteases released from hepatocytes.
- The subscript "a" designates the activated form of a factor
  - e.g., “XIII" is versus "XIIIa"
    - Anti-coagulants (protein C, protein S)
    - Non-enzymatic protein cofactors (factors VIII, V, and tissue factor)
    - Calcium ions

- Vitamin K
- Fibrinogen

What was mentioned in the lecture:

so let's talk about coagulation, which is basically the biochemical reactions that take place in order to form blood clots.
there are a number of players in the process of blood coagulation, some are small like calcium ions and vitamin K, others are large like platelets themselves and you have molecules in between, first, we have the organizing surface of platelets which we already discussed how it is formed, you have also proteolytic zymogens, the term " zymogens" means enzymes that require proteolytic cleavage in order for them to be active, in GI physiology, we talked about a number of zymogens, including trypsinogen, chymotrypsinogen, pro-elastase, etc. all these require proteolytic modification generating the active enzymes, which are termed trypsin, chymotrypsin, elastase and so on, zymogens of the process of blood coagulation include pre-kallikrein, pro-thrombin as well as a number of proteins known as factors which are designated with roman numbers and these are known as factors VII, IX, X, XI, XII, XIII, these factors are mainly serine proteases (cleave proteins at the site of serine residues), they are released from hepatocytes once they're activated, clotting factors are designated with the subscript "a" once activated through proteolytic cleavage, for example, factor XIII, is called factor XIIIa once activated.
We have other proteins that function in the clotting cascade as anticoagulants, so they are inhibitors of blood coagulation and these include protein C and protein S, there are also non-enzymatic protein cofactors including factors VIII, V and a protein known as tissue factor, as we said calcium ions and vitamin K are also involved, and then we have fibrinogen which forms the fibrin network.

Molecular components of coagulation

Clotting factor number	Clotting factor name	Function	Plasma half-life (h)
I	Fibrinogen	Clot formation	90
II	Prothrombin	Activation of I, V, VII, VIII, XI, XIII, protein C, platelets	65
III	TF	Co factor of VIIa	-
IV	Calcium	Facilitates coagulation factor binding to phospholipids	-
V	Proacclerin, labile factor	Co-factor of X-prothrombinase complex	15
VI	Unassigned		
VII	Stable factor, proconvertin	Activates factors IX, X	5
VIII	Antihaemophilic factor A	Co-factor of IX-tenase complex	10
IX	Antihaemophilic factor B or Christmas factor	Activates X: Forms tenase complex with factor VIII	25
X	Stuart-Prower factor	Prothrombinase complex with factor V: Activates factor II	40
XI	Plasma thromboplastin antecedent	Activates factor IX	45
XII	Hageman factor	Activates factor XI, VII and prekallikrein	
XIII	Fibrin-stabilising factor	Crosslinks fibrin	200
XIV	Prekallikerin (F Fletcher)	Serine protease zymogen	35
XV	HMWK- (F Fitzgerald)	Co factor	150
XVI	vWf	Binds to VIII, mediates platelet adhesion	12
XVII	Antithrombin III	Inhibits IIa, Xa, and other proteases	72
XVIII	Heparin cofactor II	Inhibits IIa	60
XIX	Protein C	Inactivates Va and VIIIa	0.4
XX	Protein S	Cofactor for activated protein C	

HMWK – High molecular weight kininogen; vWf – Von Willebrand factor; TF – Tissue factor

What was mentioned in the lecture:

so this is a list of the different clotting factors, their names and what their functions are, as you go through this lecture, you can go back to this slide to again to sort summarize what the process is, note that factor II is the prothrombin, factor I is fibrinogen, however, factor IV is calcium ions themselves, so these factors do not necessarily indicate a protein.
Try using this mnemonic: foolish people try climbing long slopes after Christmas, some people have fallen.

Factor I = fibrinogen

Factor II = prothrombin

Factor III = thromboplastin

Factor IV = calcium ions

Factor V = labile factor

Factor VI = there is no factor VI

Factor VII = stable factor

Factor VIII = anti-hemophilic factor A

Factor IX = Christmas factr

Factor X = Stuart-Prower Factor

Factor XI = plasma thromboplastin antecedent

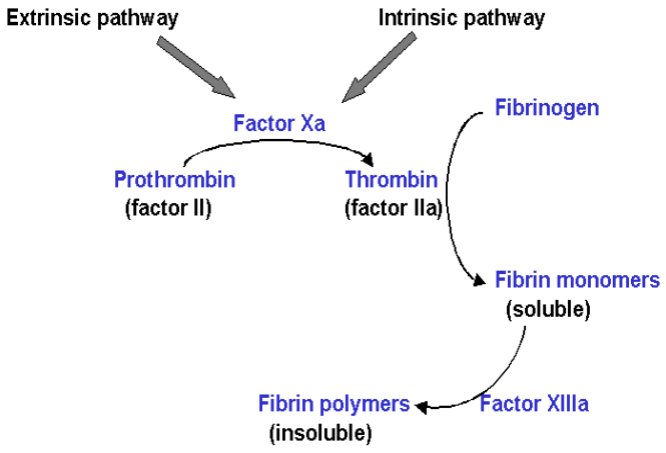
Factor XII = Hageman factor

Factor XIII = fibrin stabilizing factor

The two pathways

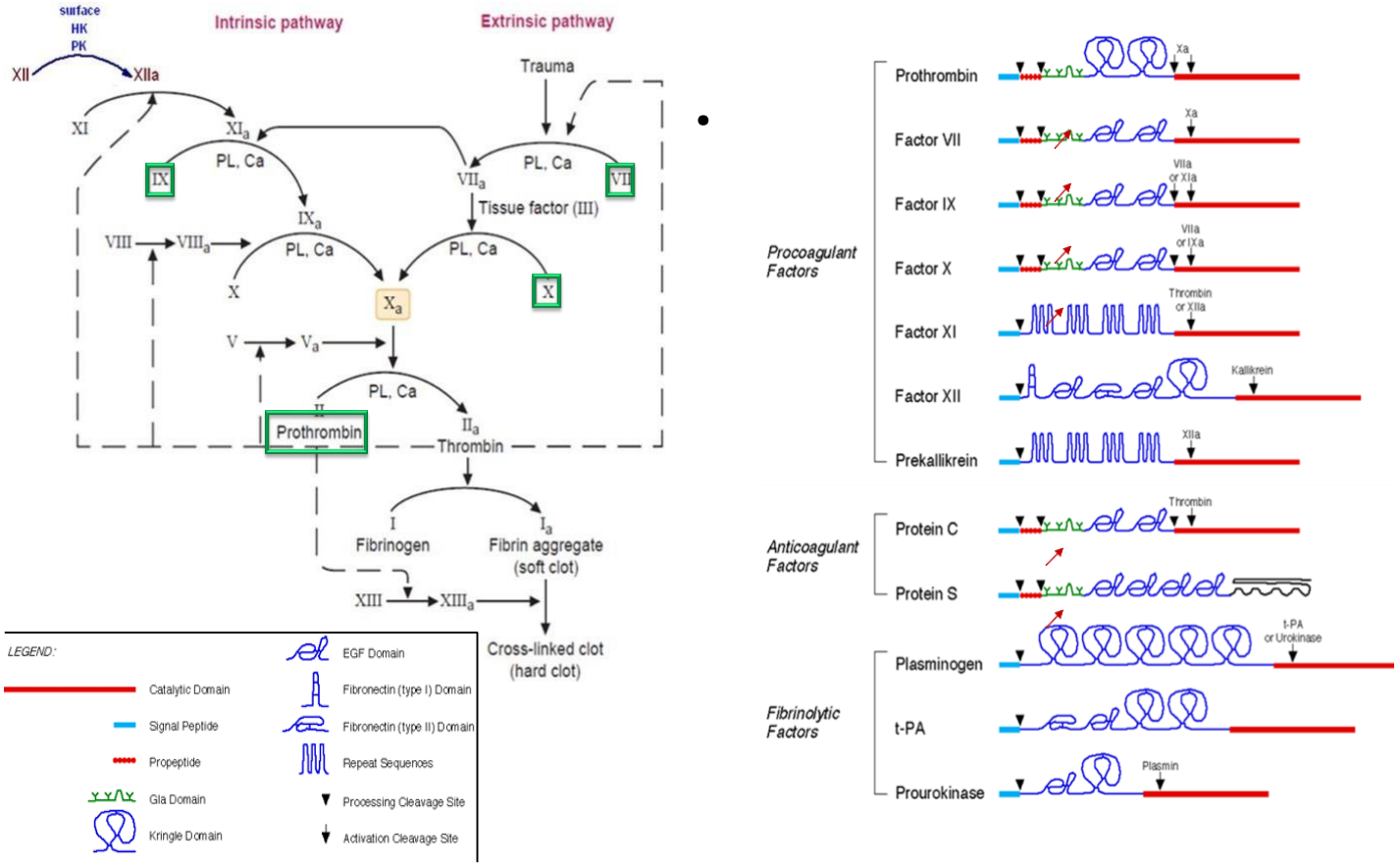
What was mentioned in the slide:

