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Hemodynamic Disorders 2: Hemostasis and Thrombosis

Outline:

I. Normal Homeostasis
- A. Purpose
- B. Components
  1. Vascular wall (endothelium and basement membrane)
  2. Platelets
  3. Coagulation cascade
- C. Response to Vascular Injury
  1. Local Response
  2. Primary hemostatic Plug
  3. Secondary hemostatic Plug
  4 Counter-regulation

II Endothelial Cell Contributions to Homeostasis
- A. Anti-thrombotic activity in the health vessel
  1. Inhibition of platelets
  2. Inhibition of coagulation
- B. Prothrombotic activity in the damaged vessel
  1. Promotion of platelet adherence
  2. Promotion of coagulation cascade

III. Platelet Contributions to Homeostasis
- A. Prothombotic activity

IV. Coagulation Cascade
- A. Nomenclature of factors
- B. Proteolytic cleavage
- C. Components of the cascade
  1. Intrinsic
  2. Extrinsic
- D. Inhibitors of the cascade

V. Thrombosis
- A. Causes of pathological thrombosis
  1. Endothelial injury
  2. Abnormal flow
  3. Hypercoagulability of blood
- B. Types of thrombi
  1. Cardiac (mural) thrombus
  2. Arterial thrombus
  3. Venous thrombus
- C. Fate of thrombus

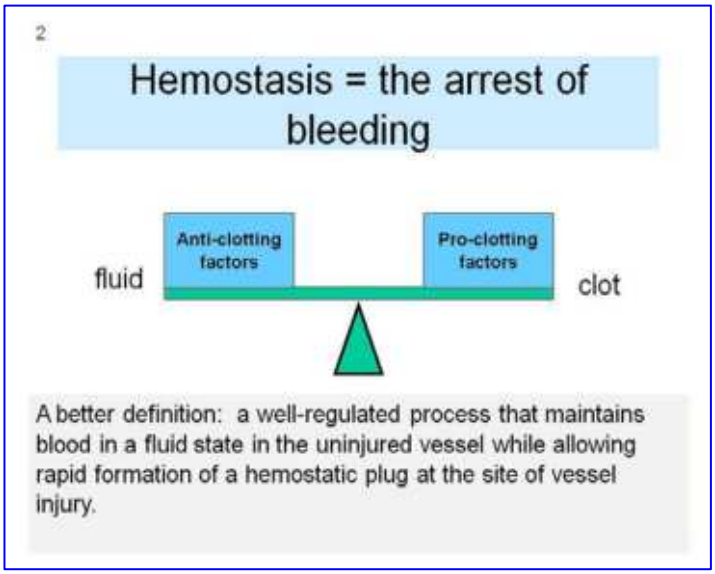
Suggested Reading:

Robbins Basic Pathology by Kumar, Abbas and Aster, Chapter 4 (101-119)

Objectives:

1. Define hemostasis and describe the 3 components of normal hemostasis.
2. Describe events in the 4 steps of formation and regulation of the permanent hemostatic plug.
3. Describe the differing roles of endothelial cells in both the injured and non-injured vessel.
4. Describe the role of platelets in development of the primary and secondary plug.
5. Detail the factors which influence (stimulate or inhibit) initial platelet attachment and their source.
6. Describe the various sources and types of factors in the coagulation cascade.
7. Develop a basic understanding of the cascade and list the requirements for activation of both the extrinsic and intrinsic pathways.
8. Describe how the 2 pathways relate to clinical testing of clotting times (PT and PPT).
9. Identify the components of the common pathway.
10. Describe three possible causes of thrombosis.
11. Describe 2 mutations that lead to hypercoagulability states.
12. Compare and contrast the differences between mural, arterial, and venous thrombi in terms of cause, morphology, and complicating outcomes.

I. Normal Homeostasis:

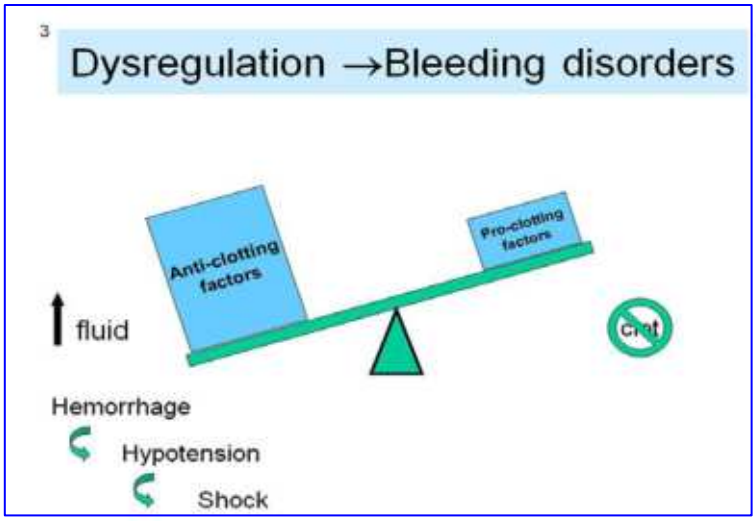


A. Purpose -

Hemostasis is simply defined as the **arrest of bleeding**. A better definition would include consideration of the dynamic interactions between formed elements and other blood components.

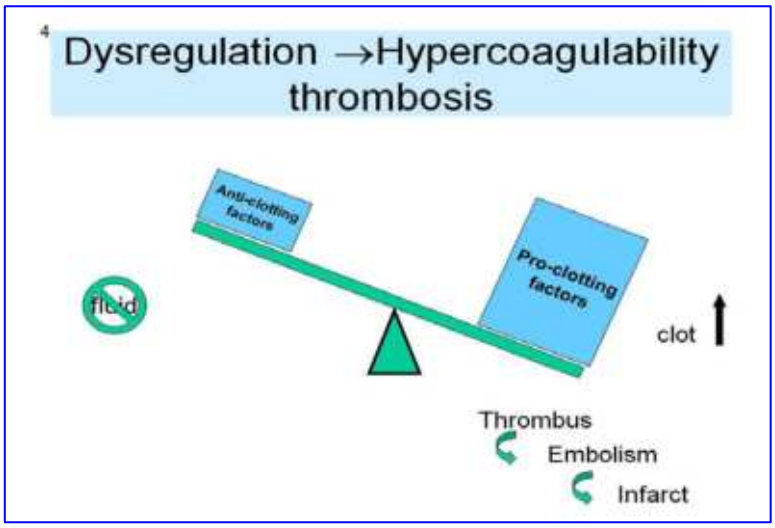
For example: Hemostasis is a well-regulated process that maintains blood in a fluid state in the uninjured vessel while allowing rapid formation of a hemostatic plug at the site of the vessel injury (*Basic Pathology*).

Dysregulation of hemostasis accounts for a variety of pathological conditions.

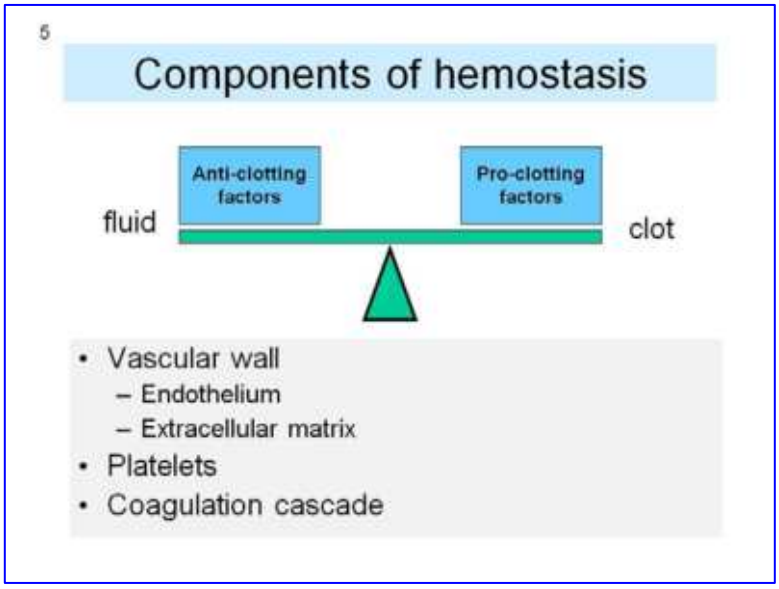


In **bleeding disorders** there is a relative increase in anti-clotting or fibrinolytic activity and decrease in clotting activity.

The result is hemorrhage which may lead to hypotension and shock.



