HEMATOLOGY
&LYMPH SYSTEM

physiology sheet

Number	7
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Correction	tasneem mountaj
Doctor	saleem

* Subjects of this lecture :

- Hemostasis
- Platelets, general information, their ultrastructure and role in hemostasis.
- Definitions: Thrombus, Embolus, Arteriosclerosis and Atherosclerosis.

***NOTE**: Prof Khreisha's slides were the main source of information. Hemostasis in new textbooks is a little bit different than from how he presented it, hence I recommend reading a few pages of Robbins Basic Pathology, 9th edition (79-86) or at least taking a look at them, as hemostasis is important from a pathological perspective rather than
physiological.

-Hashim ahmed is the one who wrote this sheet . I just edited it according to our record.

Corrector's note:
-Don't let the number of pages scare you, the sheet is simple, direct, and comprehensive. It's been reviewed a gazillion times. You got this.

-At the beginning of the lecture prof. Khreisha asked whether this statement is medically correct or not “does iron absorption occur mostly in the jejunum?”
Answer: it is correct we all know that the duodenum is the main site for iron absorption, but because the jejunum length is longer than the duodenum; jejunum capacity for iron absorption is much greater.

Hemostasis

- **Hemostasis means to stop bleeding.**
    - If a large blood vessel (e.g. internal carotid artery) was injured, no physiological mechanism exists to stop bleeding, so applying pressure and suturing the blood vessel is the only choice we have.

    - However, small blood vessels (I.e. arterioles, venules, and capillaries) can be sealed by platelet plug formation or *clotting*. This is where hemostasis works.

***So, hemostasis aims to stop bleeding from small blood vessels once injured*.**

Before talking about hemostasis, purposefully, we are going to revise the normal structure of a blood vessel:

- A blood vessels is composed of three layers, arranged from the lumen outwards as the following:
  1-Endothelium and basement membrane (contains collagen).
  2-Smooth muscles for vasodilation and vasoconstriction.
  3-Layer of connective tissue composed mainly of collagen

The presence of endothelium is important to maintain the integrity of blood vessels, and thus deliver the blood to tissues. So, when blood vessels are damaged,
there *must* be something which repairs them. This is what hemostasis is for.

-If the cut in the blood vessel is very small, this can be simply sealed by *platelet plug*.
However, if the cut is large, there must be a *blood clot* to seal it.

- **Hemostasis is divided into two stages**: **primary** and **secondary** hemostasis.

  ***In **primary hemostasis** we form a **platelet plug,but** this may be too weak or loose to seal a large cut in a blood vessel. So, **secondary hemostasis** exists to stabilize the weak, loose platelet plug, forming a **blood clot.**

  ****Primary Hemostasis** = **Formation of a weak platelet plug**. This is mediated by interactions between the **endothelium** and **platelets**.

  ** **Secondary Hemostasis** = **Stabilization of the weak platelet plug, forming a stable insoluble blood clot (fibrin clot).** This is mediated by the **coagulation cascade.**

So, the three elements of hemostasis are:
1- Endothelium
2- Platelets
3- Coagulation cascade

**Before we start explaining the details of hemostasis, we want to introduce the whole story briefly and discuss Platelets. This is an overview to the whole subject. I know it might be overwhelming, but be patient. read the rest of the sheet and re-read it again until it sinks in.*

- If we have a large cut in a small blood vessel, we have to form a clot. Now, how
  can this clot be formed?

    - First, we have to **reduce the blood flow to the injured area by vasoconstriction.**
    - Then, we have to call for platelets to come and form a plug **(Platelet adhesion and aggregation).** By that, we have formed the weak platelet plug of primary hemostasis.

    - Now, we have to form a stable blood clot and this is achieved by the formation of **insoluble cross-linked fibrin. This is mediated by the coagulation cascade.**

- let's talk about the platelets, which play an essential role in this process.

Platelets

- Platelets are developed from the giant cells called "megakaryocytes" in the bone marrow, which diameter is usually around 100 micrometers. A single megakaryocyte can give rise to about 4000 platelets.
- platelets are *anucleated* cells.
- The **differentiation time "**thrombopoiesis" is **10 days**. Remember that RBCs and WBCs need 6-7 days for maturation in the bone marrow.
- The hormone which controls their formation in the bone marrow is **thrombopoietin,** produced in the **kidney and to a lesser extent from the liver**.
- **Life span** is **10 days**.
- Platelet Count (150,000-450,000).
- High count = Thrombocytosis.
- Low count = Thrombocytopenia.

