HEMOSTASIS AND BLOOD COAGULATION

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CBU SCHOOL OF MEDICINE

❑Hemostasis means *stoppage of bleeding and prevention of blood loss* when a blood vessel is injured. It occurs by 4 mechanisms :

(A) Vasoconstriction (V. C.) of the injured vessel

This occurs as a result of the following :

1- Nervous reflexes (which are initiated by the pain of injury).
2- Myogenic contraction of the injured vessel (as a direct effect of trauma)
3- Release of serotonin and thromboxane A2 from platelets (both are V.C).

❑The greater the trauma, the stronger the V.C. and vice versa, so bleeding is severe in sharply-cut vessels (due to weak V.C. as a result of minor trauma).
❑V.C. is also weaker if the cut was longitudinal or irregular.

(B) Formation of a platelet plug (= temporary hemostatic plug)

❑The membranes of the platelets contain receptors for *collagen and ADP (the latter has 3 types of receptors)* as well as for *fibrinogen, thrombopoietin and the Von Willebrand factor*

- vWF is produced by the endothelial cells in the blood vessel walls and also by the platelets

❑The platelet plug is formed as follows:

1- As a result of injury of the blood vessel wall, the subendothelial collagen fibres are exposed.

2- The platelets adhere to collagen as well as to the Von Willebrand factor in the blood vessel wall via receptors on the platelet membrane (=platelet adhesion or binding)

3- Such binding of platelets initiates *platelet activation.* The activated platelets swell and develop pseudopodia, become sticky and release the contents of their granules, which contain ADP.

4- The released *ADP* binds to the nearby platelets (at the ADP receptors in their membranes) leading to their activation

5- The newly activated platelets stick to the originally-activated platelets *(= platelet aggregation)* and also release ADP which, in turn activates more and more platelets.

❑Thus a *vicious circle of platelet activation* is elicited leading to formation of a *loose platelet plug* (which can usually stop bleeding, but only from small wounds)

In addition to ADP, platelet aggregation is also promoted by

(1) A *platelet activation factor* (= *PA F)* which is secreted by the platelets and by the neutrophils and monocytes

(2) Thrombin

(3) Thromboxane A 2

(C) Formation of a blood clot (= definitive hemostatic plug)

- The platelet plug becomes firm *when fibrin threads* are deposited (discussed later), and this is essential for stoppage of bleeding from large vessels.

Clot retraction (=syneresis): A few minutes after formation of the blood clot, it starts to shrink and within 20-60 minutes,

- It becomes about one half its original size, and a clear non-coagulable yellow fluid called serum is squeezed out.
- As the clot retracts, it pulls the edges of the injured vessle together which helps hemostasis
- Clot retraction was believed to be produced by a substance secreted by the platelets called retractozvme.

- However, it was found that the *platelets themselves contract* when they attach to the fibrin threads (leading to clot retraction) due to activation of their contractile proteins *(actin, myosin and thrombostenine)* by *thrombin and Ca2+*

(D) Repair of the damaged blood vessel

- Fibroblasts plus the vascular endothelial and smooth muscle cells grow and proliferate

- This is by the effect of a growth factor secreted by the *platelets, macrophages and endothelial cells* (= *platelet-derived growth factor, PDGF*).

- This cellular growth invades the blood clot till it is organized into *fibrous tissue* within 1-2 weeks, which repairs the injured vessel

THE PLATELETS (THROMBOCYTES)

- These are small granulated oval or round bodies 2-4 microns in diameter.
- They have no *nuclei* and are formed as detached bits from giant cells in the bone marrow called *megakaryocytes.*
- Their normal count averages *300000/mm^3* and their half life averages *4 days.*
- Their production is regulated by (I) The colony-stimulating factors that control the production of megakaryocytes (2) Thrombopoietin.
- This factor is produced by the liver and kidneys and it facilitates maturation of the megakaryocytes (and there are also thrombopoietin receptors on the platelets).
- About 60-75 % of the platelets are present in the circulating blood while the remainder is present mostly in the spleen

STRUCTURE OF THE BLOOD PLATELETS

(A) The platelet membrane :

- This is extensively invaginated with a complex canalicular system in contact with the ECF.
- It contains *phospholipids* that include *platelet factor 3* (which plays an important role in blood clotting).
- There is also a coat *of glycoprotein* on its surface, which favours its *adhesion to injured endothelium* (but not to normal endothelium)

(B) The platelet cytoplasm :

- This contains the following :

(1) Glycogen, lysosomes, mitochondria, residuals of endoplasmic reticulum and Golgi apparatus (which synthesize enzymes and store Ca2+)

(2) *Contractile proteins* (actin, myosin and thrombosthenine).

(3) Dense granules that contain non-protein substances (specially ADP and serotonin)

(4) Alpha granules that contain protein substances (PDGF. Clotting factor XIII , Von Willebrand factor and PAF)

FUNCTIONS OF THE PLATELETS

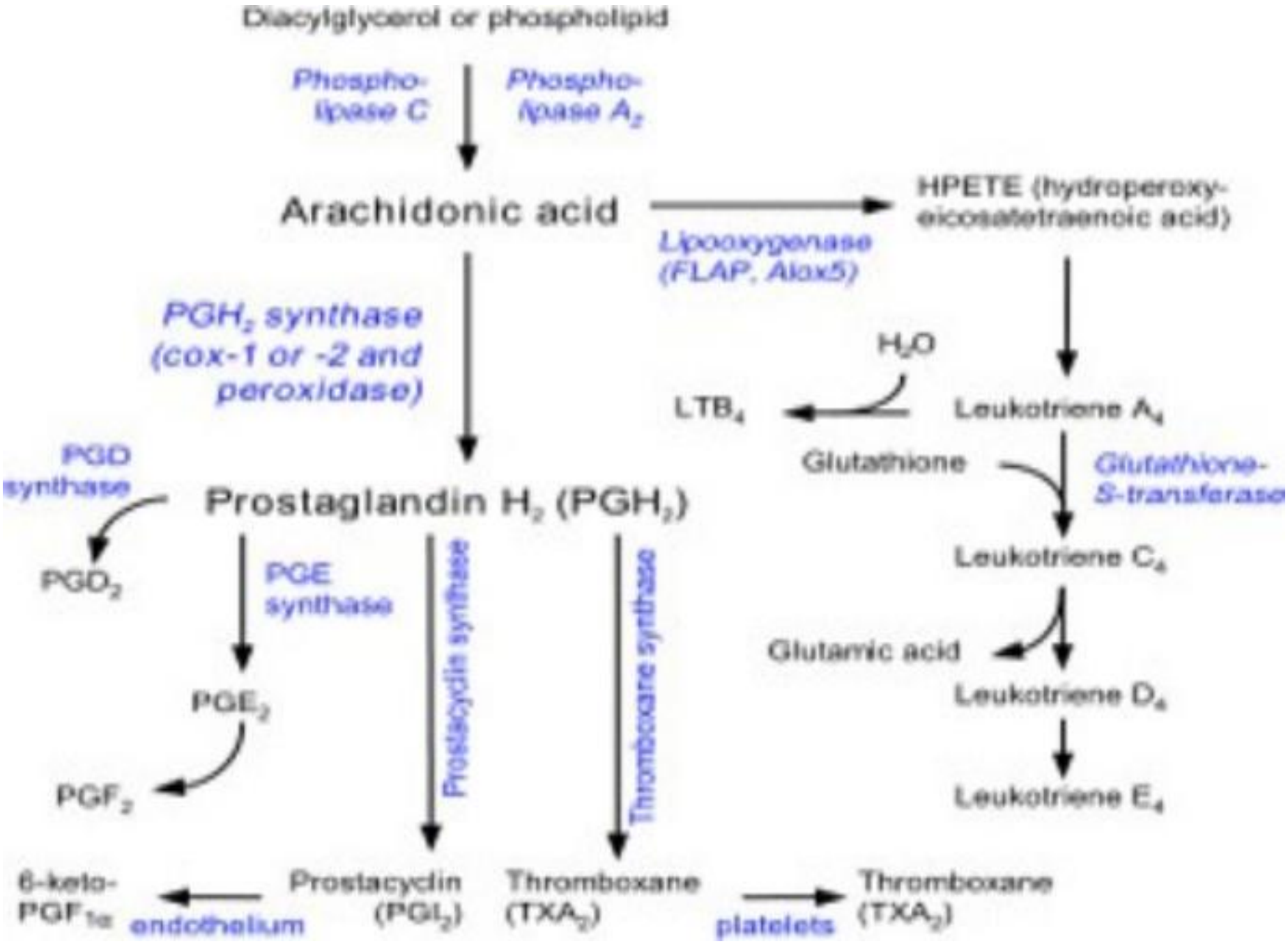
❑The platelets arc primarily concerned with *hemostasis* through performing the following functions :