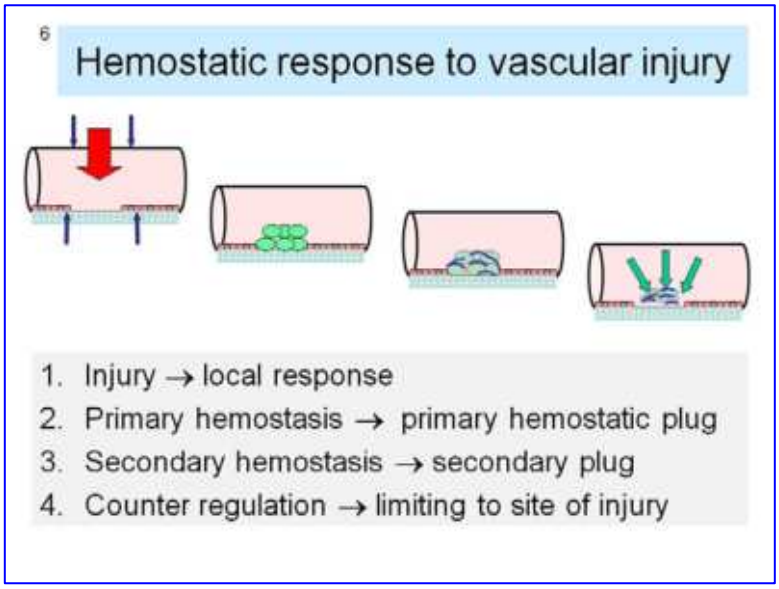
The converse may be seen in **hypercoagulability states** that lead to formation of thrombi. Here pro-clotting activity greatly exceeds anti-clotting activity resulting in inappropriate thrombus formation which may lead to embolism and infarct. In this lecture we will concentrate on the normal components of hemostasis so that you will form a knowledge base to better understand the pathology of specific bleeding disorders presented in future lectures.



B. Components –

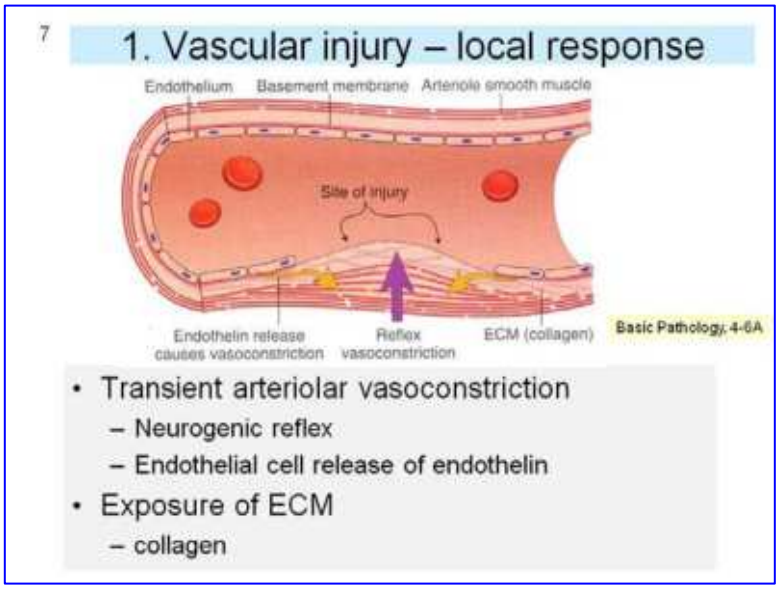
The **3 components** of normal hemostasis include the vascular wall (endothelium and extracellular matrix), platelets, and the coagulation cascade.

C. Normal Hemostatic Response to Vascular Injury:

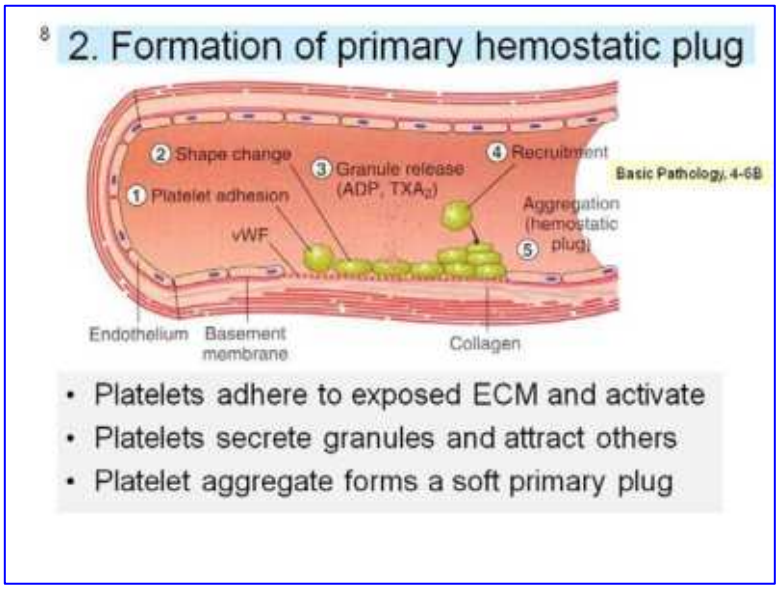


Following vascular injury a series of events results in formation of a hemostatic plug which effectively stops or prevents hemorrhage until the vessel is repaired.

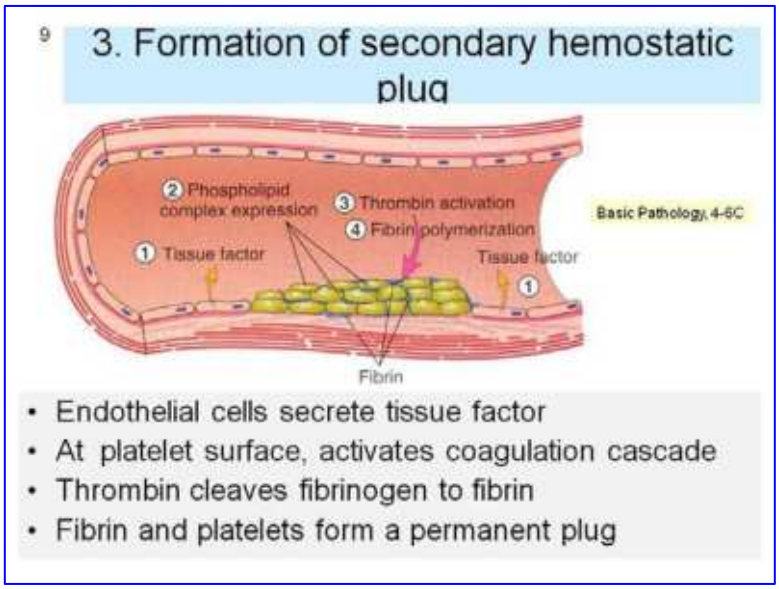
These events are described in 4 steps illustrated in the figures below and Figures in your textbook:



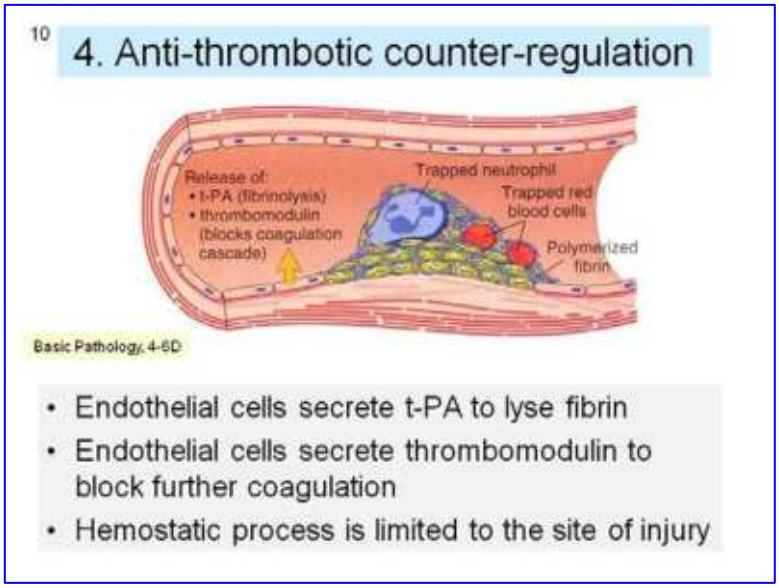
1. Local response - Injury evokes a neurogenic response of transient arteriolar vasoconstriction and endothelial cells secrete **endothelin** which is a powerful vasoconstrictor. This early, but brief, vasoconstriction helps to limit initial hemorrhage. Note that endothelial cell loss at the site of injury results in exposure of blood to the extracellular matrix. This will initiate subsequent steps in this process.



2. Formation of primary hemostatic plug - Primary hemostasis involves exposure and adherence of platelets to the extracellular matrix. Contact with the matrix causes platelet activation, secretion of granules containing substances that attract additional platelets, and the resulting platelet aggregate forms a soft primary plug.

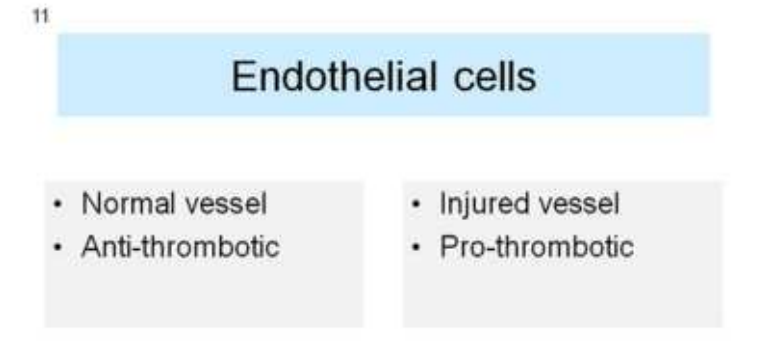


3. Formation of secondary hemostatic plug - Secondary hemostasis is a longer process and involves activation of the coagulation cascade. Injured endothelial cells secrete tissue factor which interacts with platelet factors and the platelet surface to initiate a cascade of reactions ending in the activation of thrombin. Activated thrombin cleaves the soluble protein fibrinogen to insoluble fibrin which then forms a mesh within the platelet aggregate. The platelet-fibrin complex is the secondary plug.



