

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)



HLS Physiology | MID 7

Blood Clotting



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Omaima hjaj

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بسم الله الرحمن الرحيم،

نرجو منكم الدعاء لزميلنا في الدفعة
عون العدوان، الذي وافته المنية.

اللهم ارحمه واغفر له، وأسكنه فسيح جناتك، وأنر قبره وأنس وحشته، واجعل قبره روضةً من رياض الجنة.

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رحمه الله رحمةً واسعةً وأسكنه الفردوس الأعلى من الجنة...

Hemostasis

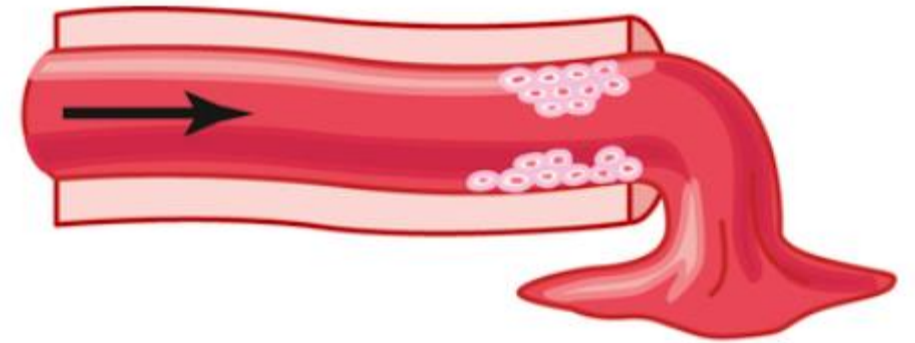
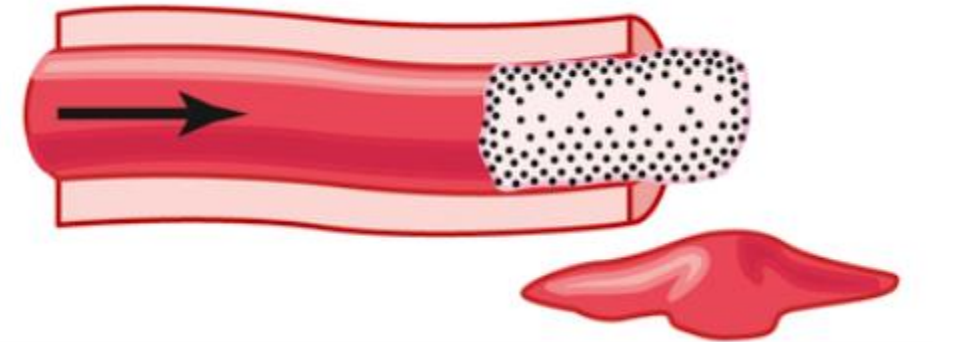
Events of hemostasis



Hemostasis means prevention of blood loss and includes the following events:

It is composed of multiple steps, but ultimately, all these systems work together in a synergistic and coordinated way.

1. Vascular constriction. (**Vasoconstriction**)
2. Formation of a platelet plug.
3. Formation of a blood clot as a result of blood coagulation. (**clotting**)
4. Fibrous organization or dissolution of the blood clot **Fibinolysis (Clot Dissolution)**



Hemostasis

Events of hemostasis



❑ Step 1: Vascular Constriction (Vasoconstriction):

- **Mechanism:** When a blood vessel (especially a small blood vessel or arteriole) is damaged or injured, it immediately constricts.
- **Effect:** This constriction decreases the diameter of the blood vessel, which in turn decreases blood flow.
- **Causes of Vasoconstriction:** Local myogenic spasm, Local nervous reflexes, Factors released from injured tissues and platelets (such as Thromboxane A₂).

❑ Step 2: Platelet Plug Formation:

- **Overview:** Platelets play a major role in forming a plug to seal the vascular injury.
- **Sub-steps of Platelet Plug Formation:**
 1. **Platelet Adhesion:** The attachment of platelets to exposed collagen and other damaged structures in the vessel wall.
Involves von Willebrand factor (vWF) and platelet receptors binding to subendothelial components.
 2. **Platelet Activation:** Activated platelets release their granule contents (such as ADP, Thromboxane A₂, and Calcium ions).
 3. **Platelet Aggregation:** The attachment of platelets to each other (platelet-to-platelet adhesion) as well as to the endothelium (adhesion).

Collectively, these processes form the platelet plug.



❑ Step 3: Blood Coagulation (Clotting) & The Clotting Cascade:

- **Definition of a Blood Clot:** A blood clot is composed of a **fibrin mesh network** (fibers) in which blood cells (such as Red Blood Cells – RBCs, platelets, and plasma proteins) are trapped.
- **The Clotting Cascade:**
 - ✓ Coagulation occurs via a sequence (cascade) of reactions where inactive clotting factors are sequentially activated (e.g., factor X becomes active, turning another inactive factor to active, and so on) until the final product is formed.
- **Source & Synthesis of Clotting Factors:**
 - ✓ Most clotting factors are plasma proteins synthesized primarily by the **liver**.
 - ✓ They circulate in the blood in an **inactive form** under normal, healthy physiological conditions (without injury).
 - ✓ When an injury occurs, a specific signal triggers the cascade, turning inactive factors into active forms successively until reaching the final product: **Fibrin** (fibrin fibers).

Hemostasis

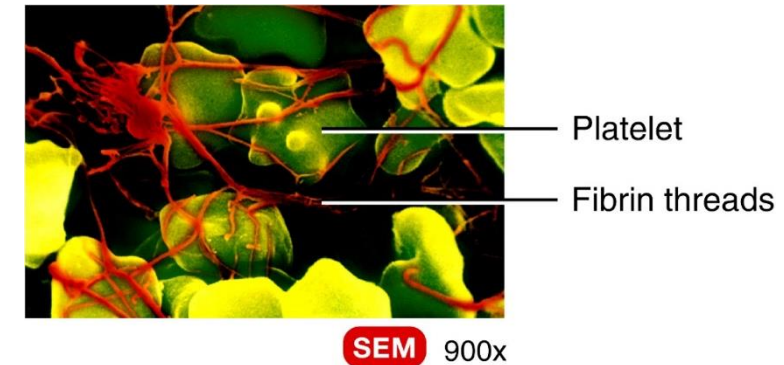
Blood clot

- Series of chemical reactions culminating in formation of fibrin threads.
- Clotting (coagulation) factors – Ca^{2+} , several inactive enzymes, various molecules associated with platelets or released by damaged tissues.

**** The clot begins to develop in 15 to 20 seconds if the trauma to the vascular wall is severe and in 1 to 2 minutes if the trauma is minor.**

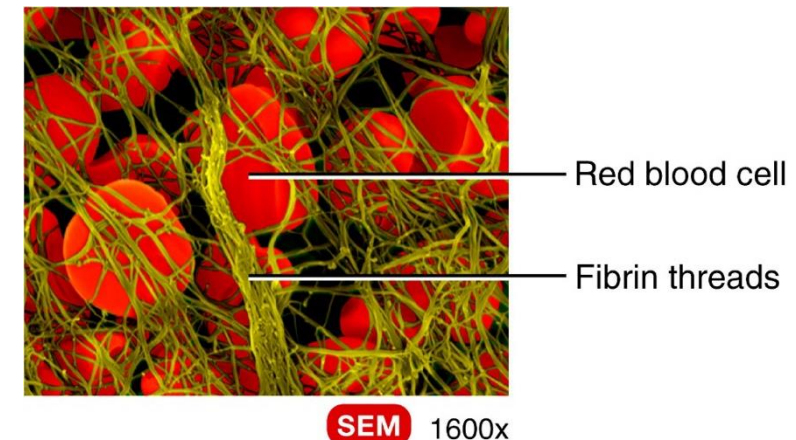
**** Within 3 to 6 minutes after rupture of a vessel, the entire opening or broken end of the vessel is filled with clot if the vessel opening is not too large.**

