# *Lec (16)*

# Normal hemostases

The term ***haemostasis*** means Mechanisms which help to prevent of blood loss.

Which included four mechanisms:

1- **Vasoconstriction.**

2- **Formation of platelet plug**

3- **Blood coagulation**

4- **Formation of fibrous tissue**

**1- Vasoconstriction.**

This will help to reduces blood flow from injured vessel.

**Cause by:**

**1-** Sympathetic reflex

2- Release of vasoconstrictors ($TXA_2$ and serotonin) from Platelets that adhere to the walls of damaged vessels.

**2- Formation of platelet plug**:

**Mechanism:**

1-Platelet adherence

2-Platelet activation

3-Platelet aggregation

**Platelets**

Produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes .1/3 of marrow output of platelets is trapped in spleen. Normal count: 150,000-450,000/µL

Life span 7-10 days. Removed from circulation by tissue macrophage system mainly in spleen.

Thrombopoietin: major regulator of platelet production (produced by liver and kidney).

## Functional characteristics of platelets

The cell membrane of platelets contains:

A coat of glycoprotein (receptors) that cause adherence to injured endothelial cells and exposed collagen.

Phospholipids, that plays an important role in blood clotting.

## Their cytoplasm Contains

1- Contractile proteins (actin & myosin).
2- Dense granules, which contain substances that are secreted in response to platelet activation including serotonin & ADP.
3- α-granules, which contain secreted proteins e.g. platelet-derived growth factor (PDGF) which stimulates wound healing, fibrin stabilizing factor (factor XIII) and other clotting factors.

## Mechanism of platelet plug formation

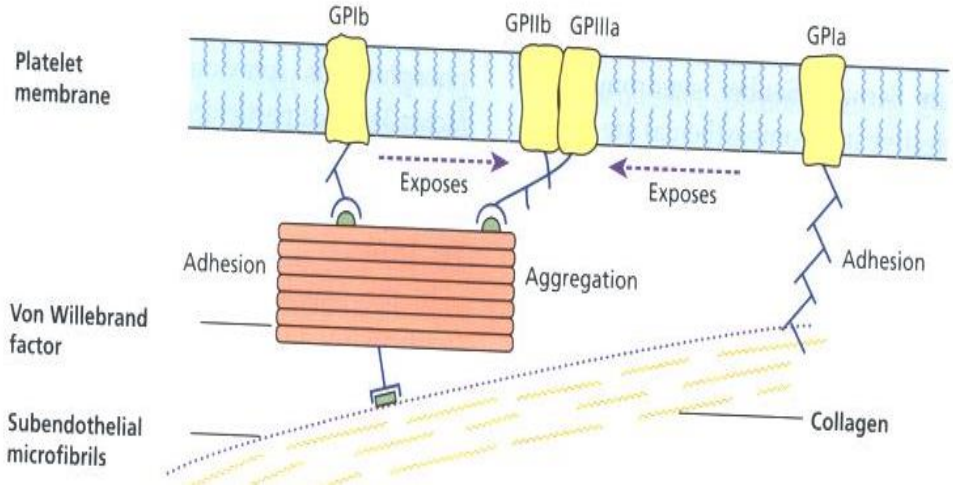
### 1- Platelet adhesion:

When a blood vessel wall is injured, platelets adhere to the exposed collagen and von Willebrand factor in the wall via platelet receptors.

The binding of glycoprotein (GP) Ib (which consists of four proteins: GPIb:, GPIb, GPIX; GPV to von Willebrand factor leads to adhesion to the subendothelium and also exposes the GPIIb/IlIa binding sites to fibrinogen and von Willebrand factor leading to platelet aggregation. The GPIa site permits direct adhesion to collagene

### 2- Platelet activation.

Activated platelets release the contents of their granules including ADP and secrete TXA2 →activates platelets to produce further accumulation of more platelets (platelet aggregation) and forming a platelet plug.



