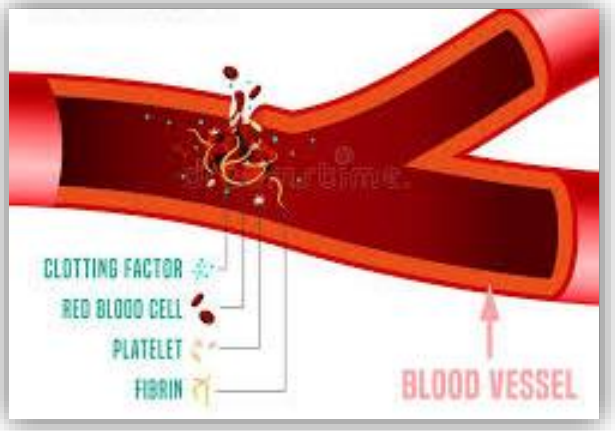
University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

Coagulation Profile:
Bleeding Time and Clotting Time Tests



Dr. Hasnaa Ahmed & Dr. Asmaa A. Ajwad

January 18th 2021

Outlines

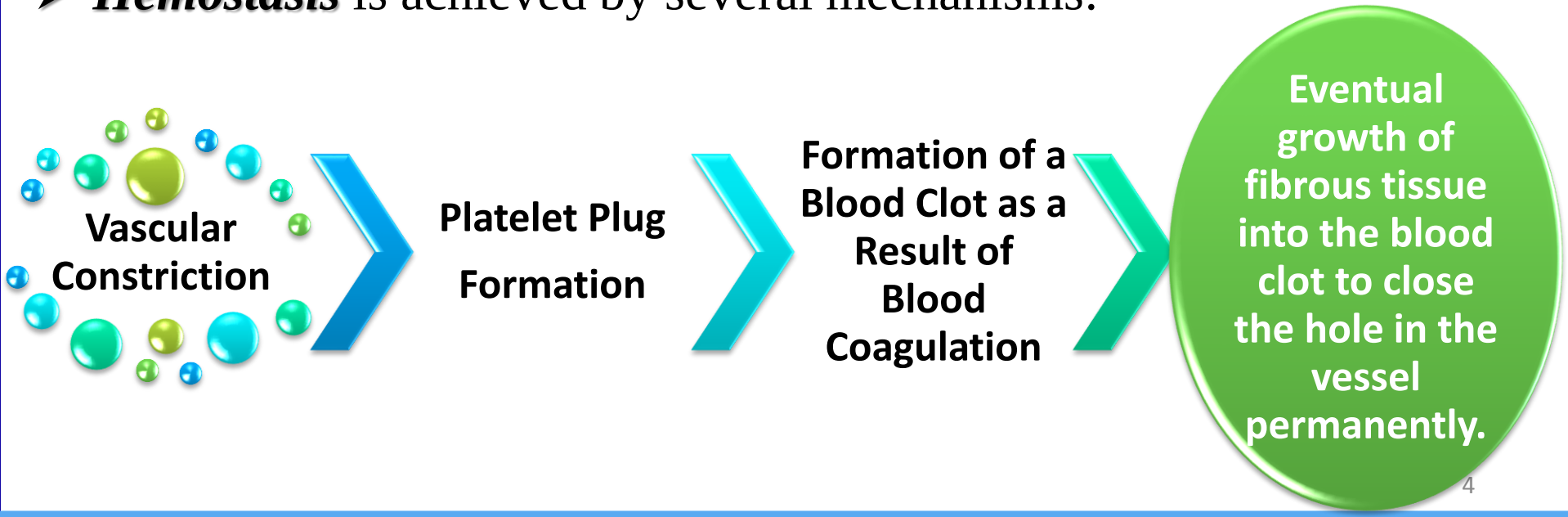
- *Hemostasis:* All you need to know about hemostasis process!
- ***Bleeding Time Test :*** Principle, Methods, Procedure, and Clinical applications.
- ***Clotting Time Test :*** Principle, Methods, Procedure, and Clinical applications.

Objectives

1. Students will learn the different phases required in hemostasis process in details.
2. Know about different methods to perform bleeding test with some clinical applications.
3. Perform coagulation test in the lab and discuss some medical implications.

Introduction

- Blood must be maintained in a fluid state in order to function as a transport system, but must be able to solidify to ***form a clot*** following vascular injury in order to prevent excessive bleeding, a process known as ***hemostasis***.
- ***Hemostasis*** is a sequence of events that leads to bleeding cessation via the formation of a fibrin-platelet hemostatic plug.
- ***Hemostasis*** is achieved by several mechanisms:



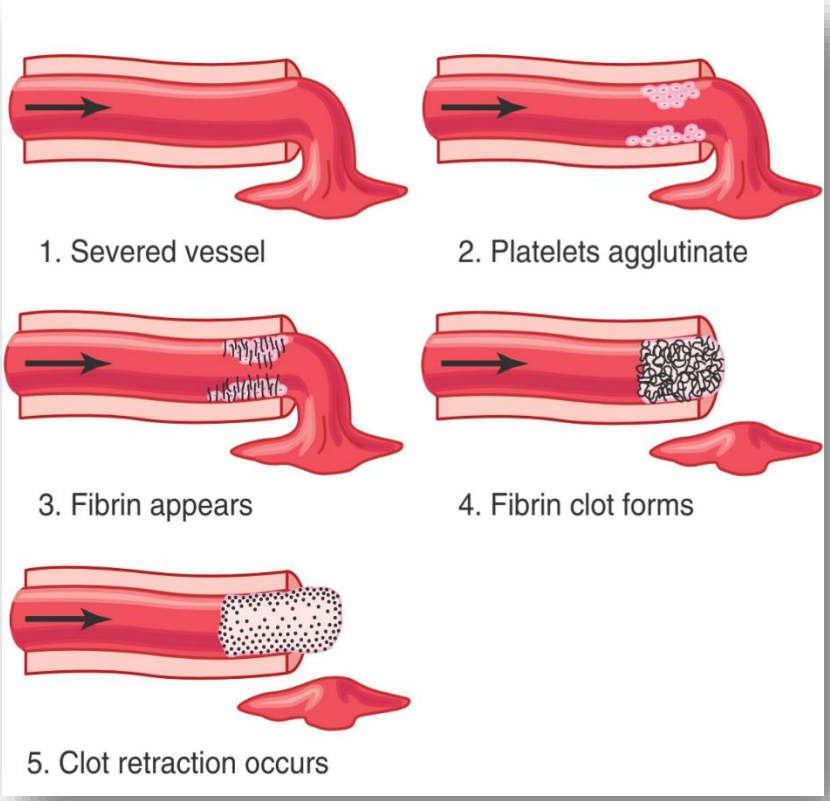
Phases of Hemostasis Process

I. Vascular Phase → **II. Platelets Phase** → **III. Coagulation Phase**

This phase is followed by:

A. Clot Retraction

B. Fibrinolysis



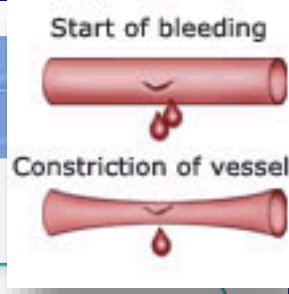
Phases of Hemostasis Process

- ***Successful hemostasis*** localized to the area of tissue damage and is followed by removal of the clot and tissue repair. This is achieved by complex interactions between the vascular endothelium, platelets, von Willebrand's factor, coagulation factors, natural anticoagulants and fibrinolytic enzymes. Dysfunction of any of these components may result in hemorrhage or thrombosis.
- ***Hemostasis process depends on :***

- Vessel Wall Integrity
- Adequate Number of Platelets
- Proper Functioning Platelets
- Adequate Levels of Clotting Factors
- Proper Function of Fibrinolytic Pathway

Now let's go over each phase of the hemostasis process:

Vascular Phase (Vascular Constriction)



- Immediately after an injury, the trauma to the vessel wall causes smooth muscle in the wall to contract; this reduces the flow of blood from the ruptured vessel.

- The contraction results from (1) local myogenic spasm,(2) local autacoid factors from the traumatized tissue and blood platelets such as thromboxane A2, and (3) nervous reflexes.

- The spasm can last for many minutes or even hours, during which the processes of platelet plug formation and blood coagulation can take place.

Platelets Phase :Platelet Plug Formation

- ***Platelets*** (also called thrombocytes) are an essential part of the blood-clotting process or hemostasis. They are not cells; rather, they are very small, irregularly shaped, non-nucleated fragments from large megakaryocytes.
- Though they do not have nuclei, In their cytoplasm there are:

- *Actin, myosin & thrombosthenin, which are contractile proteins.*
- *Mitochondria and enzyme systems for forming ATP and ADP*
- *Enzyme systems for producing prostaglandins*
- *An important protein called fibrin-stabilizing factor and*
- *Vascular endothelial, smooth muscle, and fibroblasts growth factors.*

- On the platelet cell membrane surface is a coat of glycoproteins that only adhere to injured vessel wall, especially injured endothelial cells and even more to any exposed collagen. additionally, the platelet membrane contains phospholipids.

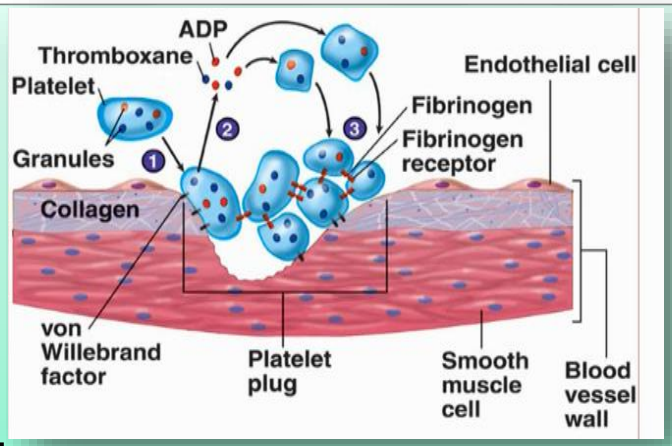
Mechanism of Platelet Plug

When platelets come in contact with a damaged vascular surface especially with collagen, they become activated:

Their contractile proteins contract and cause the release of granules that contain multiple active factors

They become sticky so they adhere to collagen and to a protein called von Willebrand factor

They secrete ADP; and their enzymes form thromboxane A2. The ADP and thromboxane in turn act on nearby platelets to activate them as well, & they adhere to the original activated platelets thus forming a platelet plug which is loose at first, but it is then reinforced by fibrin clot.



