HLS

MODIFIED NO6

PHYSIOLOGY

كتابة: سيرين باسل و
جنى الخريسات

تدقيق: تم التدقيق

الدكتور إباء زيادنة

UNIT VI

Hemostasis and Blood Coagulation

Ebaa M Alzayadneh, PhD

Associate Professor of Physiology

Color code

- Slides
- Doctor
- Additional info
- Important

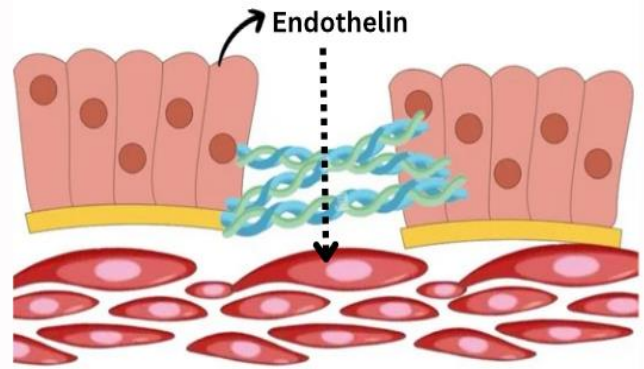
Five Steps of Hemostasis

1) Vascular Spasm
2) Platelet Plug Formation
3) Coagulation
4) Clot Retraction
5) Fibrinolysis

The following slides are not part of the lecture
Not for exam purposes
We made them so you could connect all the information together

1) Vascular Spasm

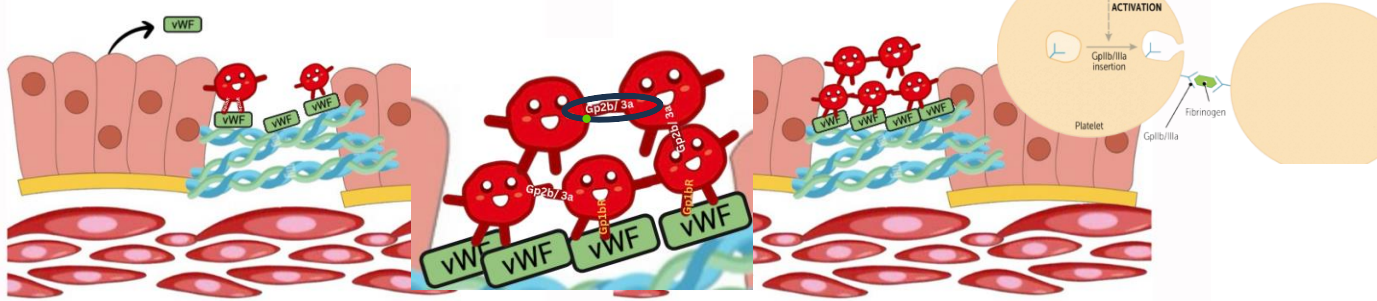
- **Endothelin**
  - Secreted by injured endothelial cells
  - Binds on to receptor on smooth muscle
  - Smooth muscle contracts
- **Myogenic**
  - Direct contact or injury to smooth muscle causes smooth muscle contraction
- **Nociceptor Activation**
  - Inflammatory chemicals are released when there's inflammation
  - E.g., histamine, leukotrienes, prostaglandins
  - These chemicals stimulate the nociceptors
  - Nociceptors (pain receptors) will initiate pain
  - Pain reflex induces vasoconstriction



Platelet Plug Formation

AKA Primary Hemostasis

- Platelets in the blood stream are inactivated by factors released from endothelial cells, such as NO and prostaglandins.
- When endothelial cells are damaged, NO and prostaglandin production is decrease, and the von Willibrand factor (vWF) gets release.
- vWF attaches to the exposed collagen fibers, and the platelets bind to vWF via glycoprotein Ib receptor.
- When platelets are activated, they release ADP and thromboxane.
- ADP causes further activation to other plateles by binding to the receptor P2Y12.
- Platelets bind with other platelets via their GP2b/3a, with fibrinogen bridging them together.