4. Counter regulation - Once the permanent plug is formed, counter-regulatory measures are in place to ensure that the hemostatic process is limited to the site of injury. Endothelial cells secrete t-PA (tissue plasminogen activator) which initiates steps to lyse fibrin. When thrombin binds thrombomodulin (an endothelial receptor) inhibition of the coagulation cascade is initiated.

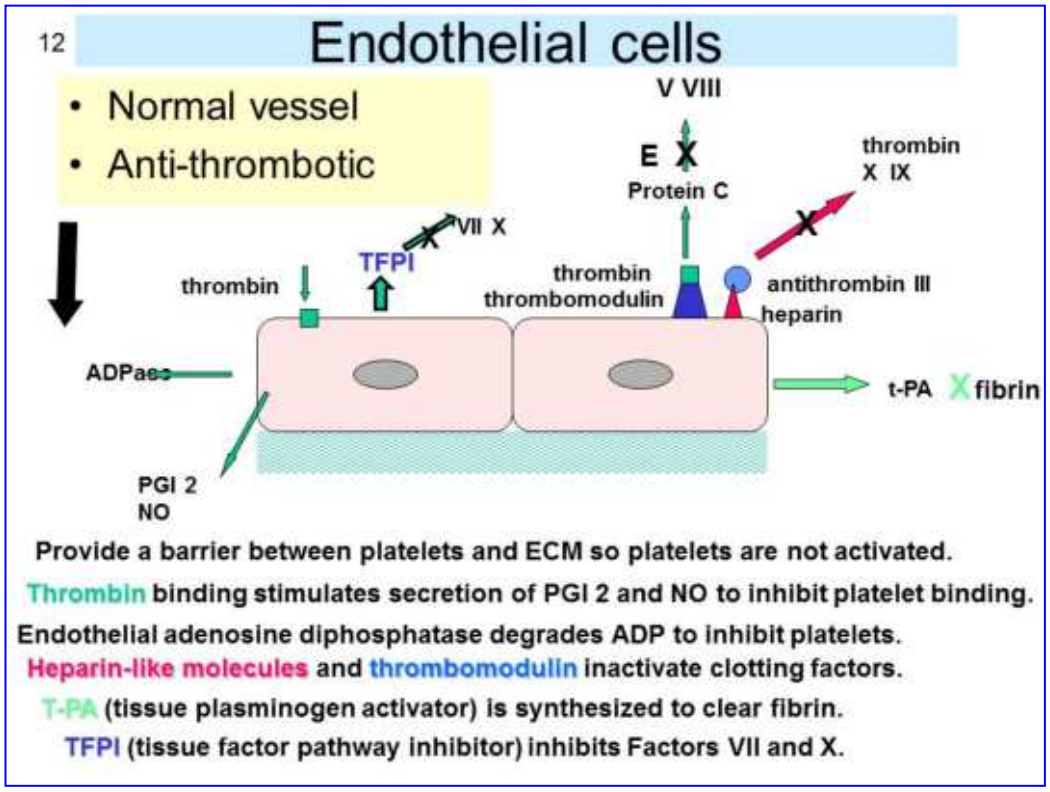
From this brief description of the events of hemostasis you can appreciate the complex interaction between endothelial cells, platelets, and the components of the coagulation cascade. We will now look at each in a more comprehensive manner.



II. Endothelial Cell Contributions to Hemostasis:

Endothelial cells respond to injury by promoting coagulation. In the normal vessel, however, they act to inhibit this process preventing inappropriate clotting. They are the **regulators** of thrombus formation, propagation and resolution.

A. Endothelial anti-thrombotic activity in the normal vessel - Endothelial cells inhibit thrombus formation in the following ways:



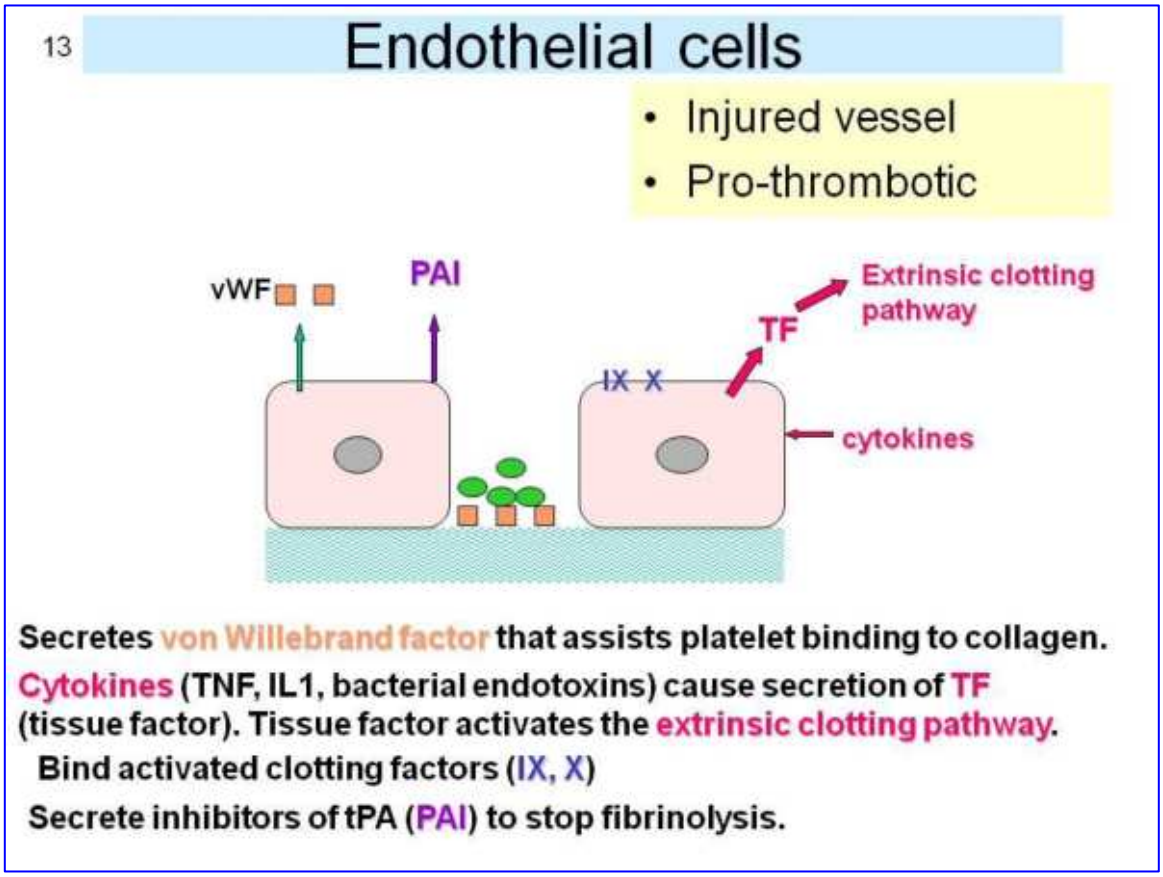
1. Inhibition of platelets-

- Endothelial cells provide a **physical barrier** between platelets and the extracelluar matrix to stop activation of the former.
- Even in the presence of activated platelets, thrombin and cytokine bind to endothelial cells and stimulate their secretion of **PGI-2** and **nitric oxide (NO)**. These substances are potent vasodilators and inhibitors of platelet binding to healthy endothelium.
- Endothelial cells secrete adenosine phosphatase (**ADPase**) which degrades ADP (a platelet aggregation factor).

2. Inhibition of coagulation-

- **Heparin-like molecules** on the surface of the endothelial cell, bind **anti-thrombin III** which inactivates thrombin and Factors X and IX of the coagulation cascade.
- Binding of thrombin to the **thrombomodulin receptor** on the endothelial cell alters the thrombin to activate Protein C which enzymatically cleaves and inactivates Factors V and VIII in the presence of Protein S (also secreted by the endothelial cell).
- Endothelial cells secrete **tPA** (tissue plasminogen activator) that activates plasmin to lyse and clear fibrin from the cell surface. Endothelial cells secrete **TFPI** (tissue factor pathway inhibitor) that inhibits Factor VII and Factor X complex activity in coagulation.

B. Endothelial pro-thrombotic activity in the damaged vessel - In the event of vessel damage, endothelial cells are capable of switching to pro-thrombotic activity.