Phase of Blood Clot Formation

- Begins 30 seconds to several minutes after phases I and II have commenced. The overall process involves the formation of the insoluble protein Fibrin from the plasma protein Fibrinogen through the action of the enzyme Thrombin. Fibrin forms a network of fibers which traps blood cells and platelets forming a thrombus or clot

This phase consists of a series of reactions in which blood is transformed from a liquid to a gel. The main three steps of this series of reactions are:

- 1) Formation of Prothrombin activator complex: through intrinsic and extrinsic pathways.
- 2) Conversion of Prothrombin into thrombin.
- 3) Thrombin catalyzes the conversion of fibrinogen into a fibrin threads.

Phase of Blood Clot Formation

Coagulation (Clotting) Factors

I	Fibrinogen	Half-life (h): 96
II [K]	Prothrombin	72
III	Tissue thromboplastin	
IV	Ionized calcium (Ca^{2+})	
V	Proaccelerin	20
VII [K]	Proconvertin	5
VIII	Antihemophilic factor A	12
IX [K]	Antihemophilic factor B; plasma thromboplastin component (PTC); Christmas factor	24
X [K]	Stuart–Prower factor	30
XI	Plasma thromboplastin antecedent (PTA)	48
XII	Hageman factor	50
XIII	Fibrin-stabilizing factor (FSF)	250
–	Prekallikrein (PKK); Fletcher factor	
–	High-molecular-weight kininogen (HMK); Fitzgerald factor	

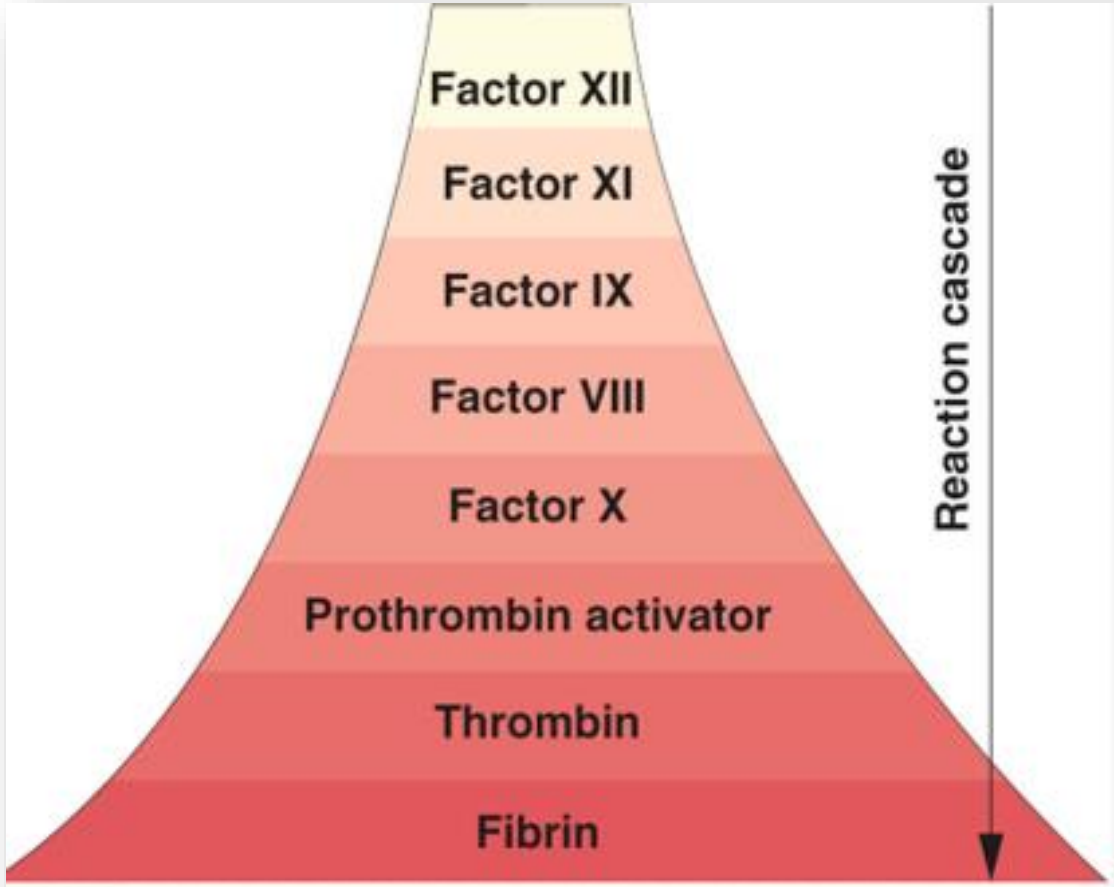
Most of them are plasma proteins (β globulin) formed in the liver

Vitamin K-dependent clotting factors are: II, VII, IX, X

Most of them are present as proenzymes (inactive)