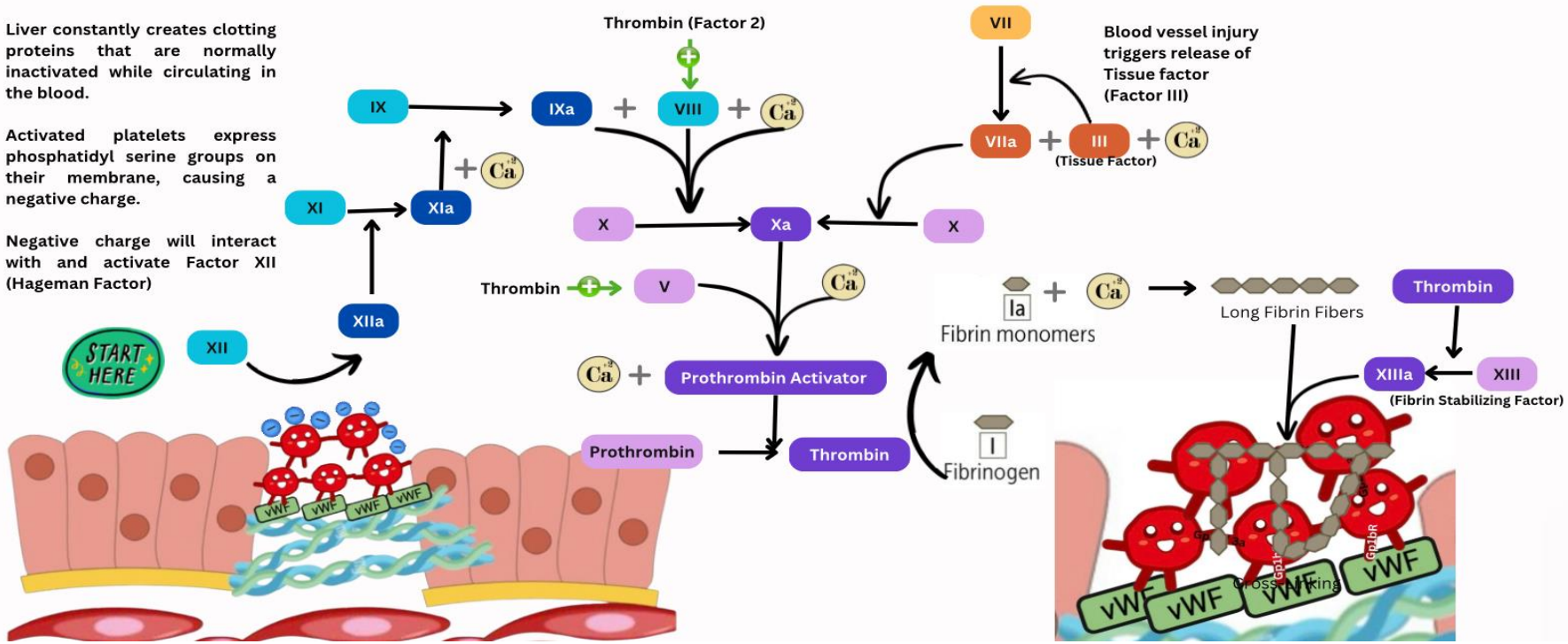


Coagulation Pathway

AKA Secondary Hemostasis

- Intrinsic Pathway: Factors 12, 11, 9 & 8
- Common Pathway: Factors 10, 5, 2, 1
- Extrinsic Pathway: Factors 3, 7

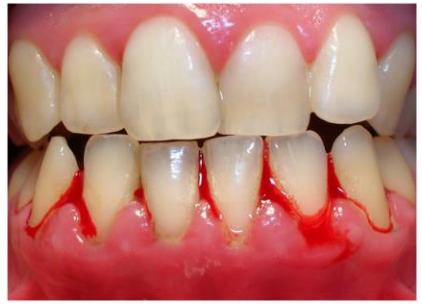
- Liver constantly creates clotting proteins that are normally inactivated while circulating in the blood.
- Activated platelets express phosphatidyl serine groups on their membrane, causing a negative charge.
- Negative charge will interact with and activate Factor XII (Hageman Factor)



Formation of the Platelet Plug

- **Contact with damaged endothelium**
  - **Assume irregular forms**
  - **Contract and release granules (ADP, thromboxane A_2)**
- **Adhere to collagen and vWF**
- **Other platelets accumulate, adhere, and contract, form plug, initiate clotting**
- **Very low platelets → petechiae, bleeding gums**

The platelet-plugging mechanism is extremely important for closing tiny ruptures in very small blood vessels that occur thousands of times daily. People with low platelets have thousands of small hemorrhagic areas that develop each day under the skin and throughout the internal tissues, manifesting as petechiae.



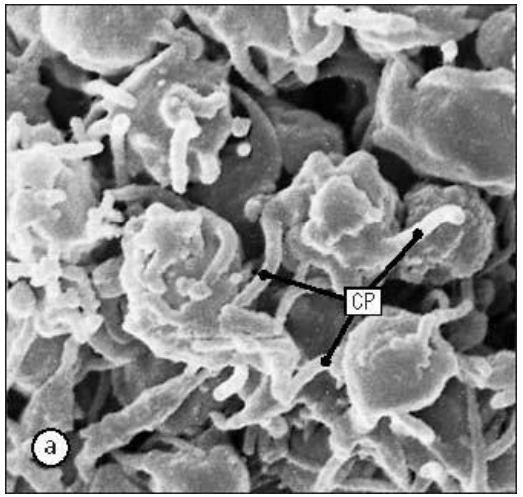
When platelets encounter a damaged vascular surface, especially with collagen fibers in the vascular wall, the platelets rapidly change their own characteristics. They begin to swell; they assume irregular forms with numerous pseudopods protruding from their surfaces; their contractile proteins contract forcefully and cause the release of granules that contain multiple active factors (such as ADP, thromboxane, serotonin)

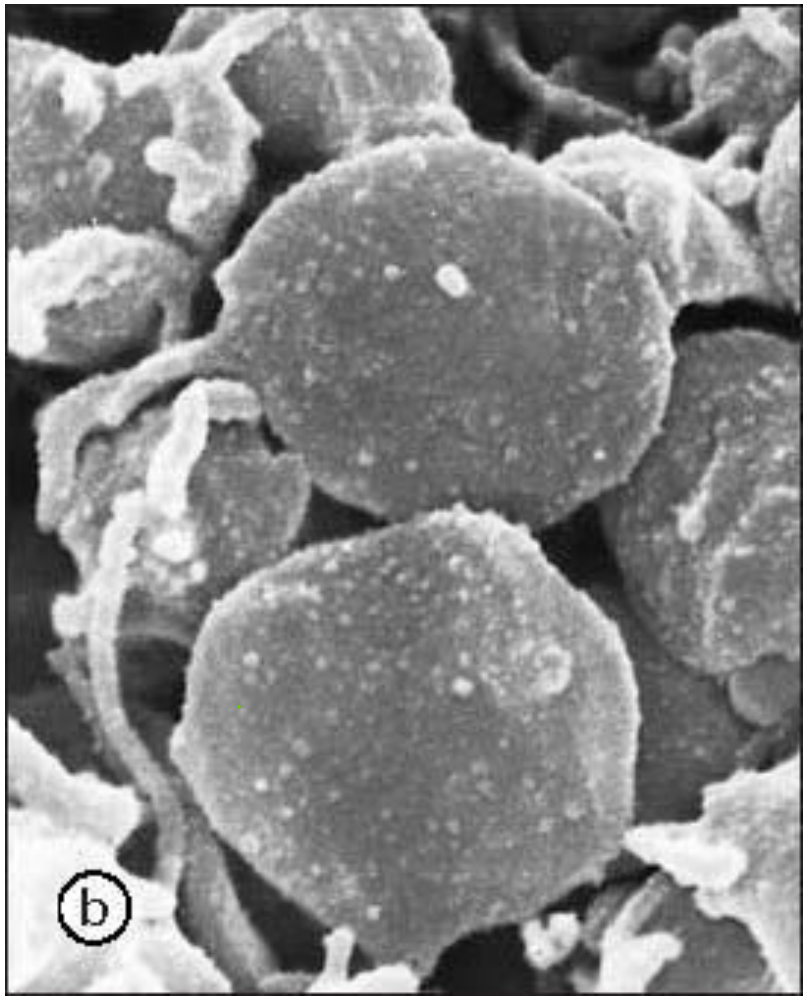
The activated platelets become sticky so that they adhere to collagen in the tissues and to a protein called von Willebrand factor that leaks into the traumatized tissue from the plasma. They secrete large quantities of ADP; The platelets also form thromboxane A2 since they have COX-1.

