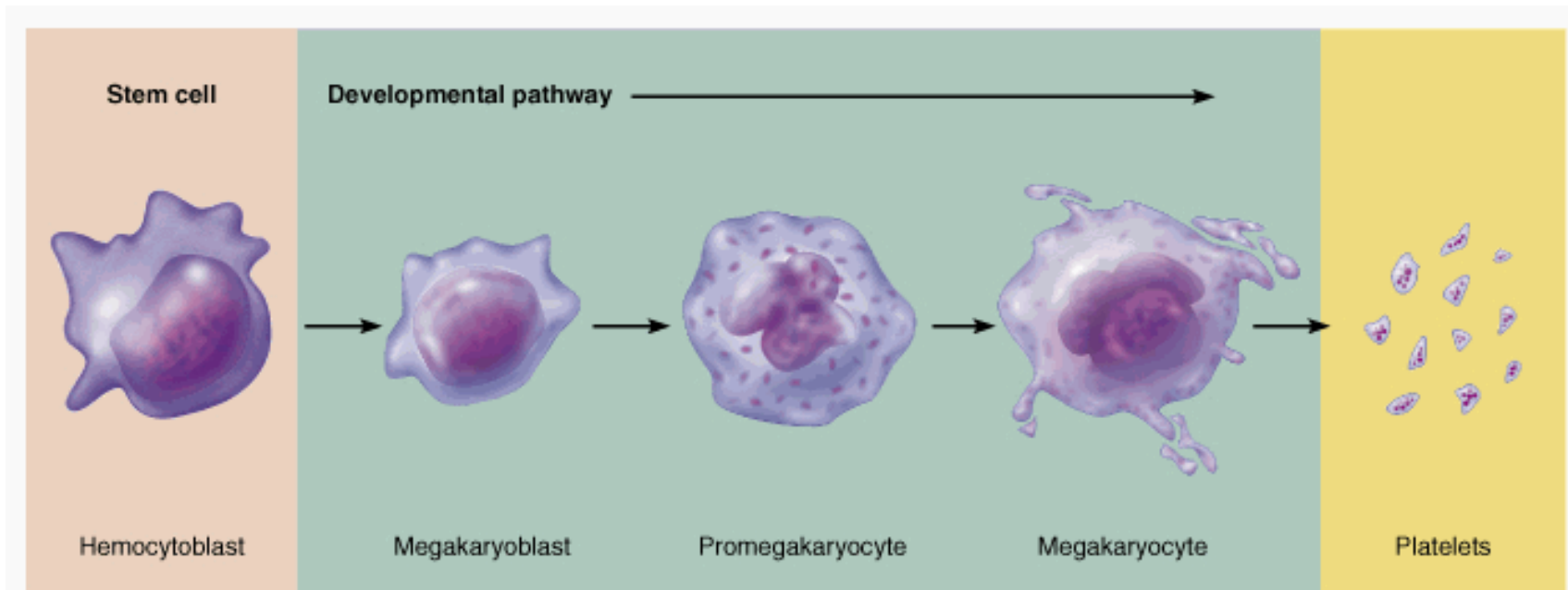
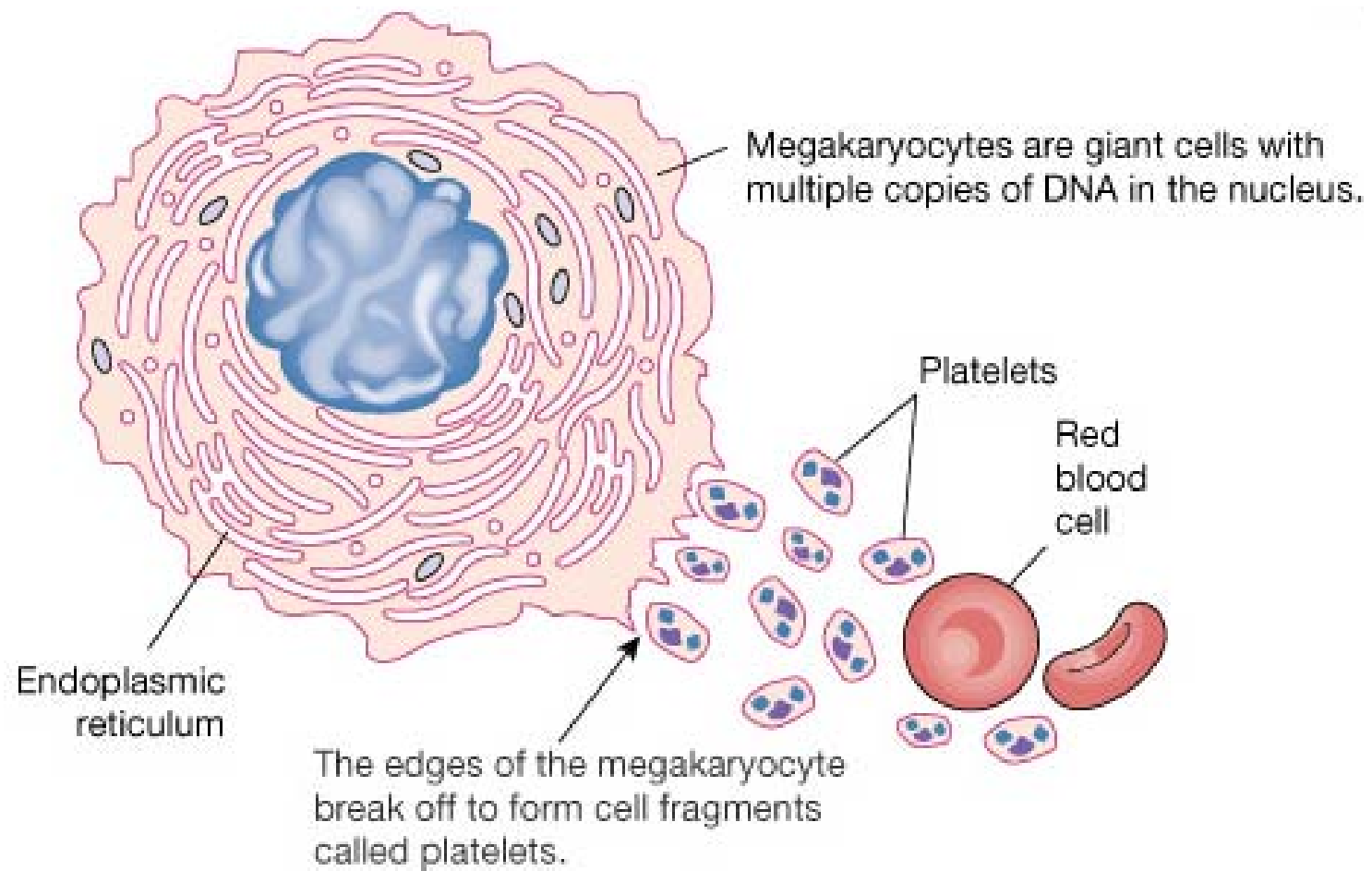