**** After 20 to 60 minutes, the clot retracts, which closes the vessel still further.**



(a) Early stage

Figure 19.10a Tortora - PAP 12/e
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(d) Red blood cells trapped in fibrin threads

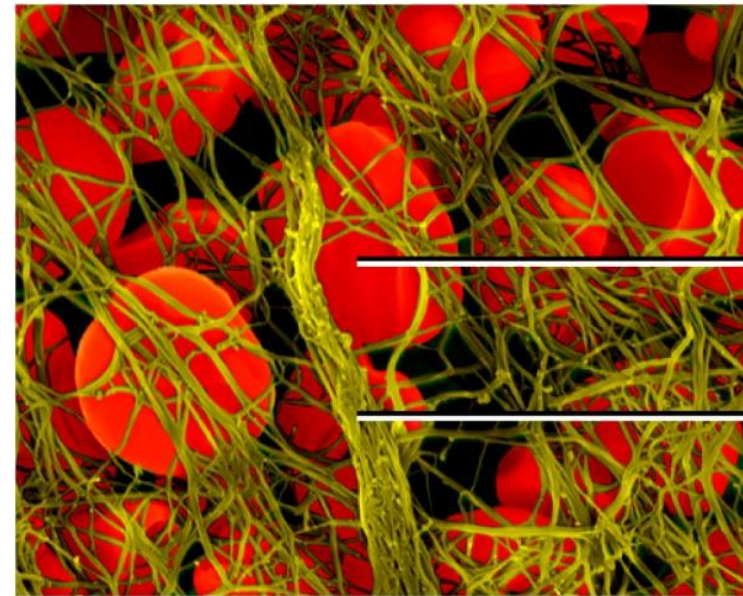
Figure 19.10d Tortora - PAP 12/e
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Hemostasis

Blood clot

- A **meshwork of fibrin** fibers running in all directions and entrapping **blood cells, platelets, and plasma**.
- The fibrin fibers also **adhere to damaged surfaces** of blood vessels, thereby **prevents** further **blood loss**.

A successful hemostatic response must achieve three things: it must act rapidly, remain localized to the damaged area, and eventually allow the clot to be removed as the vessel heals.



Red blood cell

Fibrin threads

SEM 1600x

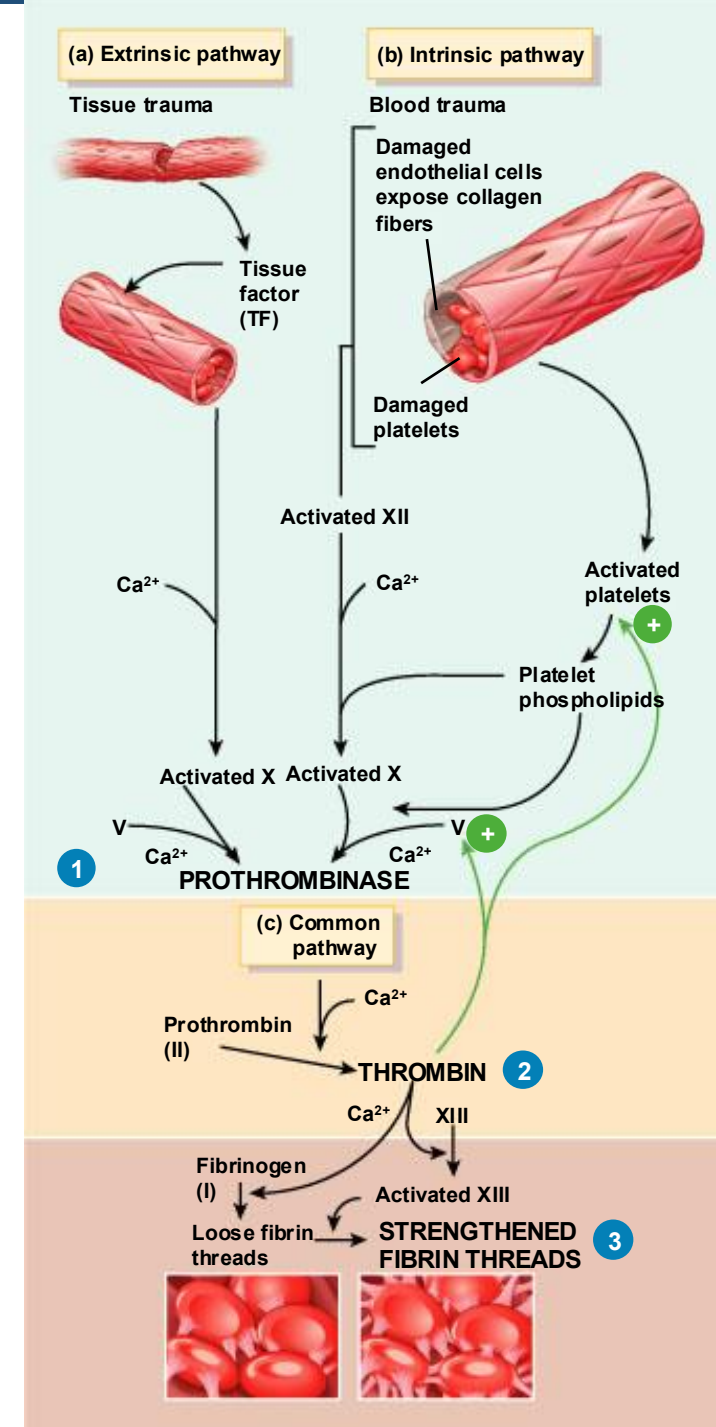
(d) Red blood cells trapped in fibrin threads

Figure 19.10d Tortora - PAP 12/e
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Hemostasis

Blood coagulation

1. **Extrinsic or intrinsic pathways** lead to formation of **prothrombinase**.
2. Prothrombinase converts **prothrombin** into **thrombin**
3. Thrombin converts **fibrinogen** (soluble) into **fibrin** (insoluble) forming the threads of the clot



Hemostasis

Blood coagulation

Blood coagulation involves a cascade of pathways that ultimately lead to the **Common Pathway**.

The main pathways are (**Extrinsic Pathway** / **Intrinsic Pathway** / **common pathway**)

Common Pathway (The central/core pathway where both extrinsic and intrinsic pathways merge)

Coagulation Definition: Refers to the coagulation of the blood.

Extrinsic: Triggered by something **outside** of the blood.

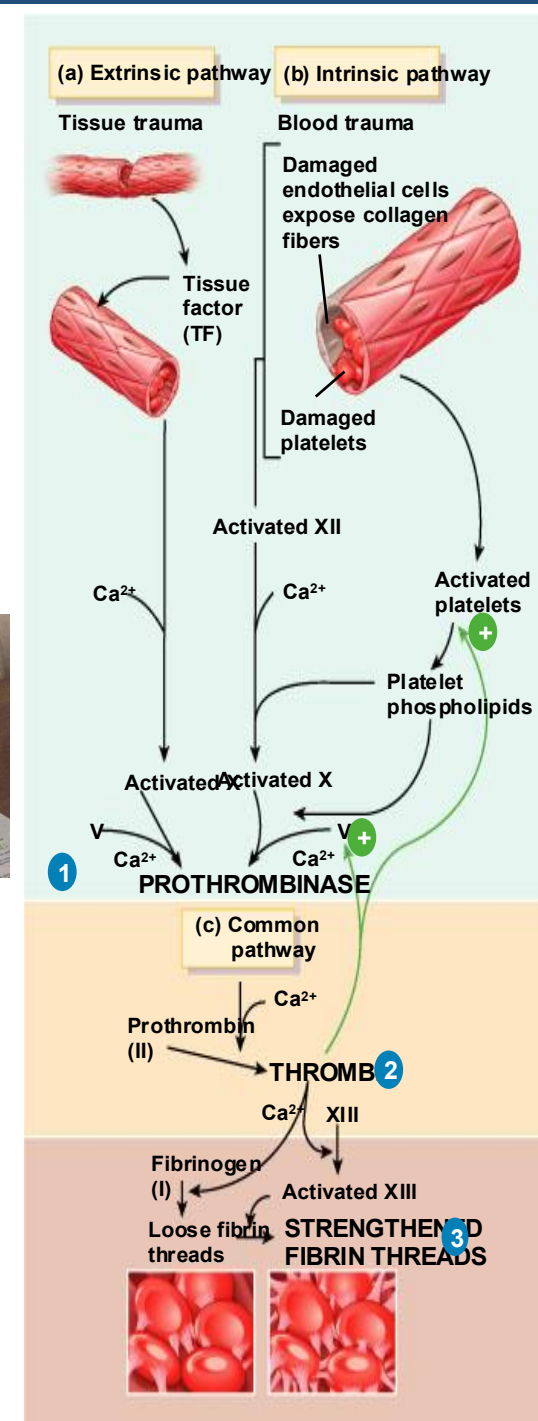
Intrinsic: Triggered by something **within** the blood itself.