## 3- Platelet aggregation

It is characterized by cross-linking of platelets through active GPIIb/IIIa receptors with fibrinogen bridges. A resting platelet has about 50-80 000 GPIIb/IIIa receptors, which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb /IIIa molecules, due to binding of a-granule membrane (rich in receptors) with the plasma membrane, activation of surface-exposed GPIIb /IIIa, enabling platelet cross-linking with fibrinogen bridges. Binding brings about molecular conformational changes resulting in a firm Connection and further activation of the platelet.

**3-*Blood Coagulation***

The clotting mechanism involves a cascade of reactions in which clotting factors are activated.

Most of them are plasma proteins synthesized by the liver (vitamin K is needed for the synthesis of factor II, VII, IX and X).They are always present in the plasma in an inactive form.

When activated they act as proteolytic enzymes which activate other inactive enzymes.

Several of these steps require Ca++ and platelet phospholipid.

**Table 18.1** The coagulation factors

| Factor number | Descriptive name |
|---|---|
| I | Fibrinogen |
| II | Prothrombin |
| III | Tissue factor |
| V | Labile factor |
| VII | Proconvertin |
| VIII | Antihaemophilic factor |
| IX | Christmas factor |
| X | Stuart–Prower factor |
| XI | Plasma thromboplastin antecedent |
| XII | Hageman (contact) factor |
| XIII | Fibrin-stabilizing factor |
| | Prekallikrein (Fletcher factor) |
| | HMWK (Fitzgerald factor) |

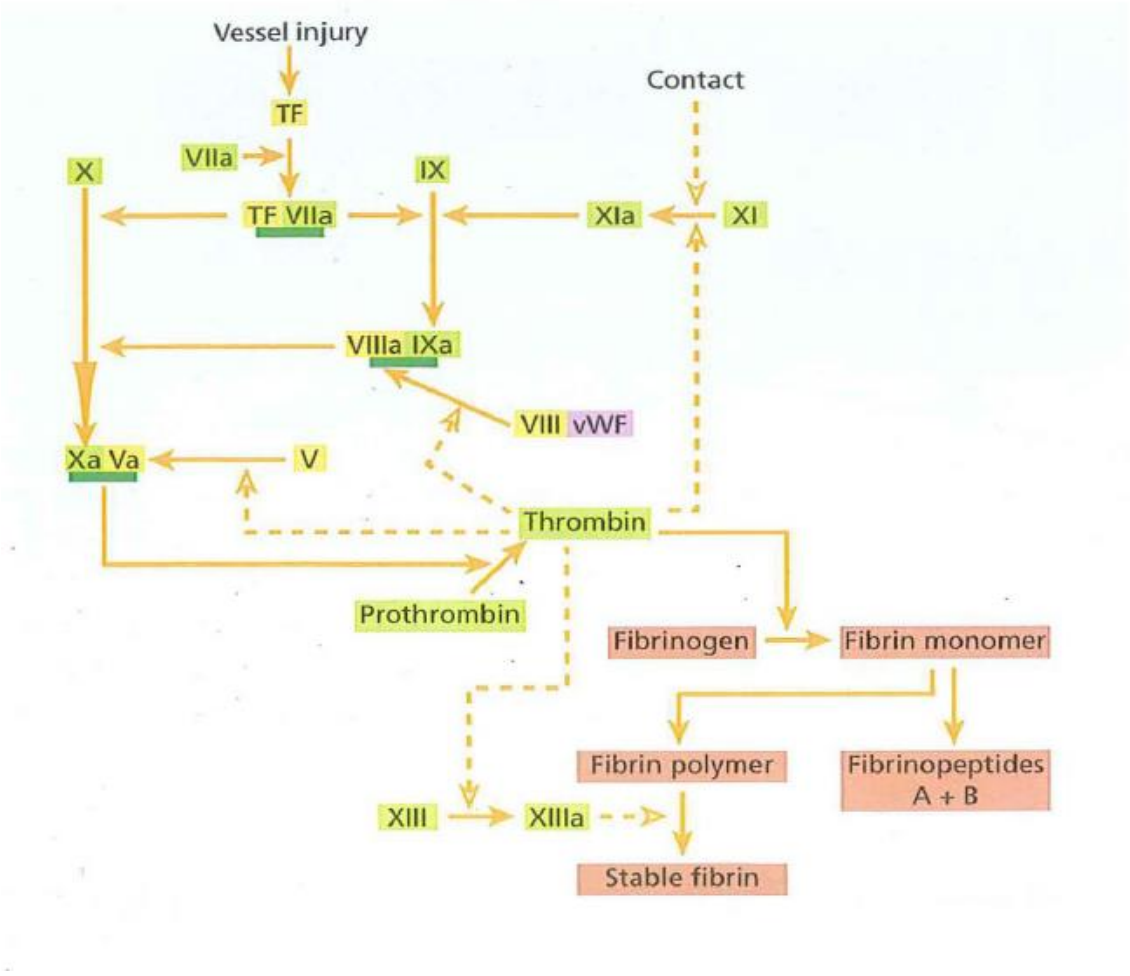
*Active without proteolytic modification.
HMWK, high molecular weight kininogen.