The ADP and thromboxane in turn act on nearby platelets to activate them as well, and the stickiness of these additional platelets causes them to adhere to the original activated platelets.

Entrapped RBCs

Platelet Plug





Platelet aggregates

This plug is loose at first, but it is usually successful in blocking blood loss if the vascular opening is small. Then, during the subsequent process of blood coagulation, fibrin threads form. These threads attach tightly to the platelets, thus constructing an unyielding plug.

Figs. 7.15a and 7.14b, *Stevens & Lowe Human Histology,* 4th edition

Clot Formation and Progression

- Begins in 15- 20 seconds in severe vascular trauma
- Occlusive clot within 3-6 minutes unless very large vascular defect
- 20-60 minutes: Clot retraction
- 1- 2 weeks : Invasion by fibroblasts
  Organization into fibrous tissue

Clotting speed depends on the severity of the injury. The more severe the injury is, the faster the clotting.
Injuries that we are referring to are of intermediate severity, not the very severe wounds with completely damaged tissues.

The clot begins to develop in 15 to 20 seconds if the trauma to the vascular wall has been severe. If the trauma has been minor, the clot forms in 1 to 2 minutes.

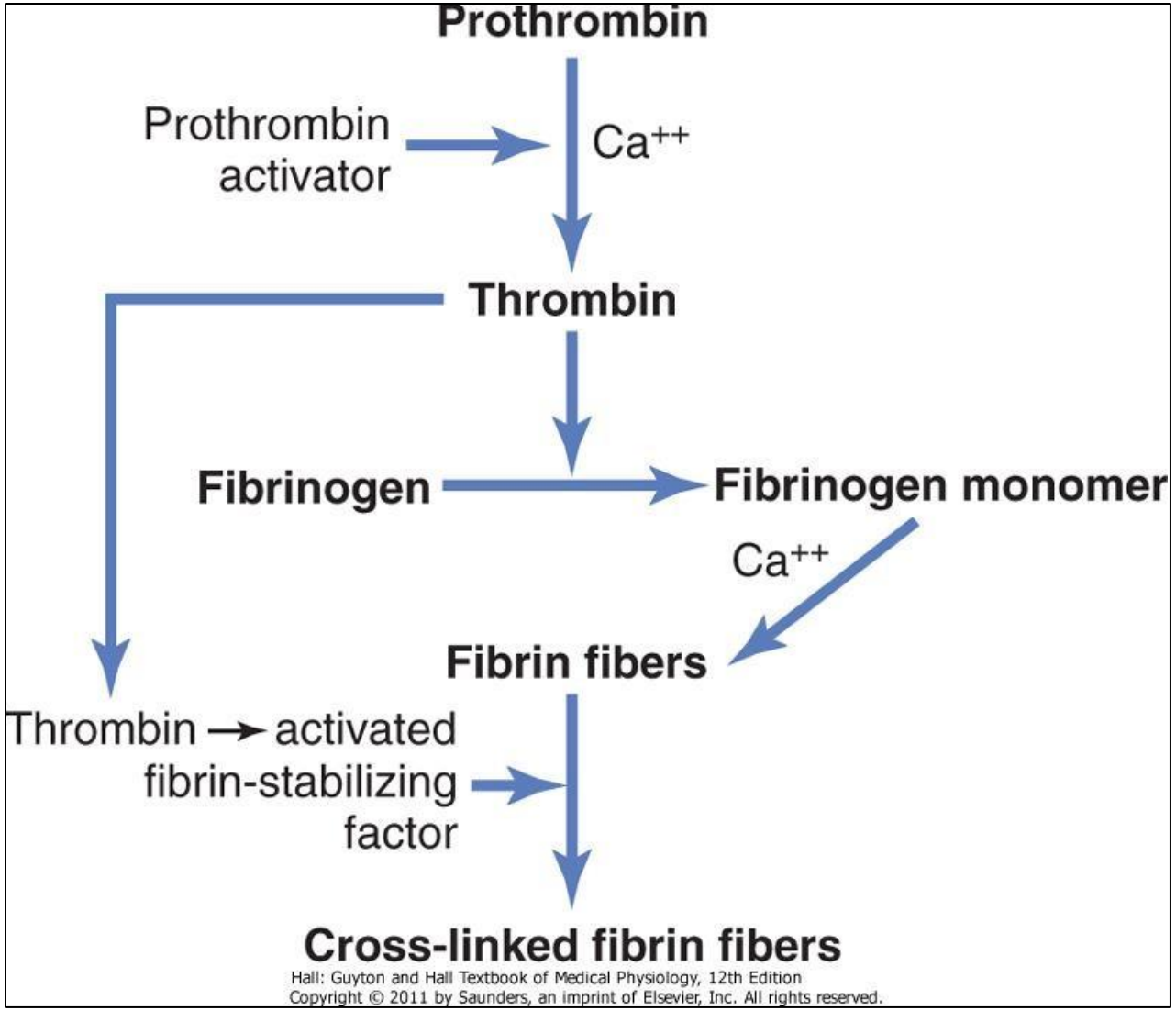
Activator substances from the traumatized vascular wall, from platelets, and from plasma proteins initiate the clotting process.

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. If the defect is too large, the injured individual must go to the ER for medical intervention.

After 20 minutes to an hour, the clot retracts with the aid of contractile proteins in the platelets, which closes the vessel, enhancing the healing process. The contraction expels serum. The fluid expressed is called serum because all its fibrinogen and most of the other clotting factors have been removed; in this way, serum differs from plasma. Serum cannot clot because it lacks these factors.

1-2 weeks after the injury, fibroblasts will invade the injury site and synthesize collagen and fibers, forming scar tissue.

Key Steps in Blood Clotting



The most important step of the coagulation pathway is the conversion of prothrombin to thrombin. The thrombin acts as an enzyme to convert fibrinogen into fibrin monomers. Those monomers polymerize to form fibers that enmesh (wrap, enclose) platelets, blood cells, and plasma to form the clot.