Once activated, it induces a cascade reaction

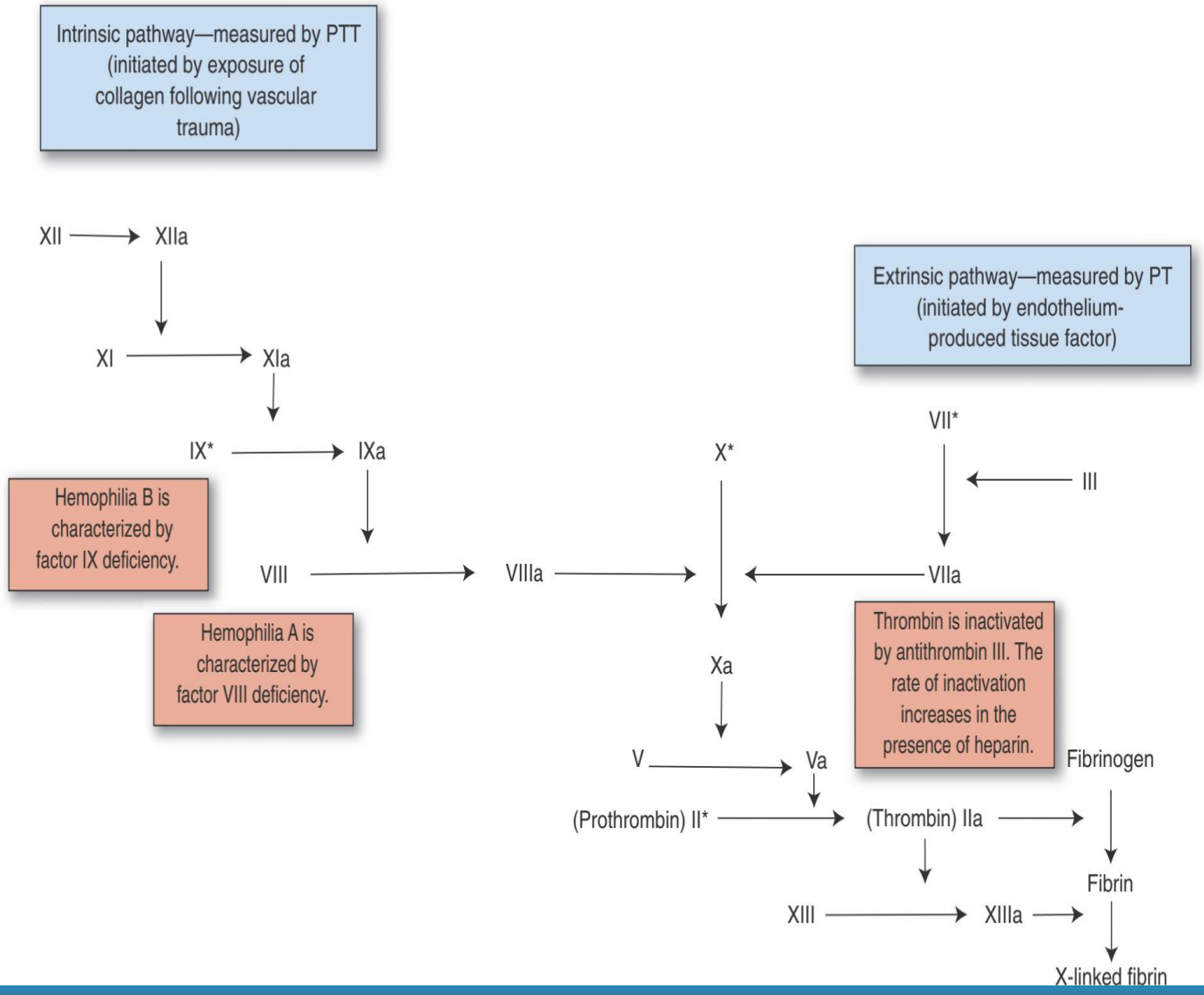
Phase of Blood Clot Formation



- In the formation of the clot, an enzyme called thrombin converts *fibrinogen* into insoluble protein, *fibrin*
- Fibrin aggregates to form a meshlike network at the site of vascular damage

Phase of Blood Clot Formation

Coagulation mechanism is composed of an extrinsic and intrinsic pathway, which eventually merge into one



Phase of Blood Clot Formation

Extrinsic pathway:

1. When blood comes in contact with injured tissue – tissue thromboplastin (F III) interacts with proconvertin (F VII), and Ca^{2+} activating Stuart factor (F X).

Intrinsic pathway:

2. Exposed collagen activates Hageman factor (F XII). Activated F XII activates plasma enzyme – plasma thromboplastin antecedent (PTA; F XI, which in the presence of Ca^{2+} activates Christmas factor (F IX). F IX interacts with antihemophilic factor (F VIII), Ca^{2+} to form a complex that activates Stuart factor (F X).