1. Extrinsic Pathway (Tissue-Initiated)

Trigger: Triggered by tissue injury. When tissue surrounding blood vessels is damaged (tissue injury), it releases **Tissue Factor** (also known as tissue thromboplastin), which is a complex of phospholipid and membrane protein.

Process: Because the signal originates outside the blood (from the surrounding tissues), it initiates the **Extrinsic Pathway**.



Hemostasis

Blood coagulation



2. Intrinsic Pathway (Blood Trauma-Initiated):

Trigger: Triggered by blood trauma (it can happen without physical trauma).

Mechanism: Normally, platelets, red blood cells (RBCs), and white blood cells (WBCs) flow smoothly through the circulation. The innermost layer lining the blood vessels is the **endothelium** (consisting of smooth endothelial cells).

Activation: Anything that alters or disrupts the smooth endothelial lining—exposing the underlying subendothelial collagen to the blood—constitutes a trigger.

Alternative Triggers: Certain factors produced by the liver in an inactive form can also cause changes upon interacting with injured endothelium, initiating the pathway.

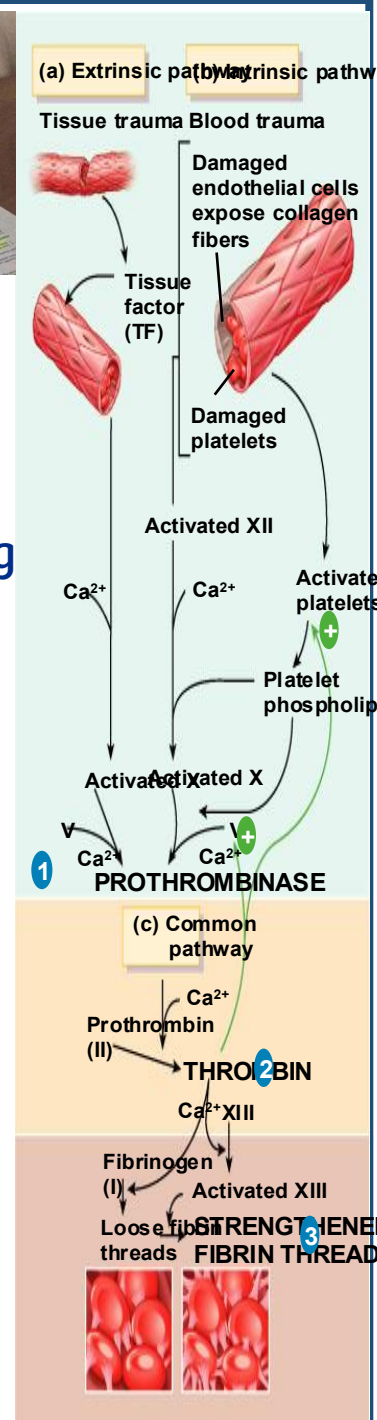
Summary: Both pathways ultimately merge to activate the common pathway. While in-vitro (laboratory) tests can distinguish and measure each factor individually, in vivo they are highly interconnected.

✓ The Prothrombinase Complex & Conversion to Thrombin

Prothrombinase (Factor X activator): Not just a single enzyme, but a **complex** (often referred to as prothrombinase or prothrombin activator).

Function: Prothrombinase acts on **prothrombin** (inactive) and converts it into **thrombin** (active).

Role of Thrombin: Thrombin serves as the central enzyme (the core) of the coagulation pathway.



****In both the extrinsic and the intrinsic pathways, a series of different plasma proteins called blood-clotting factors plays a major role.**

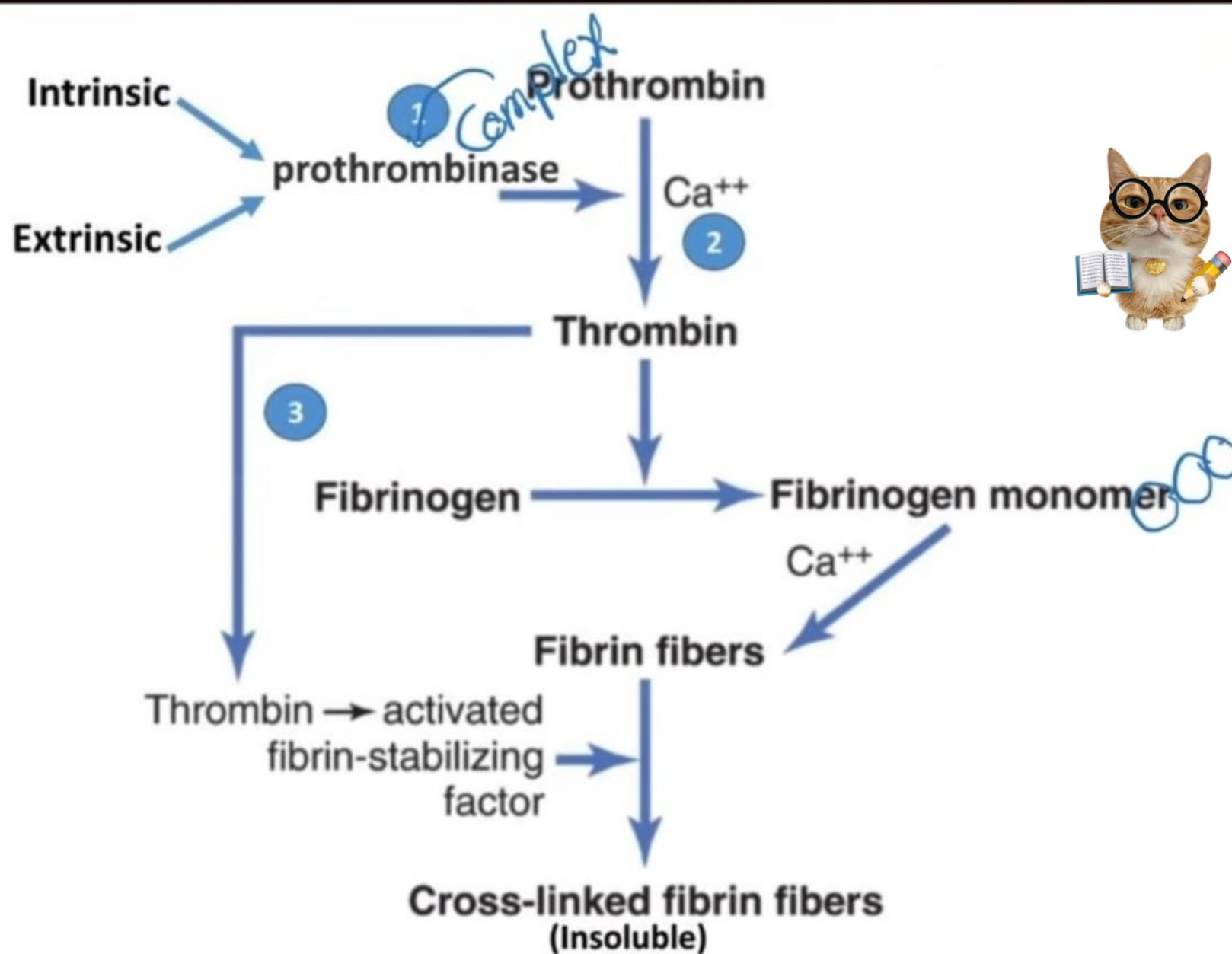
****Most of these proteins are inactive forms of proteolytic enzymes.**

When converted to the active forms, their enzymatic actions cause the successive, cascading reactions of the clotting process

****These factors are designated by roman numerals in the order in which the factors were discovered, not the order in which they participate in the clotting process.**

****Prothrombin activator is generally considered to be formed in two ways, although, in reality, the two ways interact constantly with each other.**





** Thus, the rate-limiting factor in causing blood coagulation is usually the formation of prothrombin activator and not the subsequent reactions beyond that point, because these terminal steps normally occur rapidly to form the clot.