Prothrombin can be activated to thrombin through two pathways:
1) Intrinsic and 2) Extrinsic
They will be discussed in the coming slides

Thrombin is a protein 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 that has the automatic capability to polymerize with other fibrin monomer molecules to form fibrin fibers. Therefore, many fibrin monomer molecules polymerize within seconds into long fibrin fibers

In the early stages of polymerization, the fibrin monomer molecules are held together by weak noncovalent hydrogen bonding, and the newly forming fibers are not cross-linked with one another; therefore, the resultant clot is weak and can be broken apart with ease.
However, another process occurs during the next few minutes that greatly strengthens the fibrin reticulum. This process involves a substance called fibrin-stabilizing factor, also known as Factor 13, that is present in small amounts in normal plasma globulins but is also released from platelets entrapped in the clot. Factor 13 then operates as an enzyme to cause covalent bonds between the fibrin monomer molecules, as well as multiple cross-linkages between adjacent fibrin fibers, thus adding tremendously to the three-dimensional strength of the fibrin meshwork.

Similar to albumin in weight

Effector Proteins for Clotting

- **Prothrombin**
  - **α 2 globulin, MW 68,700; 15 mg/dl in plasma**
  - **Vitamin K-dependent synthesis in liver**
  - **Cleaved by PT (Prothrombinase) activator to thrombin, MW 33,700**
- **Fibrinogen**
  - **MW 340,000; 100-700 mg/dl in plasma**
  - **Synthesized in the liver (acute phase reactant)**
  - **Usually intravascular; can extravasate with increased vascular permeability**

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

Therefore, either lack of vitamin K or the presence of liver disease that prevents normal prothrombin formation can decrease the prothrombin to such a low level that a bleeding tendency results.

Extravasate : leak out from a vessel

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.

So, fibrinogen is mainly found intravascularly. But when inflammation occurs, the permeability of capillaries is increased which allows fibrinogen to leak into the tissue fluids in sufficient quantities that allow clotting of these fluids in the same way that plasma and whole blood can clot.

Fibrin Production

- **Thrombin (weak protease) cleaves four small peptides from fibrinogen**

  → fibrin monomer → spontaneous polymerization

- **Long fibers form clot reticulum**

- **Fibrin stabilizing factor**
  - **In plasma and released from platelets**
  - **Activated by thrombin**
  - **Covalent bonds, and cross-linking of fibrin monomers and adjacent fibrin fibers**

Thrombin cleaves 4 molecules off of the soluble fibrinogen to form insoluble fibrin monomer. The fibrin monomers polymerize spontaneously to form fibrin threads.

Fibrin Stabilizing Factor AKA Factor 13 is further activated by thrombin.
Its function is to make the fibrin monomers stronger by forming covalent bonds, and by cross-linking of fibrin

Refer to slide 13 for more details

Clot Extension

- **Thrombin is bound to platelets and trapped in the clot**
- **Can act on prothrombin to generate more thrombin (positive feedback)**
- **Thrombin also produces more prothrombin activator by acting on other clotting factors**
- **Additional fibrin monomers and polymers are generated at the periphery of the clot**

Thrombin binds to receptors on platelets, which keeps the platelets in the injured area and prevents thrombin from going to other areas and forming unnecessary clots.

Thrombin converts prothrombin to thrombin through positive loops which will be discussed in the coming slides. So once thrombin is activated, it causes an **explosive coagulation** because of the positive feedbacks.

The positive feedback also causes **more clotting and extends the fibers**. But the fiber extension is limited

Clot Retraction

- **Begins within 20-60 minutes**
- **Fibrin binds to damaged vessel wall**
- **Platelets bind to multiple fibrin fibers**
  - **contract via actin, myosin, thrombosthenin, and FSF (Factor XIII), Ca++ from organelles**
- **Clot tightens, expressing serum, and closing the vascular defect**

Platelets contribute directly to clot contraction by activating platelet thrombosthenin, actin, and myosin molecules, which are all contractile proteins in the platelets.

This action helps compress the fibrin meshwork into a smaller mass.

So, again, what's the rate-limiting or key step?
The rate-limiting step is the **generation of the prothrombin activator complex**. This complex is responsible for **converting prothrombin into thrombin**, initiating a rapidly continues process. why? **Thrombin activates multiple pathways**, Once Thrombin is, activated we start a positive feedback loop.
Let's take a moment to briefly discuss the upcoming slides to provide a better understanding before delving into the details.
How is the prothrombin activator complex generated?
Prothrombin activator is generally considered to be formed in two ways, although, in reality, the two ways interact constantly with each other:
(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.**

The names of these pathways are associated with the **location of the injury**.
When the injury is only in the **blood or exposure of the blood to collagen** from a traumatized blood vessel wall, this is what initiates the **intrinsic pathway**.

However, When tissue underneath is injured, a factor known as **Tissue Factor** AKA factor III , is released from the tissues. This initiates the **extrinsic pathway**, which is **faster** than the intrinsic pathway. This explains why clotting is faster in more severe injuries as tissues are involved, releasing **tissue factor**.

All in all, tissue involvement determines whether there is an extrinsic, intrinsic, or both pathways.
When can it be both? When the injury involves both blood components and the tissue underneath.

- **Two pathways** **Generating Prothrombin Activator**
  - **Extrinsic pathway – Trauma to vessel wall and adjacent tissues**
  - **Intrinsic pathway – Trauma to the blood or exposure of the blood to collagen**

- **Both pathways involve "clotting factors"—mostly inactive proteases that are activated in cascades**

The main difference between extrinsic and intrinsic is the speed and coagulation factors involved

Involving Tissue Factor AKA factor III

Both pathways activate different inactive coagulation factors, which eventually activates prothrombin into thrombin.

I understand it may seem confusing now, but we'll clear it up soon!