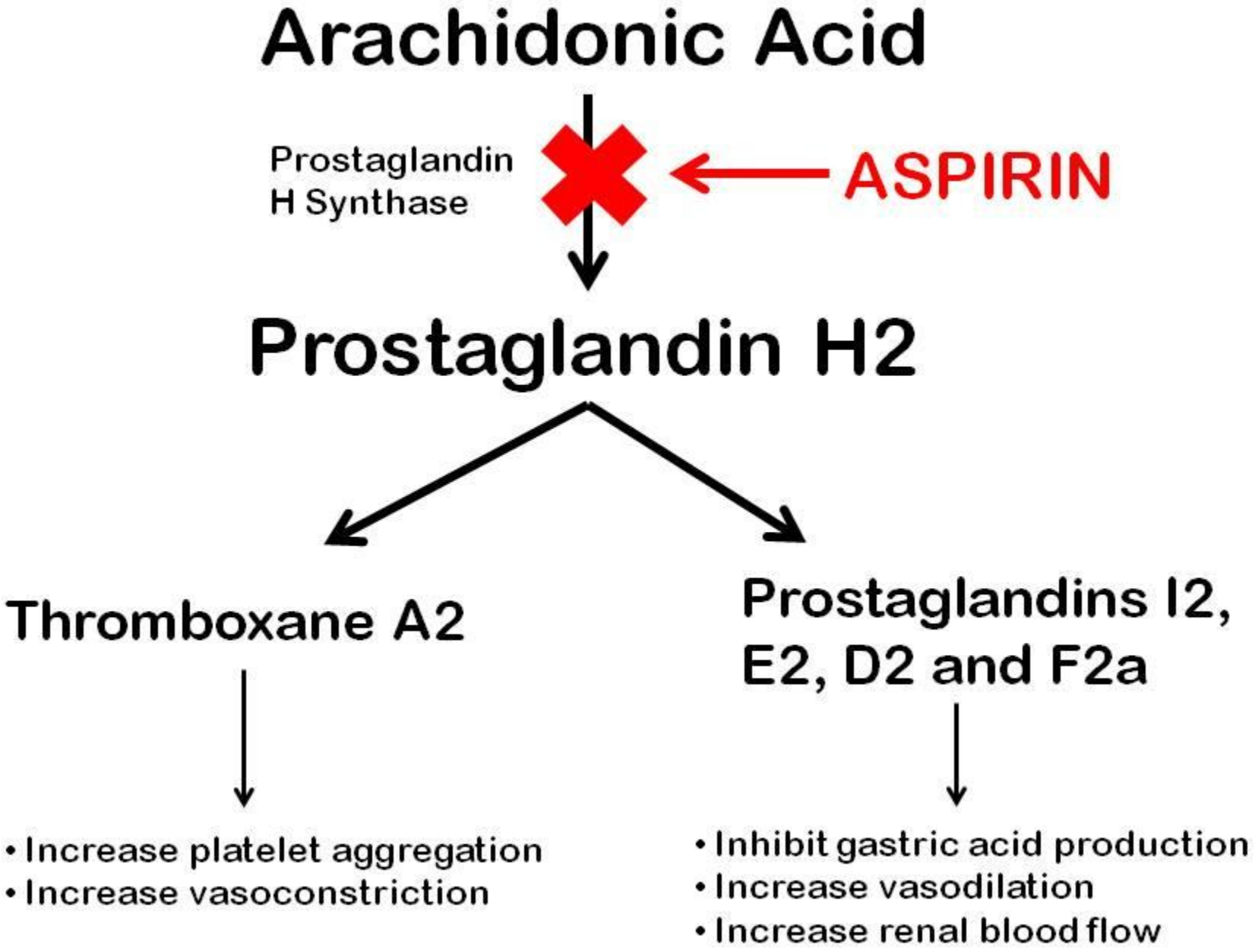
(1) Formation of the *primitive hemostatic plug*.
(2) Release of VC substances specially *serotonin and thromboxane A 2* .
(3) Release of *platelet factor 3,* which is essential for blood clotting.
(4) induction of *clot retraction* by its contractile proteins.
(5) Stabilization of the blood clot by *factor XIII.*
(6) Release of the *PDGF* which repairs of the damaged vessel wall.
(7) Helping conversion of prothrombin to thrombin in sites of clotting because much of the prothrombin first attaches to the platelets that are already bound to the damaged tissues (Guyton, 2006)

THROMBOXANE *A 2*

- This *is* a *prostaglandin-related substance* that causes *V.C. and promotes platelet aggregation.*
- It is formed from *arachidonic acid* as follows :
- This acid is converted to prostaglandin H2 (PGH2) by action of the cyclooxygenase enzyme.
- PGH2 is then converted into thromboxane A2 by action of the thromboxane synthetase enzyme.
- In the vessel walls, PGH_2 is also formed from arachidonic acid, but here, it is converted into PGI_2 (prostacyclin) by action of the prostacyclin synthetase enzyme.
- PGI_2 inhibits platelet aggregation and produces V.D. (opposite actions to those of thromboxane A2)

- The balance between the actions of thromboxane A2 and prostacyclin normally favours localized platelet aggregation and clot formation.
- This prevents excessive extension of the blood clot, which *keeps the blood vessels open and maintains the blood flow*
- Aspirin blocks the cyclooxygenase enzyme, so the production of both thromboxane A2 and prostacyclin is decreased.
- However, the endothelial cells can readily produce new cyclooxygenase while the platelets cannot.
- the formation of prostacyclin is much more rapidly restored than thromboxane A2 , thus platelet aggregation is inhibited and clot formation is reduced.
- For this reason, small doses of aspirin taken for prolonged periods can prevent intravascular clotting