- The intrinsic pathway is initiated when subendothelial surface (i.e., collagen) is exposed.
- The extrinsic pathway is initiated in response to tissue injury.
  - Tissue factor (TF) protein is released.
- However, the two pathways converge on a common pathway.



What was mentioned in the lecture:

Classically, blood coagulation has been classified into two pathways that were thought to be independent, these are known as the extrinsic pathway and the intrinsic pathway, basically, the extrinsic pathway is activated in response to tissue injury while the intrinsic pathway can be activated as a result of an internal effect like inflammation, but it has been found that actually these pathways are not totally independent, rather, there is a bridge that connects both pathways to each other and when the extrinsic pathway is activated you have activation of the intrinsic pathway and vice versa, so they are really interconnected and not totally independent of each other.
both pathways converge at a single point which is the activation of factor X, and factor X is responsible for activating factor II which is prothrombin, when factor II (prothrombin) is activated to form factor IIa (thrombin), it can form the fibrin mesh.



What was mentioned in the lecture:

Now we'll cover the clotting pathway, we have the extrinsic pathway, which is activated when you have vascular injury due to trauma or other extrinsic causes, vascular injury results in exposure of collagen as well as von Willebrand factor, this exposure results in the activation of factor VII (stable factor) into factor VIIa, then, with the help of tissue factor, factor VIIa activates factor X (Stuart-Prower factor) into factor Xa.
the intrinsic pathway is activated is response to tissue injury due to inflammation or other intrinsic causes, the tissue factor also plays an important role in the intrinsic pathway, as it forms a bridge whereby factor VIIa which is also activated in the extrinsic pathway, can activate factor IX

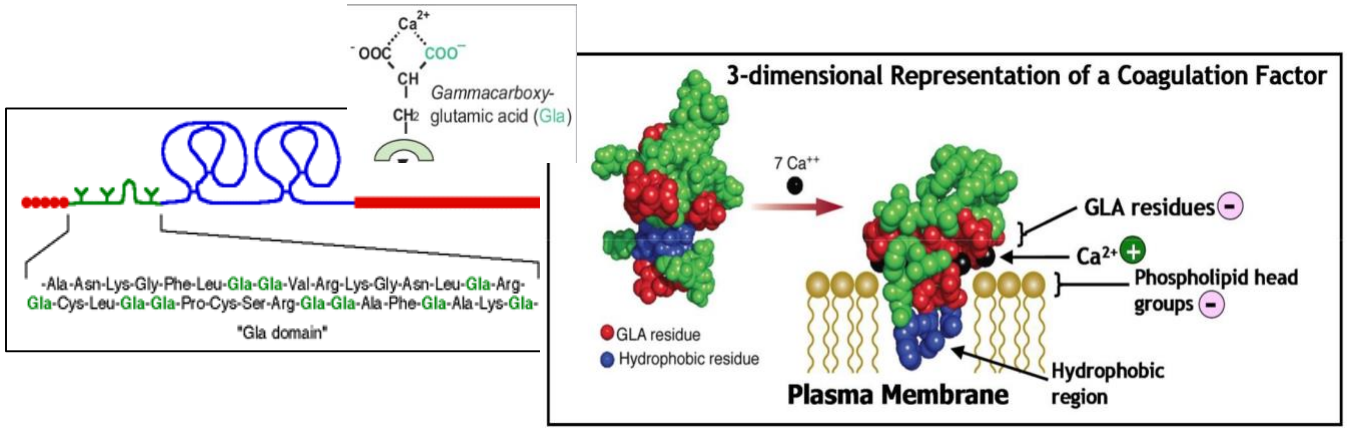
(Christmas factor) forming factor IXa, then factor XIa can also activate factor X (Stuart-Prower factor).
so as we said, you have the both pathways converge at this point right here with the activation of factor X into factor Xa, then, factor Xa activates prothrombin (factor II) into thrombin (factor IIa) which then forms the fibrin network.
in case of inflammation, you have activation of the kalliherin pathway, in which factor XII (Hageman factor) is activated into factor XIIa , which in turn activates factor XI (fibrin-stabilizing factor) into factor XIa activates factor IX (Christmas factor), which then as we said, activated prothrombin and the clotting proceeds.
there are a number of co-factors that play an important role in this process, these are the non-enzymatical factors, they include tissue factor, factor VIII (antihemophilic factor A), factor V (labile factor) and so on, pay attention also to the involvement of phospholipids that is present at the surface of platelets and calcium ions in the process of activation.

The figure on the right is an illustration of the different domains of the different proteins of the clotting cascade what we really care about is the presence of a domain in some clotting factors known as the Gla domain (glutamate domain), this glutamate domain presents in prothrombin (Factor II), factor VII (stable factor), factor IX (Christmas factor), factor X (Stuart-Prower factor) as well as protein C and protein S that are the anticoagulants.

Gla domain

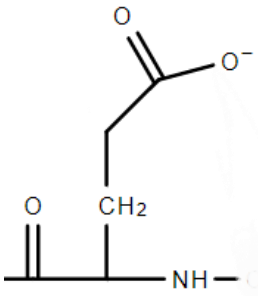
What was mentioned in the slide:

- An ER/Golgi carboxylase binds to prothrombin and factors IX, VII, and X and converts 10≥ glutamate (Glu) residues to γ-carboxyglutamate (Gla), followed by a small (10 a.a.) hydrophobic region.
- The Gla residues bind calcium ions and are necessary for the activity of these coagulation factors and formation of a coordinated complex with the charged platelet surface to localize the complex assembly and thrombin formation to the platelet surface.