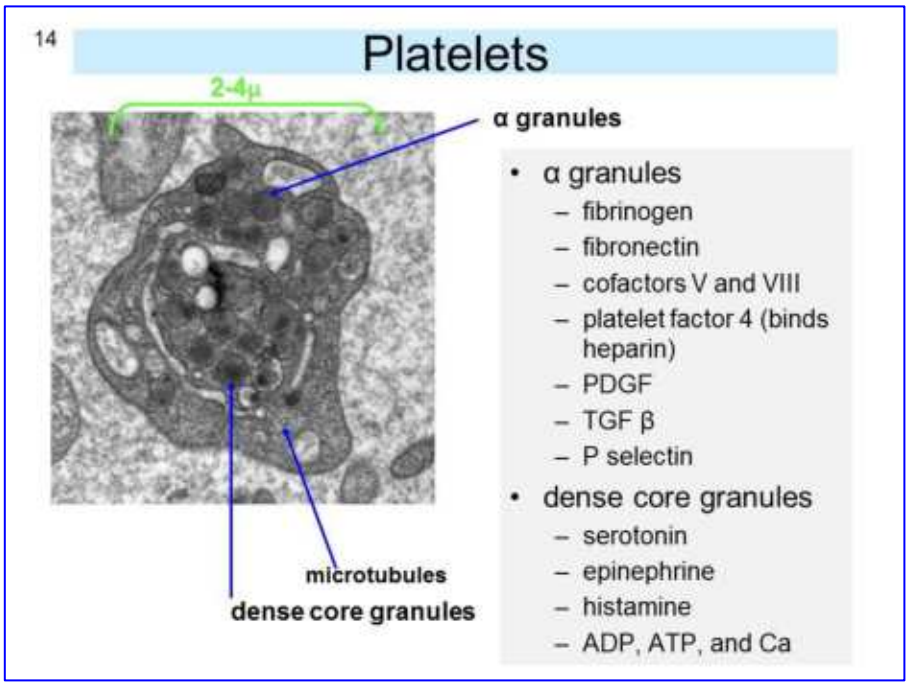
1. Promotion of platelet adherence

- von Willebrand factor (vWF) is a normal product of endothelial cells. When extracellular matrix is exposed during injury, vWF binds platelets to exposed collagen. The strength of this binding exceeds the sheer force of blood flow due to specific binding to GP1b glycoprotein on the platelet.

2. Promotion of the coagulation cascade

- Injury and cytokines (TNF, IL1, and bacterial endotoxins) stimulate endothelial secretion oftissue factor which activates the extrinsic clotting pathway.
- Endothelial cells bind certain clotting proteins (Factor IX, X) and enhance their activity.
- Endothelial cells secrete inhibitors of tPA (plasminogen activator inhibitor, PAI) which inhibits the lysis of fibrin.

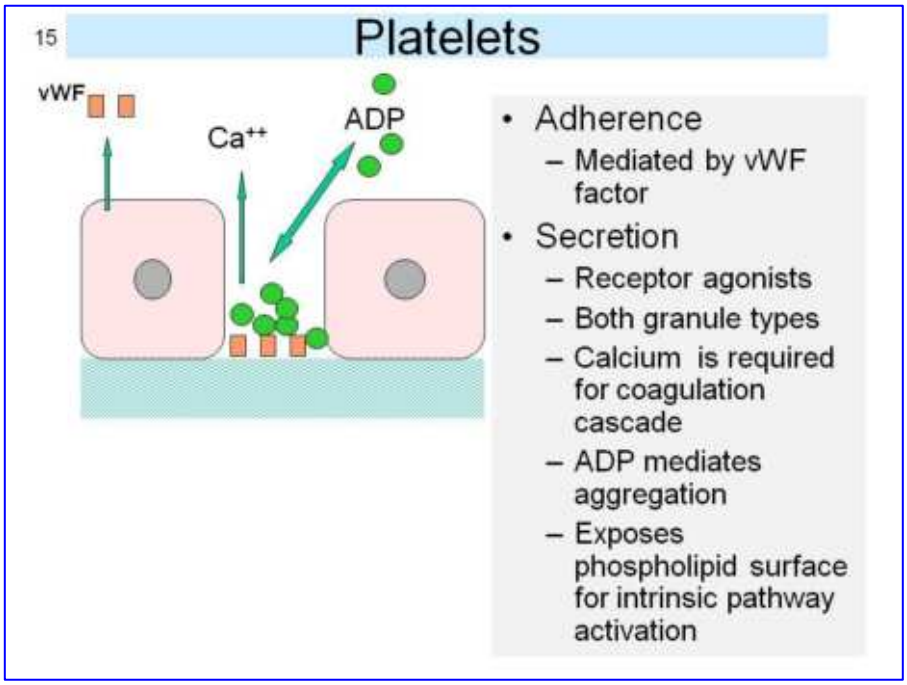
III. Platelet Contributions to Hemostasis:



Platelets are formed from 2-4 micron cytoplasmic fragments of megakaryocytes. They do not contain a nucleus but do contain 2 specific granule types in addition to lysosomes and peroxisomes. α granules contain fibrinogen, fibronectin, factors V and VIII, platelet factor 4, PDGF, TGF β and P selectin. Dense core granules contain serotonin, epinephrine, histamine, ADP, ATP, and Ca^{++}. Platelets participate in hemostasis by promoting formation of the plug.

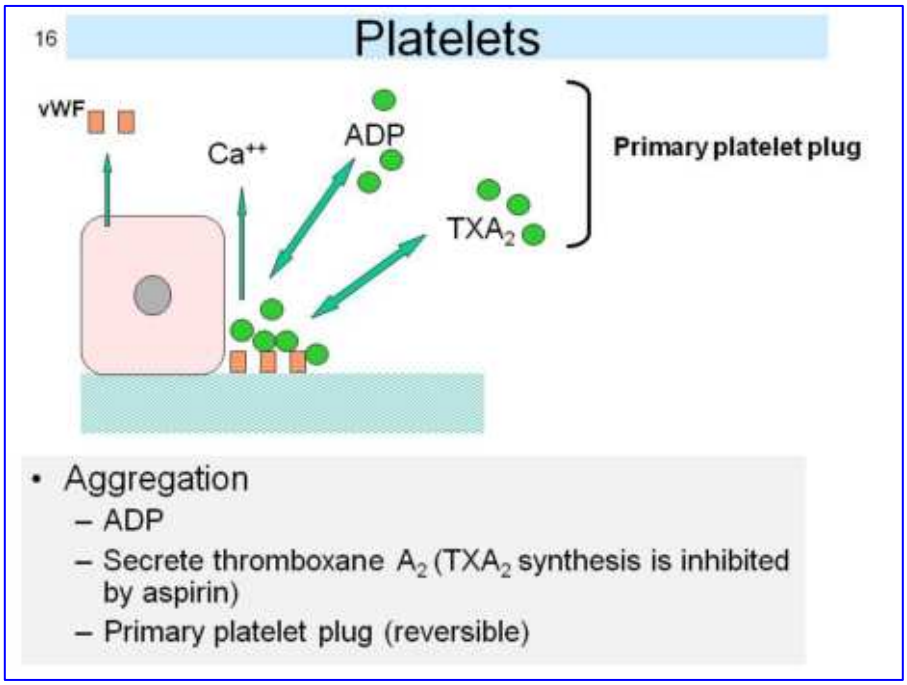
Unlike endothelial cells, they do not have an inhibitory side. The steps in plug formation after the platelet contacts the extracellular matrix include: adhesion, secretion, and further aggregation and contraction and are described as follows.

A. Prothrombotic Activity

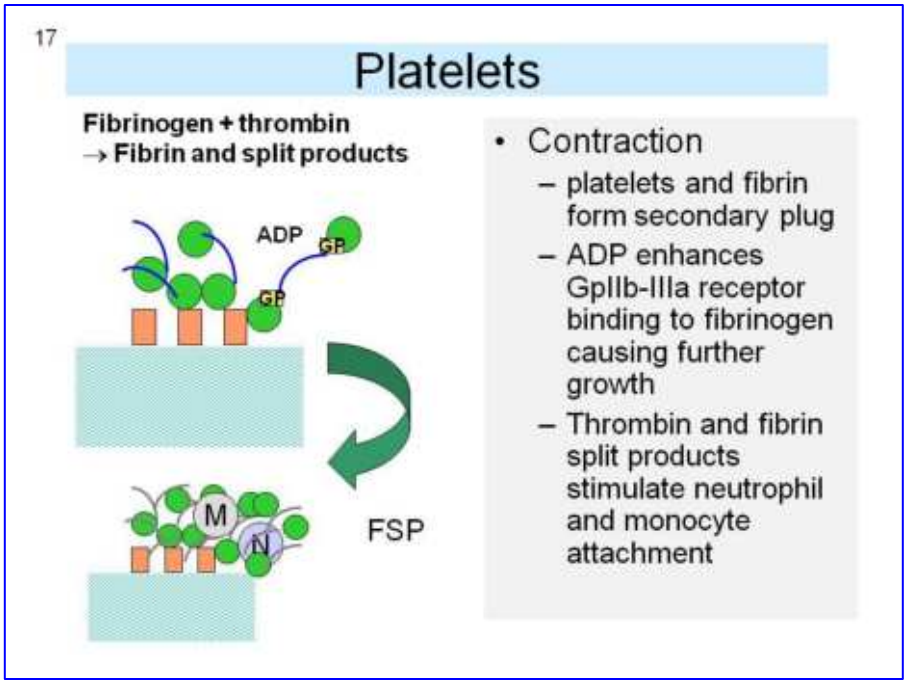


1. Adherence- As described earlier, platelets adhere to the extracellular matrix collagen and other proteins via the mediator vWF. (Note that genetic deficiencies in vWF contribute to the most common bleeding disorder.)

2. Secretion- Adherence stimulates secretion of both types of platelet granules. ADP stimulates platelet aggregation while Ca^{++} is essential in many steps of the coagulation cascade. The process of degranulation makes phospholipid surfaces of the platelet membrane available to proteins of the intrinsic coagulation pathway which require a surface for activation.



3. Aggregation- The process of further platelet aggregation increases the size of the platelet plug. In addition to ADP, the platelets secrete thromboxane 2 (TXA2). TXA2 stimulates platelet aggregation. (Note that aspirin inhibits TXA2. It is also interesting to note that endothelial PGI-2 inhibits platelet attachment. So platelet attachment is largely regulated by the balance of these two substances.