Diacylglycerol or phospholipid
Phospho-lipase C
Phospho-lipase A2
Arachidonic acid
HPETE (hydroperoxy-eicosatetraenoic acid)
Lipooxygenase (FLAP, Alox5)
PGH2 synthase (cox-1 or -2 and peroxidase)
H2O
LTB4
Leukotriene A4
PGD synthase
Prostaglandin H2 (PGH2)
Glutathione
Glutathione-S-transferase
PGD2
PGE synthase
Leukotriene C4
Glutarnic acid
PGE2
Leukotriene D4
PGF2
Prostacyclin synthase
Thromboxane synthase
Leukotriene E4
6-keto-PGF1α
Prostacyclin (PGI2)
Thromboxane (TXA2)
endothelium
platelets
Thromboxane (TXA2)


Arachidonic Acid
Prostaglandin H Synthase
ASPIRIN
Prostaglandin H2
Thromboxane A2
Prostaglandins I2, E2, D2 and F2a
• Increase platelet aggregation
• Increase vasoconstriction
• Inhibit gastric acid production
• Increase vasodilation
• Increase renal blood flow

Differences between plasma and serum

- The composition or the plasma and serum is almost identical except that *serum lacks fibrinogen* (so serum is called *defibrinated plasma*)

- *It lacks most clotting factors specially factors V & VIII* (because they are consumed during the clotting process (so serum doesn't clot while plasma do)

- Serotonin is also more in the serum due to its release from the platelets.

BLOOD COAGULATION (CLOTTING)

FACTORS REQUIRED FOR BLOOD CLOTTING

Factor I	***Fibrinogen***
Factor II	***Prothrombin***
Factor III (TPL)	***Thromboplastin or Tissue factor***
Factor IV	***Ca^{2+} (Calcium ions)***
Factor V	***Proaccelerin, labile factor***
Factor VII	***Proconvertin, stable factor***
Factor VIII	***Antihemophylic globulin(AHG) or factor***
Factor IX	***Christmas factor***
Factor X	***Stuart Prower factor***
Factor XI	***Plasma thromboplastin antecedent (PTA)***
Factor XII	***Hageman factor (glass factor)***
Factor XIII	***Fibrin-stabilizing factor (Laki-Lorand factor)***
High molecular weight kininogen	**HMWK *or Fitzgerald factor***
Prekallikrein	***Fletcher factor***
Platelet factor 3 (PL)	***Platelet phospholipid***
Von Willebrand factor	***Factor VIII-related antigen***

THE CASCADE MECHANISM OF BLOOD CLOTTING

- The process of blood clotting involves a *cascade(= series) of reactions* which proceed in 2 different systems (or pathways).

(A) THE EXTRINSIC SYSTEM (PATHWAY)

This occurs when there is *trauma (damage) to the vascular wall and the surrounding tissues.* It is a *rapid process* that may start and terminate in as little as 15 seconds, and it proceeds as follows

(1) The damaged tissues release *TPL (factor III):* This is a protein phospholipid mixture that *activates factor VII*

(2) Active factor VII (VIIa) in presence of TPL, PL and Ca2+ activates factor X (an alpha-globulin that is synthesized by the liver).

(3) Active factor X (Xa) acts as a prothrombin activator that converts prothrombin to thrombin (in presence of factor V, Ca 2+ and PL)

(4) Thrombin converts the soluble fibrinogen into *insoluble fibrin* (by release of 2 pairs of polypeptides from each fibrinogen molecule).

▪Many fibrin molecules polymerize in a few seconds into *long fibrin threads* which run in all directions entrapping various blood cells forming a *clot reticulum*

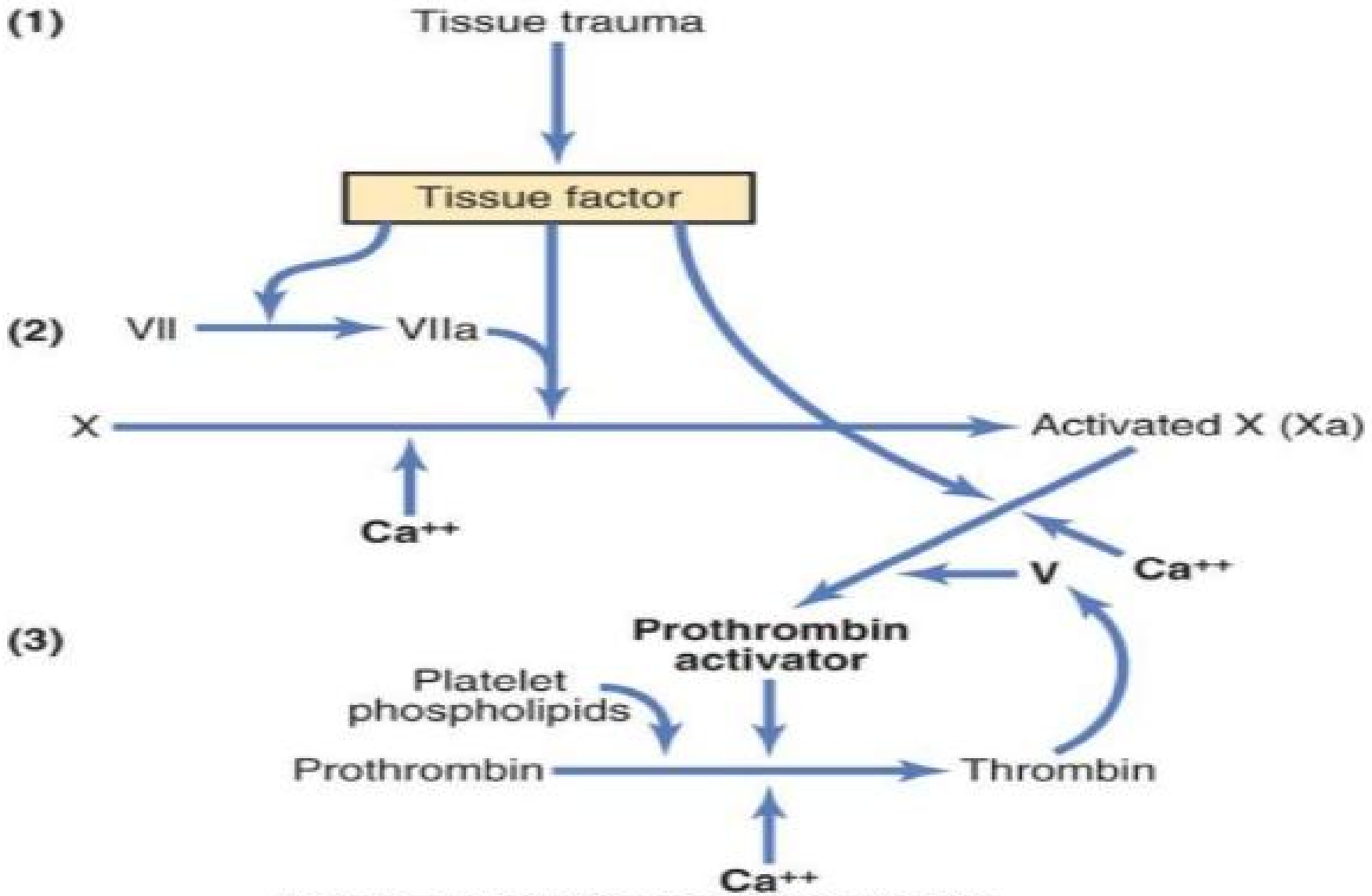
*** Prothrombin is an alpha-globulin synthesized by the liver (m.w 68700) and its normal plasma concentration is about 15 mg %