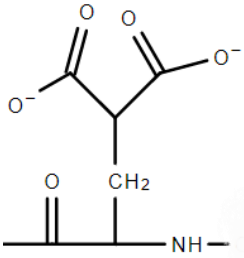


What was mentioned in the lecture:

what is the Gla domain or the glutamate domain? this domain is a part of the primary structure (amino acid structure) of factors II (prothrombin), VII (stable factor), IX (Christmas facor) and X (Stuart-Prower factor), protein S anticoagulant and protein C anticoagulant, this domain is rich with glutamate residues, (remember: glutamate is an amino acid having the structure illustrated in the figure), so you have about 9 to 12 glutamate residues within this domain, those glutamate residues are the substrates for an enzyme known as a carboxylase enzyme what this enzyme does is that it adds another carboxyl group, forming a glutamate with two carboxyl groups known as γ-carboxyglutamate, γ-carboxyglutamate is highly negatively charged, these charges facilitate interaction between γ-carboxyglutamate with the positively charged calcium ions, this helps in binding of these proteins with the plasma membrane of platelets, in the plasma membrane of the platelets, you have the phospholipid head groups which are negatively charged, and calcium ions are possibly charged, so calcium ions sort of mediate the interaction of these proteins with the plasma membrane, the insertion of these proteins with plasma membrane is also solidified by having a hydrophobic region in these proteins, so that helps inserting the proteins into the plasma membrane and that what makes the coagulation biochemical process takes place on the surface of platelets.



Structure of glutamate

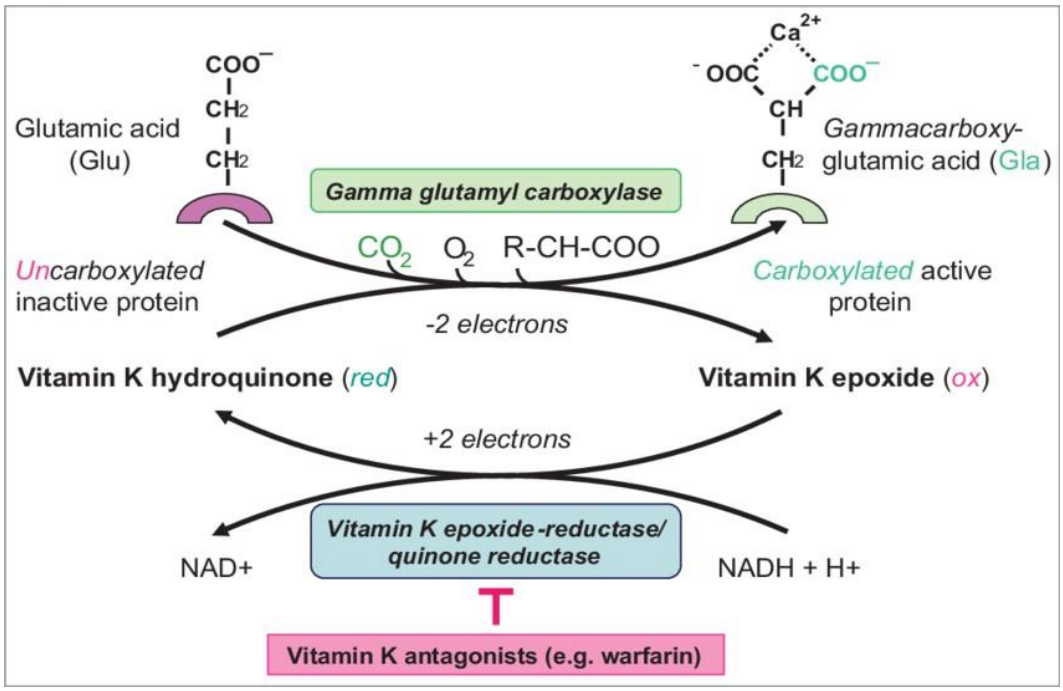


Structure of γ-carboxyglutamate

The role of vitamin K

What was mentioned in the slide:

- Vitamin K participates in conversion of Glu to γ-Gla.
- Vitamin K becomes oxidized and must be regenerated.



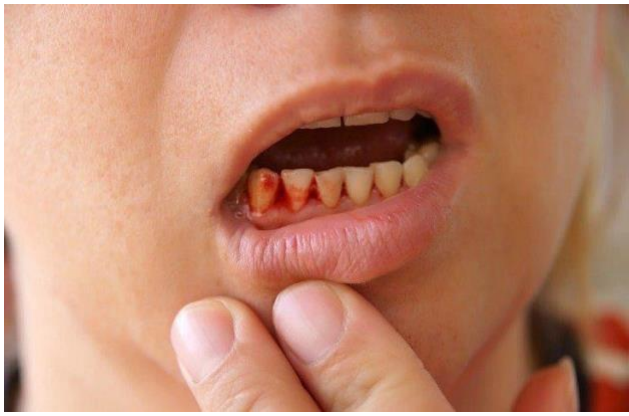
What was mentioned in the lecture:

Carboxylation of glutamate residues in factors II, VII, IX, X, protein S and protein C requires vitamin K as a source of electrons, so during this reaction, the active form of vitamin K, known as vitamin K hydroxyquinone, is oxidized (electron are withdrawn from it) into what is known as vitamin K epoxide, it's very important for vitamin K epoxide to be reduced to regenerate the active form of vitamin K (that is vitamin K hydroxyquinone) and this happens enzymatically through an enzyme known as vitamin K epoxide reductase enzyme, vitamin K epoxide reductase requires NADH as a source of electrons, Vitamin K epoxide reductase is the target of the antagonist known as Warfarin, which inhibits regeneration of Vitamin K, thus inhibiting synthesis of factors II, VII, etc. and preventing clotting.

Newborns and vitamin K deficiency

What was mentioned in the slide:

- Newborns are at risk for early vitamin K deficiency bleeding. Why?
- The placenta is a poor passage channel for fat-soluble compounds, including vitamin K.
- Neonates are born with an immature liver that impairs coagulation factor synthesis and GLA modifications.
- Breast milk is a poor source of vitamin K.
- Intestinal flora, the main source of vitamin K, is not established yet.



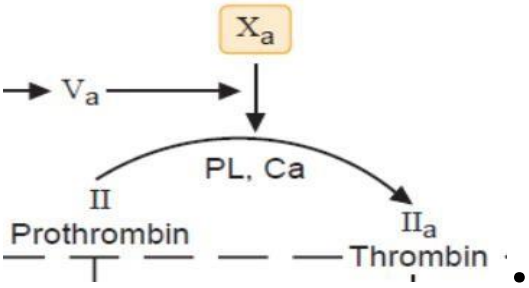
What was mentioned in the lecture:

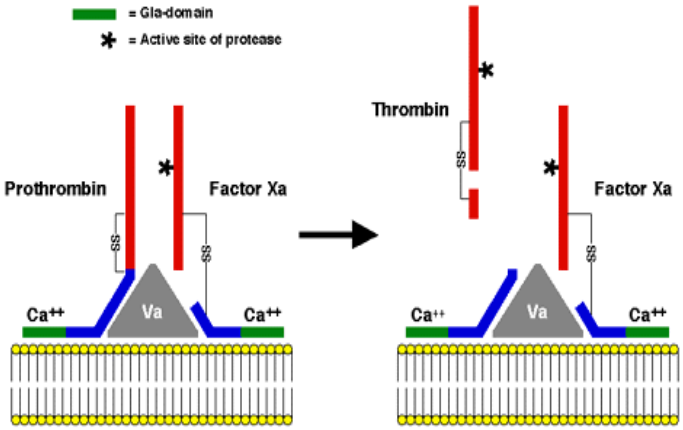
a side note on vitamin K is that newborns are at risk for early vitamin K deficiency and that may lead to bleeding, there are four reasons for that: the first is that the placenta is really a poor passage channel for fat soluble compounds such as vitamin K, neonates are also born with immature liver that is not able to produce these coagulation factors and to modify the factors at the glutamate residues, something else is that breast milk is a poor source of vitamin K, and the last reason is that the intestinal flora which are the main source of vitamin K in humans is not really mature in newborns, it's not yet established.
how do we know that these newborns have vitamin K deficiency? there are a number of signs or manifestations, one of them is gum bleeding as well as formation of bruises.