Common pathway

Activated F X in the presence of Ca^{2+} forms complexes with accelerin (F V) to form prothrombin activator

Phase of Blood Clot Formation

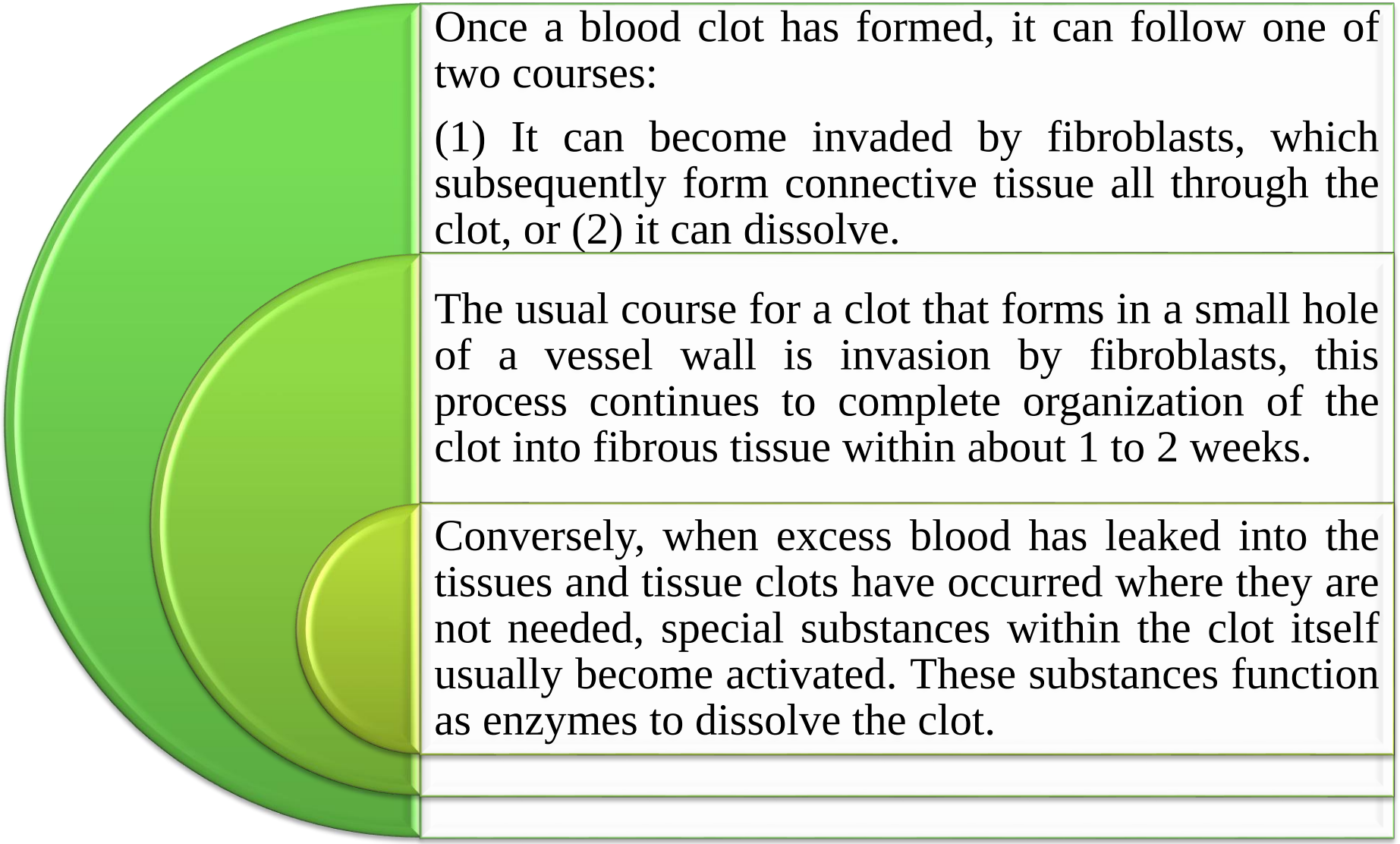
Conversion of prothrombin to thrombin

- Prothrombin - inactive precursor of enzyme thrombin
- In the presence of prothrombin activator and Ca^{2+} prothrombin is converted to thrombin
- Thrombin itself increases its own rate of formation (positive feedback mechanism)

Conversion of fibrinogen to fibrin

- Fibrinogen - plasma protein produced by the liver
- Thrombin converts fibrinogen to fibrin
- Thrombin also activates fibrin-stabilizing factor (F XIII), which in the presence of Ca^{2+}, stabilizes the fibrin polymer through covalent bonding of fibrin monomers

Fibrous Organization or Dissolution of Blood Clot



Once a blood clot has formed, it can follow one of two courses:

(1) It can become invaded by fibroblasts, which subsequently form connective tissue all through the clot, or (2) it can dissolve.

The usual course for a clot that forms in a small hole of a vessel wall is invasion by fibroblasts, this process continues to complete organization of the clot into fibrous tissue within about 1 to 2 weeks.

Conversely, when excess blood has leaked into the tissues and tissue clots have occurred where they are not needed, special substances within the clot itself usually become activated. These substances function as enzymes to dissolve the clot.

Coagulation Tests

1. **Test of the Vascular Platelet Phase of Hemostasis:**
   - ***Bleeding Time (BT) …..Today Lab***

2. **Tests of the Coagulation Cascade:**
   - ***Clotting Time (CT) or Coagulation time …..Today Lab***
   - Activated Partial Thromboplastin Time (APTT).
   - Prothrombin Time (PT).