** This cross-linkage is catalyzed by a clotting factor known as factor XIII (fibrin-stabilizing factor)

❑ Formation of Fibrin Mesh (Final Steps)

Fibrinogen to Fibrin: Thrombin acts on **fibrinogen** (a large, high-molecular-weight protein present in blood circulation).

Monomer Production: Thrombin performs proteolysis on fibrinogen, cleaving it into **fibrin monomers**.

Spontaneous Polymerization: Once converted into monomers, these molecules spontaneously aggregate (bonds form between them) to produce polymers, forming loose fibrin strands.

Stabilization (Cross-Linking):

Monomers alone in a loose state are unstable (analogous to loose matchsticks that easily scatter).

To make the network strong and stable, an active enzyme called **Factor XIIIa (Activated Fibrin-Stabilizing Factor)** steps in.

Factor XIIIa creates covalent bonds between the fibrin monomers, cross-linking the fibrin fibers together to form a tight, stable mesh (the final clot network).

Role of Platelets in Coagulation:

Platelets actively contribute to the coagulation pathway.

Without platelets and the **Factor XIII-fibrin clot complex**, proper blood clotting cannot be fully achieved.

❑ Naming and Numbering of Coagulation Factors

Coagulation factors are numbered using Roman numerals (Factor I through Factor XIII, noting that Factor VI was later reclassified/removed, so it goes from V to VII).

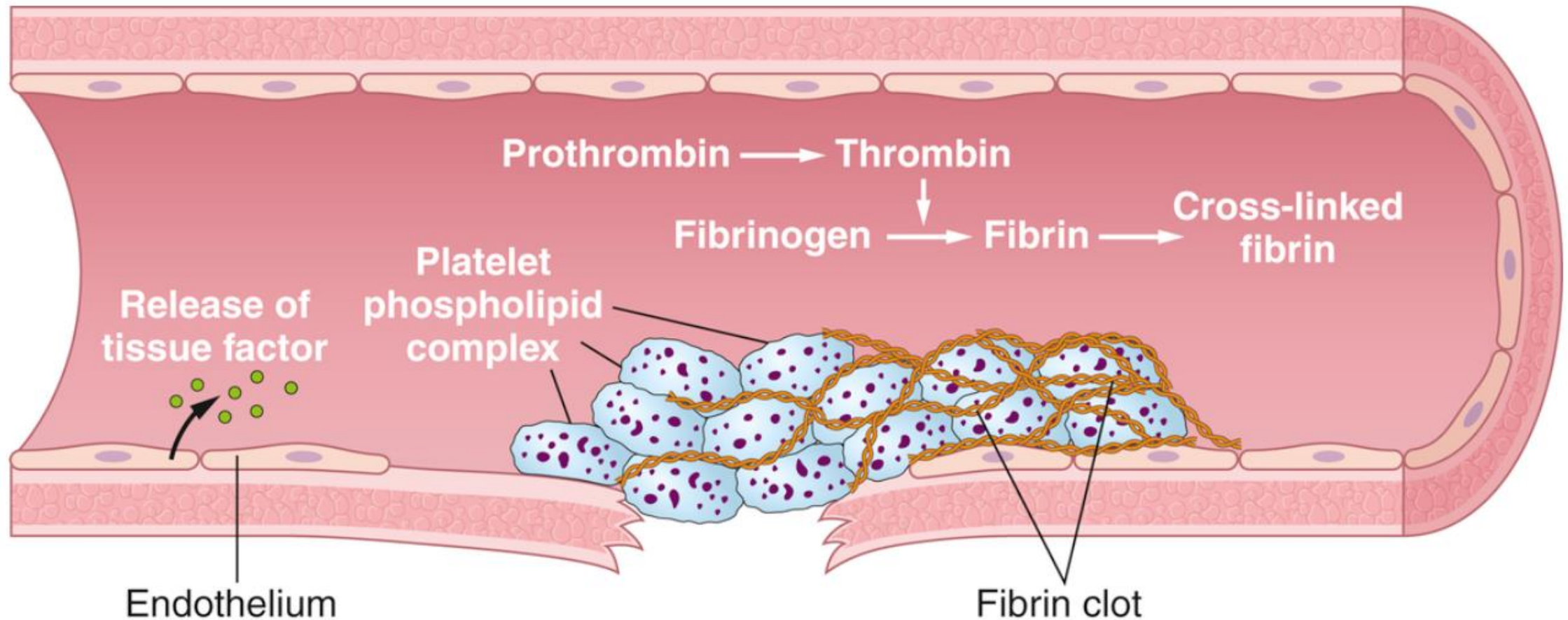
Many factors also have common descriptive names (e.g., Factor I is Fibrinogen, Factor II is Prothrombin, etc.).

doc prefers using names over numbers when possible, as names often hint at the protein's physiological function, making them easier to remember, though clinical contexts or exams may utilize numbers.



Hemostasis

Common pathway



Hemostasis

Prothrombin and Thrombin

Prothrombin is a plasma protein (**α 2-globulin**).

It is an **unstable protein** that can split easily into smaller compounds, one of which is thrombin (half MW).

Prothrombin is **formed continually** by the **liver**, and it is **continually** being **used** throughout the body for blood clotting.

Vitamin K is required by the liver for normal activation of prothrombin, as well as a few other clotting factors.

Vitamin K is important for the synthesis of certain clotting factors, which are factors **II, VII, IX, and X**.

These are the **vitamin K dependent coagulation factors**. The doctor mentioned that remembering them as “1972” is easier

If there is a vitamin K deficiency, the levels of all these clotting factors will decrease.

Much of the prothrombin first attaches to **prothrombin receptors** on the **platelets** already bound to the damaged tissue.

- Thrombin is an enzyme with weak proteolytic capabilities.
- It acts on **fibrinogen** to remove four low-molecular-weight peptides from each molecule of fibrinogen, forming one molecule of **fibrin monomer**.
- Fibrin monomer has **the automatic capability** to **polymerize** with other fibrin monomer molecules to form **fibrin fibers**.

- In the early stages of polymerization, the fibrin monomer molecules are held together by **weak noncovalent** hydrogen bonding (weak clot).
- **Fibrin-stabilizing factor** that is present in small amounts in normal plasma **globulins** but is also released from **platelets** entrapped in the clot (**inactive**).
- **Thrombin** activates the fibrin-stabilizing factor which cause **covalent bonds** between more and more of the fibrin monomer molecules, as well as **multiple cross-linkages** between adjacent fibrin fibers, thus adding **strength** of the fibrin meshwork.