Prothrombin activation

What was mentioned in the slide:

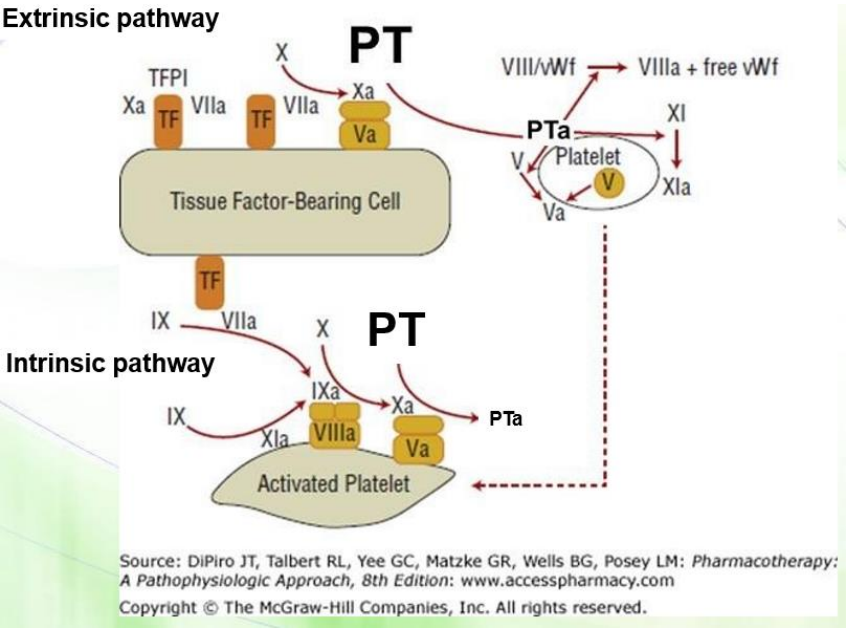
- The complex of factor Xa/Va is the “prothrombinase complex”.
- Factor Xa converts prothrombin to thrombin, which is accelerated by Va, platelets (or phospholipids), and calcium ions.
- Binding of calcium alters the conformation the Gla domains of these factors, enabling them to interact with a membrane surface of platelets. Aggregated platelets provide the surface upon which prothrombin activation occurs.





What was mentioned in the lecture:

Both the intrinsic and the extrinsic pathways converge at the activation of factor X, once activated, factor Xa forms a complex with factor V known as the prothrombinase complex, calcium ions are involved in the formation of this complex, also prothrombin (factor II) interacts with factor V with the help of calcium ions, so what factor V does is that it brings both factor Xa and prothrombin (factor II) close to each other, and then factor Xa cleaves prothrombin forming the active enzyme thrombin.

- 

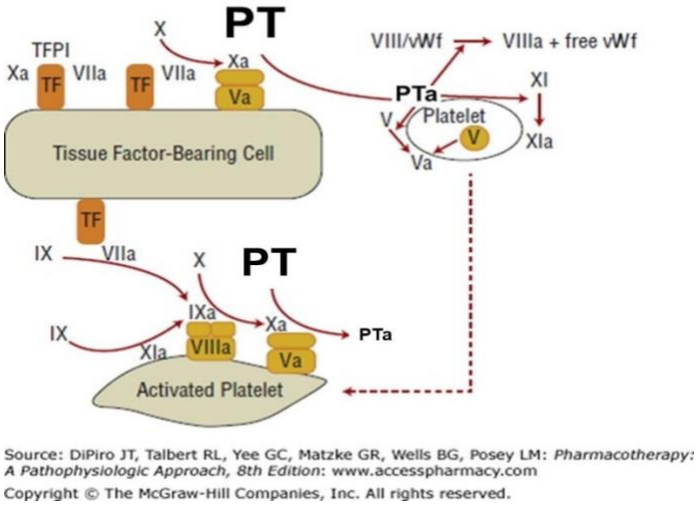
What was mentioned in the lecture:

As we've said, we have the involvement of several factors in the extrinsic pathway and the intrinsic pathway, in the extrinsic pathway, prior to activation of factor X, you have activation of factor VII with the help of tissue factor, however, in the intrinsic pathway, you have also the involvement of tissue factor which activates factor IX, factor IX in turn activates factor X by associating with factor VIII, so both factor VIII and IX participate in the activation of factor X through the intrinsic pathway.

Factors V and VIII

What was mentioned in the slide:

- Va and VIIIa are cofactors that increase the proteolytic efficiency of Xa and IXa, respectively.
- Factor Xa/VIIIa is known as the "tenase
- complex".
- Factors V and VIII are activated by thrombin.
- Factor VIII circulates in plasma bound to von Willebrand factor, which increases VIII



half-life, and, when released, it gets activated.

- von Willebrand factor deficiency is associated decrease in the plasma concentration of factor VIII.

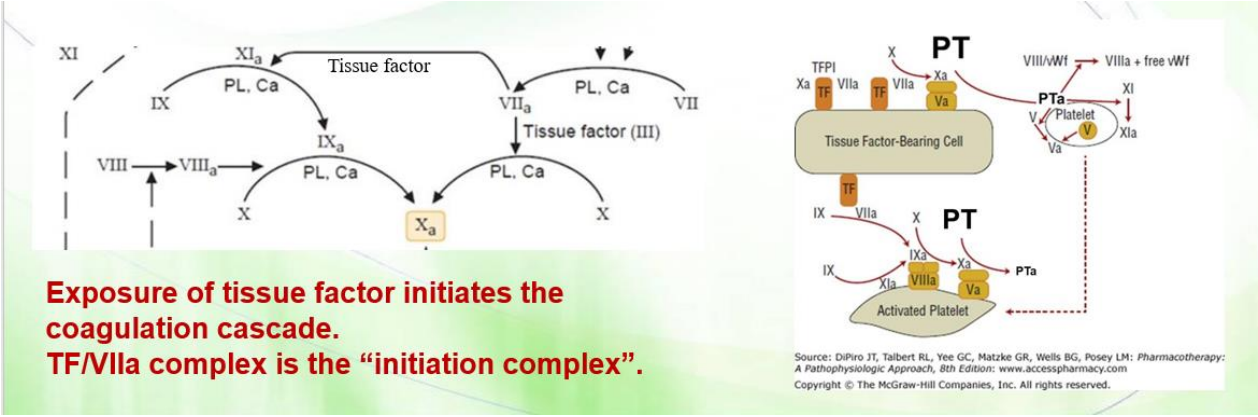
What was mentioned in the lecture:

Both factor V and factor VIII are cofactors that increase the proteolytic efficiency of factors X and IX respectively, however, factor VIII can interact with factor X as well, the complex of factor X and factor VIII is known as the tenase complex.
Both factors V and VIII are activated by thrombin (factor IIa) even though that thrombin comes later (downstream) in the cascade, what is the significance of that, it is sort of a positive feedback mechanism that amplifies clotting.
Factor VIII is found circulating in the plasma, but not free, rather, it is bound to von Willebrand factor, and as we said, von Willebrand factor is usually attracted to site of injury, so this von Willebrand factor bring factor VIII to the site of injury, moreover, the presence of von Willebrand factor increases the half-life of factor VIII greatly, thus, in the absence of von Willebrand factor, the half-life of factor VIII decreases greatly.
there is a condition that is related to deficiency of von Willebrand factor, and it is associated with decreased plasma concentration of factor VIII, individuals with von Willebrand factor suffer from excessive bleeding and inability to coagulate blood.