3. **Test of Fibrinolysis and the Mechanisms That Control Hemostasis:**
   - Fibrin Degradation Products (FDP)

Bleeding Time Test

Definition

- Bleeding time (BT) is a clinical laboratory test performed to evaluate platelet function/number. It involves the creation of a standardized incision and timing the cessation of bleeding.

Depends on

The bleeding time is dependent upon:

The efficiency of tissue fluid in accelerating the coagulation process ,capillary function and the number of blood platelets present and their ability to form a platelet plug.

Prolonged bleeding time is generally found when:

- The platelet count is below 50,000/µL
- When there is platelet dysfunction

Bleeding Time Test

Applications

- A bleeding time evaluation is most helpful in a patient with clinical bleeding whose platelet count and results of coagulation studies (PT/INR, aPTT) are normal. In this setting, the BT will help recognize dysfunctional platelets.
- It is very useful as a screening test in the outpatient setting before invasive procedures, especially in patients with known hemorrhagic disorders, in order to predict the probability of perioperative bleeding.

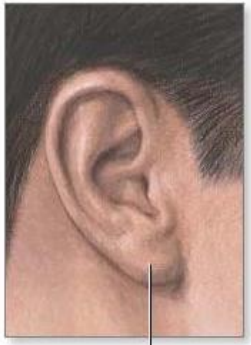
Methods

- Ivy method (more accurate)
- Duke's method ***(the one we are going to use in the lab)***

The patient should not take aspirin, NSAIDs, or alcohol for 7 days prior to the test, since they will prolong the bleeding time and lead to false-positive results.

Duke Method

- ✓ With the Duke method, the patient is pricked with a special needle or lancet, preferably on the earlobe or fingertip, after having been swabbed with alcohol.
- ✓ The prick is about 3–4 mm deep. The technician then wipes the blood every 30 seconds with a filter paper.
- ✓ The test ceases when bleeding ceases.
- ✓ Normal range is 2-6 minutes. The usual time is about 1–3 minutes.
- ✓ The test causes nervousness in the patient.
- ✓ This test method is the easiest to perform, but is the least standardized and has the less precision and accuracy.

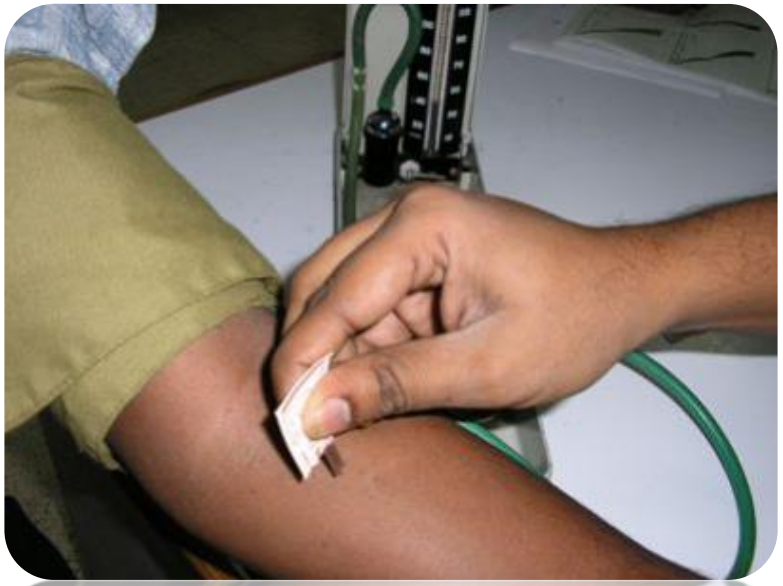

Normal ear lobe

Ivy Method

- A blood pressure cuff is placed on the upper arm & inflated to 40 mmHg.
- A lancet or scalpel blade is used to make a stab wound (1 mm deep and 10 mm long) on the forearm (avoid superficial or visible veins, because they may have longer bleeding times due to their size).
- A stopwatch starts recording time.
- Every 30 seconds, a filter paper is applied gently over the wound. Whenever the paper absorbs blood, it means that the bleeding is active and has not stopped. This is repeated every 30 seconds until the bleeding stops completely (ie, no more blood is being absorbed by the filter). After the bleeding stops, the blood pressure cuff should be deflated. The bleeding time is defined as the time from the incision until all bleeding has stopped.
- *The normal BT for the Ivy method* is less than 8 minutes.

Ivy Method

- ✓ A prolonged BT may be a result from decreased number of thrombocytes or impaired blood vessels. However, it should also be noted that the depth of the puncture or incision may be the source of error.
- ✓ The greatest source of variation in this test is largely due to difficulty in performing a standardized puncture. This usually leads to erroneously low results.



Interpretation of Bleeding Time Results

A bleeding time evaluation is used to measure the primary phase of hemostasis, which involves platelet adherence to injured capillaries and then platelet activation and aggregation. The bleeding time can be abnormal when the platelet count is low or the platelets are dysfunctional. Causes of abnormal bleeding time can be hereditary or acquired.