**Factor X can be activated by reactions in either of 2 systems**

***An Intrinsic pathway and An Extrinsic pathway in formation of clot***

## Intrinsic pathway

The initial reaction is the conversion of inactive factor XII to active factor XIIa. Factor XII is activated in vitro by exposing blood to foreign surface (glass test tube). Activation in vivo occurs when blood is exposed to collagen fibers underlying the endothelium in the blood vessels**.**



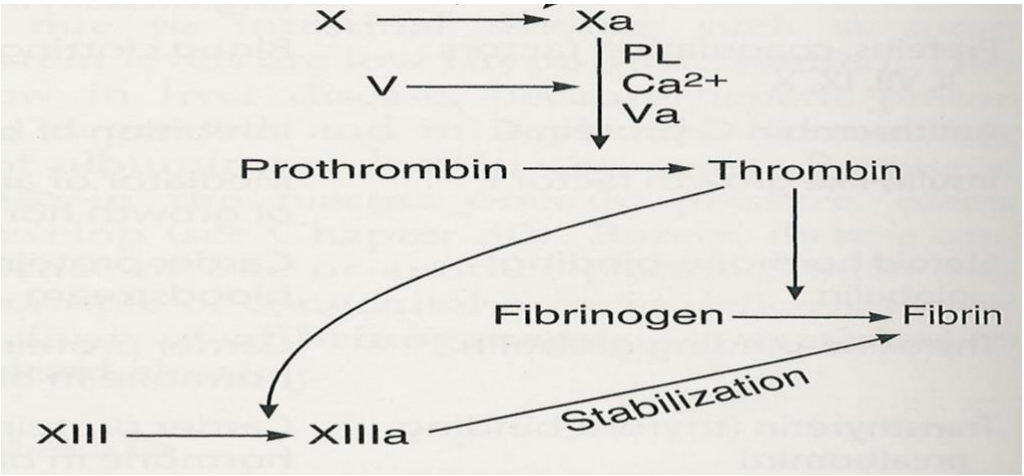

## Extrinsic pathway

Requires contact with tissue factors external to blood.

This occurs when there is trauma to the vascular wall and surrounding tissues. The extrinsic system is triggered by the release of tissue factor (thromboplastin from damaged tissue), that activates factor VII.

The tissue thromboplastin and factor VII activate factor X.

-The ultimate step in clot formation is the conversion of fibrinogen → fibrin.