- **Normally, the bone marrow contains only about one day's reserve of platelets. Therefore, human beings are susceptible to develop thrombocytopenia more quickly than granulocytopenia or erythrocytopenia.**

- **Platelets maintain the integrity of blood vessels as mentioned early, BUT HOW?**

  Every day, very small ruptures in the walls of blood vessels occur very frequently. In this case, platelets form a plug and seal these cuts calmly *without* activating the coagulation cascade and clot formation.
  This hemostatic process *needs platelets,* that's why **platelets maintain the integrity of blood vessels**.
  This is evidenced, as platelets deficiency results in letting these ruptures open, and thus RBCs migrate from the slightly injured blood vessel into the tissues, forming hemorrhagic areas under the skin.

- **Ultrastructure of Platelets (under the microscope):**

  - Function of platelets in hemostasis:

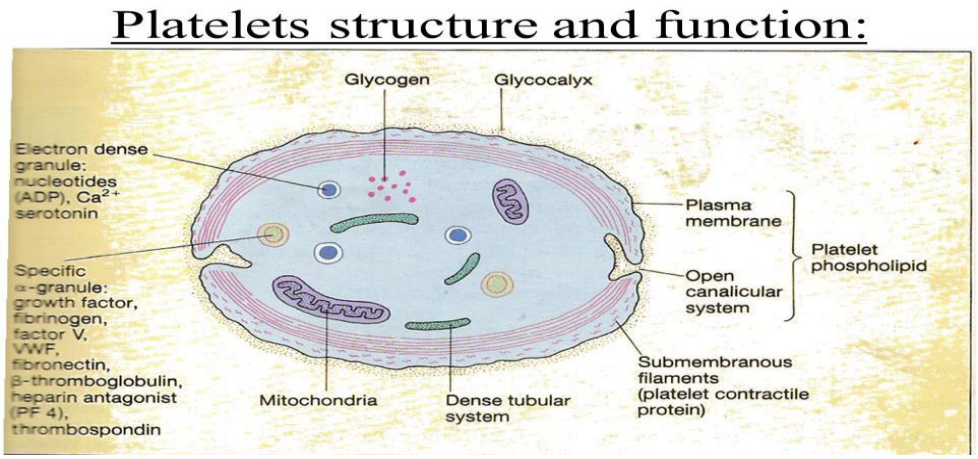
    a- Formation of the weak, loose hemostatic plug in primary hemostasis.
    b- Providing a phospholipid surface that recruits and concentrates coagulation factors (don't consume your time in this point; it will be clearly discussed later).

- To understand how platelets contribute to these functions, we have to understand their ultrastructure.

<u>Platelets have:</u>
a- Plasma Membrane
b- Granules:

1- **Electron dense granules (delta granules):***Contain*:
ADP and ATP, Ca++, serotonin, histamine, and epinephrine.
2- **Specific granules (alpha granules):***Contain*:
Acid hydrolases, growth factor, fibrinogen, factors 5 and 8, fibronectin, beta-thromboglobulin, and platelet factor-4 (a heparin antagonist).

All these components play a role in hemostasis.



As we mentioned before, primary hemostasis is the formation of a weak hemostatic plug, composed of platelets.

In order to do that, we have first to reduce the blood flow by vasoconstriction. This is followed by the attraction of platelets to the site of endothelial injury, where platelets can adhere (**Platelet Adhesion**), release their granular content (**Platelet Activation** or **Release Reaction**), then aggregate (**Platelet Aggregation**), and then fusion, as well as their procoagulant activity occurs.

Steps of Primary Hemostasis:

Step 1: Vasoconstriction

- **Goal**: To reduce the blood flow.
- **Mechanism**:
  - There are multiple things that cause vasoconstriction at the site of endothelial injury. These are:
    1-Myogenic Constriction (Local Myogenic Spasm). *Due to the injury,the smooth muscle of the blood vessel contracts.*
    2- Endothelin, a vasoconstrictor derived from the endothelium.
    3- Serotonin, derived from platelets.
    4- Thromboxane A2.

Step 2: Platelet Adhesion

Now, we have to bring platelets to the site of damage and there must be something to bind these platelets. How can we do that?

→ When the endothelium is injured, the subendothelial collagen will be exposed. vWF binds to collagen and then a glycoprotein known as Gp1b on the platelet plasma membrane binds to vWF.