Hereditary Causes	Acquired Causes
• Von Willebrand disease • Glanzmann thrombasthenia (GP IIb/IIIa deficiency) • Bernard-Soulier syndrome (GP1b deficiency) • Connective tissue diseases like Ehlor-Danlos syndrome & hereditary hemorrhagic telangiectasia.	• Medications like aspirin, nonsteroidal anti-inflammatory drugs [NSAIDs], anticoagulants [eg, heparin, streptokinase] • Vitamin C deficiency • Uremia • Liver failure • Leukemias • Myelodysplastic syndromes

Clotting (Coagulation)Time

Definition

- It is the time required for a sample of blood to coagulate in vitro (outside the vascular system) under standard conditions.

Principle

- The basis for this test is that the whole blood will form a solid clot when exposed to a foreign surface such as a glass tube.

- It is a rough measure of all intrinsic clotting factors in the absence of tissue factor.

Methods of Clotting Time Test

- ***There are two methods:***

1. Glass Container Method (Lee and White Method)
2. Capillary Tube Method

1. Glass Container Method

- ***Materials:*** Syringe for withdrawal of blood, glass test tube, stopwatch, and water bath (37°C)

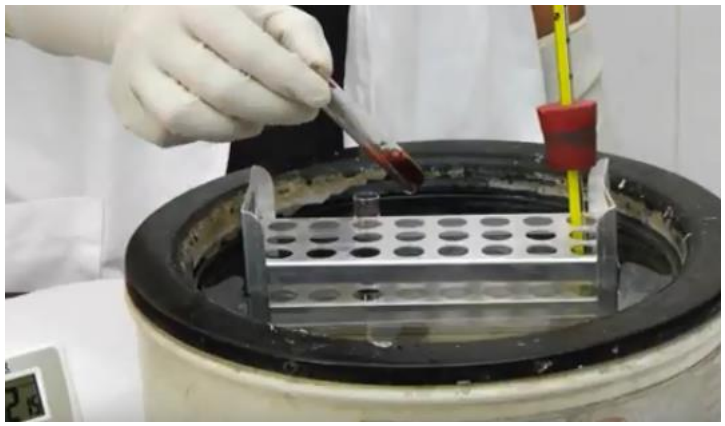
- ***Specimen:*** Fresh whole blood (4 ml).

Procedure of Glass Container Method

- Perform a venipuncture and withdraw 4 ml of blood, use 3 test tubes & fill each of them with 1 ml of blood.
- Start the stopwatch as soon as the blood enters the syringe.
- Place the three test tubes in the 37 C water bath.
- At exactly 3 minute, remove the first tube from the water bath and tilt it gently to 45 angle to see if the blood has clotted.
- If there is no clotting, return it to the water bath and check again at 30 seconds intervals.
- If the blood in the first tube has clotted, examine the second tube immediately and then the third one.
- Record the time it took the blood in the third tube to clot.
- *Normal range: 5-15 min.*
- It is important to control the temperature because the clotting time increases with the temperature. It is about twice as fast at 37°C than that at room temperature of 20°C.

Procedure of Glass Container Method

- The size of the test tube must be standardized, blood clots faster in the narrow tubes (*why ??? Think about it*)
- At the end of the test, one tube should remain in the water bath to be checked for clot retraction, also it may be allowed to remain in the water bath overnight and checked the next day for clot lysis.



Methods of Clotting Time Test

2. Capillary Tube Method

Principle

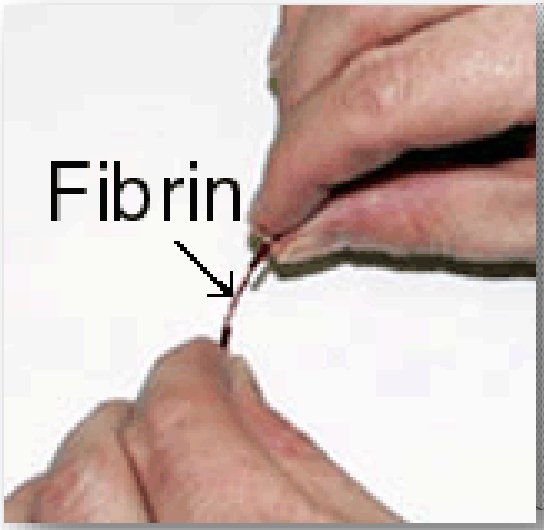
- Puncture the skin, blood is taken to a plain capillary tube and stop watch started.
- Formation of fibrin strings is noted by breaking the capillary tube at regular intervals.
- The time taken for the first appearance of the fibrin string is recorded.