4. Contraction - Up to this point, the plug is a primary plug and the formation is reversible. With activation of the coagulation cascade and the addition of fibrin to the clot, the secondary plug is formed and this is a permanent structure. The binding of thrombin (the product of the coagulation cascade) to the platelet surface results in the conversion of fibrinogen to fibrin. Fibrin forms a meshwork around the platelets to stabilize the aggregate. Note that fibrinogen also binds the platelets at specific GpII receptors and forms bridges between platelets to enhance further growth of the aggregate. The conversion of fibrinogen results in both fibrin and fibrin split products (FSP). The FSP's along with thrombin attract monocytes and neutrophils into the plug (i.e. inflammation).

IV. Coagulation Cascade: Before discussing the coagulation cascade we should acknowledge that it involves a number of proteins (enzyme and non-enzymes).

A. Nomenclature - The following chart lists the factors of the coagulation pathway, their Roman numeral designation, common name, and the source of the protein or ion. The hepatocyte is the major site of synthesis for clotting factors. So it is easy to see how the individual with major liver disease would be deficient in clotting proteins. Also note that both endothelial cells and platelets produce some of the factors. We have already referred to many of them in our prior discussion. Finally note that each of the starred factors is subject to known genetic defects which result in alterations in the proteins leading to bleeding disorders. For example mutations in Factor VIII, the most commonly mutated factor, can be responsible for Hemophilia.

18

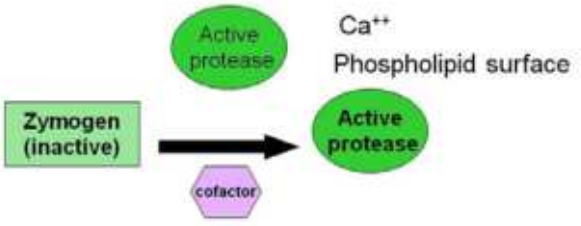
Coagulation Factors

Factor	Standard Name	Type	Source
I	Fibrinogen	Pro-form of fibrin	platelet – alpha granules
II	Prothrombin	Enzyme	hepatocytes
III	Tissue Factor	Non-enzyme CoF	Endothelial cells (not in plasma)
IV	Calcium Ions	ION	Platelets / plasma
V	Proaccelerin	Non-enzyme CoF	Platelet – alpha granules
VII	Proconvertin	Enzyme	hepatocytes
VIII	Antihemophilic factor (AHF)	Non-enzyme CoF	Platelet – alpha granules In plasma, bound to vWF
IX	Plasma thromboplastin	Enzyme	hepatocytes
X	Stuart factor	Enzyme	hepatocytes
XI	Plasma thromboplastin antecedent	Enzyme	hepatocytes
XII	Hagemann factor	Enzyme	hepatocytes
XIII	Fibrin stabilizing factor	Enzyme	

19

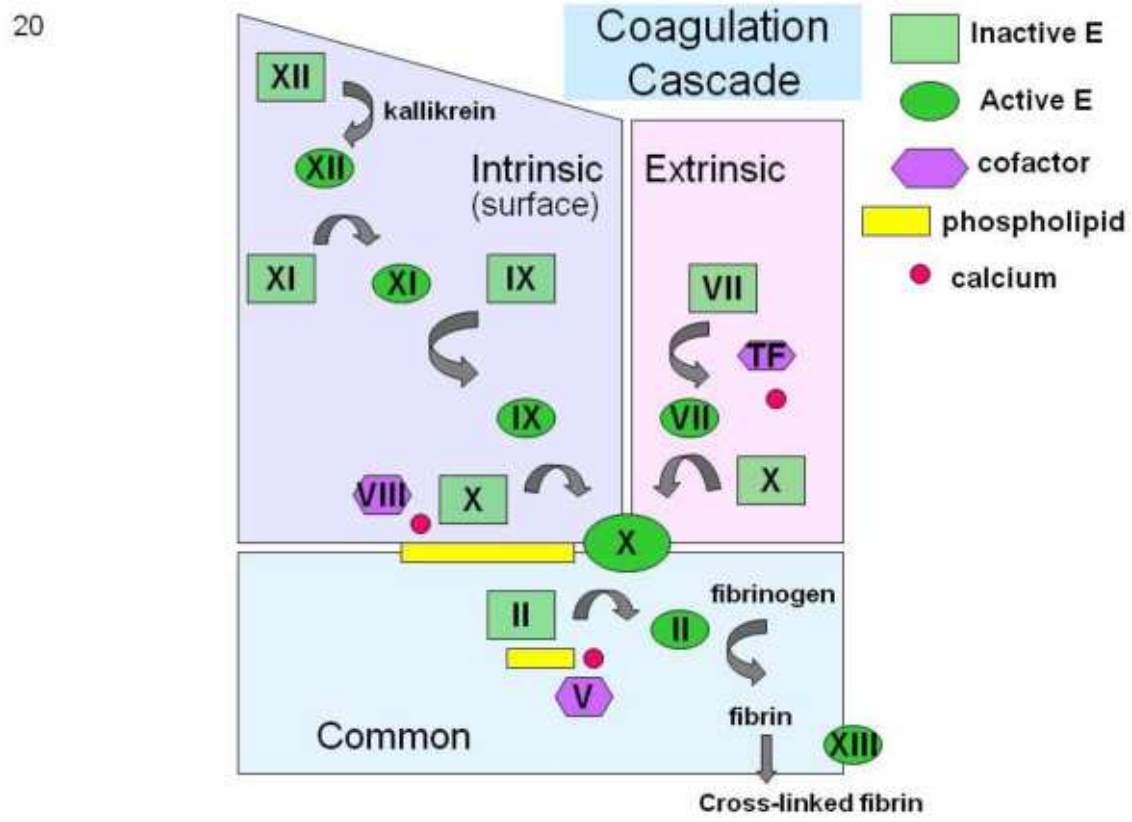
Coagulation Cascade

- A series of enzymatic conversions that turns proenzymes into active enzymes culminating in the formation of thrombin.



B. Coagulation reactions- The cascade is comprised of a series of reactions involving the factors listed above. The reactions involve the conversion of inactive forms of one factor into an active enzyme. That enzyme, in turn, catalyzes the next conversion. Non-enzymatic factors also serve as essential co-factors in some of the conversions and the presence of Ca^{++} and phospholipid surfaces are also essential.

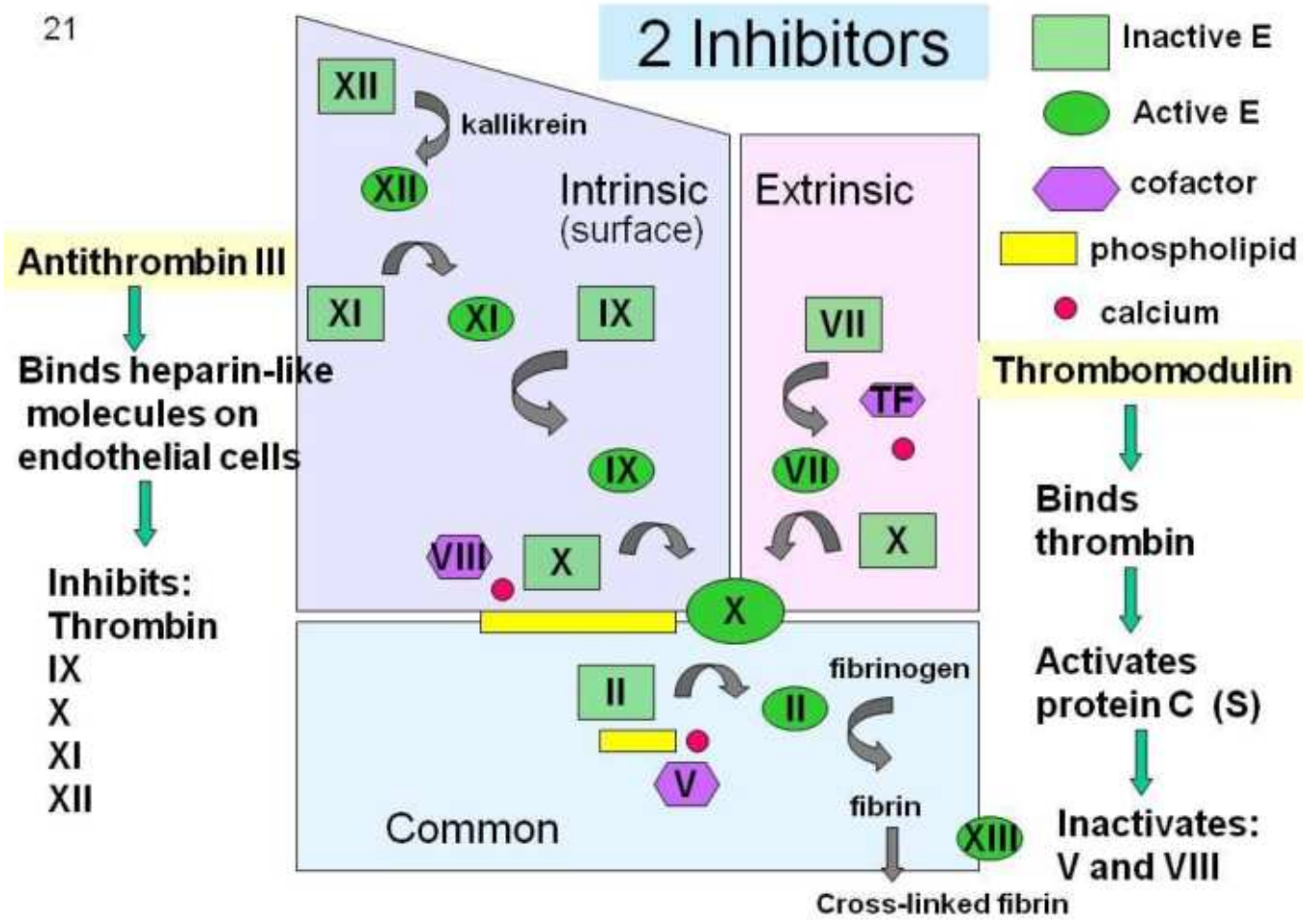
C. The coagulation cascade- For the purpose of description, the cascade has traditionally been divided into an extrinsic component, intrinsic component and common component. We now realize that there is greater interaction between certain parts of the extrinsic and intrinsic components than first thought. The extrinsic pathway is more physiologically relevant.