Hemostasis

Fibrinogen

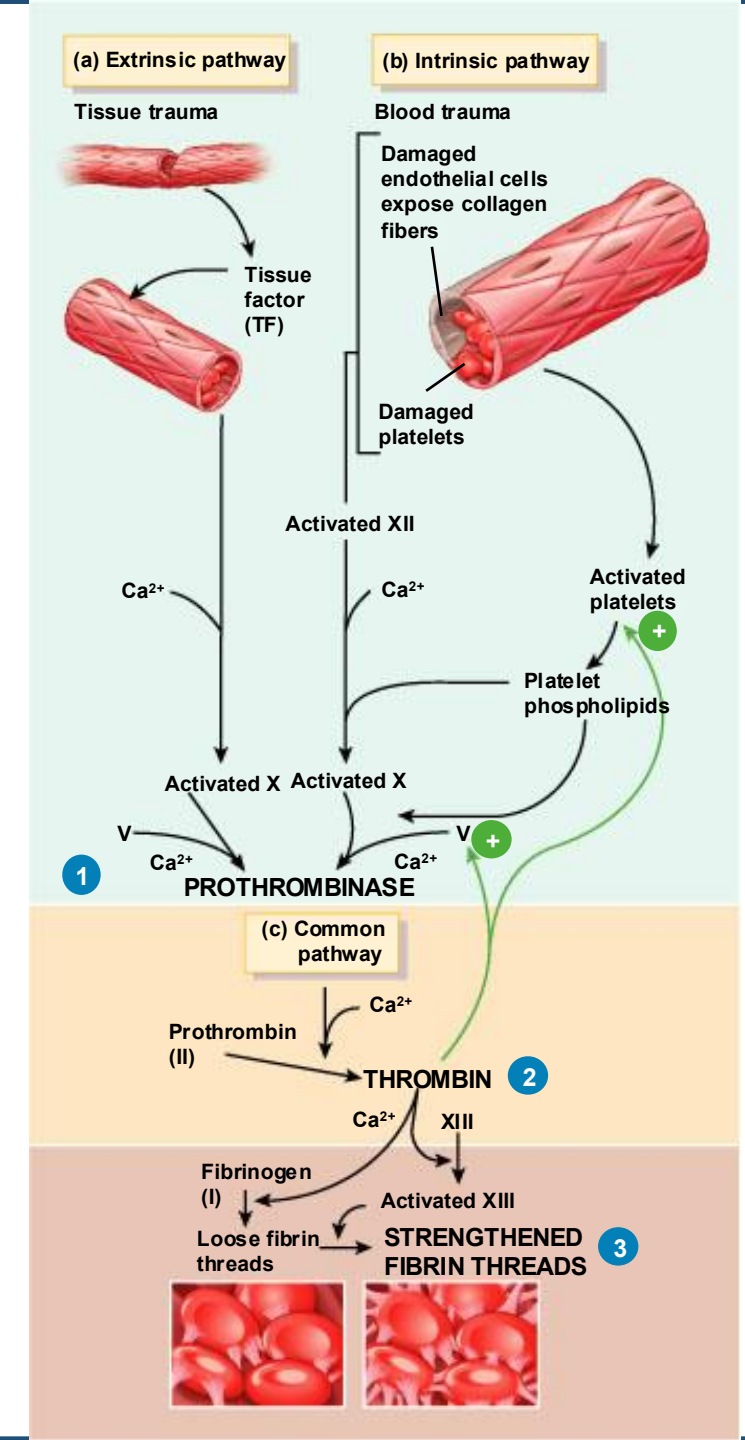
- Fibrinogen is a high-molecular-weight protein that occurs in the **plasma**.
- Fibrinogen is **formed in the liver**.
- Because of its **large molecular size**, little fibrinogen normally leaks from the blood vessels into the interstitial fluids, and because fibrinogen is one of the essential factors in the coagulation process, **interstitial fluids ordinarily do not coagulate**. Fibrinogen is normally present in the circulation, so coagulation usually occurs within the intravascular system. However, in cases of major tissue damage, fibrinogen may leak into the interstitial fluid.

****Yet, when the permeability of the capillaries becomes pathologically increased, fibrinogen does then leak into the tissue fluids in sufficient quantities to allow clotting of these fluids in much the same way that plasma and whole blood can clot.**

Hemostasis

Blood coagulation

- Prothrombin activator is generally considered to be formed in two ways:
 - (1) by the **extrinsic pathway** that begins with trauma to the vascular wall and surrounding tissues
 - (2) by the **intrinsic pathway** that begins in the blood itself.



Common Pathway: Prothrombinase (Prothrombin Activator)

When we discussed the common pathway, we mentioned that we need to reach a stage called prothrombinase, or prothrombin activator. This is the rate-limiting step in the coagulation process. Once prothrombin activator is formed, it drives the coagulation cascade forward, leading to the formation of fibrin fibers.

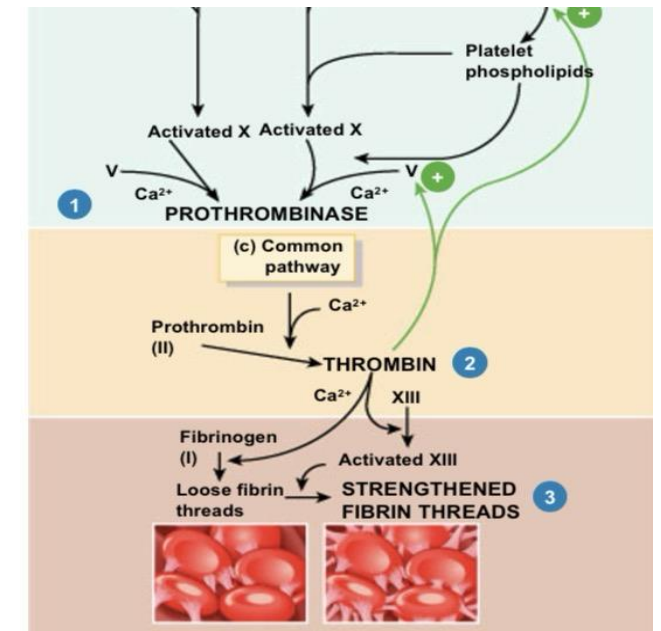
The prothrombinase complex consists of:

- Activated factor X (Xa)
- Factor V
- Calcium ions (Ca^{2+})
- Platelet phospholipids

Which Component Is Responsible for the Actual Proteolytic Activity?

The actual proteolytic activity of the prothrombinase complex is carried out by activated factor X (Xa) **Why?** Because factor X is activated, whereas factor V initially acts as a cofactor in the complex. Once prothrombinase is formed, activated factor X converts prothrombin into thrombin. Thrombin then activates factor V, enhancing its activity as a cofactor. The activated factor V joins the prothrombinase complex and increases its ability to convert more prothrombin into thrombin.

This creates a positive feedback loop, in which thrombin promotes further thrombin generation, accelerating the coagulation process.



Ca^{2+} and platelet phospholipids provide the surface on which coagulation takes place.

This is the second role of platelets in coagulation. The first was related to factor XIII, the fibrin-stabilizing factor

*Extrinsic pathway

Fewer steps than intrinsic and occurs rapidly

Tissue factor (TF) leaks into the blood from cells *outside (extrinsic to)* blood vessels and initiates formation of prothrombinase

*Intrinsic pathway

More complex and slower than extrinsic

Activators are either in direct contact with blood or contained *within (intrinsic to)* the blood

Outside tissue damage not needed

Also forms prothrombinase

**The intrinsic and extrinsic mechanisms usually operate simultaneously. When a blood vessel ruptures during tissue injury, the intrinsic mechanism stops blood in the injured vessel, and the extrinsic mechanism clots blood that escaped into the tissue before the vessel was sealed off. Typically, clots are fully formed in 3 to 6 minutes.

** the extrinsic pathway can be explosive, limited only by the amount of **tissue factor** released from the traumatized tissues and by the **quantities of Factors X, VII, and V in the blood.**

Hemostasis

3. Extrinsic pathway

Tissue factor is a protein associated with the phospholipid part of the cell membrane. When tissue damage occurs, tissue factor is exposed and acts as the trigger for the extrinsic pathway.