→ vWF and Gp1b are essential for platelet adhesion, and if they were genetically deficient, the patient shows increased bleeding tendency.

THAT IS WHAT SCIENCE SAYS.

However, Prof. Khraisha added information which I couldn't prove from any resource. Prof Khreisha said, quote: "For adhesion to occur, we need both factor *VIII:vWF and* glycoprotein I *on the plasma membrane of platelets".*

"Factor VIII is produced by endothelial cells and platelets and is made up of several functional parts. Functional Parts of Factor VIII are:
1- Factor VIII:C refers to the coagulant portion of the molecule and represents the ability of the molecule to correct coagulation.
2- Factor VIII:AG makes possible platelet aggregation.
3- Factor VIII:vWF that is required for normal platelet adhesion in hemostasis."

Step 3: Platelet Activation or Release Reaction

After adhesion, platelets are activated (i.e. can release their granules contents).
- Activated platelets release ADP, serotonin, lysosomal enzymes, heparin neutralizing factor (PF4) and calcium ions.
- *The most important ones are ADP and Ca++*.

Activated platelets also synthesize thromboxane A2 which causesvasoconstriction as well as stimulation of platelet aggregation.

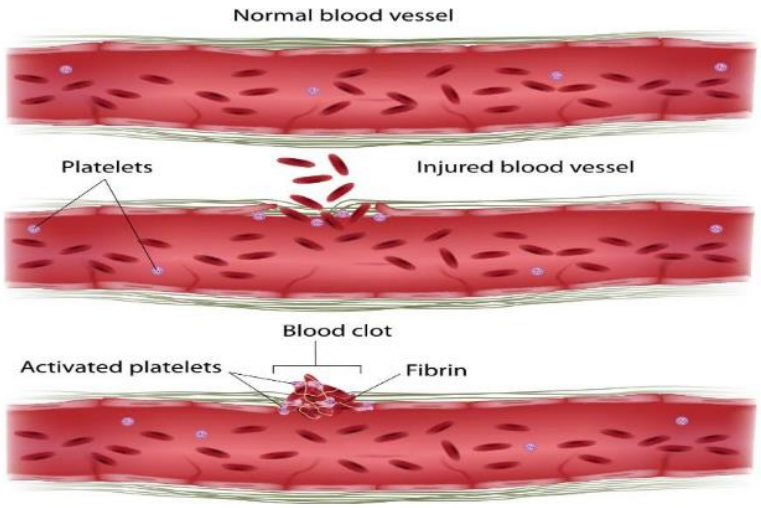
Note: Aspirin inhibits cyclooxygenases which produce TXA2 in platelets, and thus inhibits hemostatic plug formation; hence its effectivity in prevention of abnormal blood clotting.

<u>Once a blood vessel is injured, we want platelets to adhere and aggregate only at</u>
<u>the site of injury but not in adjacent areas.</u> To achieve that, TXA2 is produced from platelets during the release reaction, causing more vasoconstriction and further platelet aggregation ONLY at the site of action.

-On the other hand, **PGI2 (Prostacyclin) and N.O** are produced in adjacent endothelial cells, causing vasodilation and inhibition of platelet aggregation to the surrounding
intact areas of the blood vessel.

Step 4: Platelet Aggregation

- Released ADP and thromboxane A2 cause additional platelets to aggregate at the site of vascular injury.
- ADP causes platelets to swell and encourages the platelets membranes of adjacent platelets to adhere to each other.