Tissue factor

What was mentioned in the slide:

- Tissue factor is an integral membrane protein that is expressed on the surface of "activated" monocytes, subendothelial cells, and other cells.
- Tissue factor greatly increases the proteolytic efficiency of VIIa.



What was mentioned in the lecture:

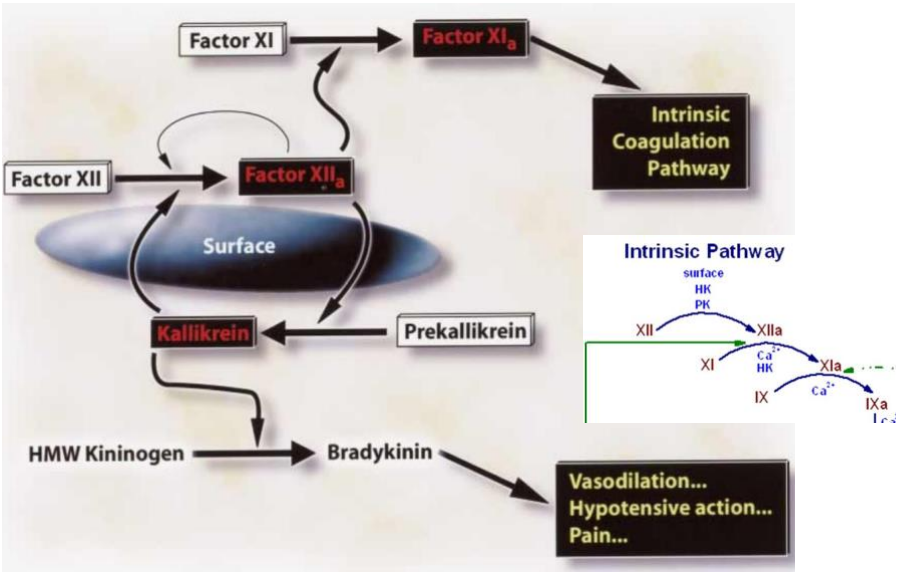
tissue factor is an integral membrane protein that is expressed on the surface of activated monocytes, sub-endothelial cells as well as other cells, in physiological conditions, blood cells and platelets are not exposed to sub-endothelial cells unless there is tissue injury, then, you can have

platelets binding to the tissue factor, once you have this interaction with tissue factor, the proteolytic activity of factor VIIa is greatly enhanced, because the tissue factor forms a complex with factor VII known as the initiation complex, thus, you can have activation of factor X through the extrinsic pathway.
you also have the involvement of tissue factor in the intrinsic pathway, whereby it is important for activating factor IX which activates then factor X through the intrinsic pathway.

The kallikrein-kinin system

What was mentioned in the slide:

Factor XII binds to exposed collagen at the site of vessel wall injury and is activated by high-MW kininogen and kallikrein

- 

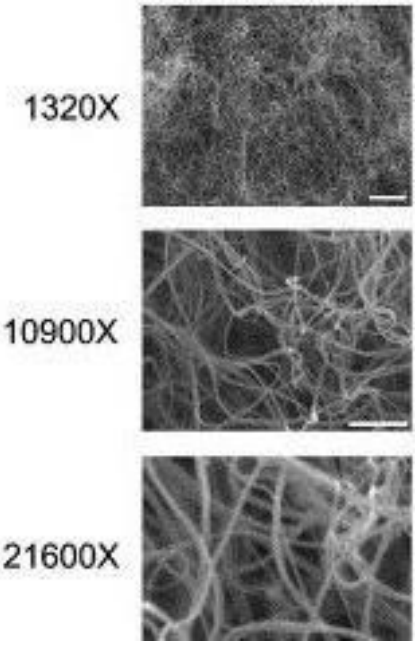
What was mentioned in the lecture:

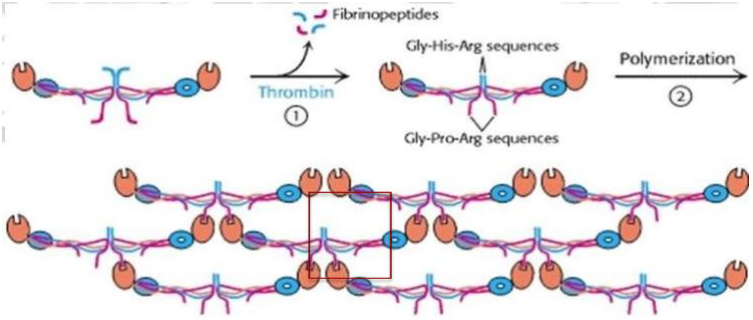
In the intrinsic pathway, upstream of factor IX, you have factors XI and XII, and you have activation of what is known as the kallikrein-kinin system, these are inflammatory molecules and they do have an effect on the coagulation process.
Kallikrein is a protease that is released in inflammation, when activated, it can activate factor XII into factor XIIa, Factor XIIa can then activate factor XI into factor XIa, when then is able to further activate factor IX that is involved in the intrinsic pathway.
Kallikrein as well has other effects, by cleaving kininogen producing bradykinin, which as an inflammatory molecule that has a vasodilation effect, a hypotensive action, it is also involved in the molecular mechanism of pain.

Formation of a fibrin clot

What was mentioned in the slide:

- Thrombin cleaves fibrinogen releasing fibrinopeptides.
  - Fibrinogen is a two triple-stranded helical protein held together by disulfide bonds.
- Fibrin molecues create electrostatic attractions between the central domain and the end domains facilitating the aggregation of the monomers into a gel consisting of long polymers.
- The clot resulting from aggregation of fibrin monomers is referred to as the "soft clot".