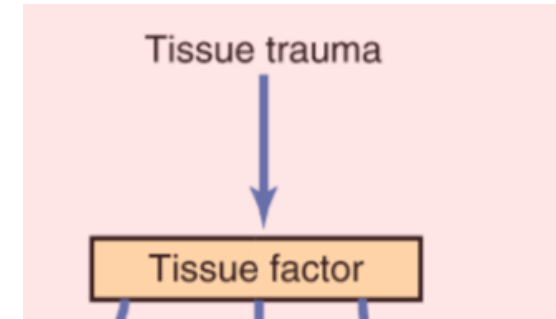
1. Release of tissue factor

2. Activation of factor X—role of factor VII and tissue factor.

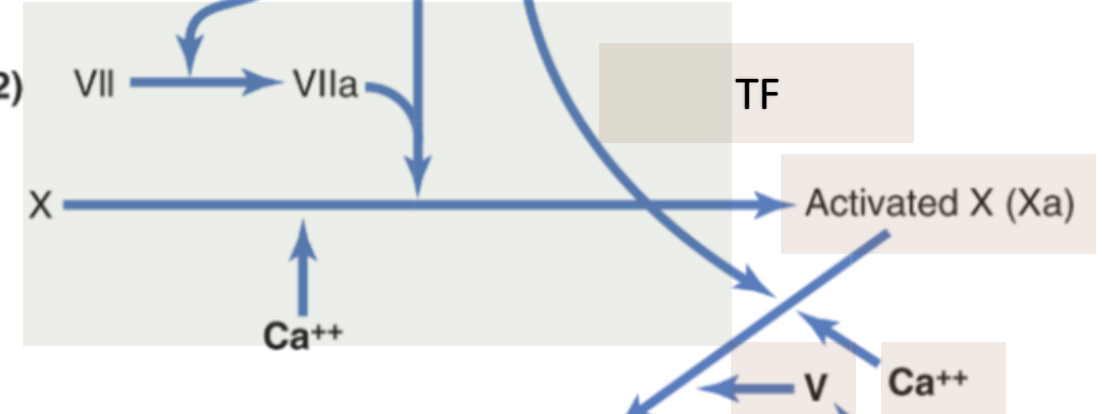
3. Effect of Xa to form prothrombin activator—role of factor V

The doc mentioned that the exact order of the factors may not be the main focus, but remember that the extrinsic pathway involves tissue factor, factor VII, and factor X (with factors X and V being part of the prothrombinase complex.)

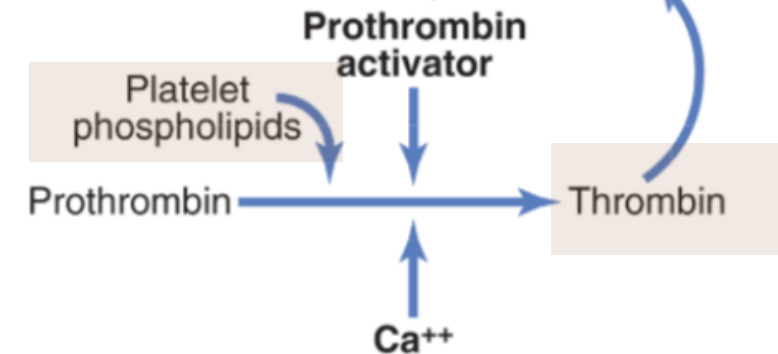
(1)



(2)



(3)



**** Tissue factor:** Traumatized tissue releases a complex of several factors called tissue factor. This factor is composed especially of **phospholipids** from the membranes of the tissue plus **a lipoprotein** complex that functions mainly as a **proteolytic enzyme**.

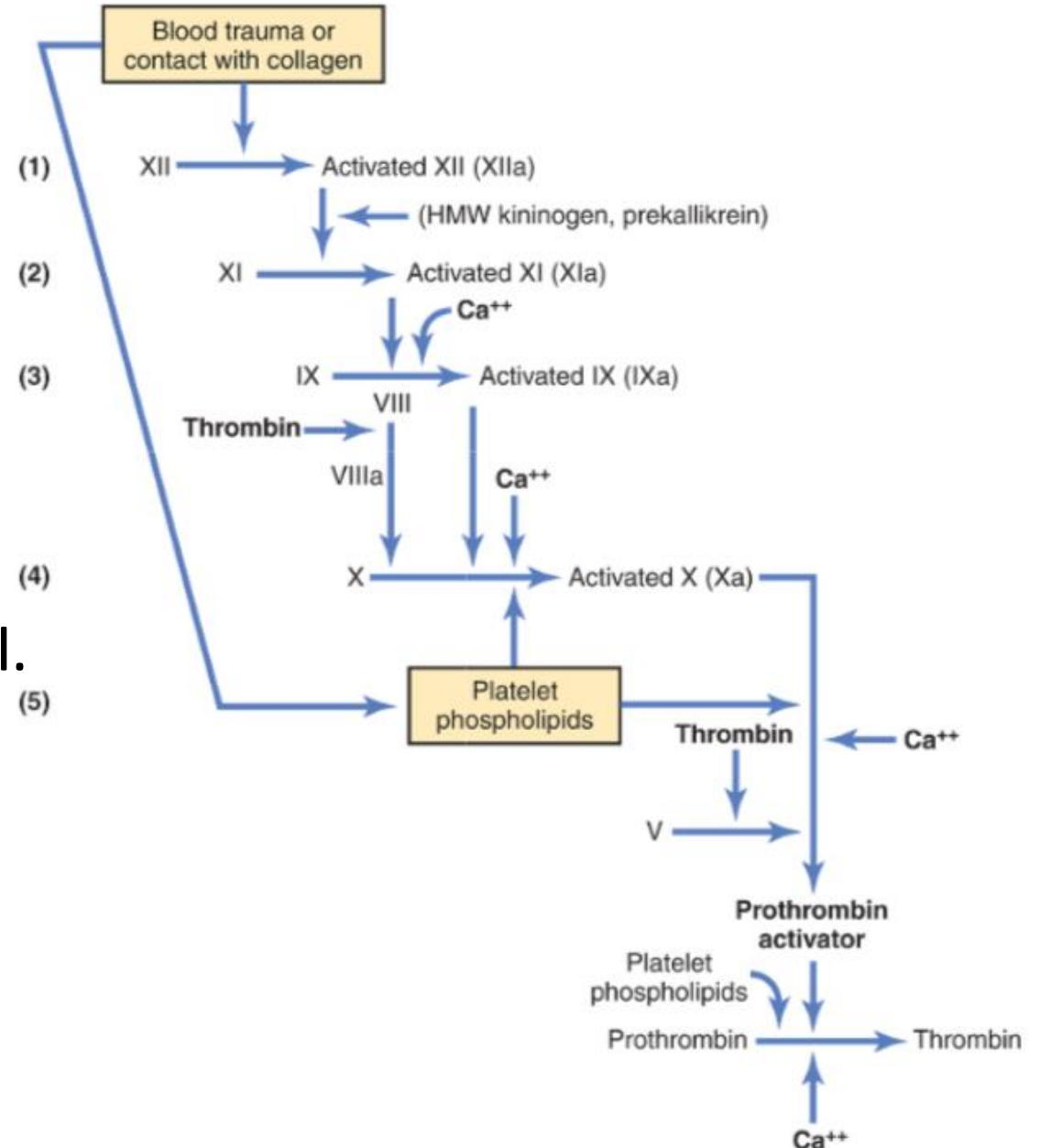
**** 2.** The **activated Factor X** combines immediately with tissue phospholipids that are part of **tissue factors** or with additional **phospholipids** released from **platelets**, as well as with **Factor V** to form the complex called **prothrombin activator**.

**** 4.** Thus, in the final prothrombin activator complex, **activated factor X** is the **actual protease** that causes splitting of prothrombin to form thrombin. **Activated factor V** greatly **accelerates** this protease activity, and **platelet phospholipids** act as a **vehicle** that further accelerates the process. Note especially the **positive feedback effect of thrombin**, acting through factor V, to accelerate the entire process once it begins.

3. Intrinsic pathway

1. Blood trauma causes activation of factor XII and release of platelet phospholipids
2. Activation of factor XI
3. Activation of factor IX
4. Activation of factor X—role of factor VIII.

The intrinsic pathway begins with factor XII, which circulates in the blood. When factor XII comes into contact with exposed collagen following endothelial injury, or with a glass surface in a blood collection tube, it undergoes a conformational change and becomes activated.