Secondary Hemostasis

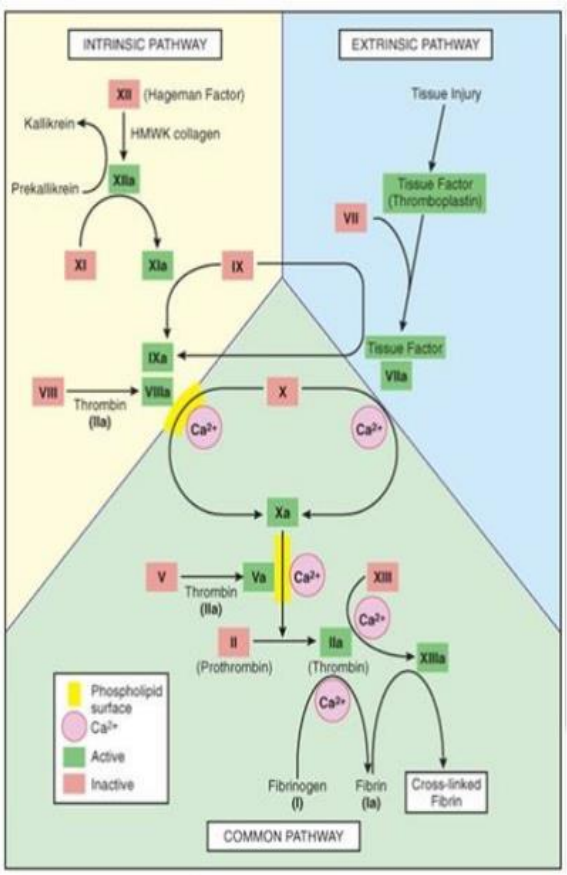
* Secondary hemostasis aims **to stabilize the primary hemostatic plug**. This is mediated by the **coagulation cascade**.
* The coagulation cascade is a cascade which involves a group of proteins synthesized in the liver in inactive forms (proenzymes).

* The coagulation cascade means that at each step, a proenzyme is cleaved to become an active enzyme that then cleaves the next proenzyme to become an active enzyme and so on.

* Clotting factors are synthesized in the liver in inactive forms that circulate in the blood. So, when are these going to be activated?
1- Exposure to a foreign surface (subendothelial collagen) like factor 12.
2- Phospholipid surface:

- The complex of enzymes, substrates and calcium needs a phospholipid surface to assemble on. This surface is provided by platelets or endothelial cells.

3- Calcium



The Role of Calcium in Hemostasis:
-Without calcium, the blood doesn't coagulate. Calcium ions are required for each step in the clotting process **except for the first two reactions of the intrinsic pathway**.(to be discussed in a moment).
-Adequate levels of calcium are therefore *necessary* for normal clotting. In reality, plasma calcium levels never fall low enough to impair the clotting process, since

death would have resulted from other causes (most notably tetany of respiratory muscles) long before.

***EDTA** (EthyleneDiamineTetraacetic Acid) or **citrate**, which bind calcium and other ions and it is used to treat blood clotting .HOW ? we know that Calcium is necessary for the coagulation of the blood , so EDTA binds Ca and inhibits it in forming the blood clot .
* EDTA is used in lead poisoning ,because it can bind lead and prevents its action.

* The coagulation cascade has two pathways:
    a- The **intrinsic** pathway: **all** of its components are **in** the blood.
    b- The **extrinsic** pathway: **not all** of its components are **in** the blood.

A. Intrinsic Pathway

- When the blood is exposed to a foreign surface, such as the subendothelial collagen exposed after vascular endothelial injury, factor XII gets activated and thus converted to factor XIIa.

- Then, factor XIIa acts on factor XI to activate it. This reaction needs prekallikrein and HMWK.
- Factor XIa activates factor IX.
- Factor IXa activates factor VIII.
- Factor IXa + Factor VIIIa + Phospholipid surface + Calcium form an activating complex called **Tenase** which activates Factor X (substrate of Tenase).

Note: Each step in the coagulation cascade occurs using an activating complex. Each activating complex is composed of an enzyme (the earlier clotting factor), substrate (the latter clotting factor), calcium ions and a phospholipid surface.

- E.g: When we convert factor 9 to factor 8, factor 9 is the enzyme and factor 8 is the substrate.

- It's a delayed pathway (not very fast). It needs minutes (~6 min) to start working, but it's long lasting and more important than the extrinsic pathway.
- Platelets can activate factor XI, even in the absence of factor XII, prekallikrein and HMWK. So, there's no abnormality resulting from the absence of these three factors, unlike platelets deficiency.

B. Extrinsic Pathway

- Fast (needs only seconds to start working).
- Phospholipids and proteins from the injured tissue (thromboplastin) combine with calcium to activate factor VII, forming factor VIIa.
- Then, factor VIIa with calcium form a complex (also called Tenase) to activate factor X.
- Activated Factors X, V can cleave prothrombin, forming thrombin.
- Thrombin cleaves fibrinogen, forming fibrin.
- To cross-link fibrins, factor XIII in the presence of calcium and thrombin acts on fibrin threads to stabilize them.
- *Factor XIII* is also called *fibrin-stabilizing factor*.

****Functions of Thrombin:**

1- Activation of fibrinogen.
2- Activation of factors V, VIII and XIII.
3- Activation of platelets.
4- Activation of protein C, an anticoagulant.