## Clot retraction

Clot formation is fully developed in 3-6 min Contraction of platelets trapped within the clot shrinks the fibrin meshwork pulling the edges of the damaged vessel closer together. During clot retraction serum is squeezed from the clot

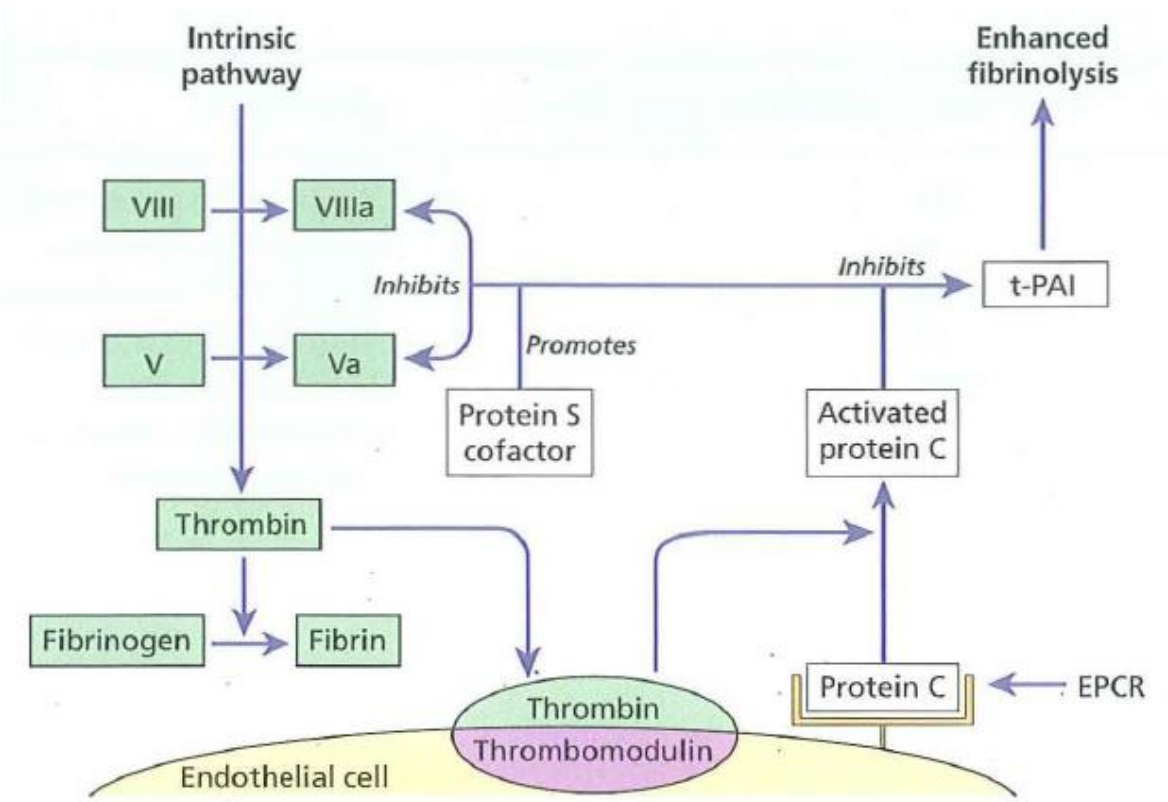
## Coagulation factor inhibitors

It is important that the effect of thrombin is limited to the site of injury. The first inhibitor to act is tissue factor pathway inhibitor (TFPI) which is synthesized in endothelial cells and is present in plasma and platelets and accumulates at the site of injury caused by local platelet activation. This inhibits Xa and Vila and tissue factor to limit the main *in vivo* pathway by forming the quaternary complex. There is direct inactivation of thrombin and other serine protease factors by other circulating inhibitors of which antithrombin is the most potent. It inactivates serine proteases by combining with them by peptide bonding to form high molecular weight stable complexes
Heparin potentiates its action markedly. Another protein, heparin cofactor II, also inhibits thrombin. α2-Macroglobulins, α2-antiplasmin, C1 esterase inhibitor and αl-antitrypsin also exert inhibitory effects on circulating serine proteasess

## Protein C and protein S

These are inhibitors of coagulation cofactors V and VIII. Thrombin binds to an endothelial cell surface receptor, thrombomodulin. The resulting complex activates the vitamin K-dependent serine protease protein C which is able to destroy activated factors Vand VIII, thus preventing further thrombin generation. The action of protein C is enhanced by another vitamin K-dependent protein, S, which binds protein C to the

platelet surface .An endothelial protein C receptor localizes protein C to the endothelial surface, promoting protein C activation by the thrombin-thrombomodulin complex.
In addition, activated protein C enhances fibrinolysis.