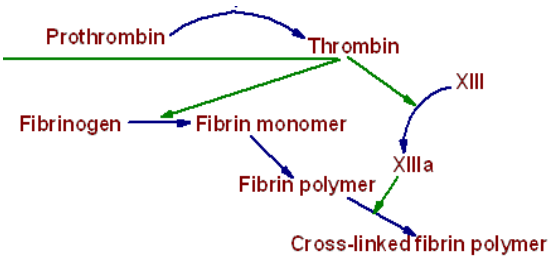
What was mentioned in the lecture:

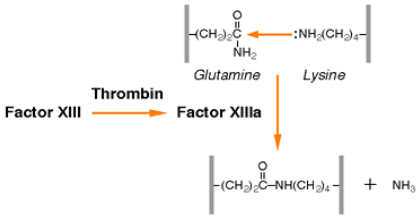
After that you had activation of all of these molecules, we're getting to the climax of the coagulation process, so after you have activation of factor X, factor Xa activates prothrombin (factor II) into thrombin (factor IIa), thrombin acts on fibrinogen, it converts fibrinogen into fibrin, how does it do that? By removing a number of peptides of the protein, this results in the ability of fibrin molecules to form electrostatic interactions between the head and the tails of two other molecules, so you have the head (the blue line in the figure) forming electrostatic interactions with the tails (the orange balls) of two other fibrins and so on, so what happens here is that you have aggregation of the fibrin molecules together forming a clot, this clot is known as a soft clot because the interaction between all of these molecules is based on electrostatic interactions which is non-covalent.

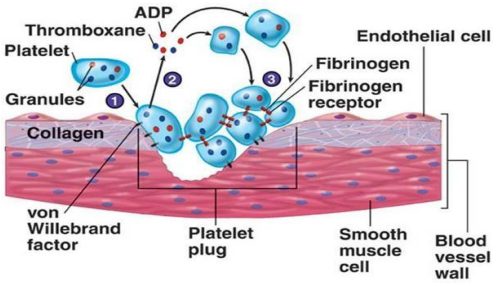
Factor XIII

What was mentioned in the slide:

- Factor XIII is a transglutaminase that is activated by thrombin.
- Factor XIIIa catalyzes a transglutamination reaction that catalyzes covalent cross-linking reaction between a glutamine of one fibrin monomer to a lysine of an adjacent fibrin monomer.
- It also cross-links the fibrin clot to adhesive proteins on the endothelial tissue and to the platelet surfaces strengthening the platelet plug.
- The cross-links strengthen the fibrin mass, forming the “hard clot”







What was mentioned in the lecture:

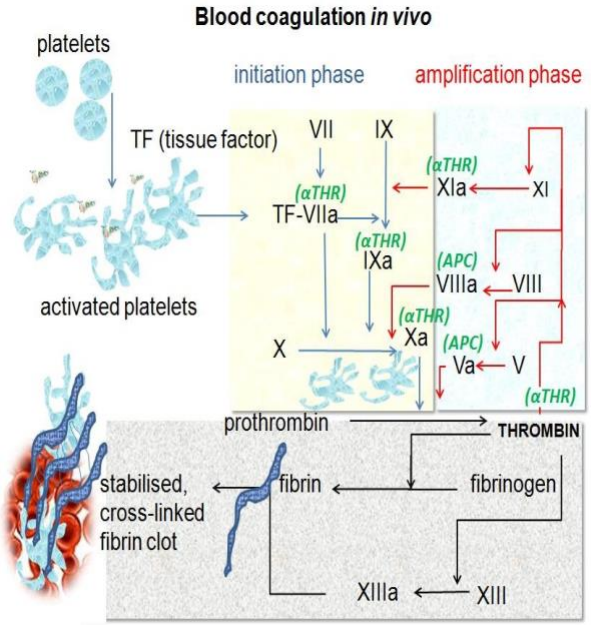
in addition to cleaving the fibrinogen into fibrin, thrombin can act on another protein and it is known as factor XIII forming factor XIIIa, factor XIIIa is a transglutaminase, and what it does is that it forms a covalent interaction (cross-linking) between a glutamine residue on one fibrin monomer to a lysine residue on another fibrin monomer, so now, you have a formation of a hard clot, this clot, not only that it contains the fibrin network, it also entrap platelets inside it, strengthening the platelet plug.

Amplification of coagulation reactions

What was mentioned in the slide:

- The sequential enzymatic activation allows for amplification.
- Amplification also results from positive feedback reactions.
- These include activation of V, VII, VIII, and XI by thrombin.

What was mentioned in the lecture:

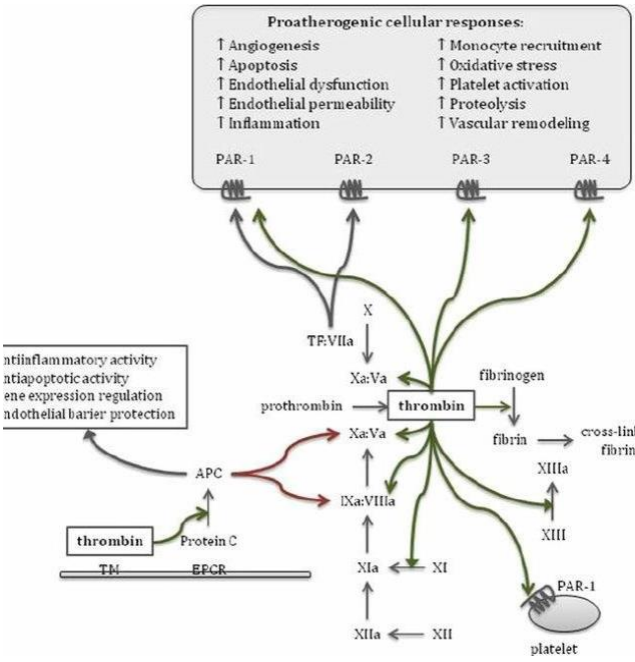


At this point, we're really getting to the climax of the symphony, what happens now is that you have activation of all of these molecules and it's getting even amplified, the reason is that you have positive feedback activation, so that activation of prothrombin leads to activation of more prothrombin molecules.
once prothrombin is activated into thrombin, an in addition to acting on fibrinogen and factor XIII, thrombin acts back on all of these factors, so what it does is that it activates more factor V (which activates factor X), more of factor VIII (which activates factors X and IX) more of factor XI (which is produced in response to kallikrein and leads to activation of factor IX), and it can also act on factor VII (which is involved in activation of factor X through the intrinsic pathway) as well, so you have more amplification of these zymogens, getting to factor X that activates more and more pro-thrombin to thrombin.

Roles of thrombin

What was mentioned in the slide:

- Platelet recruitment
- Amplification of the coagulation complex
- Formation of soft clot
  - Proteolytic cleavage of fibrinogen
- Formation of hard clot
  - Activation of factor XIII
- Attenuation of its own activity
  - Activation of protein C
- Other actions
  - Binding to its receptor on surface of platelets induces vascular remodeling (e.g. angiogenesis) and inflammation



What was mentioned in the lecture:

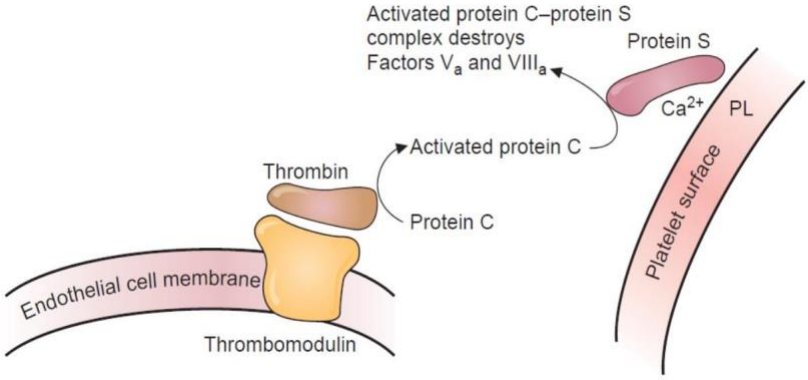
thrombin is important for a number of reasons, firstly, it has its own receptor on platelets, what this receptor does upon activation is that it recruits platelets to the site of injury and activates them.
In addition, thrombin amplifies the coagulation complex, it forms the soft clot with the fibrin monomers forming a network, it also forms the hard clots by activating factor XIII, In addition, what thrombin does is that it terminates coagulation and this is the next topic of this lecture.
In addition, thrombin itself can bind to what is known as protease activated receptors, these receptors are present on the surface on of number of cells including endothelial cells, and what they do is that they can induce vascular remodeling (angiogenesis) as well as inducing inflammation.