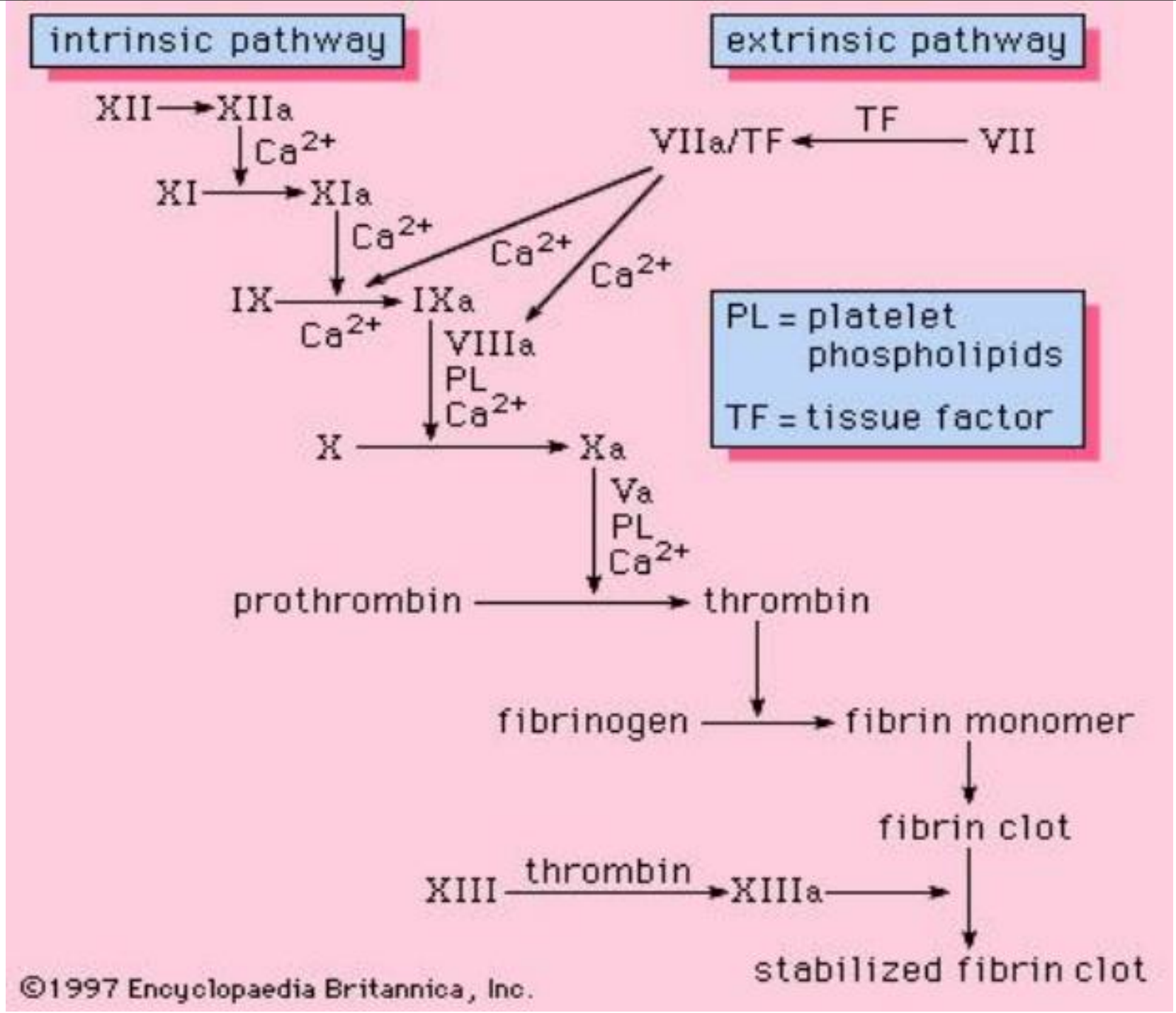
*** Factor V greatly increases the effect of factor Xa on prothrombin but it is inactive at first. However, it becomes activated by the small amount of thrombin that is initially formed. Thrombin then acts in a positive feedback way through activating factor V to accelerate the entire process once it begins.

*** The extrinsic system can be inhibited by a tissue factor inhibitor (= TFI) which forms a complex with TPL, VIIa and Xa. EXAMS QUESTION

(5) The formed clot is soft and loose, and it becomes firm and tight by factor XIII (which is released from the platelets and becomes activated by thrombin) in presence of Ca2+ The clot adheres to the injured part of the vessel and then retracts, thus preventing further blood loss.

