MEDICINE 438's

GIT PHYSIOLOGY

LECTURE VII: Coagulation Mechanisms

EDITING FILE

IMPORTANT | MALE SLIDES | EXTRA | FEMALE SLIDES | LECTURER'S NOTES

1 COAGULATION MECHANISMS

Lecture Seven

OBJECTIVES

- Recognize the different clotting factors
- Understand the role of calcium ions during clotting cascades.
- Describe the cascades of intrinsic and extrinsic pathways for clotting.
- Recognize process of fibrinolysis and function of plasmin.
- Recognize some conditions causing excessive bleeding.
- Understand some important anticoagulants and their mechanism of action.

Introduction

A crucial physiological balance exists between factors promoting coagulation "**procoagulants**" (eg; platelets, phospholipids, clotting factors) and factors inhibiting coagulation "**anticoagulants**" (eg; natural inhibitors, antithrombin III, protein C and S, fibrinolysis)(discussed later)

- Coagulation depends on **balance** between these two factors.
- Disturbances in this balance leads to **thrombosis** or **bleeding.**

Hemostasis

Hemostasis is defined as spontaneous arrest or stoppage of blood loss.

Coagulation: Formation of fibrin meshwork or threads to form a clot.

Blood clot: is composed of a meshwork of fibrin fibers running in all directions and entrapping blood cells, platelets, plasma.[1]

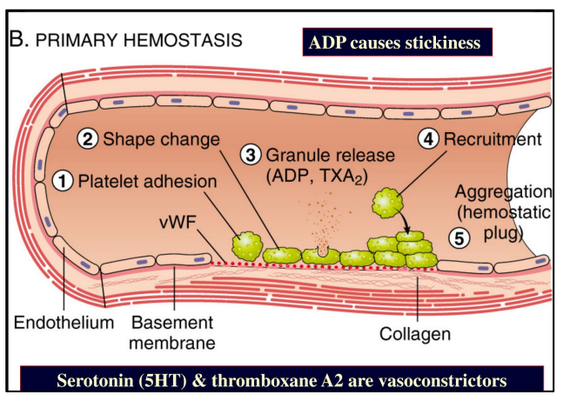

Figure 7-2

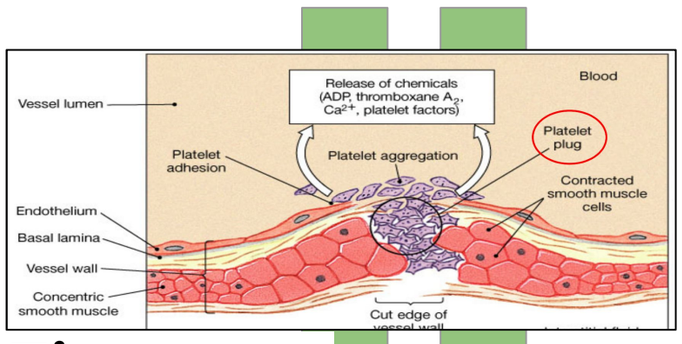

Figure 7-1

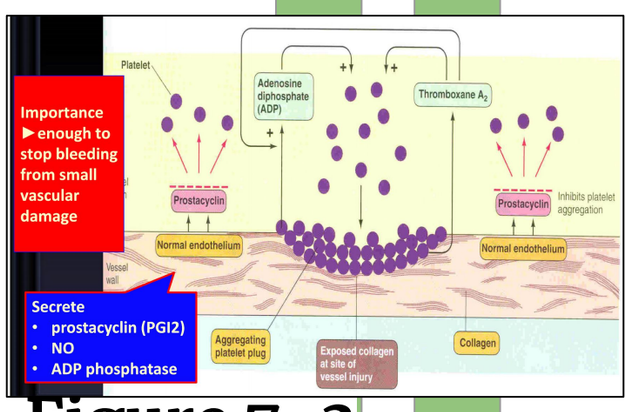

Figure 7-3

Mechanism of Hemostasis:

1. Vessel wall (vasoconstriction)

Causative Factors are three:

A) Nervous reflexes.
B) Local myogenic spasm
C) Local humoral factors (Platelets release Thromboxane A2 which is a vasoconstrictor). It's inhibited by aspirin, as it is a product of COX enzymes action on arachidonic acid.