## BIood flow

At the periphery of a damaged area of tissue, blood flow rapidly achieves a dilution and dispersal of activated factors before fibrin formation has occurred. Activated factors are destroyed by liver parenchymal cells and particulate matter is removed by liver Kupffer cells and other reticuloendothelial cells.

## Fibrinolysis

Fibrinolysis (like coagulation) is a normal haemostatic response to vascular injury. Plasminogen, a B globulin proenzyme in blood and tissue fluid, is converted to the serine protease plasmin by activators either from the vessel wall (intrinsic activation) or from the tissues (extrinsic activation).

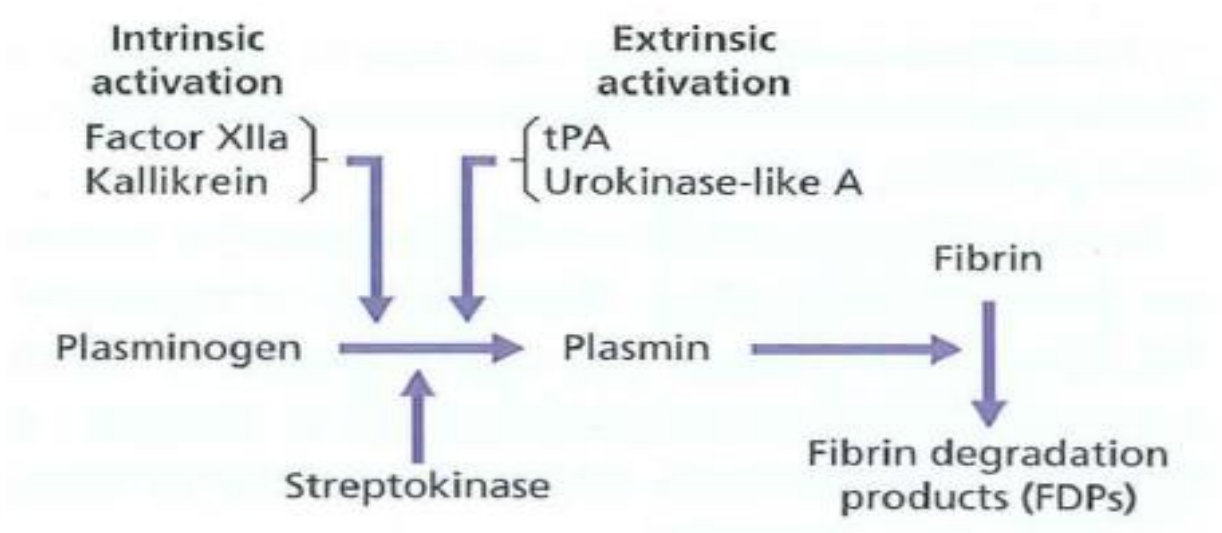
The most important route follows the release of tissue plasminogen activator (tPA) from endothelial cells. tPA is a serine protease that binds to fibrin. This enhances its capacity to convert thrombus bound plasminogen into plasmin. This fibrin dependence of tPA action strongly localizes plasmin generation by tPA to the fibrin clot. Release of tPAoccurs after such stimuli as trauma, exercise or emotional stress. Activated protein C stimulates fibrinolysis by destroying plasma inhibitors of tPA thrombin inhibits fibrinolysis by activating thrombin-activated fibrinolysis inhibitor (TAFl). Plasmin generation at the site of injury limits the extent of the evolving thrombus. The split products of fibrinolysis are also competitive inhibitors of thrombin and fibrin polymerization.

Fibrinolytic agents are widely used in clinical practice .Therapeutic recombinant tPA has been synthesized using recombinant *DNA* technology.

Urokinase is atPA initially isolated from human urine.

Plasmin is capable of digesting fibrinogen, fibrin, factors V and VIII and many other proteins. Cleavage of peptide bonds in fibrin and fibrinogen produces a variety of split (degradation) products.