**** (1)** When factor XII is disturbed, such as by coming into contact with collagen or with a wettable surface such as glass, it takes on a new molecular configuration that converts it into a proteolytic enzyme called activated factor XII. Simultaneously, the blood trauma also damages the platelets because of adherence to collagen or to a wettable surface (or by damage in other ways); this releases platelet phospholipids which also plays a role in subsequent clotting reactions.

**** (4)** The activated factor IX, acting in concert with activated factor VIII and the platelet phospholipids, activates factor X.

Hemostasis

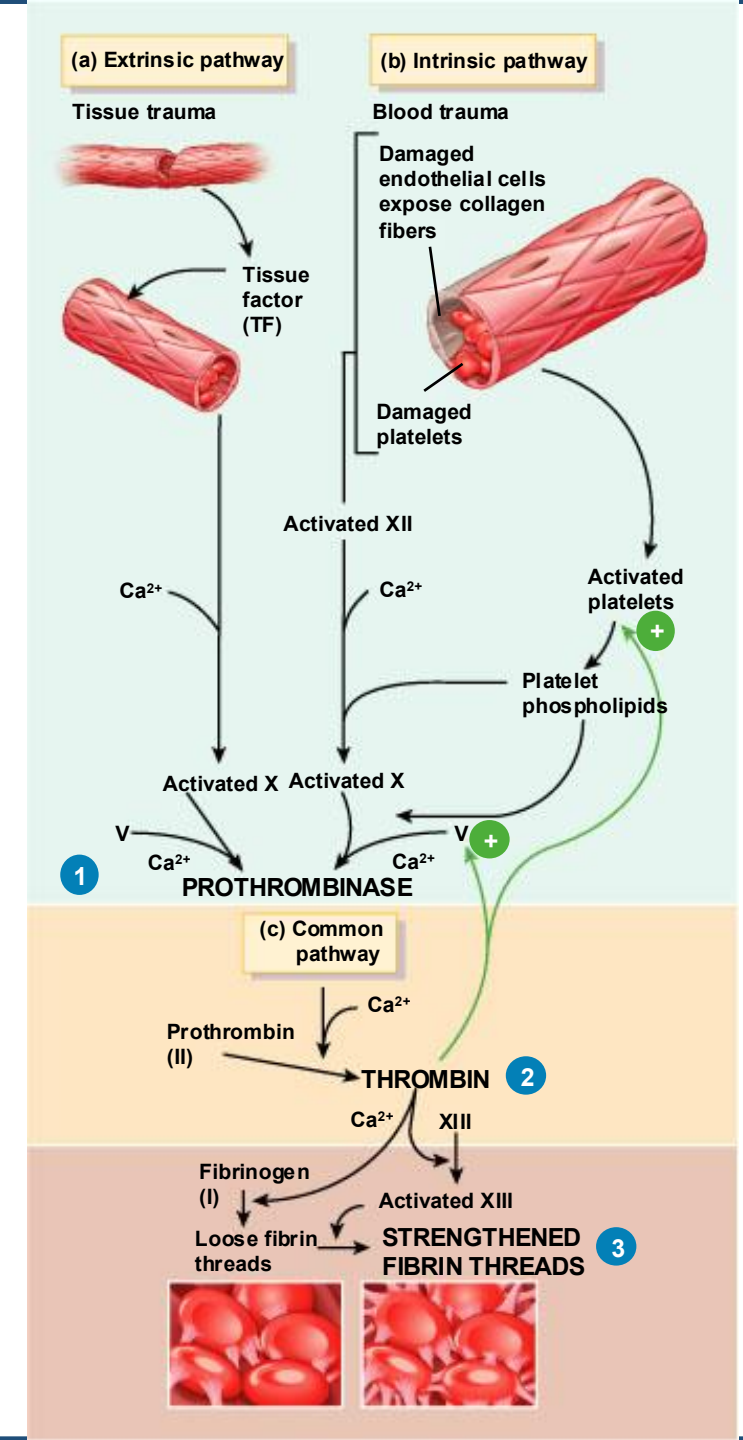
Thrombin

Thrombin has 2 positive feedback effects:

- Accelerates formation of prothrombinase (V)
- Thrombin activates platelets

*****Roles of Thrombin Multitasking thrombin, in addition to

- (1) converting fibrinogen into fibrin,
- (2) activates factor XIII (fibrin-stabilizing factor) to stabilize the resultant fibrin mesh
- (3) acts in a positive-feedback fashion to facilitate its own formation,
- (4) enhances platelet aggregation, which in turn is essential to the clotting process



Summary: Differences Between the Intrinsic and Extrinsic Pathways

1. Initiator:

- **Extrinsic pathway:** Tissue damage (tissue trauma) exposes tissue factor.
- **Intrinsic pathway:** Blood trauma, such as endothelial injury, exposes collagen, causing a conformational change in factor XII.

2. Number of Steps and Speed:

- **Intrinsic pathway:** More steps → slower.
- **Extrinsic pathway:** Fewer steps → faster.

****The extrinsic pathway depends on the amount of tissue factor. More tissue damage can expose larger amounts of tissue factor, creating a risk of explosive clotting at multiple sites. This will be discussed in the next lecture.**

Think!

Why do we use Ethylenediaminetetraacetic acid (EDTA) tube to collect blood for CBC?

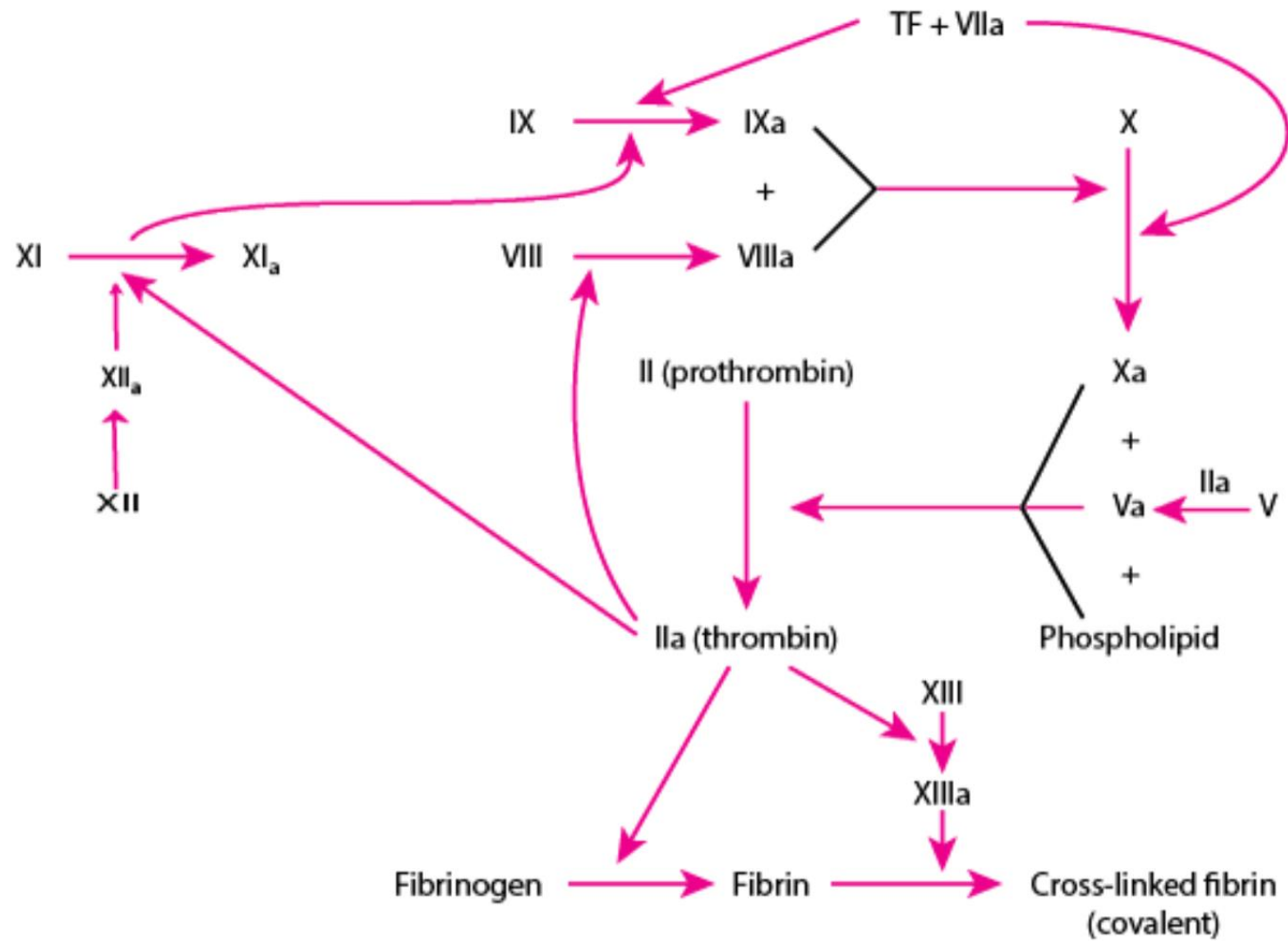
When a blood sample is collected in a normal glass tube, contact with the glass can activate Factor XII, initiating the intrinsic pathway and leading to coagulation. To prevent coagulation in a CBC sample, we collect the blood in a purple-top tube containing EDTA (ethylenediaminetetraacetic acid), which acts as an anticoagulant.