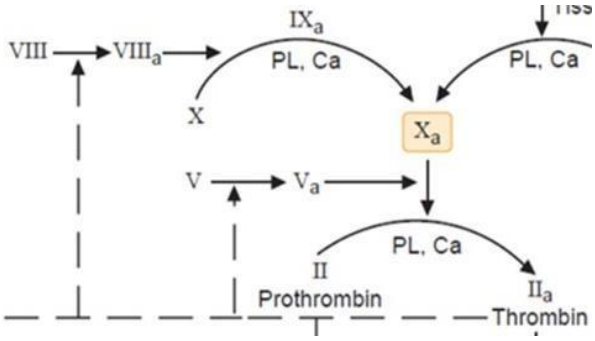
Anti-clotting factors

Protein C and protein S

What was mentioned in the slide:

- Thrombin binds to thrombomodulin in the surface of endothelial cells.
- Thrombin can then activate protein C, which forms a complex with protein S, both of which are vitamin K-dependent cofactors.
- The complex degrades factors V and VIII.





What was mentioned in the lecture:

now the symphony is going down, so let's talk about the anti-clotting factors.
once thrombin is activated, what it does is that it binds to a protein known as thrombomodulin, this protein is present on the surface of endothelial cells, this thrombomodulin brings thrombin closer to a protein known as protein C, protein C is activated by thrombin forming activated protein C, then this activated protein C forms a complex with protein S which is present on the surface of platelets, this complex of protein C protein S degrades factors V and VIII so you have termination of activation of prothrombin and factor X.

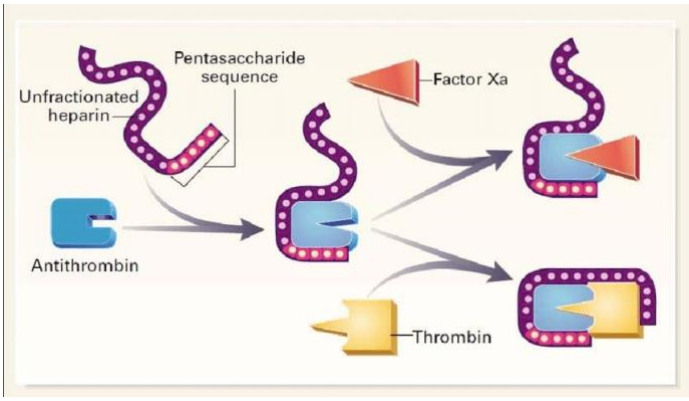
Antithrombin III

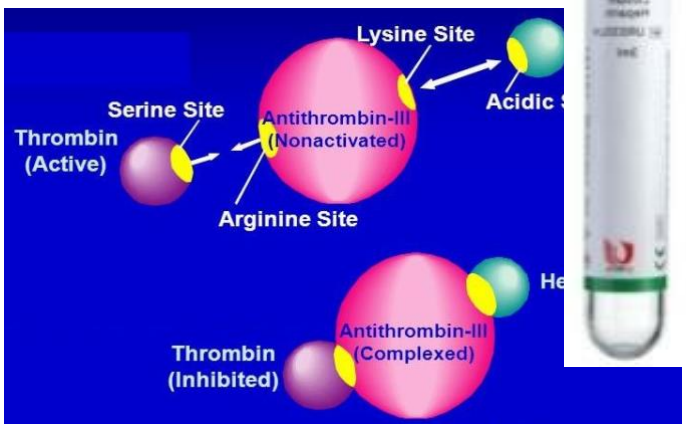
What was mentioned in the slide:

In the clinic, phlebotomy tubes are often treated with heparin in order to inhibit clot formation.

Antithrombin III is a serine protease inhibitor of thrombin as well other clotting factors (IXa, Xa, XIa, and XIIa).

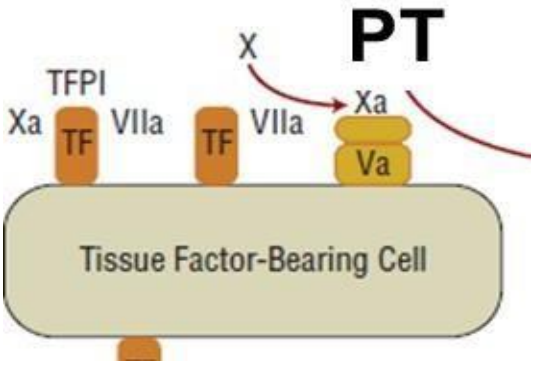
Heparin sulfate, a polysaccharide synthesized by mast cells and present on surface of endothelial cells, binds to antithrombin III, promoting binding to its substrates.





What was mentioned in the lecture:

another inhibitor of coagulation is known as antithrombin III, antithrombin III is a serine protease inhibitor, it inhibits not only thrombin, but it can also inhibit other proteases such as factors IX, X, XI and XII, so what antithrombin does is that it binds to heparin sulfate, heparin sulfate is a polysaccharide synthesized by mast cells, released and is bound to the surface of endothelial cells, once antithrombin binds to heparin sulfate, the confirmation of antithrombin III changes allowing it to bind to thrombin and the other clotting factors inhibiting them, the interaction between antithrombin III and the clotting factors and with heparin sulfate is specific.
what's important about heparin is that it is used in laboratories in clinical laboratories in phlebotomy tubes, so what it does is that it prevents blood clotting, so once blood is drawn, it's collected into these tubes and it stays in a liquid form, otherwise it coagulates immediately, heparin is also used as a drug.

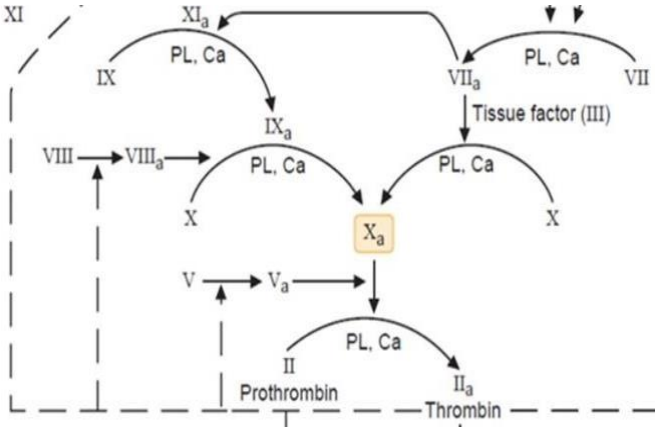


Tissue Factor pathway inhibitor

What was mentioned in the slide:

- Tissue factor pathway inhibitor (TFPI) is a protein found in plasma lipoproteins and bound to the vascular endothelium.
- It binds to and inhibits factor Xa.