****Functions of Ca+2 inside the platelets:**

1- Contraction of Actin & Myosin.
2- Secretion of granules content.
3- Phospholipase activation.

- ***Regulation of Clotting:***

- Blood clotting is an essential process in the human body that prevents blood loss when blood vessels are injured. However, if it were to be turned on in an irregular manner, pathological problems result. For example, if clotting takes a long time to occur, hemorrhage results, and if the opposite, thrombosis will occur.

To avoid this, there must be certain things that maintain the blood in its fluid form.

what causes the normal fluidity of the blood/ how do we maintain blood's fluidity?

1- Heparin from basophils and mast cells. It acts as an anticoagulant.
2- Clotting factors, mainly prothrombin and fibrinogen, are present in the blood in **the inactive** form. During the circulation, some of them are removed by theliver.
3- **Minor clottings** which occur **normally** and dissolve quickly.

From this process, there are two advantages:

a- First, clotting factors are reduced to some extent as they are used for these clottings.

b- Second, the end products of degradation of the minor clottings. (fibrin/fibrinogen degradation products) function as anticoagulants.

4- Endothelial lining of vessel is *smooth*, sticking of platelets to it doesn't occur. Also, both the lining & the platelets have *negative charges* repelling platelets away from
lining.

5- There is a protein (anti-coagulant) in blood called **antithrombin III**. It inhibits the
action of thrombin as well as factors IX, X, XI, and XII.

6- Thrombin is bound by a specific receptor on endothelial cells, thrombomodulin. The result of this interaction is conversion of circulating protein C to its active form, Ca, protein Ca in the presence of phospholipid, Ca+2 & a co-factor, protein S, inactivates factor V & VIII & thus limits the generation of thrombin. Proteins C & S require vitamin K for their synthesis in the liver & protein Ca also enhances fibrinolysis.

7- Two other proteins, a2 -macroglobulin & alpha-1-antitrypsin, also contribute to the antithrombin effect of plasma.

- **What happens when the clot is no longer needed (i.e. it was formed, sealed the blood vessel, and the blood vessel is now healthy again and there's no need for the clot)?**

  → The clot first retracts and then lysed by fibrinolysis.

<u>Clot Retraction:</u>

Following the coagulation of blood, the clot gradually shrinks as serum is extruded from
it. This is achieved by contraction of platelets.

- If a blood tube is left in the lab for two hours, the blood volume shrinks by 50%.
-Calcium and platelets membranes are responsible for clot retraction.
-Chemicals released from platelets also play a role, but their membranes are more important in this process.

Clot Dissolution:

- The fibrin in the clot has to be lysed. This is achieved by an enzyme called ***plasmin***.
- When the subendothelial collagen is exposed, factor XII is activated to start the coagulation cascade. At the same time, factor XII activates *fibrinolysis*. How?

Factor XII stimulates the production of kallikrein. Kallikrein promotes the conversion of plasminogen into plasmin. This plasmin digests fibrin and lyse the clot.

* Plasminogen activators:

1- Endogenous Activators:

a- Tissue plasminogen activator: produced by endothelial cells.

b- Contact phase of coagulation.

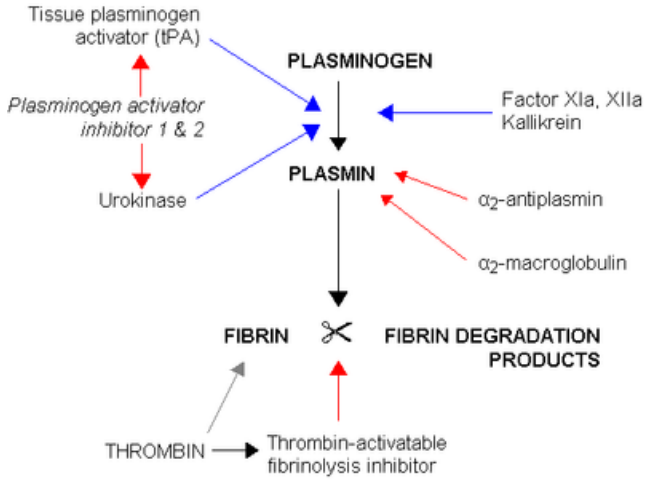
Exogenous: Urokinase (from the blood) and streptokinase (from bacteria).

* These are life injections that lyse clots.