Importance: Crushing injuries → intense spasm→ no lethal loss of blood.

2. Platelets (production and activation of platelets followed by platelet plug formation)
   - Figure 7-2, Figure 7-3
3. Blood coagulation: clot formation and retraction (intrinsic & extrinsic pathways)(discussed later)
4. Fibrinolysis (discussed later)

I	Fibrinogen
II	Prothrombin
III	Tissue factor (Thromboplastin)
IV	Calcium
V	Labile factor, proaccelerin, Ac-globulin (Ac-G)
VI	No longer used (previously thought to be present, was mistaken for factor Va)
VII	Stable factor, serum prothrombin conversion accelerator (SPCA), proconvertin
VIII	Antihemophilic factor, antihemophilic factor A, antihemophilic globulin
IX	Antihemophilic factor B, christmas factor, plasma thromboplastin component (PTC)
X	Stuart-Prower factor, Stuart factor
XI	Plasma Thromboplastin Antecedent (PTA), antihemophilic factor C
XII	Hageman factor, glass factor
XIII	Fibrin stabilizing factor
Pre-K	Fletcher factor, prekallikrein
HMWK	High molecular weight kallikrein, Fitzgerald factor

Table 7-1

FOOTNOTES

1. Platelets contain myosin and actin contractile proteins, and aggregated platelets can contract almost like a muscle within the clot to pull the blood vessel ends, and also to squeeze out the trapped plasma into a serum, since it will be lacking its clotting factors.

Features of Some Factors

Factors	Prothrombin (II)	Thrombin	Fibrinogen (I)	Fibrin-stabilizing factor (XIII)
Characteristics	Is a plasma protein, α2-globulin and its molecular weight is 68,700 Daltons Present in normal plasma in a concentration of **15 mg/dl.** It is unstable protein that can be split easily into **thrombin.**	Is a protein enzyme with weak proteolytic capabilities It acts on **fibrinogen** to form one molecule of **fibrin** monomer. **Fibrin monomers** polymerize with one another to form fibrin **fibers.** **Thrombin** is essential in platelet morphological changes to form a primary plug. **Thrombin** stimulates platelets to release **ADP** & **thromboxane A2**; both stimulate further platelets aggregation.[1] It activates **Factor XIII** and **Factor V.**	is a high-molecular weight plasma protein (nearly 340,000 Daltons). Plasma concentration 100-700 mg/dl Little or no **fibrinogen** leaks from blood vessels.[2]	is a plasma protein, it is also released from platelets that is entrapped in the clot. It must be activated before it affects the fibrin fibers. Activated **Factor XIII** operates as an enzyme causing additional strength of **fibrin** meshwork, promoting cross-linking.
Site of Synthesis	Liver	Activated form of prothrombin	Liver	Liver and megakaryocytes (platelets)

Table 7-2

Blood Coagulation

Clot formation: A series of biochemical reactions leading to the formation of a blood clot within few seconds after injury.

The duration depends on the severity of the injury:

1. 1-2 min→ Minor trauma.
2. 15-20 sec→ Severe trauma.

Prothrombin (inactive thrombin) is activated by a long ***intrinsic*** or short ***extrinsic*** pathways.

- This reaction leads to the activation of **thrombin** enzyme from inactive form **prothrombin.**
- Thrombin will change **fibrinogen** (plasma protein) into **fibrin** (insoluble protein)[3]