Extrinsic Pathway of Blood Clotting

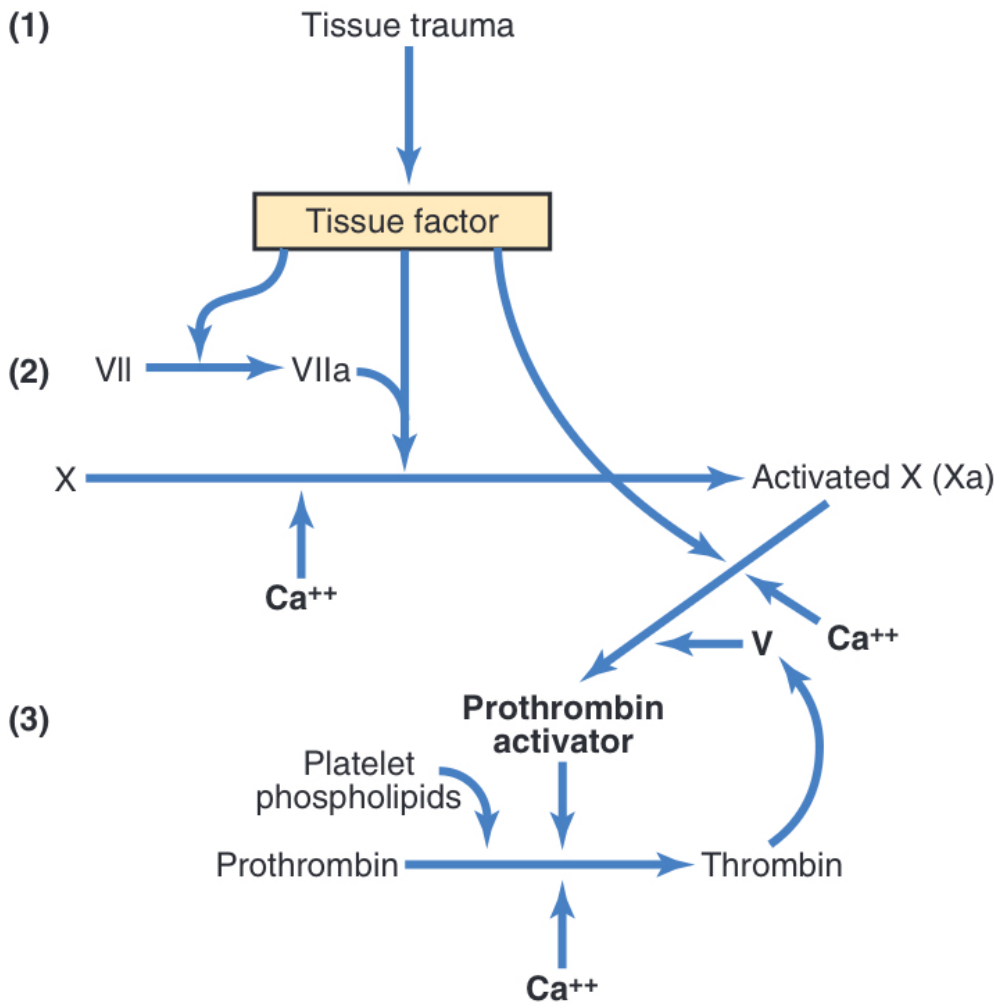


Figure 37-3. Extrinsic pathway for initiating blood clotting.

Extrinsic pathway involve tissue trauma (traumatized extravascular tissues that come in contact with the blood).

1. Traumatized tissue releases a factor called Tissue Factor. This factor is composed especially of **phospholipids plus a Protein** complex that functions mainly as a proteolytic **enzyme**.

2. **Tissue factor activates factor VII** ,then, tissue factor further complexes with activated factor VII in the presence of calcium ions to form **activated factor X (Xa)**, factor X is the main protease in the prothrombin activator complex.

Extrinsic Pathway of Blood Clotting

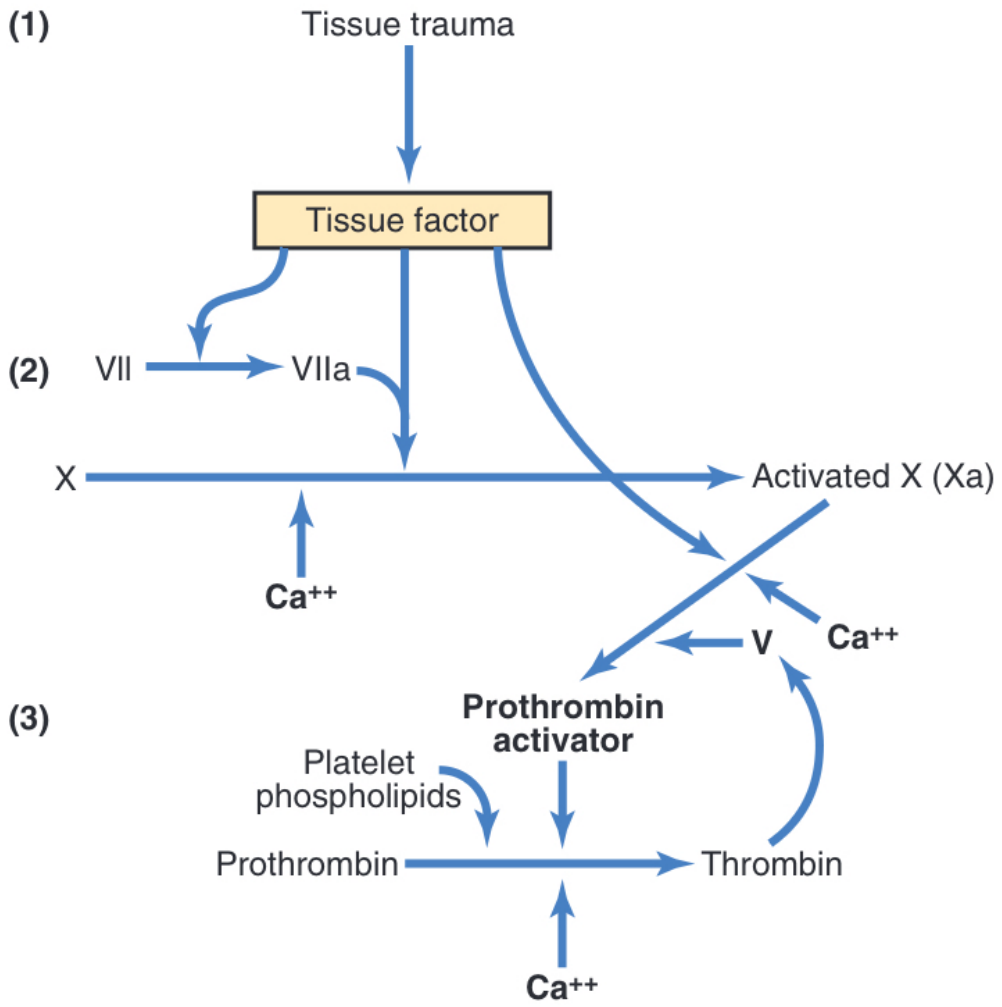


Figure 37-3. Extrinsic pathway for initiating blood clotting.