Large amounts of the smallest fragments D and E can be detected in the plasma of patients with disseminated intravascular coagulation



## Inactivation of plasmin

Tissue plasminogen activator is inactivated by plasminogen activator inhibitor (PAl). Circulating plasmin is inactivated by potent inhibitors α2antiplasmin and α2-macroglobulin.

## Tests of haemostatic function

Defective haemostasis with abnormal bleeding may result from:

1 -A vascular disorder

2 -Thrombocytopenia or a disorder of platelet function

3 -Defective blood coagulation.

***A number of simple tests are employed to assess the platelet, vessel wall and coagulation components of haemostasis.***

## 1-Blood count and blood film examination

As thrombocytopenia is a common cause of abnormal bleeding, patients with suspected bleeding disorders should initially have a blood count including platelet count and blood film examination. In addition to establishing the presence of thrombocytopenia, the cause may be obvious (e.g. acute leukaemia).

## 2-Screening tests of blood coagulation

Screening tests provide an assessment of the 'extrinsic' and 'intrinsic' systems of blood coagulation and also the central conversion of fibrinogen to fibrin.The prothrombin time (PT) measures factors VII, X, V, prothrombin and fibrinogen. Tissue thromboplastin (a brain extract) and calcium are added to citrated plasma. The normal time for clotting is 10-14 s. It may be expressed as the international normalized ratio (INR)

The activated partial thromboplastin time (APTT) measures factors VIII, IX, XI and XII in addition to factors X, V, prothrombin and fibrinogen. Three substances-phospholipid, a surface activator (e.g. kaolin) and calcium-are added to citrated plasma.

The normal time for clotting is approximately 30-40s.

Prolonged clotting times in the PT and APTT because of factor deficiency are corrected by the addition of normal plasma to the test plasma

(50 : 50 mix). If there is no correction or incomplete correction with normal plasma, the presence of an inhibitor of coagulation is suspected.

The thrombin (clotting) time (TT) is sensitive to a deficiency of fibrinogen or inhibition of thrombin.

Diluted bovine. thrombin is added to citrated plasma at a concentration giving a clotting time of 14-16 s with normal subjects.

## 3-Specific assays of coagulation factors

## 4-Bleeding time

The bleeding time is a useful test for abnormal platelet function including the diagnosis of VWF deficiency. It has largely been replaced by the platelet function analysis-IOO (PFA-lOO) test. It will be prolonged in thrombocytopenia but is normal in vascular causes of abnormal bleeding. The test involves the application of pressure to the upper arm with a blood pressure cuff, after which small incisions are made in the flexor surface forearm skin. Bleeding stops normally in 3-8 min.

## 5-Tests of platelet function

The most valuable investigation is platelet aggregometry which measures the fall in light absorbance in platelet-rich plasma as platelets aggregate. Initial (primary) aggregation is caused by an external agent, the secondary response to aggregating agents released from the platelets themselves. The five external aggregating agents most commonly used are ADP, collagen, ristocetin, arachidonic acid and adrenaline. The pattern of response to each agent helps to make the diagnosis. Flowcytometry is now increasingly used in routine practice to identify platelet glyoprotein defects.

In the PFA-lOO test, citrated blood is aspirated through a capillary tube onto a membrane coated with collagen/ADP or collagen/adrenaline. Blood flow is maintained. Platelets begin to adhere and aggregate, primarily via VWF interactions with GPIb and GPIIb/IIIa, resulting in occlusion of the aperture.The PFA-lOO analysis may give false negative results with relatively common platelet defects. Full platelet function tests and VWF screening may be required to exclude abnormal platelet function, even if the PFA-lOO test is normal.

## 6-Test of fibrinolysis

Increased levels of circulating plasminogen activator may be detected by demonstrating shortened euglobulin clot lysis times. A number of immunological methods are available to detect fibrinogen or fibrin degradation products (including D-dimers) in serum. In patients with enhanced fibrinolysis, low levels of circulating plasminogen may be detected