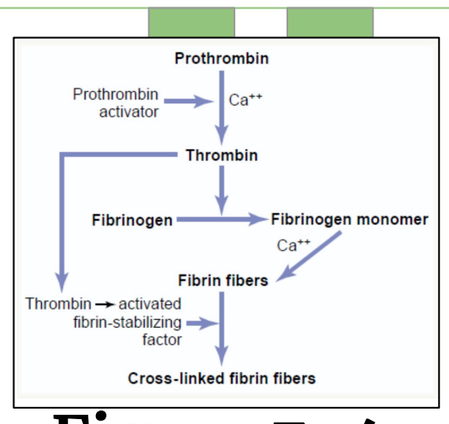

Figure 7-4

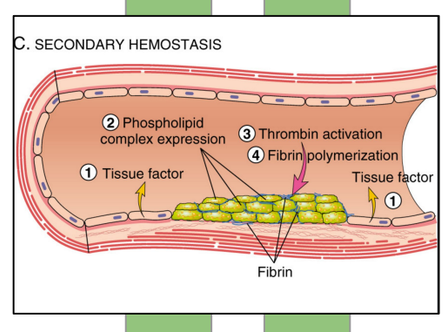

Figure 7-5

FOOTNOTES

1. Thromboxane A2 is a prostaglandin that causes vasoconstriction and platelet activation.
2. This occurs in normal conditions, in inflammatory conditions increased vascular permeability can promote extravasation of fibrinogen and possibly fibrosis.
3. A biochemical point to remember is that proteins are generally divided into structural and globular proteins, structural proteins are generally insoluble in water and mainly are not involved in metabolic reactions, like fibrin. Whereas globular proteins like enzymes perform a metabolic function and are water-soluble.

Coagulation Cascade: Classical Model

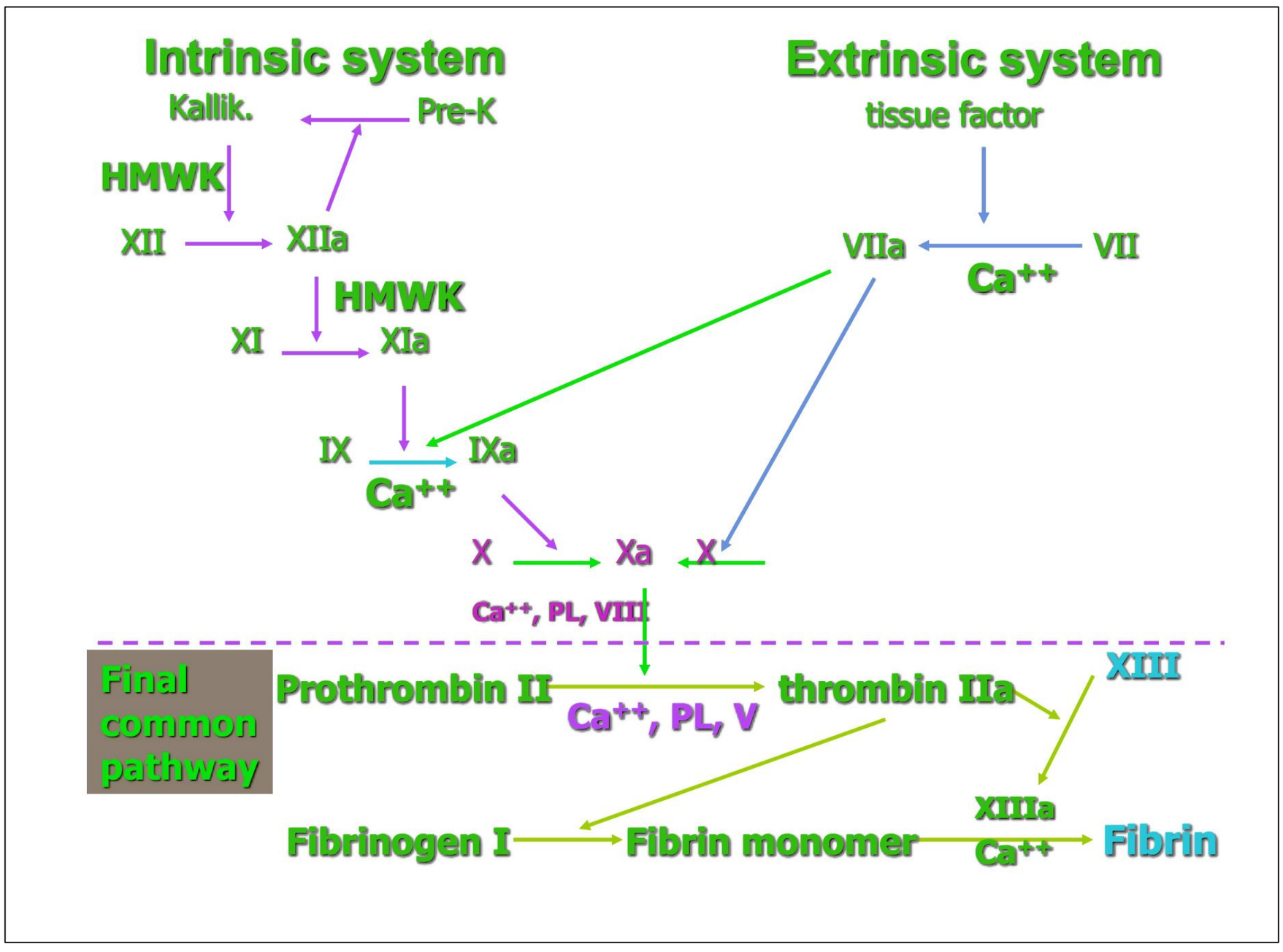

Figure 7-6 HMWK: High Molecular Weight. Kallikrein, a protease.