Extrinsic Pathway




intrinsic pathway
extrinsic pathway
XII → XIIa
Ca2+
XI → XIa
Ca2+
IX → IXa
Ca2+
VIIIa
PL
Ca2+
X → Xa
Va
PL
Ca2+
prothrombin → thrombin
fibrinogen → fibrin monomer
fibrin clot
XIII thrombin → XIIIa
stabilized fibrin clot
VIIa/TF ← TF — VII
Ca2+
Ca2+
PL = platelet phospholipids
TF = tissue factor
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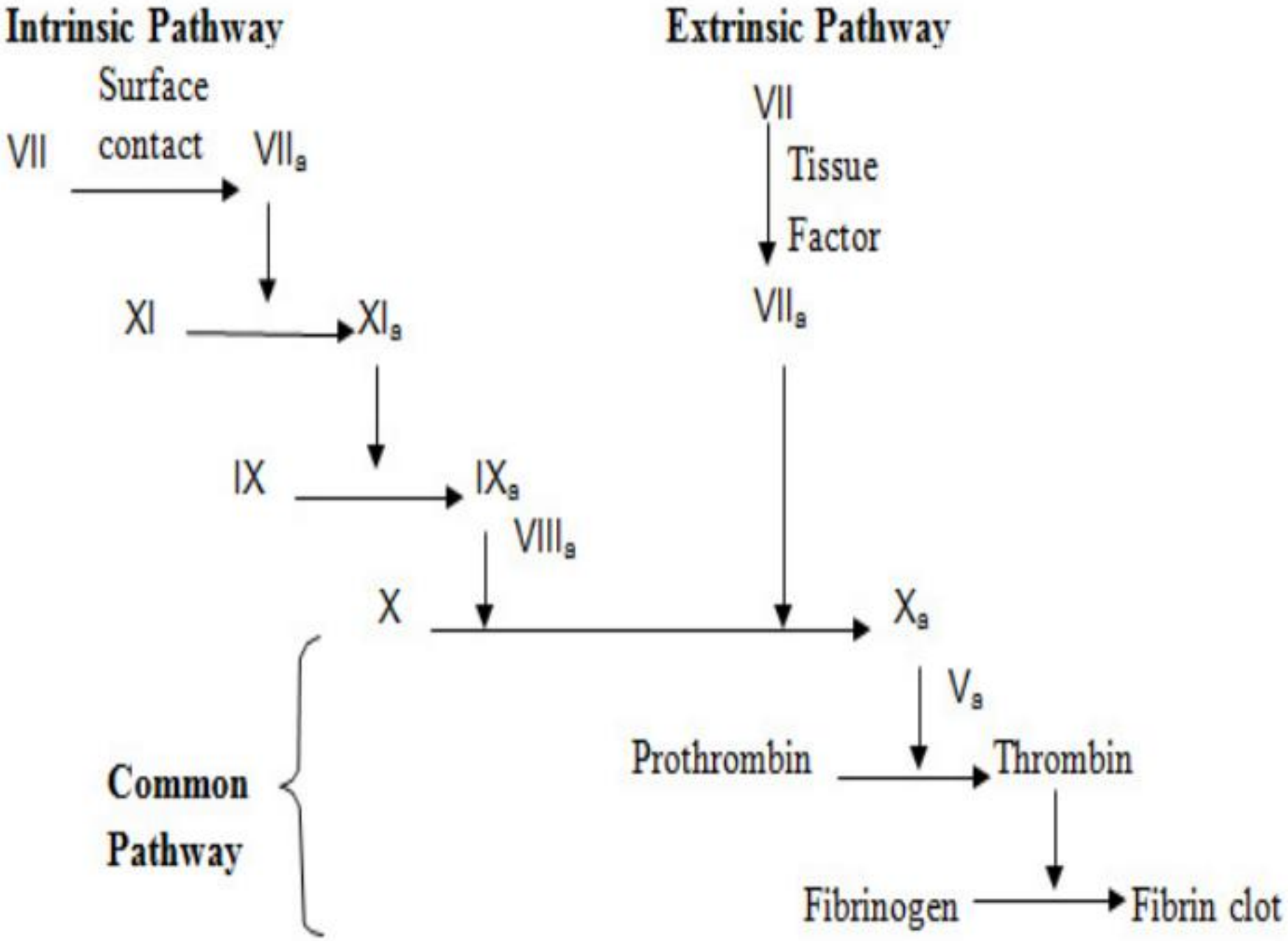

Intrinsic Pathway
Surface
contact
VII
VIIa
XI
XIa
IX
IXa
VIIIa
X
Common
Pathway
Extrinsic Pathway
VII
Tissue
Factor
VIIa
Xa
Va
Prothrombin
Thrombin
Fibrinogen
Fibrin clot

(B) THE INTRINSIC SYSTEM (PATHWAY)

- This occurs *in absence of tissue damage* both *in vitro* by exposing the blood to electronegatively charged wettable surfaces (e.g. glass) and *in vivo* e.g. in cases of *intravascular clotting* (= *thrombosis)* which occurs when blood is exposed to damaged endothelial cells or subendothelial collagen fibres .

- It is a slow process that begins to develop in 1-2 minutes and requires 6 minutes or more to form a clot. It proceeds as follows

(I) Exposure of blood to substances such as glass or collagen fibres leads to 2 effects

(a) Activation of factor XII (catalyzed by HMW kininogen and kallikrein)

(b) Platelet aggregation and release of PL

(2) Factor XIIa activates factor XJ (catalyzed by HMW kininogen).

(3) Factor XIa activates factor IX (the latter is also activated by factor VIIa formed in the extrinsic system

(4) Factor *IXa forms a complex with factor VIIIa*, and in the presence ofPL and Ca *2+,* it *activates factor X*

(5) The same occurs as in steps 3, 4 and 5 in the extrinsic pathway

NOTE:

*** The Von Willebrand factor forms a complex with factor VIII and the latter is activated when separated from this factor (and also by thrombin). Accordingly, Von Willebrand factor regulates the activity and blood level of factor VIII besides inducing platelet adherence

Question: Name two factors that activate factor VIII

Name two functions of vWF

*** All clotting factors are globulins that are synthesized by the liver except

(1) Factor III (tissue thromboplastin)
(2) Factor IV (Ca 2+)
(3) Von Willebrand factor (a protein synthesized by the platelets and vascular endothel-ium)
(4) Factor XIII (which is synthesized by the platelets)

*** *The extrinsic system occurs only in vivo* (because it needs a tissue factor) whereas the *intrinsic system occurs both in vivo and in vitro.*

*** In cases of tissue damage, blood clotting occurs by *both systems starting by the extrinsic* (due to release of factor III) then it continues by the Intrinsic pathway (due to exposure of collagen)

*** Intravascular clotting(= thrombosis) as well as clotting in test tubes occur by the intrinsic pathway.

Thrombosis may occur in:

(a) The veins (if the blood flow becomes sluggish e.g. as a result of prolonged immobilization after surgical operations and delivery)
(b) The arteries (at the sites of damaged intima e.g. due to atherosclerosis)
(c) The heart: (at damaged areas of the endocardium e.g. the mural thrombi that overlie areas of myocardial infarction)

*** Once blood clotting starts, *a vicious circle that promotes more clotting is initiated and continues by a +ve feedback mechanism till bleeding stops*.

This is produced mainly by *thrombin* which once formed it enhances the clotting process by: HOW DOES THROMBIN ENHANCE THE CLOTTING PROCESS?

(a) Helping more platelet aggregation and release of PL
(b) Activating factors V, VIII and XIII (specially factor V) and accelerating the actions of factors IX, X, X I and X II
(c) Acting on prothrombin by autoactivation, leading to more production of thrombin.

ROLE OF Ca 2+ IN BLOOD CLOTTING

- Ca 2+ is an *essential* catalyst in the process of blood clotting. *Except for the initial steps, it is required for all other steps of blood clotting by both the extrinsic and intrinsic systems*

- Its normal plasma level is 9-11 mg % , and if removed from the blood, clotting wouldn't occur.

- However, in vivo reduction of blood Ca 2+ to levels that stop blood clotting is incompatible with life.

- This is because clotting stops only when the blood Ca 2+ level is severely decreased (to about 4 mg %), and such level cannot be reached clinically since death would occur before it is reached due to tetany (severe muscle spasm) that occurs when the Ca2+ level drops just below 7 mg%

IMPORTANCE OF VITAMIN K IN BLOOD CLOTTING

- Vitamin K is one of *the fat-soluble vitamins, so it requires bile to be absorbed in the small intestine.*

- It is a necessary for *conversion of glutamic acid to gamma-carboxyglutamic acid.*

- *Six (6) of the proteins involved in blood clotting require this conversion* before they are released into the circulation.

- These include *clotting factors II, VII, X I and X as well as proteins C and S*

- Therefore, lack of vitamin K causes bleeding (so it is called antihemorrhagic vitamin).

- This can occur as a result of either

(a) *Failure of absorption e.g. due to deficient bile flow*

(b) *Deficient intestinal bacteria* (which normally synthesize vitamin K)

(c) *Severe lack of the vitamin in diet*.