1. The **intrinsic pathway** requires an activating surface which is provided by the phospholipid of exposed platelet membranes in vivo. The intrinsic pathway begins with the conversion of XII to its active form in the presence of kallikrein. Active XII then catalyzes the activation of XI. Activated XI catalyzes the activation of IX which in turn activates X in the presence of the phospholipid surface, cofactor VIII and calcium. **Note: PPT (partial thromoplastin time)** is a clinical assessment of components of the intrinsic pathway (XII, XI, IX, VIII, X, V, II, and fibrinogen). A ground glass surface and phospholipids are exposed to patients' citrated plasma. Normal clotting should occur in 28-35 seconds. This is useful in heparin therapy screening.

2. The **extrinsic pathway** is activated by the release of tissue factor (III) which induces the activation of VII. Activated VII also catalyzes the conversion of X to its active form. So it is here with activation of Factor X that the two pathways meet. The common pathway proceeds with active X catalyzing conversion of prothrombin to active thrombin (II) in the presence of cofactor V, a phospholipid surface, and calcium. Thrombin then converts fibrinogen to fibrin which takes part in the final clot formation. Fibrin is further cross-linked by XIII. **Note: PT (prothrombin time)** is a clinical assessment of components of the extrinsic pathway (VII,X, II, V, and fibrinogen). Tissue factor is added to patients' citrated plasma. Normal clotting should occur in 11-13 seconds. This is helpful in monitoring the effects of Coumadin anticoagulationtherapy. The active Factor VII is vitamin K dependent and Coumadin is used as a vitamin K antagonist.

D. Inhibitors of the cascade - The coagulation cascade can be regulated by inhibitors that inactivate certain factors or by fibrinolytic mechanisms. Both serve to limit coagulation to the site of injury



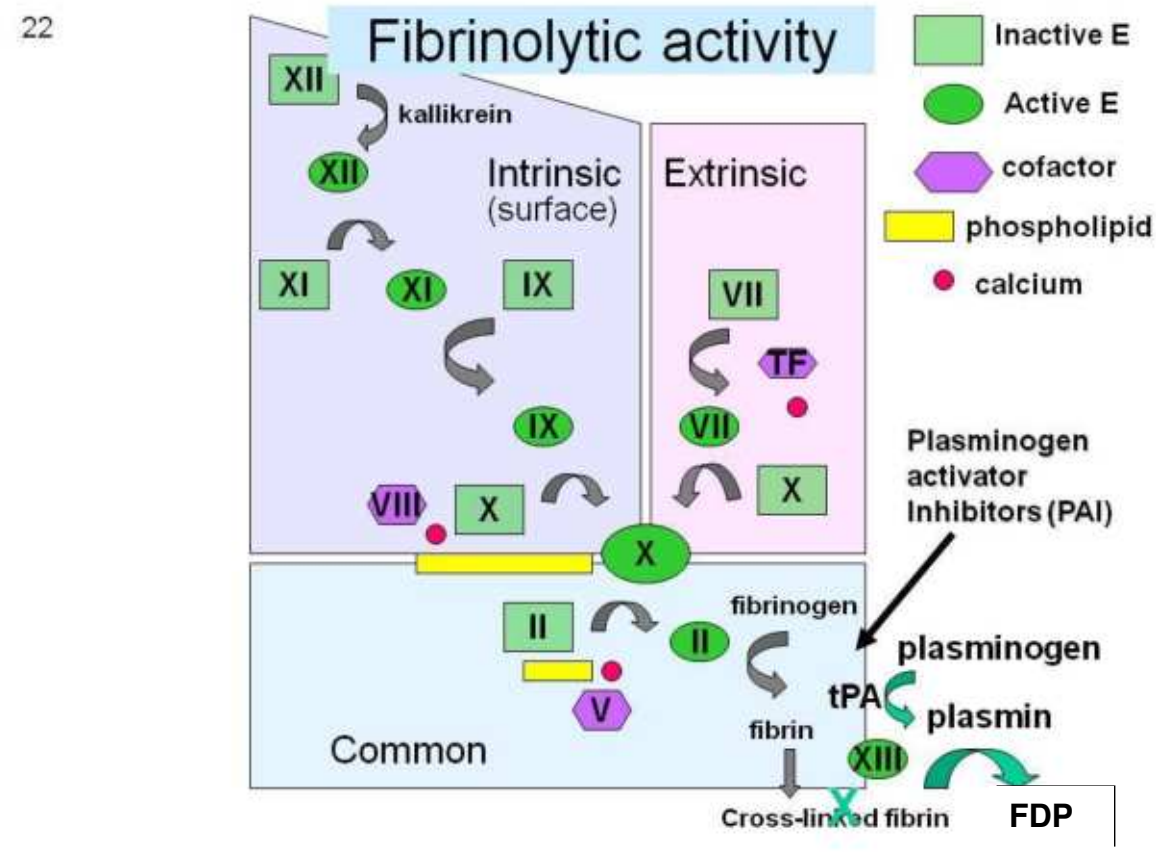
Inhibitors of coagulation -

- **Anti-thrombin III** is a natural anti-coagulant that binds to heparin-like proteins on endothelial cells and is activated resulting in the inhibition of thrombin and factors IX, X, XI, and XII of the intrinsic pathway.

- When thrombin binds thrombomodulin on the surface of endothelial cells, it changes character and actives **Protein C** which then inactivates cofactors V and VIII to inactivate the common pathway.

- **Tissue factor pathway inhibitor (TFPI)** inhibits the factor X and VII complex.

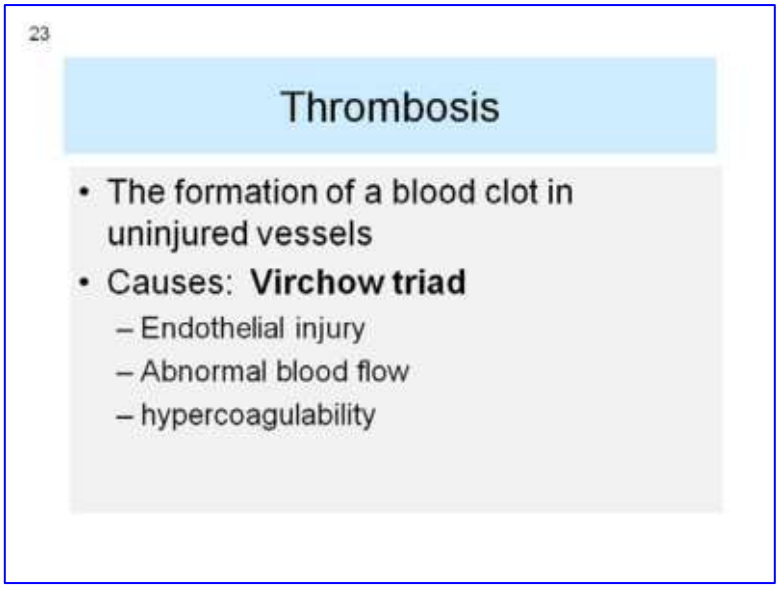
Fibrinolytic mechanisms-



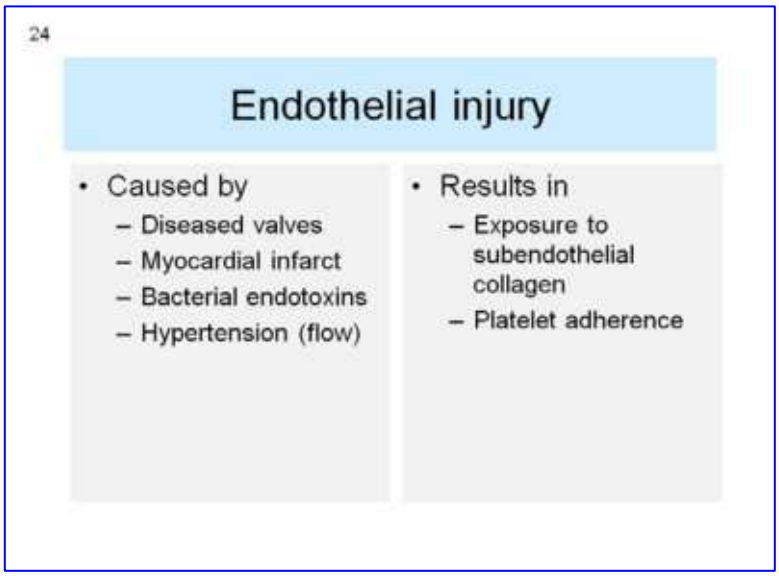
- **tPA's** are secreted by endothelial cells and serve to convert plasminogen to plasmin. The active plasmin then breaks down fibrin to limit the size of the clot.