Intrinsic Pathway

The **trigger** is the activation of **factor XII** by contact with ***foreign surface, injured blood vessel, and glass.***[1] (all clotting factors present in the blood)

1. Activated **Factor XII** will activate **Factor XI**
2. Activated **Factor Xl** will activate **Factor IX**
3. Activated **Factor IX** + **Factor VIII** + **platelet phospholipid factor (PF3)** + **Ca^{++}** activate **Factor X**

Extrinsic Pathway

The **trigger** is ***release of tissue thromboplastin from damaged tissues and it is composed of phospholipids from the membranes of the tissue plus a lipoprotein complex that functions mainly as a proteolytic enzyme.***

1. **Tissue thromboplastin** activates **Factor VII**
2. **Tissue thromboplastin** + **Factor VII** + **Ca^{++}** activate **Factor X**

TF includes phospholipids from the membranes of the tissue plus a lipoprotein complex that functions mainly as a proteolytic enzyme.

Common Pathway

Following the above steps, the pathway is common for both intrinsic and extrinsic pathways.

1. Activated **Factor X + Factor V +PF3 + Ca^{++}** activate **prothrombin activator**; a proteolytic enzyme which activates **prothrombin**.[2]
2. Activated **prothrombin** activates **thrombin.**
3. **Thrombin** acts on **fibrinogen** forming insoluble **fibrin** monomers.
4. **Factor XIII** + **Ca^{++}** causes additional strength of **fibrin** meshwork → strong **fibrin** (strong clot)

FOOTNOTES

1. As in blood clotting within a glass tube.
2. To be exact, the molecule that causes thrombin activation is Factor Xa, however, once thrombin is activated it activates Factor V, and Factor V accelerates subsequent thrombin activation by combining with Xa. This is an example of positive feedback.

Coagulation Cascade: Cell-Based Model

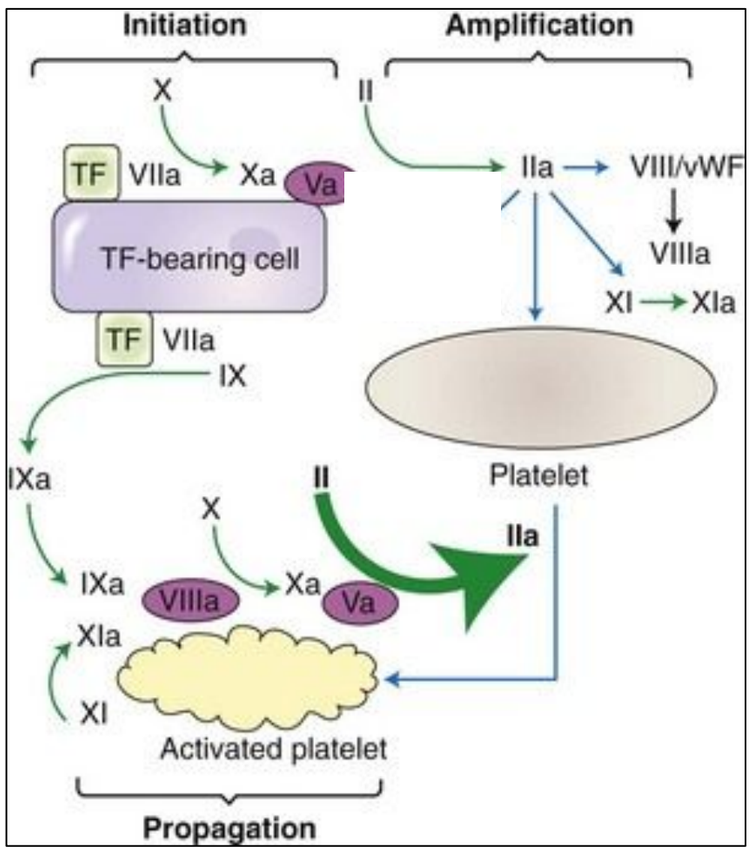

Figure 7-7 This is an alternative method to describe coagulation but in a precise, cell-based manner. **(This is a modified figure, the original slides figure can be seen here)**