CL ASSIFICATION OF THE CLOTTING FACTORS

- The clotting factors can be classified into the following 5 groups

(1) Prothrombin group: This includes factors II, VII, IX and X, all of which are synthesized *in the liver and vitamin K-dependent*

(2) Fibrinogen group: This includes factors I, V, VIII and XIII, all of which require thrombin for activation. They are all synthesized in the liver except factor XIII , and a part of factor VIII (Von Willebrand factor) which are synthesized in the platelets

(3) Contact group: This includes factors XI and XII (synthesized *in the liver)*

(4) Calcium ions (factor IV)

(5) Thromboplastin (TPL, tissue factor or factor III).

FACTORS THAT AFFECT BLOOD CLOTTING

(A) Factors that accelerate (promote) clotting

1. In vitro

(a) Warming of blood
(b) Contact of blood to -vely charged or wettable surfaces e.g. glass
(c) Addition of foreign bodies to the blood.

2. In vivo

(a) Blood stagnation
(b) Roughening or damage of the vascular endothelial lining
(c) Inject ion of vitamin K or adrenaline (the latter stimulates synthesis of fibrinogen & prothrombin and increases factor V activity)

(B) Factors that slow (inhibit) clotting

1. In vitro

(a) Cooling of blood to zero °C
(b) Collection of blood in non-wettable vessels e.g. paraflin or silicone-coated test tubes (so platelet aggregation is reduced)
(c) Blood defibrination (= removal of the fibrin threads by special methods)
(d) Addition of heparin to the blood
(e) Decreasing the blood Ca2+ concentration to low levels

2. In vivo

(a) Vitamin K deficiency
(b) Small doses of aspirin
(c) Administration or heparin or dicumarol

ANTICOAGULANTS

1. (A) Anticoagulants used in vitro

I . Sodium oxalate : This *precipitates* Ca 2+ as insoluble Ca oxalate, so the blood Ca2+ level is decreased (which prevents clotting)

2. Chelating agents e.g. Na citrate and EDTA (Ethylene-Diamine Tetra-Acetic acid) : These substances *bind Ca 2+,* so the blood Ca 2+ level is decreased. Citrate is the anticoagulant used in transfused blood

3. Heparin : This can be used both in vitro and in vivo

(B) Anticoagulants used in vivo

1- HEPARIN :

- This is a highly negatively-charged mixture of sulfated polysaccharides with a strong acid reaction.
- It is *a natural anti-coagulant* that is formed by the *mast cells and basophil leukocytes*.

- Mechanism of action : Heparin exerts its anticoagulant effect mainly by facilitating the action of antithrombin III which is a protease secreted by the liver that inhibits the active forms of factors IX , X, XI and XII (but not thrombin itself)

- Another function of heparin (the clearing effect) : Heparin acts as *a cofactor for the lipoprotein lipase enzyme* (which hydrolyzes excess lipids).

- Thus heparin indirectly decreases the level of lipids in the blood and such effect is known as the *clearing effect of heparin.*

- The antidote of heparin is protamine (a highly basic protein that forms an irreversible complex with heparin). It is used clinically to neutralize overdoses of heparin

2. DICUMAROL :

- This substance is of a *plant origin.* It is a *coumarin derivative,* and its structure is *similar to that of vitamin K.*

- Mechanism of action : Dicumarol and other coumarin derivatives (e.g *warfarin)* exert their anticoagulant effect through blocking the action of vitamin K in the liver by *competitive inhibition,* thus the *synthesis of factors II, VII, IX and X is inhibited* leading to prolongation of both the clotting and prothrombin times

- Therefore, the antidote of dicumarol is the administration of large doses of vitamin K

- Dicumarol is taken orally and it acts in vivo only (because its site or act on is the liver). Its effects appear *after a latent period of 1-2 days* (during which the clotting factors already present in the blood are consumed)

- But such effects last for *a few days* (in contrast heparin acts both in vitro and in vivo and is taken *only by* injection, and its effects appear *within a few minutes* but they last for *only a few hours).*

- Cases of thrombosis are treated *first by heparin* (due to its rapid onset of action), then treatment is continued by oral administration of dicumarol

	HEPARIN	DICUMAROL
Origin	Animal origin	Plant origin
Method of administration	By injection (specially i.v.)	Orally
Onset of action	Rapid (within a few minutes)	Slow (after 1-2 days)
Duration of action	Short (a few hours)	Long (a few days)
Antidote	Protamine	Vitamin K
Site of action	In vivo and in vitro	In vivo only
Clearing effect	Present	Absent
Mechanism of action	Mainly facilitating the action of antithrombin III	Competitive inhibition of vitamin K in the liver

MAINTENANCE OF BLOOD FLUIDITY (NATURAL ANTICLOTTING MECHANISMS)

❑Normally. blood clotting is prevented (so that the blood fluidity is maintained) by the following mechanisms :

(1) The *rapid blood flow rate and the smooth and intact endothelium* in the blood vessels. *These prevent*

(a) Accumulation and concentration of the clotting factors in the blood

(b) Platelet adherence and aggregation.

(2) The balance between the actions of *TXA2 and PGI2*.

(3) Synthesis of the various clotting factors in *inactive forms*

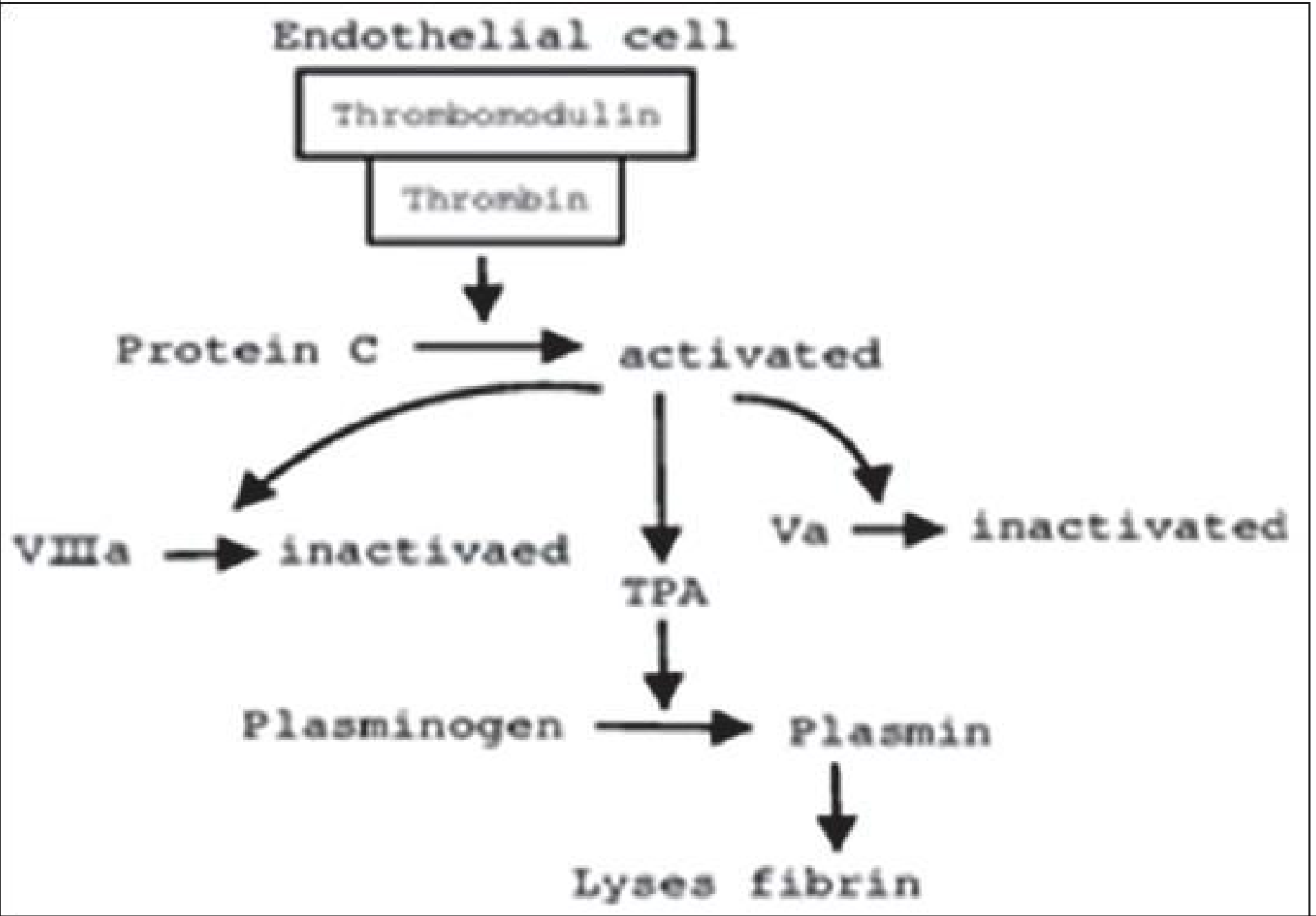
(4) The *low concentration* of the various clotting factors, and their rapid removal by the *reticuloendothelial system,* specially *in the liver.*