3. Factor X combines with Factor V in the presence of calcium ions and tissue factor to form the prothrombin activator complex. This complex then converts prothrombin into thrombin with the help of calcium ions and phospholipids. Phospholipids act as a vehicle and accelerate the process.

Factor V is inactive at the beginning of the pathway. When **Thrombin** is formed, it activates it, remember, Thrombin activates multiple processes.
When factor V is activated, it further accelerates the formation of prothrombin activator complex.

Do you mean that factor V wasn't active when Thrombin wasn't formed yet? Yes, it was in its inactive form, it performed its job, but at a slower rate.

Blood Coagulation

- **Extrinsic Pathway**

a. Release of tissue factor (Factor III)

b. Activation of Factor X- role of Factor VII and tissue factor

c. Effect of Xa (main protease) to form prothrombin activator -role of Factor V in the presence of calcium and phospholipids

to split prothrombin to thrombin

Phospholipids could originate from platelets or from the damaged endothelium; most likely from the platelets.

- Activated factor V greatly accelerates this protease activity
- platelet phospholipids act as a vehicle that further accelerates the process

Blood Coagulation

Placing a blood sample in a tube will lead to blood trauma and activate the **intrinsic pathway only.** The extrinsic pathway will not be involved because there is no tissue around or tissue factor present.

- **Intrinsic Pathway**

  a. Blood trauma causes activation Factor XII and release of platelet phospholipids

  b. Activation of Factor XI

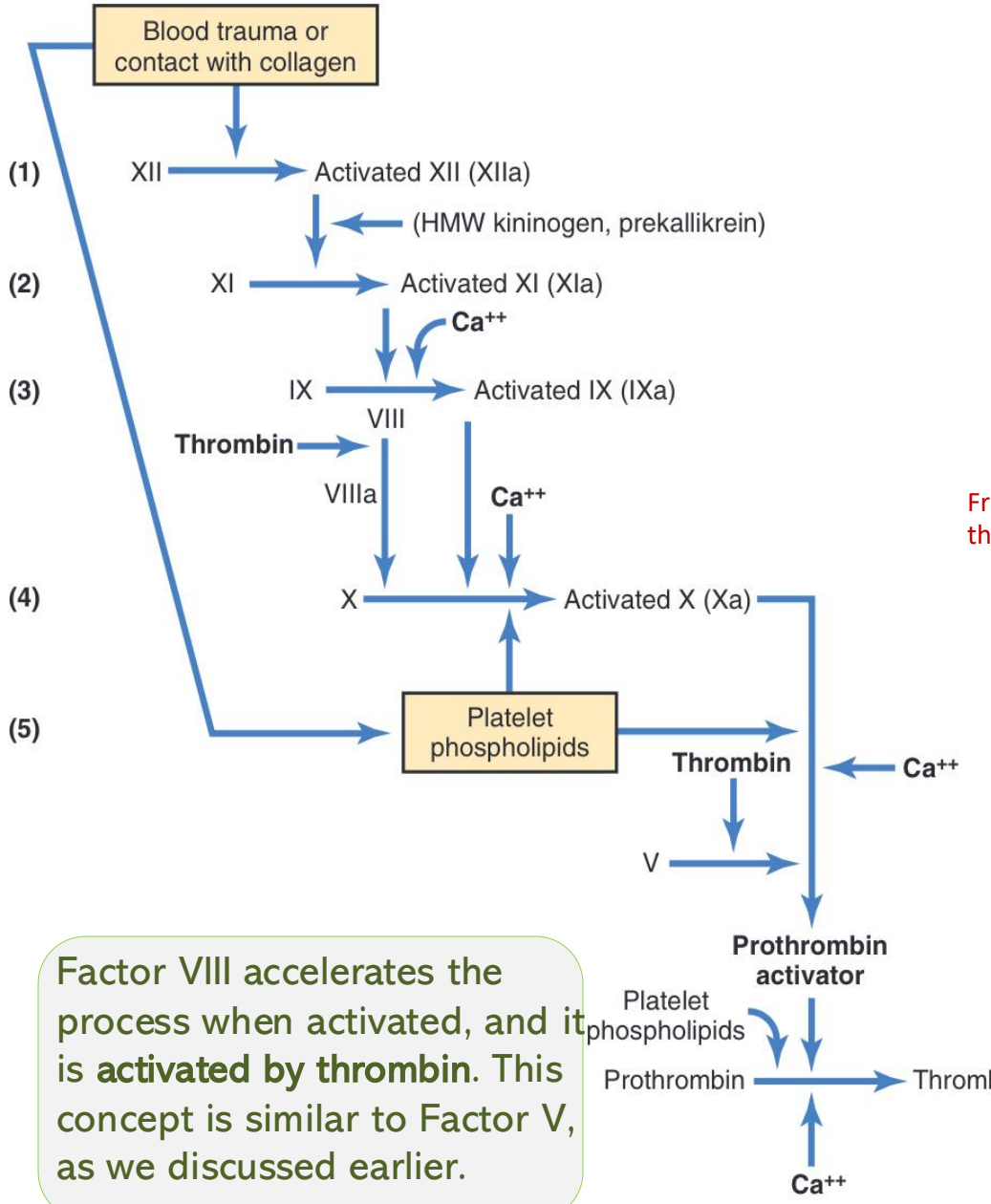
  c. Activation of Factor IX by activated XI