- Damaged tissues express tissue factor on their surfaces, tissue factor acts as a receptor and an activator for factor VII, together they form a complex that activates factor X (much like the extrinsic pathway), and a non-specific activation of small amounts of factor IX. Activated factor X is expressed on the cell surface where it attracts factor V, together they activate small amounts of thrombin, this concludes **the initiation phase.**

- Thrombin then acts on platelets causing their activation, activated platelets release chemotactic factors attracting other coagulation factors that attach to the cell's surface, like factor VIII and factor XI, these factors were shown to be activated by the released thrombin. Activated factor VIII combines with factor IX from the initiation phase, and they activate larger amount of thrombin, this concludes **the amplification phase.**

- Thrombin activates more platelets, and attracts more coagulation factors and the process of the previous stage is repeated, this is the **propagation phase.**

Clot Retraction When clot retracts (contracts), it expresses most of the fluid from the clot within 20-60 min called → Serum which cannot clot.
Serum is plasma minus clotting factors.

Fibrinolysis

Formed blood clot can either become **fibrous** or **dissolved.**

- **Fibrinolysis (dissolving):** Breakdown of fibrin by naturally occurring enzyme ***plasmin*** therefore prevent intravascular blocking.

There is a balance between clotting and fibrinolysis

- Excess clotting → blocking of blood vessels.
- Excess fibrinolysis → tendency for bleeding.

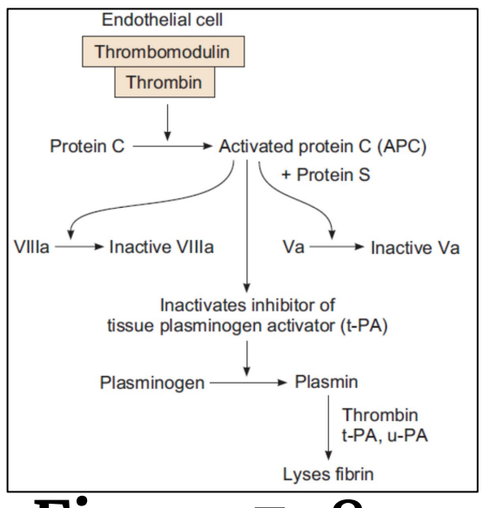

Figure 7-8

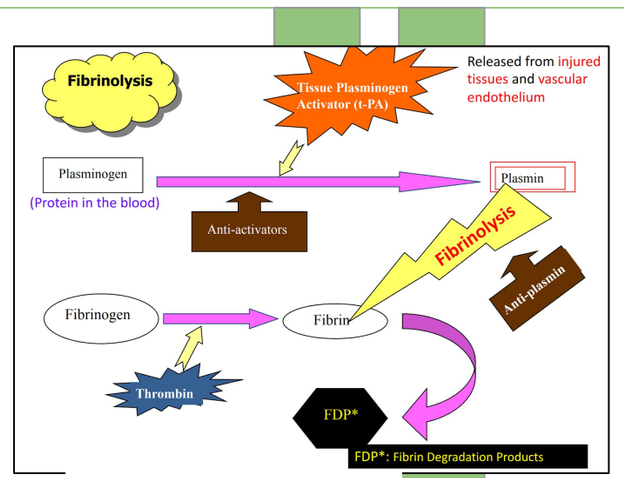

Figure 7-9

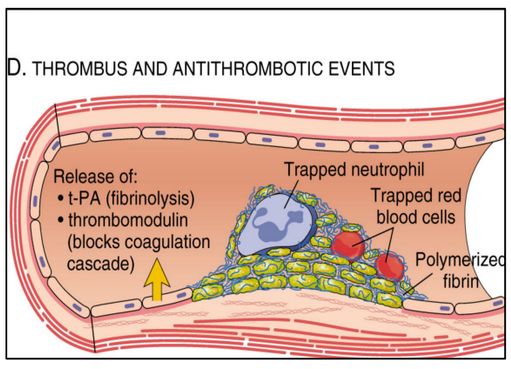

Figure 7-10

Role of Plasmin

Plasmin is present in the blood in an inactive form "**plasminogen.**"