- The Xa-TFPI complex then interacts with the tissue factor-VIIa complex and inhibits its activation of factors X and IX.
- Protein S binds to TFPI localizing it to membrane surfaces and enhancing the inhibition of Xa.
- TFPI is also able to inhibit Xa-activated Va resulting in inhibition of the pro-thrombinase complex.



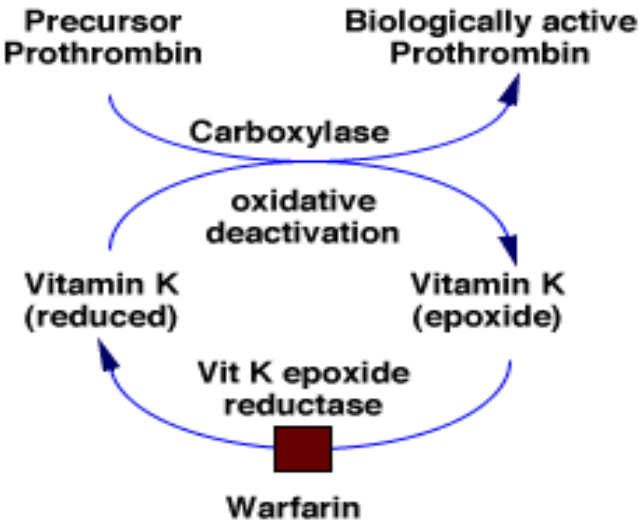
What was mentioned in the lecture:

Another coagulation inhibitor is tissue factor pathway inhibitor, what this inhibitor does is that it combines to factor X and then it can interact with the complex of tissue factor – factor VII (the initiation complex) inhibiting the whole clotting cascade, specifically, it prevents the activation of factors X as well as factor XI, the other thing about the tissue factor pathway inhibitor is that it can also prevent the prothrombinase complex (factor Xa/Va) by interacting with factor X, it can also inhibit the activation of prothrombin to thrombin.

Anti-coagulants

What was mentioned in the slide:

Blood clotting can be prevented by addition of Ca^{+2} chelators and vitamin K antagonists such as the anticoagulant drug warfarin, which inhibits reduction of vitamin K and thereby prevents synthesis of active prothrombin and factors VII, IX, and X.



What was mentioned in the lecture:

anticoagulants can be used either as drugs or in the laboratories, anticoagulants can be calcium chelators (like citrate) as well as vitamin K antagonists (warfarin).
calcium is important for the process of coagulation, as it is required for the function of factors II, VII, IX and X, by using chelators that absorb calcium ions, that compromises the efficiency of blood coagulation.
there is a drug known as warfarin and what warfarin does is that it inhibits the vitamin K epoxide reductase preventing the regeneration of the active form of vitamin K which is the reduced form, so there would be inability of the carboxylase enzyme to carboxylate glutamate and that again compromises interaction of proteins to the surface of platelets.

Degradation of the fibrin clot

Clot dissolution

What was mentioned in the slide:

- It is important to prevent clot formation when not needed by anti-clotting factors and to dissolve a clot when formed.
- Clot dissolution starts concomitant with its formation.

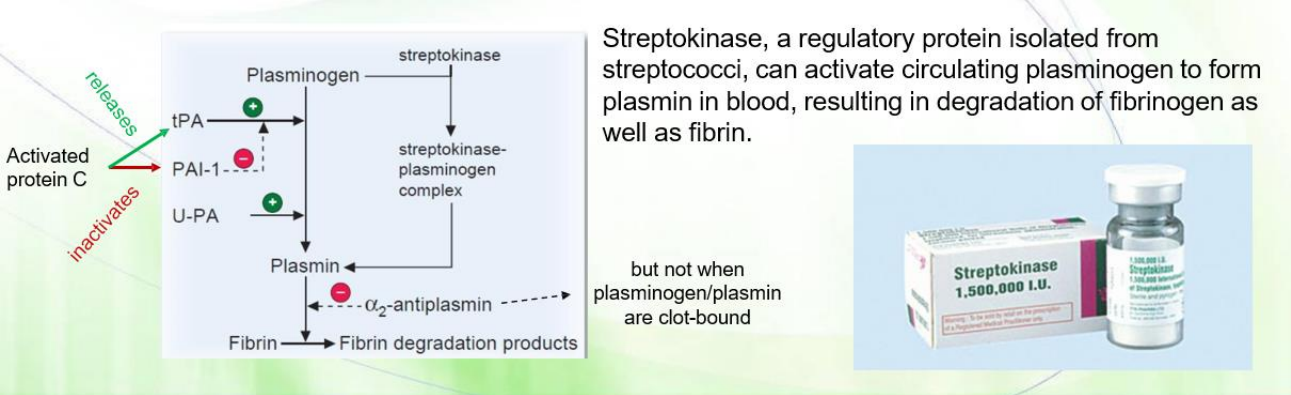
What was mentioned in the lecture:

now the music is going down, so what happens is that there's a clot, there is a reduction in cessation of bleeding, the clot must be removed and there is renewal of cells as well, activation of the removal of the clot happens simultaneously with the activation of blood coagulation by the way, so that things do not go out of control.

The fibrinolytic system

What was mentioned in the slide:

- Plasmin, a serine protease formed from plasminogen, is responsible for fibrinolysis where it catalyzes the hydrolysis of fibrin and fibrinogen to degradation products.
- Plasminogen has a high affinity for fibrin clot.



What was mentioned in the lecture:

the dissolution of blood clots is basically enzymatically dependent, it is done by a protease known as plasmin, plasmin is a serine protease, it is the activated form of its precursor that is plasminogen, plasmin is responsible for fibrinolysis, it's responsible for hydrolyzing and degrading the fibrin network, and it just happens that plasminogen has high affinity for fibrin clots, so otherwise it doesn't bind to fibrin or fibrinogen.
once plasmin is activated, it can degrade fibrin, this activation process is induced by a plasminogen activator known as tissue plasminogen activator (tPA), it is also induced by what is known as urokinase plasminogen activator (UPA), now the tissue plasminogen activator is activated by the activated protein C which is the anticoagulant that we talked about before, (the one that degrades factors V and VIII), so the result would be stimulation of the fibrinolysis process.
Plasmin is also regulated by an inhibitor known as α_2-antiplasmin, this antiplasmin keeps things in check, it inhibits plasmin, but not when plasmin is bound to the clot, this is beautiful, so if you have a plasmid that is outside the clot, it is immediately inhibited by the antiplasmin, but as long as it is bound to the clot degrading it, it's active and it cannot be inhibited by the anti-plasmin that ensures the removal of the clot without compromising or affecting the surrounding tissue and surrounding proteins.
something else is that there is a molecule known as streptokinase, streptokinase is used clinically, it is isolated from streptococci bacteria, and it can activate the circulating plasminogen to form plasmin, so what it does is that it facilitates the removal of the blood clot.

Urokinase

What was mentioned in the slide:

Urokinase, a serine protease is formed from the zymogen pro-urokinase

It is a potent plasminogen activator, and is used clinically

What was mentioned in the lecture:

in addition to tissue plasminogen activator, there is urokinase plasminogen activator, which activates plasminogen into plasmin and it can also be used clinically, notice that in the body, to ensure that everything is cool, urokinase in found in an inactive form that is pro-urokinase.