  Note: 1) **FDP's** are also formed and in the circulation indicate active thrombolytic activity which can be a useful diagnostic indicator. 2) The fibrinolytic activity can be modified by the secretion of PAI's by endothelial cells to inhibit the tPA's.

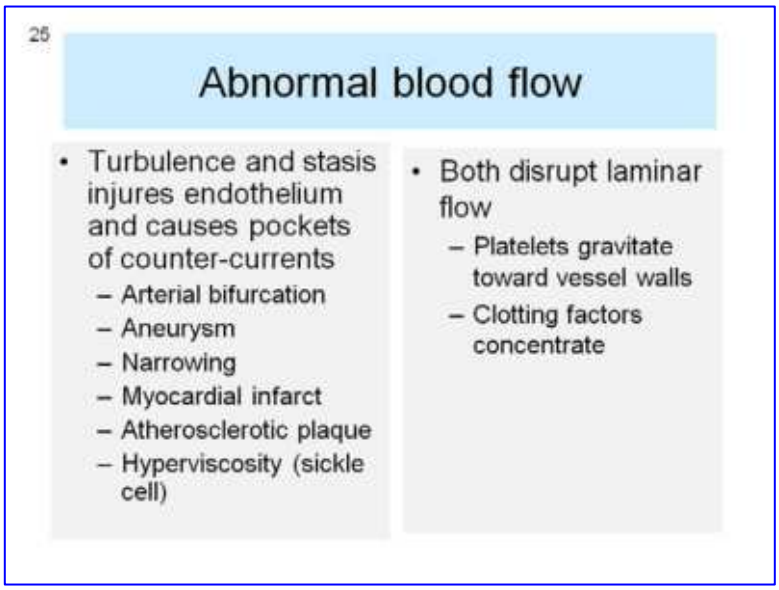
V. Thrombosis - The formation of blood clots in uninjured vessels is the result of dysregulation of hemostasis.



A. Causes of pathological thrombosis: The three cause of thrombosis are referred to as the **Virchow triad** and include endothelial injury, abnormal patterns of blood flow, and hypercoagulability of blood

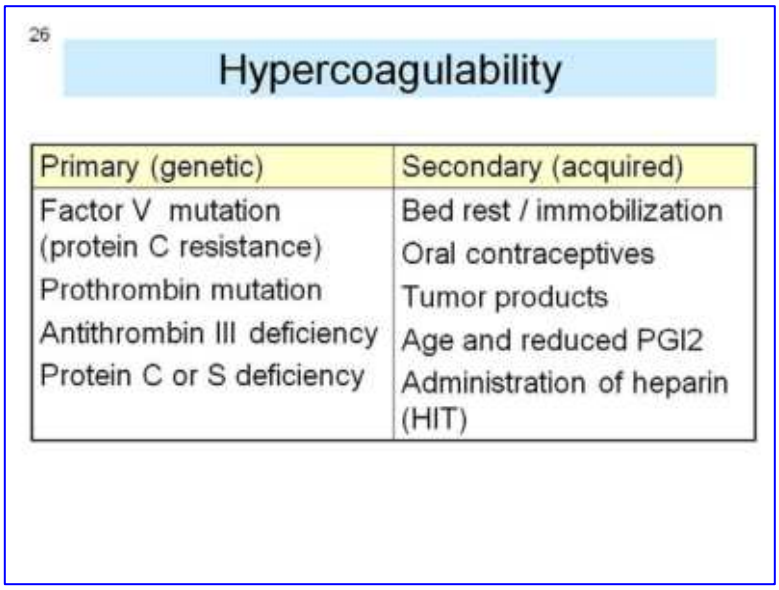


1. Endothelial injury - Injury to the endothelial lining of vessels and the heart may be caused by hypertension and damage from altered flow, bacterial endotoxins, myocardial infarct, or atherosclerotic change. Whatever the cause, the result is exposure of the subendothelial extracellular matrix and initiation of platelet attachment.



2. Abnormal patterns of blood flow - Turbulence and stasis are included in this consideration. Both injure endothelial surfaces and cause pockets of counter-currents. Areas and/or conditions that are prone to altered flow of blood include arterial bifurcation, aneurysm, narrowing of vessels, myocardial infarct, plaque, and hyperviscosity (as in sickle cell, where the altered shape of RBC's creates stasis of blood). Both turbulence and stasis disrupt laminar flow patterns. Normally, formed elements of blood (platelets and cells) will stay in the center of the vessel.

If laminar flow is disrupted, the platelets contact the wall and have better access to damaged endothelial surfaces. In addition, clotting factors may collect in stagnant areas.



3. Hypercoagulability of blood- This factor is probably the least often involved in thrombus formation but must be considered in individuals who suffer recurrent clotting problems as in deep vein thrombosis. The causes of hypercoagulability can be separated into primary (genetic) and secondary (acquired) categories. Genetic defects that cause hypercoagulability either cause increased activity of a pro- clotting factor or loss of action of an inhibitor.

Genetic:

- **Factor V mutation (Leiden Factor) -** For example mutation of Factor V causes resistance to inhibition by Protein C. Protein C cannot enzymatically cleave the mutated Factor V. This mutation is found in 2-15% of Caucasians and in 60% of individuals with recurrent DVT.

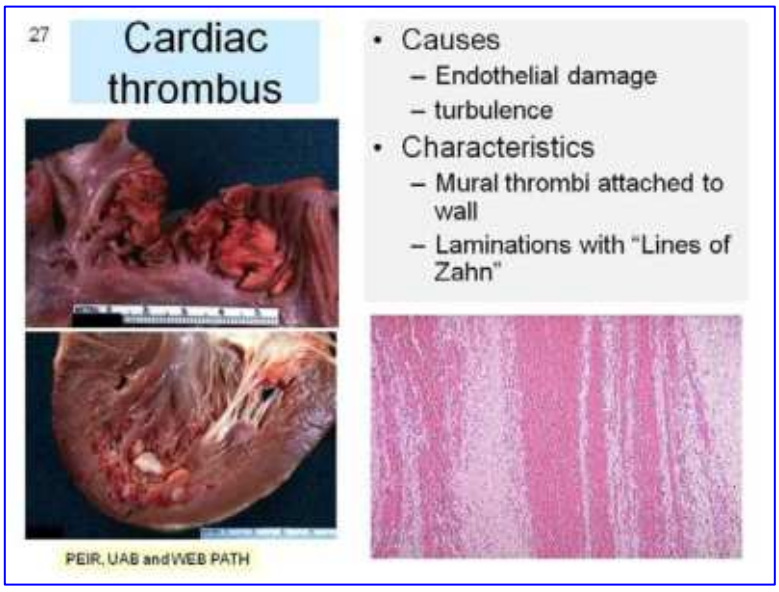
- Mutation of the prothrombin gene occurs in 1-2% of the population and results in increased transcription of prothrombin with higher levels of the protein and a three-fold increased risk of venous thrombosis.

- Other mutations cause deficiency of antithrombin III, Protein C, or Protein S which both ordinarily inhibits clotting factors.

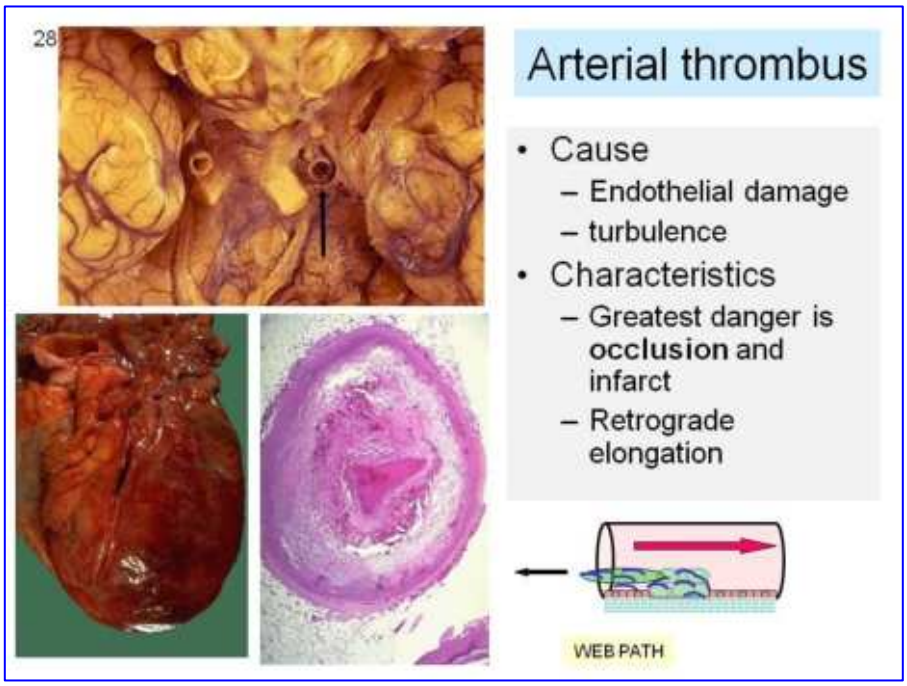
Acquired causes of hypercoagulability are diverse as are their mechanisms.