- It is activated by **tissue plasminogen activators** (t-PA) in blood.
  Uses: Tissue Plasminogen Activator (t-PA) used to activate plasminogen to dissolve coronary clots. (an example for this is streptokinase)
- Digests intravascular and extravascular deposit of **fibrin → fibrin degradation products (FDP)**
- Unwanted effect of plasmin is the digestion of clotting factors.[1]

Plasmin is controlled by:

- **Tissue Plasminogen Activator Inhibitor (t-PAI)**
- **Antiplasmin** from the liver

Conditions That Cause Excessive Bleeding

Vitamin K Deficiency

Required for synthesis of **prothrombin, factor VII factor IX,** and **factor X.**

- It leads to increased **prothrombin Time**.
- Sources are diet and synthesis by intestinal bacterial flora

Deficiency is rare, but maybe seen in:

Hepatitis, cirrhosis, acute yellow atrophy[1] and GIT disease. Malabsorption syndromes, Biliary obstruction, Broad spectrum antibiotics, Dietary def (Neonates).

- Treatment:

1. Treat the underlying cause
2. Vitamin K injections

Hemophilia

- Bleeding tendency.
- X-linked recessive disease.
- Affects males.
- It leads to increased **Activated Partial Thromboplastin Time(aPTT).**
- Types :

1. **Hemophilia A** due to deficiency of **factor VIII small component**(85%) .
2. **Hemophilia B (Christmas disease)** due to **factor IX** deficiency(15%) .
3. **Hemophilia C** (Rosenthal syndrome) due to **factor XI** deficiency and it affects both sexes.

- Clinical Features includes : Easy bruising, massive bleeding after trauma or operation, hemorrhages in joints.

Thrombocytopenia

Very low number of platelets in blood (<50,000/µl and < 10,000 is fatal)

Etiology:

1. **Decreased production** such as Aplastic anemia, Leukemias, Drugs, Infections (HIV,Measles).
2. **Increased destruction** such as Immune Thrombocytopenia purpura, Drugs, Infections (HIV)

- **Thrombocytopenia purpura:** hemorrhages throughout all the body tissues.
- **Idiopathic Thrombocytopenia:** unknown cause.

Clinical features include:

- Easy bruising, epistaxis, gum bleeding, hemorrhage after minor, trauma, petechiae.

Diagnosis:

1. PLT count decreased.
2. Bleeding time increased.

- Treatment :

1. Treatment of the underlying cause.
2. Platelets concentrates.
3. Fresh whole blood.
4. Transfusion.
5. Spleenectomy.

Von Willebrand Disease

- It is an autosomal dominant disease due to defect in **the large component of factor VIII .**
- **It leads to increased :**

1. **Activated Partial Thromboplastin Time(aPTT).**
2. **Bleeding Time.**

Table 7-3

Pseudothrombocytopenia[2]

1. Partial clotting of specimen
2. EDTA-platelet clumping
3. Platelet satellitism around WBCs
4. Cold agglutinins
5. Giant platelets

FOOTNOTES

1. A fatal condition that leads to reduction in the liver's size, and an overall degeneration of its functions in response to toxic chemicals.
2. It's an in-vitro condition when taking blood samples, the platelets clump together and appear as few large platelets thereby mistakenly giving the impression of thrombocytopenia. This is associated with the use of EDTA as an anticoagulant, and the presence of cold agglutinin (autoantibodies) against platelets. As expected, antibodies can bind to WBC, therefore satellitism around WBC is also observed.

Test	Mechanism Tested	Normal Value	Disorder
Prothrombin time (PT)	Extrinsic and common pathway	<12s beyond neonate, 12-18s in-term neonate	Liver disease, defect in vitamin K-dependent factors, disseminated intravascular coagulation (DIC)
Activated partial thromboplastin time (aPTT)	Intrinsic and common pathway	25-40s beyond neonate, 70s in-term neonate	DIC, von Willebrand disease, hemophilia
Platelet count	Platelet number	150-450 cells per millimeter cubed	Thrombocytopenia
Bleeding time (BT)	Hemostasis, capillary and platelet function	3-7 minutes beyond neonate	Thrombocytopenia, on Willebrand disease

Table 7-4 Screening tests and their significance.