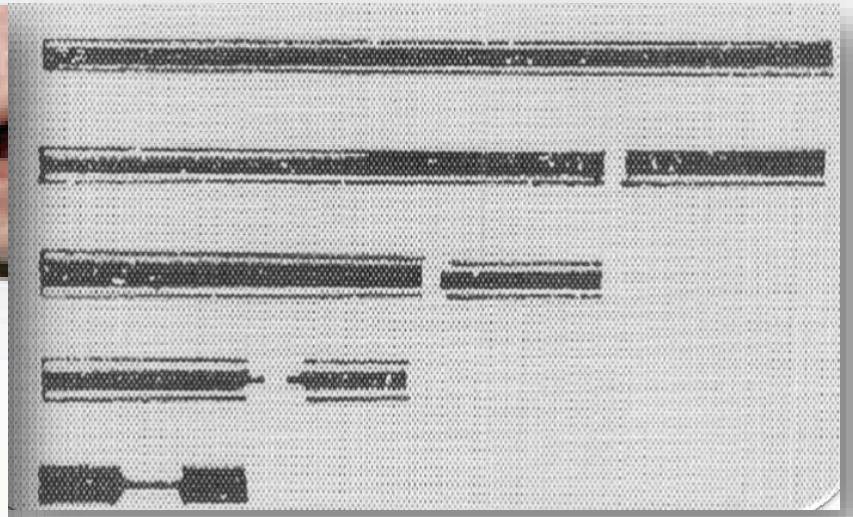
Materials

- Cotton ,alcohol, and Stop watch.
- Sterile disposable pricking needle or lancet.
- Dry glass capillary tube (narrow diameter top 2 mm, minimum 10 cm long).The tube is opened at the two ends with a blue ring (non heparinized) at one end.

Capillary Tube Method

- Clean the finger with alcohol and allow it to dry, start the stopwatch then load a capillary tube to at least ½ full.
- After about 1 to 5 minutes, take the tube between thumb and forefinger and break it gently in half, pull the ends apart to view the fibrin strands. If you don't see any strands, wait a little longer and break the tube again, usually we do a break every 30 seconds.
- Once the clot is formed, record the time.
- *Normal range is < 6 minutes.*



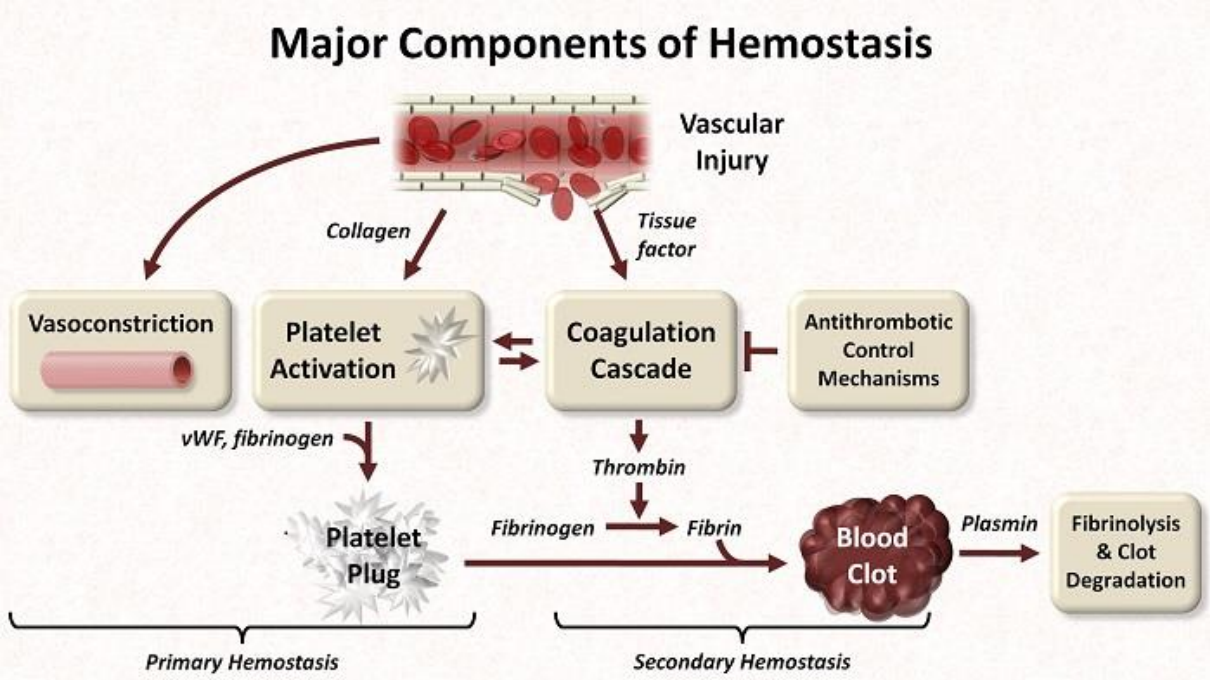


Some Medical Considerations

- Mechanism Involved is ***INTRINSIC*** Pathway.
- Clotting Time (CT) depends on presence of all clotting factors.

- ***Prolongation of clotting time may be due to:***
- Deficiencies of clotting factors like hemophilia A (factor 8 deficiency) and hemophilia B (factor 9 deficiency).
- Vitamin K deficiency
- Liver failure

- BT & CT are measured before surgery & liver or bone marrow biopsy.
- ***PURPURA :*** BT increased, CT normal.
- ***HEMOPHILIA :*** BT normal, CT increased.



Remember that:

1. People with von Willebrand disease usually experience increased bleeding time.
2. Von Willebrand factor is a platelet agglutination protein, but this is not considered an effective diagnostic test for this condition.

Thanks For Your Attention