  d. Activation of Factor X-role of Factor VIII

  e. Action of activated Factor X to form prothrombin activator-role of Factor V

Especially when it's activated

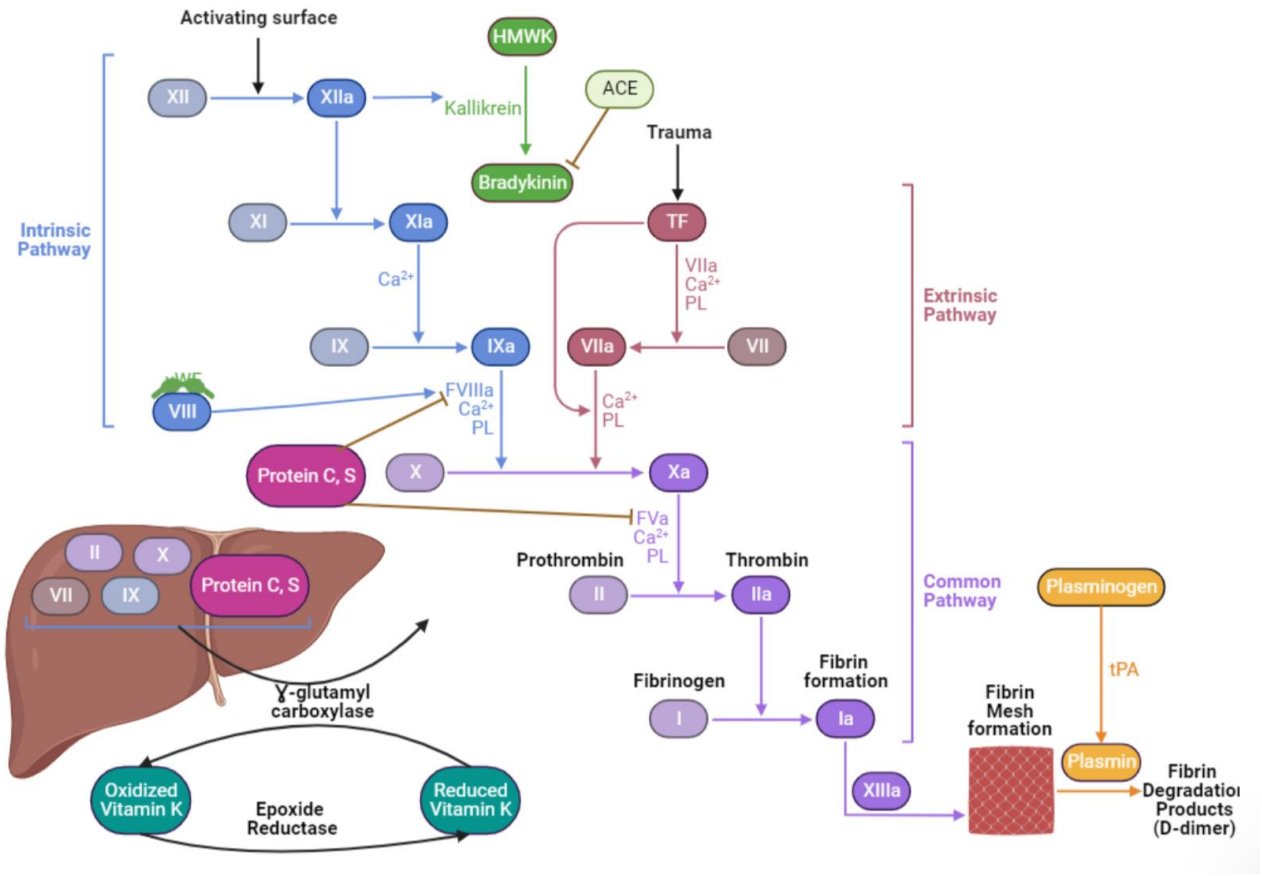
Intrinsic Pathway of Blood Clotting



From here, we start with the common pathway

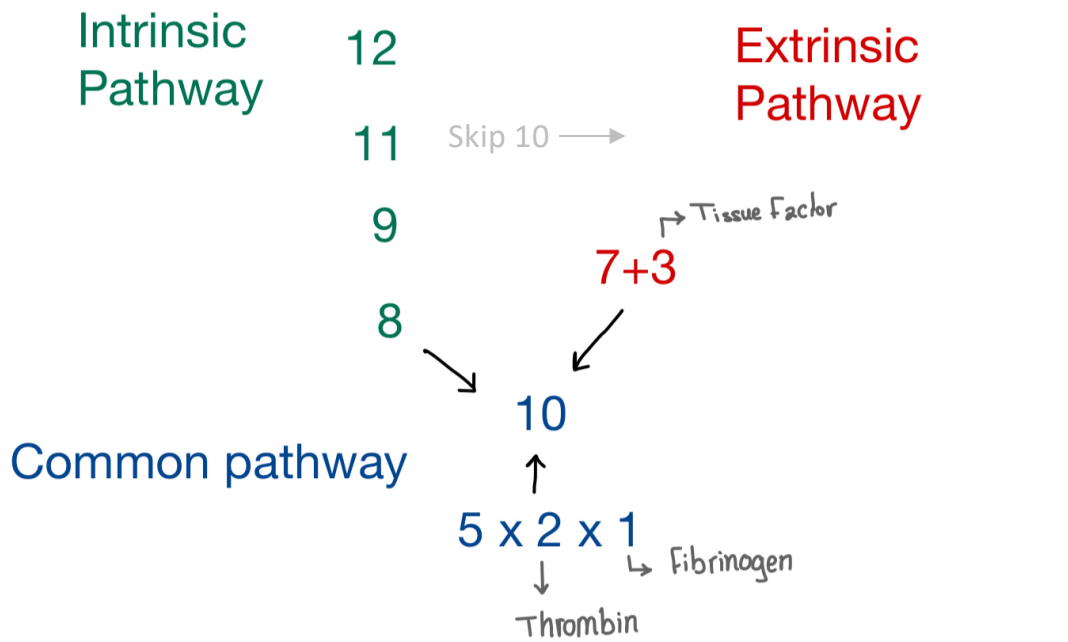
Factor VIII accelerates the process when activated, and it is **activated by thrombin**. This concept is similar to Factor V, as we discussed earlier.