Test	Hemophilia A	Hemophilia B	vW disease
Bleeding time (BT)	Normal	Normal	Prolonged
Prothrombin time (PT)	Normal	Normal	Normal
Activated partial thromboplastin time (aPTT)	Prolonged	Prolonged	Prolonged
Factor VIII	Low	Normal	Low or normal
Factor IX	Normal	Low	Normal
vW Factor	Normal	Normal	Low

Table 7-5 Hemostasis in hereditary coagulation disorders

Prevention of Blood Clotting and Anticoagulants

Endothelial Surface Factors
- Smoothness of the endothelial cell surface (ECS)
- Glycocalyx layer[1]
- Thrombomodulin protein
- Thrombomodulin binds to thrombin Activates Protein C (with Protein S) inactivates factors V & VIII and inactivates an inhibitor of tPA increasing the formation of plasmin.

Fibrin Fibers
- Adsorbs 85-90% of **thrombin** to remove it from circulation.

α_2-macroglobuIin
- Synthesized mainly in liver and acts as a binding agent for several coagulation factors.
- Inhibits thrombin.

Antithrombin III
- Combines the remaining **thrombin** (10-15%) and removes it from circulation.

Warfarin (used in vivo)
- Decreases the production of Vit. K dependent clotting factors (II, VII, IX and X) by liver (Monitored by PT time).

Heparin
- A negatively charged polysaccharide that combines with **antithrombin III** and quickly removes **thrombin** from blood.
- **Sources:** Mast cells and basophils which are abundant in liver and lungs.
- Ir increases antithrombin III effectiveness by 100-1000 fold.
- Also remove Factors XII, XI, X, and IX (Monitored by PTT time).
- Taken parenterally, can not be taken orally, since it is a large molecule and highly charged.
- It's the most widely used anticoagulant clinically, as in stroke.

Anticoagulants Used in vitro[2] No Ca++ → no Clotting (needed in many steps).
- Citrate ions → Deionization of Ca++
- Oxalate ions → Precipitate the Ca++
- Ethylenediaminetetraacetic acid (**EDTA**) → chelates (binds) calcium ions
- Heparin → Binds to AT III

FOOTNOTES

1. Glycocalyx is a mucopolysaccharide on the surface of endothelial cells that repels clotting factors and platelets, preventing coagulation.
2. These anticoagulants are useful in determining partial thromboplastin and prothrombin time, this is done in the following manner: after a blood sample is taken, EDTA, citrate or oxalate are added to prevent activation of prothrombin, since they sequester and de-ionize calcium. After that, a huge amount of calcium and tissue factor are added, this will nullify the effect of oxalate or citrate and the time taken for the blood to clot is the prothrombin time. This tests the extrinsic pathway. To test the intrinsic pathway silica or other substance that cause activation factor XII are added, and the time taken for the blood to clot is the activated partial thromboplastin time, this tests the intrinsic pathway.