- Alpha2-antiplasmin inhibits plasmin (as a way of balance).

*Plasmin causes:

- Proteolysis of fibrinogen, fibrin and factors V and VIII.
- This proteolysis produces fibrinogen degradation products which inhibit the polymerization of fibrin and platelet aggregation.



* Sometimes, unwanted clots are formed in the blood vessels & they don't move forming → **Thrombus.**
* This thrombus is either dissolved, or sometimes under the effects of the circulation is pushed & removed from its attachment and circulates in the blood vessels too. this circulating clot is called an **Embolus**.
* The embolus can sneak into narrow spaces, & if this embolus reaches the heart or the brain, this results in a serious condition because the embolus obstructs the blood supply.

*Most heart attacks are caused by either:

- **Atherosclerosis:** the **accumulation** of lipids inside the blood vessels, so they become relatively narrow.
- **Arteriosclerosis**: **losing the flexibility** of the arteries especially in old age, it's considered to be normal to some extent. However, if this happens during adulthood, then that is a sign of an abnormality.

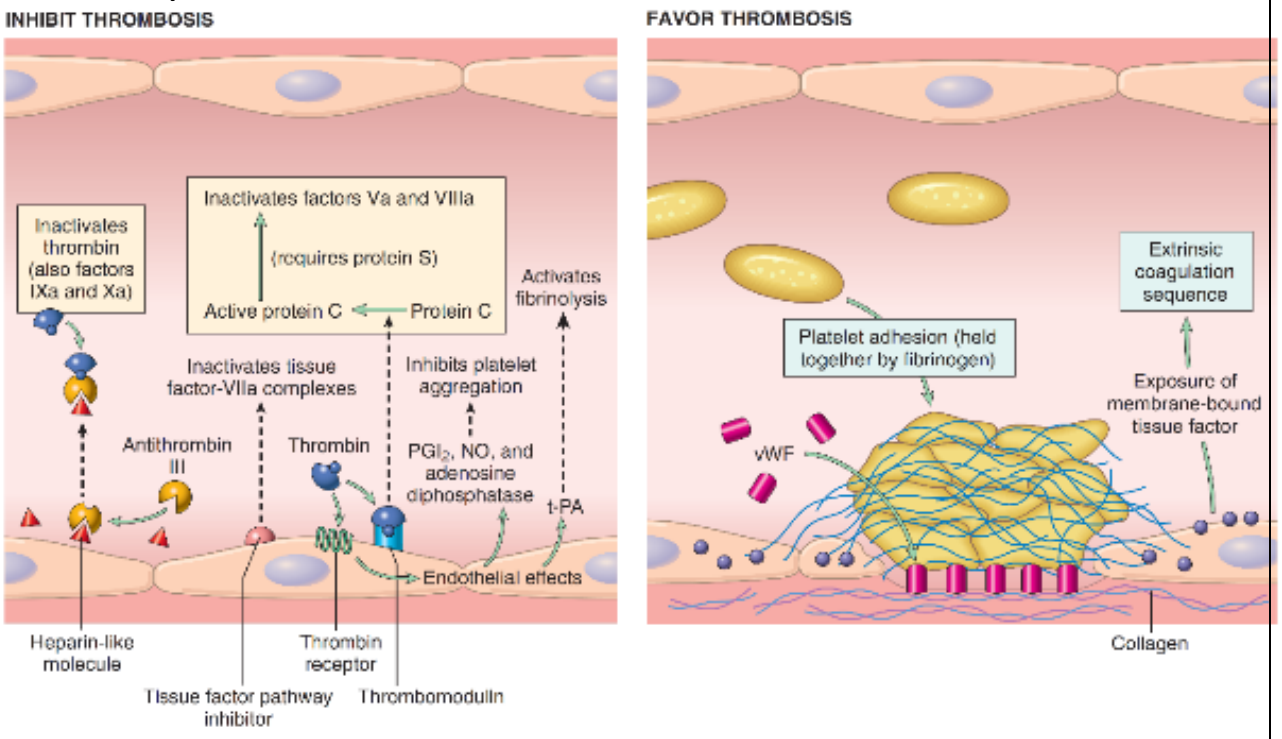


Figure 3–6 Anticoagulant properties of normal endothelium *(left)* and procoagulant properties of injured or activated endothelium *(right)*. NO, nitric oxide; PGI_2, prostaglandin I_2 (prostacyclin); t-PA, tissue plasminogen activator; vWF, von Willebrand factor. Thrombin receptors are also called protease-activated receptors (PARs).