1. Blood trauma causes **Factor XII** to be disturbed b contact with collagen or with a surface such as g converted it into a proteolytic enzyme called "act XII"
   Blood trauma causes release of platelet phospho
2. The **activated Factor XII** acts enzymatically on **Fac** activate this factor as well, which is the second st intrinsic pathway. This reaction also requires high weight kininogen and is accelerated by prekallikr
3. Activation of Factor IX by activated Factor XI in th calcium ions.
4. The activated Factor IX, acting in concert with act VIII and with the platelet phospholipids and **calci** vates Factor X. It is clear that when either Factor platelets are in short supply, this step is deficient activated Factor X is deficient . Factor VIII is the f missing in a person who has classic hemophilia, f reason it is called anti-hemophilic factor.
5. activated Factor X combines with Factor V and tis phospholipids as well as calcium to form the pro activator complex.



External image

Source: Bootcamp



An easy way to remember the factors associated with each pathway.

Synergy between the Intrinsic and Extrinsic Pathways

Which is faster than the intrinsic pathway

- **Tissue injury…**
  - **Tissue factor activates the Extrinsic Pathway**
  - **Exposure of Factor XII and platelets to collagen activates the Intrinsic Pathway**
- **Extrinsic pathway can be explosive, with clotting in < 15 seconds**
- **The Intrinsic pathway is slower**
  → **1 – 6 minutes**

وَٱسْتَغْفِرِ ٱللَّهَ ۖ إِنَّ ٱللَّهَ كَانَ غَفُورًا رَّحِيمًا ﴿١٠٦﴾

if it was alone

both of the pathways can exist the same time

How is clotting prevented under normal conditions ?

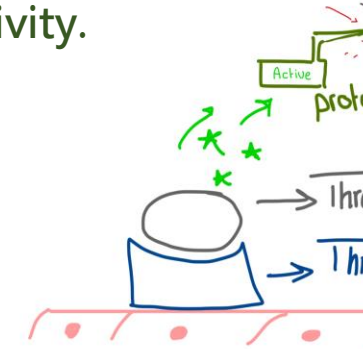
Prevention of Clotting

It is as important as preventing blood loss

A smooth endothelial surface promo
(smooth) blood flow and helps keep
their inactive state.

- **Smoothness of the endothelial surface**
- **Mucopolysaccharide coating (glycocalyx) repels platelets and clotting factors**
- **Thrombomodulin bound to endothelium binds (competes for) thrombin**
- **Thrombin-thrombomodulin activates Protein C→ inactivates factors V and VIII**
- **Damage to glycocalyx activates factor XII, platelets (intrinsic pathway). If collagen is exposed→ even more robust**

It limits the amount of thrombin that functions in the coagulation cascade, thus reducing its activity.



Anti-hemophiliac factor

Having an intact glycocalyx (Normal condition) helps to prevent clotting.

To prevent uncontrolled clot formation

Negative Feedback

- **Fibrin fibers bind 85-90% of thrombin and localize it to the clot**
- **Antithrombin III combines with the remainder and inactivates it over 12-20 minutes**

Fibrin threads absorb thrombin to localize it at the site of injury, preventing it from spreading throughout the blood vessel.

Of Thrombin

Antithrombin III is not efficient alone; it must bind to heparin, a polysaccharide, to perform its full activity (100-1000x more active)

Prevents further thrombus growth.

Normally Produced by the body

- **Physiologically, availability is limited**
- **Used therapeutically**
- **Highly negatively charged**

Heparin

used to treat certain blood vessel, heart, and lung conditions.

Heparin is given intravenously

- **Binds anti-thrombin III and increases its effectiveness 100- to 1000-fold**
- **Heparin-antithrombin III removes free thrombin from the blood almost instantly**
- **Also removes XIIa, XIa, Xa, and IXa**
- **Mast cells, basophils particularly abundant in pericapillary regions of liver and lung**

12a , 11a ,10a & 9a from intrinsic pathw

Where there is a tendency for bloo

Heparin is abundant around capillaries where small ruptures may occur

After a clot is formed, when healing begins, the clot
needed because it could block blood flow if it remai
That's why clot lysis takes place .

(Fibrinolysis)
(anticoagulant)

Clot Lysis

- **Plasminogen is trapped in the clot**

Plasminogen is the most crucial factor in cl
present in plasma and synthesized in the li
inactive form.

- **Over several days**(Of coagulation) **, injured tissues release tissue plasminogen activator (tPA)**
- **Plasminogen is activated to plasmin, a protease resembling trypsin**
- **Plasmin digests fibrin fibers and several other clotting factors**
- **Often results in re-opening repaired small blood vessels**

Practice Questions

1) Which coagulation pathway begins with tissue thromboplastin?

A) Extrinsic pathway
B) Intrinsic pathway
C) Common pathway
D) Fibrin stabilization

2) What is the primary mechanism by which hep
vents blood coagulation?

A) Antithrombin III activation
B) Binding and inhibition of tissue factor
C) Binding available calcium
D) Inhibition of platelet-activating factor

3) Which of the following causes some malnourished patients to bleed excessively when injured?

A) Vitamin K deficiency
B) Platelet sequestration by fatty liver
C) Serum bilirubin that raises neutralizing thrombin
D) Low serum protein levels that cause factor XIII problems

1) **A)** The extrinsic pathway begins with the release of tissue thromboplastin in response to vascular injury or contact between traumatized extravascular tissue and blood. Tissue thromboplastin is composed of phospholipids from the membranes of tissue.

2) **A)** The primary function of heparin is to bind to and activate antithrombin III.

3) **A)** Several clotting factors that are formed in the liver require vitamin K to be functional. Vitamin K is a fat-soluble vitamin, and absorption is dependent on adequate fat digestion and absorption. Therefore, any state of malnutrition could have decreased fat absorption and result in decreased vitamin K absorption and decreased synthesis of clotting factors.

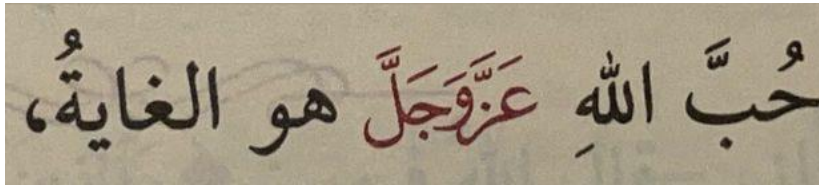
Additional sources

1. Book pages :

   Guyton & Hall physiology book “pages 483-490”

2. Youtube videos

   https://youtu.be/SGzp9wqeu84?feature=shared



VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2	13	Prothrombin	Prothrobinase
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!