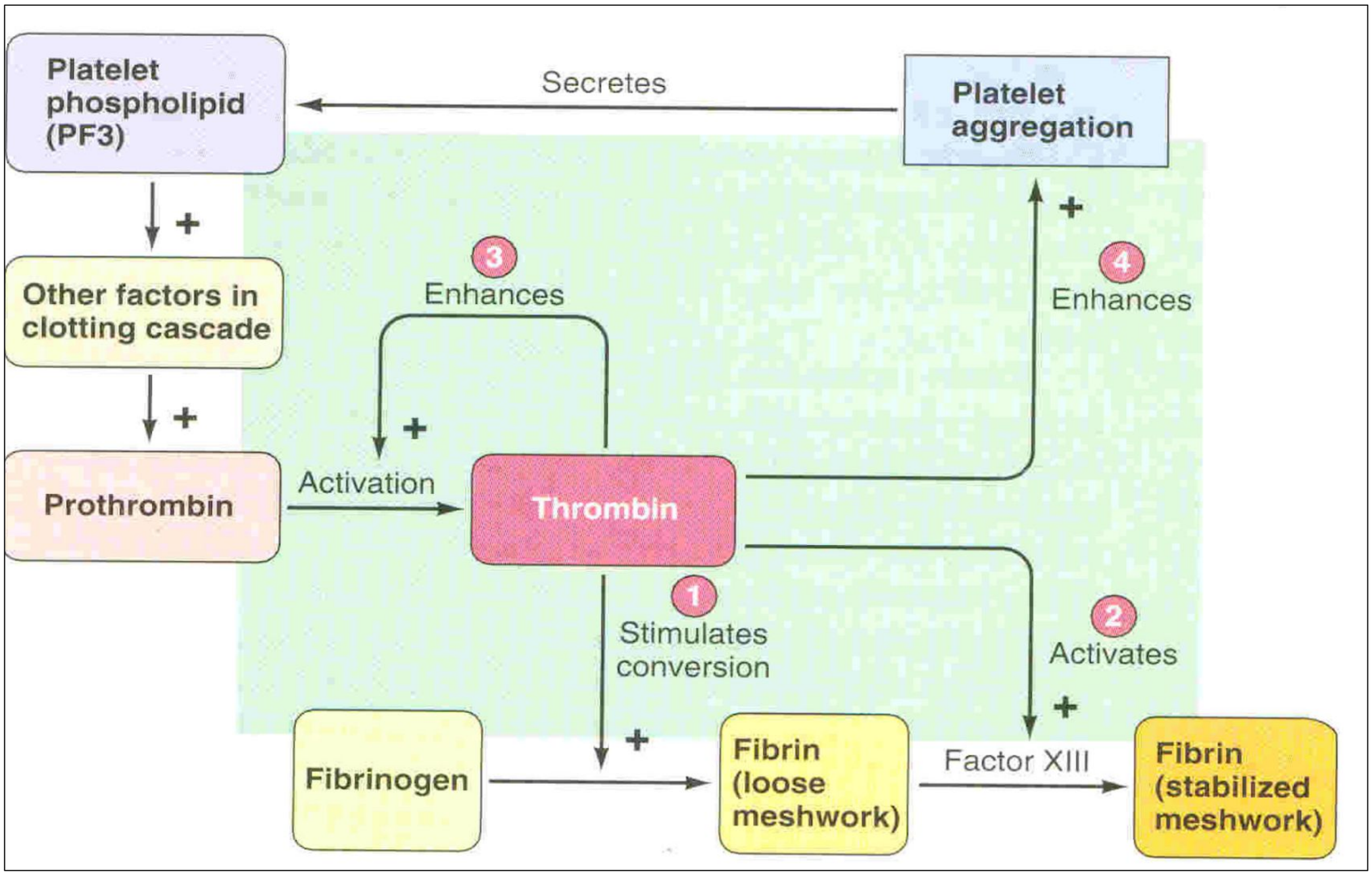

Figure 7-11 Summary of thrombin's actions

QUIZ

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1. Which of the following minerals found naturally in the body plays an essential role in the coagulation cascade?

A) Gold
B) Aluminium
C) Magnesium
D) Calcium

2. An essential vascular protective mechanism that is affected in smokers and hyperlipidemic patients and serves to protect against excessive activation of the coagulation cascade:

A) Smoothness of endothelial surface layer
B) Increased activity of plasmin
C) Deficiency of christmas factor
D) Vitamin K deficiency

3. A vasoconstricting prostaglandin playing an important role in initiating the hemostatic process:

A) Prostaglandin E2
B) Thromboxane A2
C) ADP
D) Prothrombin

4. The initiating step of the extrinsic pathway is:

A) Release of tissue thromboplastin
B) Conversion of prekallikrein to kallikrein
C) Activation of Factor VI
D) Activation of Factor XII

5. The most common cause for hemophilia is an X-linked deficiency in encoding which of the following factors:

A) Factor IV
B) Antihemophilic Factor B
C) Factor VIII
D) Factor III

SHORT ANSWER QUESTIONS

1. Name two factors activated by thrombin.
2. Name three physiological anticoagulants.

ANSWERS

1. Factor V and XIII
2. Heparin, protein C, antithrombin III

ANSWER KEY: D, A, B, A, C

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REFERENCES

- Guyton and Hall Textbook of Medical Physiology
- Ganong's Review of Medical Physiology