- **HIT (heparin-induced thrombocytopenia syndrome)** is an interesting treatment-induced condition. It can be initiated by administration of whole heparin to inhibit coagulation. This may result in auto-antibody formation against heparin and platelet membrane protein complexes. Subsequently the auto-antibodies then bind when platelets adhere to endothelium and activate the platelets, ultimately create a hypercoagulability state. To avoid this auto-antibody response fractionated heparin may be used to reduce clotting in patients. The low MW forms in the fractionated heparin retain anti-coagulation activity but do not bind platelets.

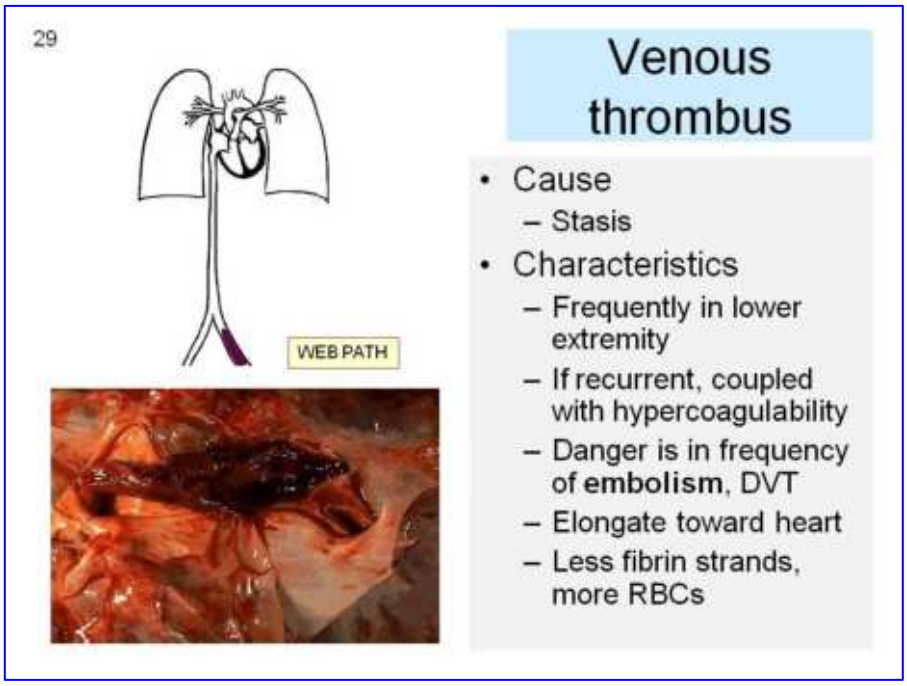
B. Types of thrombi: Thrombus formation can occur in any part of the vascular system, but location will determine the morphology of the thrombus.



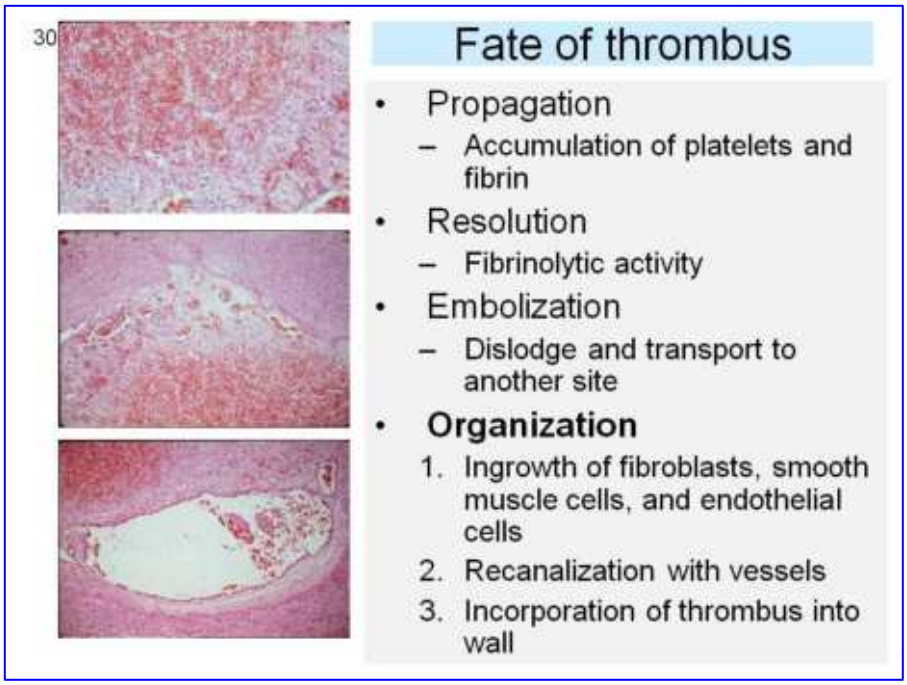
1. Cardiac thrombus - Thrombus formation in the heart is frequently caused by endothelial damage or turbulence related to myocardial infarct. In the heart the thrombus will attach to the wall, hence the name, mural thrombus. The characteristics of the mural thrombus include macroscopic and microscopically detectible Lines of Zahn. The lines or striations are formed by alternating bands of fibrin and platelets with RBC's. These are characteristic of thrombi that develop in areas of rapid blood flow so you will also see this in arterial thrombi.



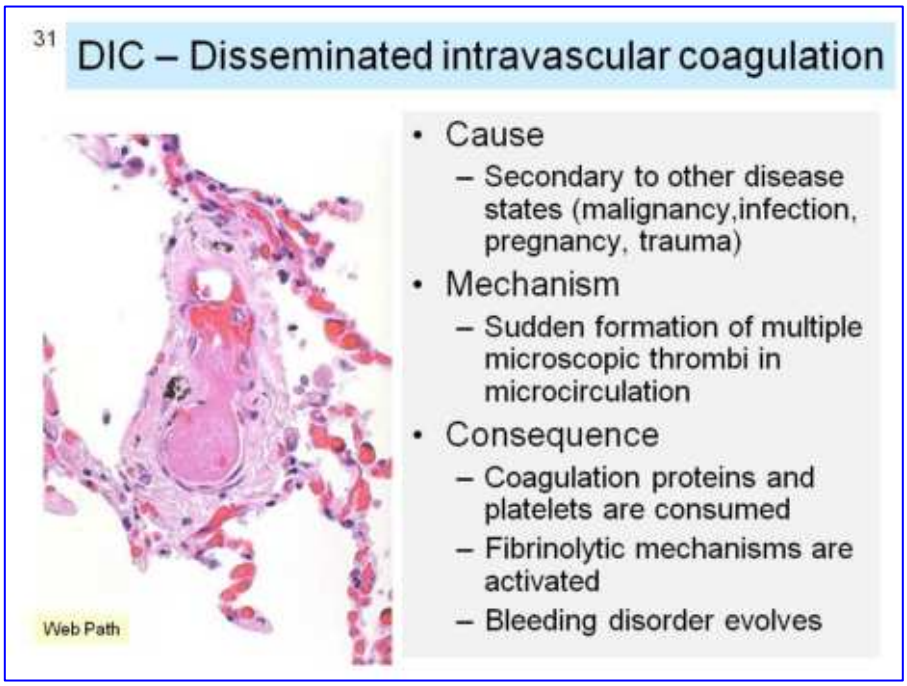
2. Arterial thrombus - These thrombi are also caused by endothelial damage or turbulence. The greatest danger of arterial thrombi is their tendency to occlude causing infarct. Embolization is less of a concern. Note that arterial thrombus growth from the attachment base is retrograde (opposite the direction of blood flow).



3. Venous thrombus - Venous thrombi are most often caused by stasis and occur frequently in the lower extremity. When they are recurrent they are often associated with hypercoagulability. The danger in venous thrombus is their tendency to embolize. Elongation of the venous thrombus is in the direction of blood flow and the venous thrombus is soft and gel-like. Because venous thrombi form in more low flow environments, the lines of Zahn may be less prominent than those of arterial thrombi. However, it is important to note that they **<u>are present</u>** as compared to a post-mortem clot that would not have lines of Zahn. Embolization of DVT to the pulmonary trunk and arteries is called a saddle embolism and is fatal.



C. Fate of the thrombus: Thrombi can continue to grow (propagate), undergo fibrinolytic resolution, embolize, or organize. You will learn more about embolization in the next lecture. Organization of the thrombus includes the infiltration of the original fibrin/platelet clot with endothelial cells, fibroblasts, and smooth muscle cells. As a result, small vessels will form within the clot and actual re-establish small flow patterns through the structure. With time the small vessel-like canals may coalesce and the thrombus may become so connective tissue-like that it is incorporated as part of the wall of the vessel.



D. Disseminated intravascular coagulation (DIC):

DIC is an interesting but often fatal complication of a number of disease states including malignancy, infection, pregnancy, or trauma. DIC involves the sudden formation of multiple microscopic thrombi in the microcirculation of all tissues and organs. Because of the widespread formation, coagulation proteins and platelets are depleted. At the same time the body activates widespread fibrinolytic activity as evidenced by high levels of circulating FSP's. The ironic consequence is that the patient will develop an uncontrolled bleeding disorder.