EDTA acts as a chelating agent, binding calcium ions and making them unavailable for coagulation. As a result, the coagulation cascade is inhibited, and the blood remains in a fluid state.

Except for the first two steps in the intrinsic pathway, calcium ions are required for promotion or acceleration of all the blood-clotting reactions.

→ Therefore, in the absence of calcium ions, blood clotting by either pathway does not occur.



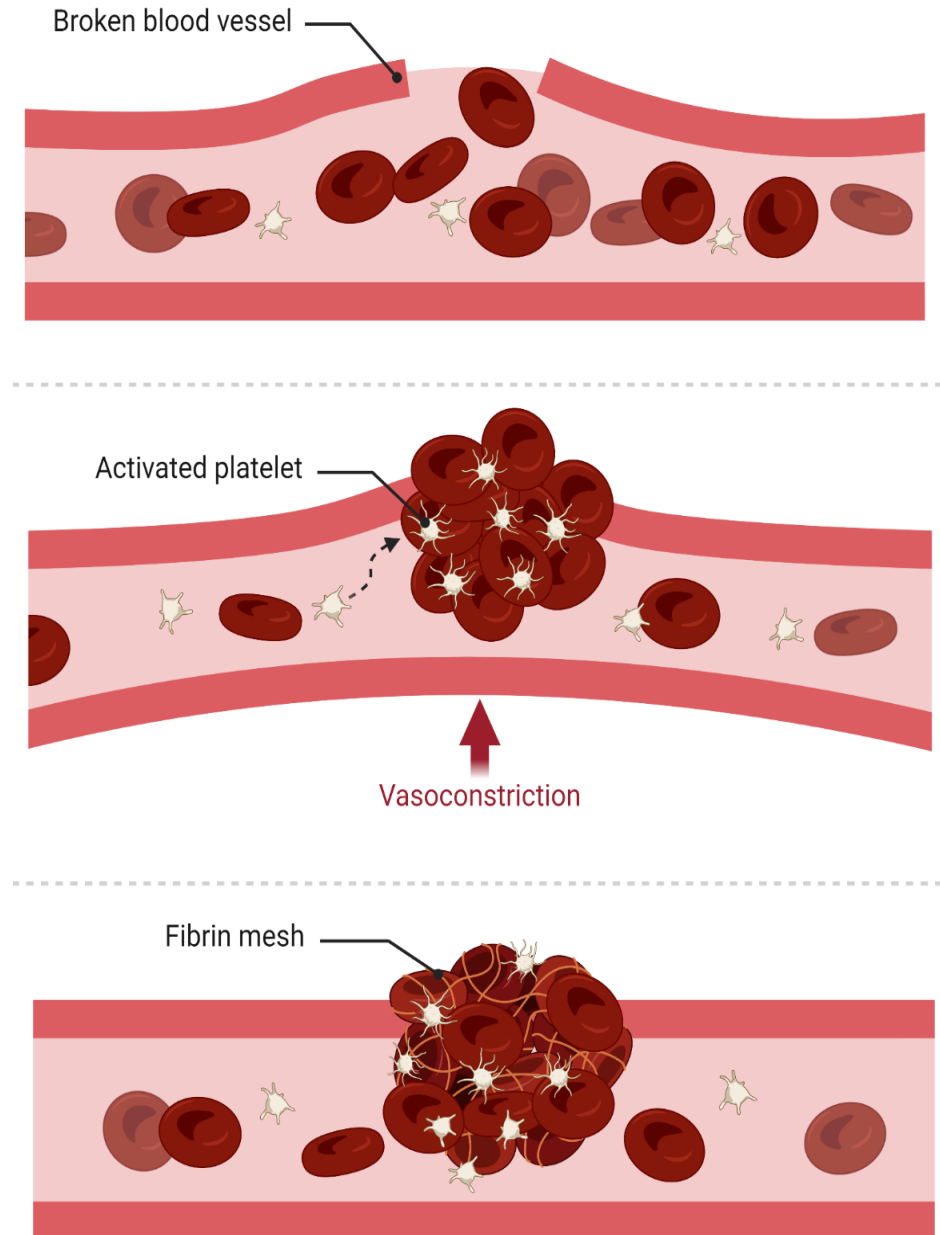


Hemostasis

Clot retraction

- Within a few minutes after a clot is formed, **platelets** begin to **contract** and usually **express** most of the **fluid** from the clot **within 20 to 60 minutes**.
- As the clot retracts, **the edges** of the broken blood vessel **are pulled together**, thus contributing still further to hemostasis.
- **Serum:** is blood plasma minus its **fibrinogen** and most of the other clotting factors.

**** The platelets contribute directly to clot contraction by activating platelet thrombosthenin, actin, and myosin molecules.**



The fibrin mesh, or blood clot, consists of insoluble fibrin fibers that trap red blood cells (RBCs), platelets, and some plasma components.

Platelets have the ability to contract due to the presence of three contractile proteins:

- Actin
- Myosin
- Thrombosthenin

These proteins enable platelets to contract and pull the edges of the clot closer together. This process is called clot retraction. As the clot contracts, it squeezes out a fluid called serum.

Differences Between Blood, Plasma, and Serum

- Blood: Consists of plasma and formed elements, including red blood cells (RBCs), white blood cells (WBCs), and platelets.
- Plasma: The liquid component of anticoagulated blood. It contains water, proteins, electrolytes, nutrients, and clotting factors, including fibrinogen.
- Serum: The fluid remaining after blood has clotted. It is similar to plasma but lacks fibrinogen and has altered levels of certain coagulation factors due to the clotting process.

رسالة من الفريق العلمي:

دعواتكم الصادقة لزميلنا عون العدوان بالرحمة

اللهم اغفر له وارحمه، وثبّته عند السؤال، وأكرم نزله ووسّع مدخله، واغسله بالماء
والتلج والبرد، ونقّه من الخطايا كما يُنقى الثوب الأبيض من الدنس، واربط على قلب
أهله وصبرهم، واجبر مصابهم، واجعل قبره نورًا وضياءً، وروضةً من رياض الجنة
اللهم آنس وحشته، وارحم غربته، وثبّته عند السؤال، واجعل الفردوس الأعلى مستقرّه
ومأواه، واجمعه مع النبيين والصديقين والشهداء والصالحين، وحسن أولئك رفيقًا
اللهم ارحمه رحمةً تطمئنّ بها روحه، وترفع بها درجته، واجعل ما أصابه تكفيرًا
لذنوبه ورفعَةً له في جنات النعيم

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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			