Final note: This is taken from the doctor's handout. He said that you should know them.
He also mentioned that almost all factors are produced in the liver, and hence any liver
disease will affect clotting.

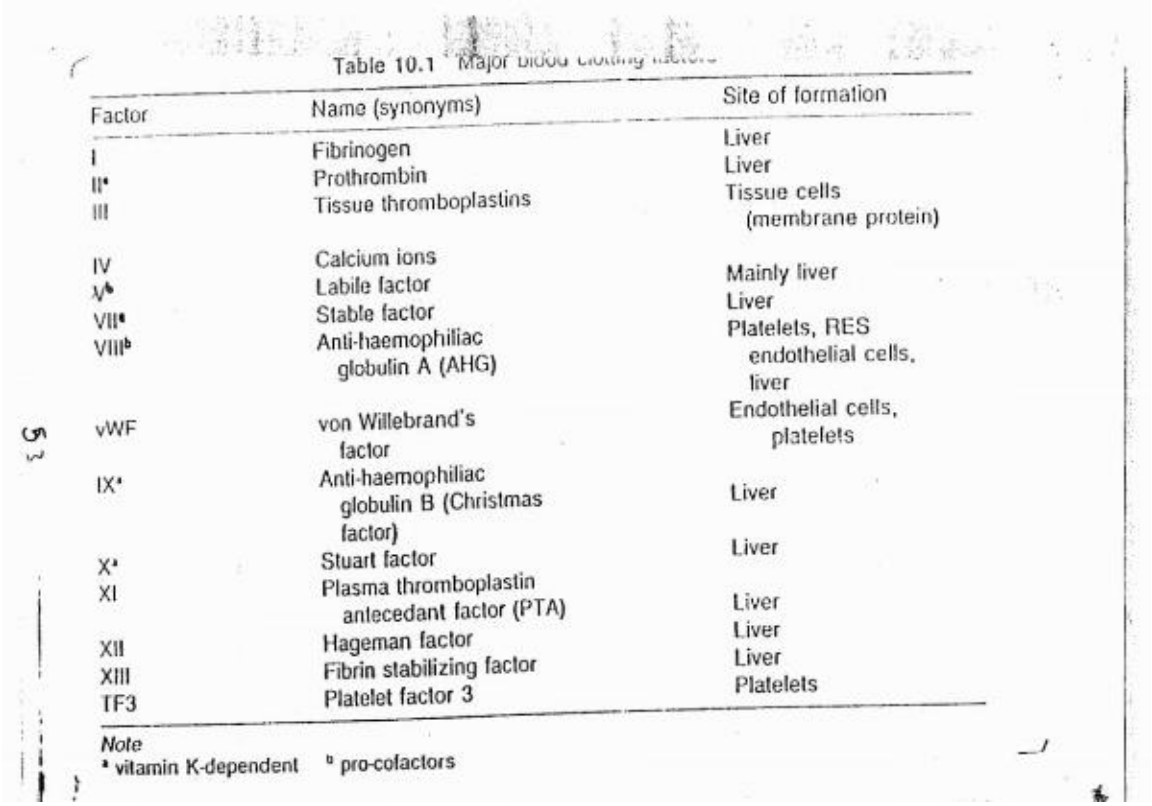


Table 10.1 Major blood clotting factors

Factor	Name (synonyms)	Site of formation
I	Fibrinogen	Liver
II[a]	Prothrombin	Liver
III	Tissue thromboplastins	Tissue cells (membrane protein)
IV	Calcium ions	Mainly liver
V[b]	Labile factor	Liver
VII[a]	Stable factor	Platelets, RES endothelial cells, liver
VIII[b]	Anti-haemophiliac globulin A (AHG)	Endothelial cells, platelets
vWF	von Willebrand's factor	Liver
IX[a]	Anti-haemophiliac globulin B (Christmas factor)	Liver
X[a]	Stuart factor	Liver
XI	Plasma thromboplastin antecedant factor (PTA)	Liver
XII	Hageman factor	Liver
XIII	Fibrin stabilizing factor	Liver
TF3	Platelet factor 3	Platelets

Note
[a] vitamin K-dependent [b] pro-cofactors

*DON'T FORGET THAT:- **Vitamin K-dependent FACTORS ARE : Factors II, VII, IX, X. And proteins C & S. (very important note).**