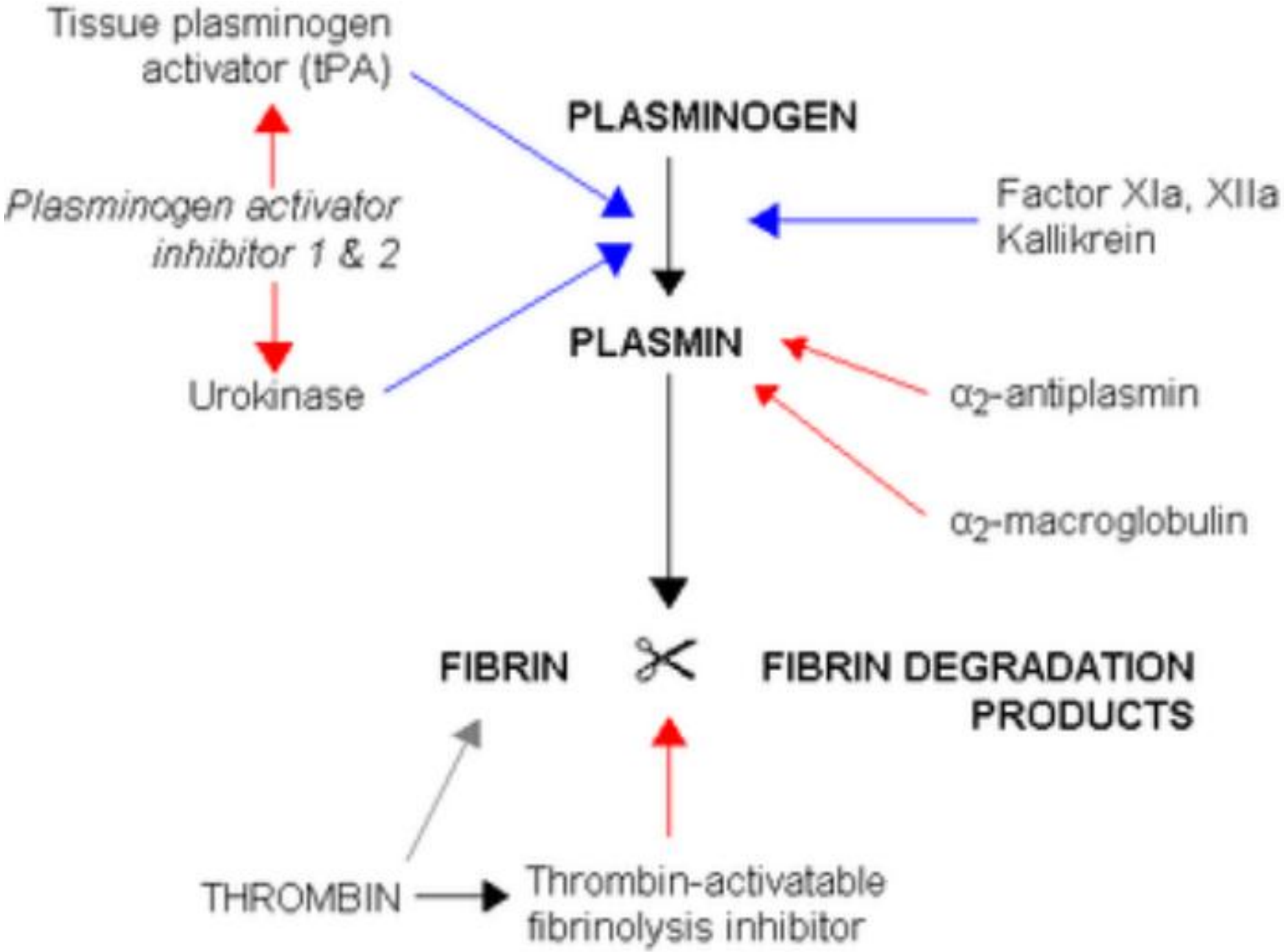
(5) Presence of *heparin and antithrombin III.*

(6) *Adsorption of thrombin to the fibrin threads* (which inhibits its effect thus preventing excessive clotting).

(7) Activity of the *fibrinolytic system,* a highly specialized system that removes the small clots that are continuously formed in the body.

THE FIBRINOLYTIC (= PLASMINOGEN) SYSTEM




Tissue plasminogen activator (tPA)
PLASMINOGEN
Plasminogen activator inhibitor 1 & 2
Factor XIa, XIIa Kallikrein
PLASMIN
Urokinase
α2-antiplasmin
α2-macroglobulin
FIBRIN
FIBRIN DEGRADATION PRODUCTS
THROMBIN
Thrombin-activatable fibrinolysis inhibitor

- The liver produces an inactive globulin called plasminogen (profibrinolysin) which can be converted to the active fibrinolytic enzyme called plasmin (= fibrinolysin) by thrombin and 2 types of activators (I) urokinase Plasminogen activator (u-PA) (2) tissue Plasminogen Activator (t-PA).

- The latter is released from injured tissues and vascular endothelium, and the liver produces an inhibitor to its action

- All vascular endothelial cells (except those in the cerebral microcirculation) produce a protein called thrombomodulin that binds to thrombin, and such thrombomodulin-thrombin complex prevents blood clotting and causes lysis of fibrin as follows :

- The complex *activates protein C*, and the activated protein C together with its co-factor *(protein S)* inactivate
(a) Clotting factors V and VIII (which blocks the clotting reactions)
(b) The inhibitor of t-PA (so increasing the formation of plasmin which helps lysis of fibrin).

- Plasmin also lyses fibrinogen into fibrinogen degradation products (FDP) which inhibit thrombin. Proteins C and S are produced in the liver and require vitamin K

FUNCTIONS OF THROMBIN

(I) Conversion of fibrinogen into fibrin.
(2) Activation of clotting factors V, VIII and XIII and acceleration of the actions of factors IX, X, XI and XII.
(3) It induces platelet activation as well as activation of the endothelial cells and leukocytes (Ganong. 2005).
(4) It helps clot retraction.
(5) It shares in activation of protein C.
(6) It shares in conversion of plasminogen to plasmin.
(7) It acts on prothrombin in a +ve feedback way to form more thrombin
Thrombin is inhibited by adsorption to fibrin and by the FDP

COMMON TESTS THAT EVALUATE HEMOSTATIC FUNCTION

(1) Clotting (coagulation) time

- Determination of the clotting time tests the *coagulative ability of the blood*.
- It is measured from the time of blood withdrawal from the body till a firm clot is formed.
- It is determined by collecting a blood sample in a test tube . placing it in a water bath at 37 °C and every 30 seconds the tube is tilted.
- Clotting is indicated when blood does not drop from the tube and the time at which clotting occurs is measured using a stopwatch.
- Normally, it is 3 - 8 (up to 10) minutes and it depends on the availability of the clotting factors required for the intrinsic system of clotting

(2) Bleeding time

- Determination of the bleeding time tests the efficiency of *hemostatic mechanisms other than blood clotting*.

- It is the time needed for bleeding to stop from an injured small blood vessel without formation of a blood clot.

- It is determined by pricking the tip of a finger (or the lobe of the ear), and the oozing blood is wiped away by a filter paper every 15 seconds without touching the skin.

- The time elapsing between the prick and stoppage of bleeding is recorded by a stopwatch. It depends on the *availability of the platelets,* and is *normally I - 3 (up to 4) minutes.*

(3) Prothrombin time

- A blood sample is obtained and immediately oxalated or citrated to prevent clotting (so that no prothrombin is converted to thrombin). Blood is then centrifuged and the plasma is separated.

- To the plasma, Ca 2+ and tissue thromboplastin (usually prepared from brain tissue) are added then it is incubated at 37 ^{o}C and the time at which clotting occurs is recorded (normally *12-16 seconds*).

- It is a test for the *extrinsic system of blood clotting*. Accordingly, it tests the quantities of *prothrombin and factor VII as well as factors I, V and X* .

- The prothrombin time is commonly determined in patients having thrombotic tendencies to determine the required dose of dicumarol

DISEASES CHARACTERIZED BY BLEEDING

Excessive bleeding may occur due to:
(A) Decreased platelet count (thrombocytopenia): This occurs in a disease called *purpura*.
(B) Deficient blood clotting : This occurs due to lack of any of the clotting factors. which may result from either :
(a) A severe liver disease (in which most clotting factors are deficient).
(b) Vitamin K deficiency (which leads to lack of factors II. VII, IX & X).
(c) A *congenital abnormality :* Any clotting factor (except factors II, III and IV) can be congenitally-deficient, leading to the following diseases that are characterized by bleeding :
1-Hemophilia, which is due to lack of factor VIII, IX or XI.
2- Parahemophilia (which is due to lack of factor V).
3- *Afibrinogenemia* (which is due to lack of factor I).
4-Hypoconvertinemia (which is due to lack of factor VII).
5- *Hageman trait, w*hich is due to lack of factor XII (here blood clotting is slow in glass only but clinically there is little bleeding tendency).
6- *Von Willebrand disease* (which is due to lack of the V.W. factor).
7- *Congenital deficiency of factors X or XIII.*

PURPURA

- This is a bleeding disease characterized by occurrence of *small punctate hemorrhages throughout all tissues*, specially in the skin where they appear as *purplish blotches* (hence the name)

- In most cases it is due to severe reduction of the platelet count (below 40000 - 50000/mm3) so the condition is often called thrombocytopenic purpura.

- However, in some cases the platelet count is normal but the platelets themselves are abnormal, so these cases are called thrombasthenic purpura

❑Lack of platelets causes spontaneous hemorrhages due to
(a) Deficient formation of platelet plugs
(b) Weak V.C. of the injured vessels
(c) Poor clot retraction. Such effects lead to *prolongation of the bleeding time while both the clotting and prothrombin times are normal*

❑Thrombocytopenia may be due to either *autoimmunity*, *hypersplenism* or *severe destruction of the bone marrow*.

*** The bleeding time is also prolonged in
(a) Scurvy *(Vitamin C deficiency)* due to weak vascular walls
(b) Deficiency of Von Willibrand factor (due to defective platelet adhesion)
(c) Prolonged use of aspirin (due to defective platelet aggregation).

HEMOPHILIA

❑This is a bleeding disease caused by *congenital deficiency of certain clotting factors*. According to the deficiency it is classified into 3 types:

(1) *Hemophilia A or classic hemophilia (85 %)* due to congenital deficiency of factor *VIII.*
(2) *Hemophilia B (15%)* due to congenital deficiency of factor *IX.*
(3) *Hemophilia C (5%)* due to congenital deficiency of factor *XI*

❑Hemophilia is inherited as a sex-linked characteristic because the deficient factors are genetically transmitted by way of the X sex chromosome as a recessive trail .

- Males are affected if one of these factors is lacking due to defect of the responsible genes in their X chromosome, while females are affected only if such defect is present in both X chromosomes.

- For this reason, *females are much less affected, and most patients are males.* However, if the female had a defect of the responsible genes in one X chromosome only (while the other has normal genes). She will be *a hemophilic carrier.*

- In this case, if the X chromosome that has the genetic defect is transmitted to one of her sons he will be hemophilic while if it is transmitted to one of her daughters, she will only be a hemophilic carrier (provided her father is normal)

- As a result of deficiency of the clotting factors, *the clotting time is markedly prolonged while both the bleeding and prothrombin times are normal.* This leads to marked bleeding after minor injuries, wounds or operations (e.g. tooth extraction)

****The prothrombin time is normal in both hemophilia and purpura.*

***Although the clotting time is normal in purpura, and the bleeding time is normal in hemophilia, yet there is *a bleeding tendency in both conditions (indicating that all mechanisms of hemostasis are required for efficient stoppage of bleeding)*

- A group of specific proteins that are related to blood clotting and fibrinolysis were recently identified.

- More than 20 of these proteins have been described and they are now called annexins.

- Annexin II forms a platform on the endothelial cells on which the components of the fibrinolytic system interact, producing fibrinolysis.

- Annexin V forms a shield around the phospholipids involved in clotting and exerts an antithrombotic effect

	HEMOPHILIA	PURPURA
Heredity	Inherited	Not inherited
Genetic abnormality	Abnormality of genes on the X chromosome	No genetic abnormality
Affected sex	Mostly males	males and females equally
Characteristics	Marked bleeding after minor injuries and operations	Spontaneous bleeding in all tissues specially the skin
Clotting time	Prolonged	Normal
Bleeding time	Normal	Prolonged
Cause	Lack of clotting factors VIII, IX or XI	Lack of platelets below 40000 - 50000/mm^3
Types	3 types (A, B and C)	2 types (see text)