Platelets

Developmental pathway of platelets





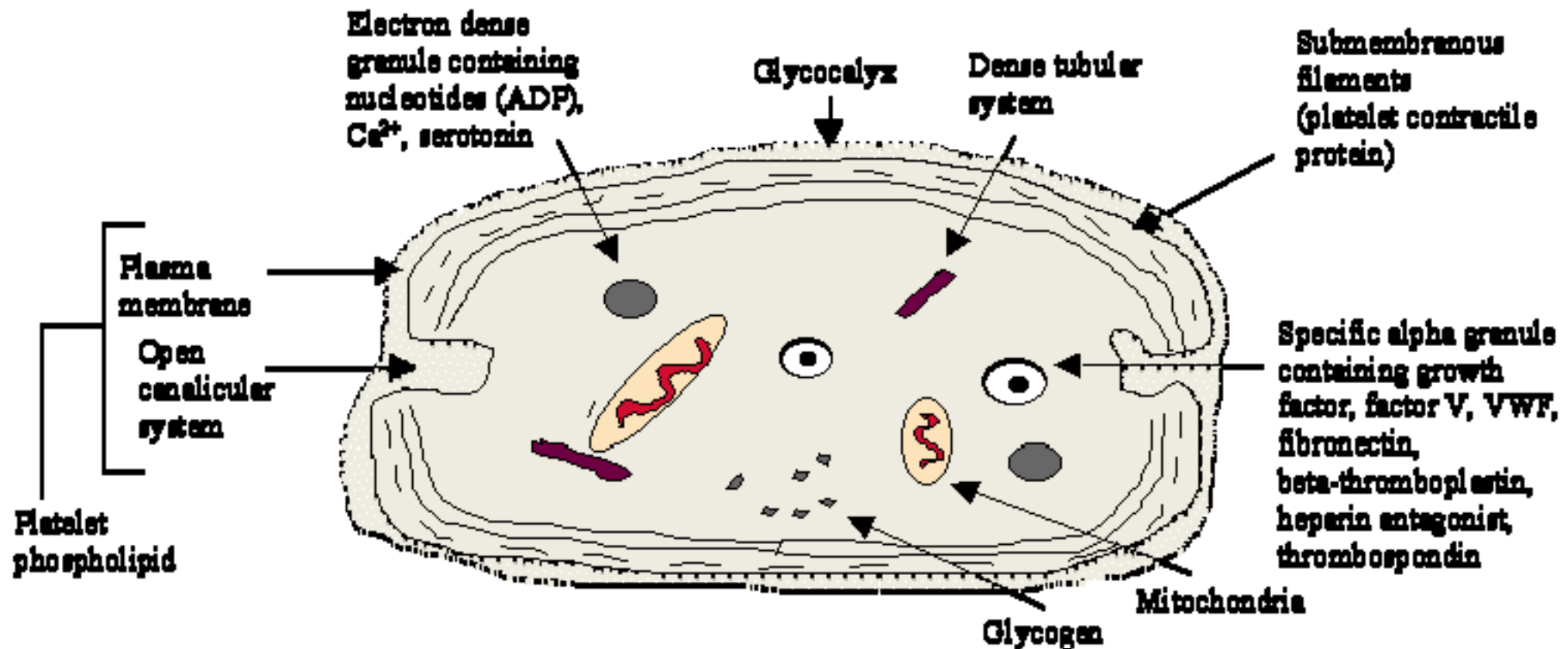
Origin and structure

- Megakaryocytes diameter 35-160 μm
- Platelet diameter 2-3 μm
- Contain an irregular ring of lobed nuclei
- Platelets are formed within the cytoplasm of megakaryocytes and released into the circulation
- Platelet survival time 8-12 days
- Destroyed mainly in spleen
- Blood count: 1.5-4lakh/ μL of blood

Morphology

- Wet preparations: colourless, moderately refractile, discoid or elliptical
- Darkfield illumination: translucent, sharp contour, few immobile granules
- Leishman's stain shows a faint blue cytoplasm with distinct reddish purple granules
- Electron microscopy reveals a cell membrane 6 nm thick which surrounds a cytoplasmic matrix containing Golgi apparatus, endoplasmic reticulum, 50-100 very dense granules, mitochondria, microvesicles, microtubules, filaments & granules

Structure of platelet



Structure of platelet...

- **Membrane structures:** surface glycoproteins serve as receptors, facilitate platelet adhesion & contraction, and determine expression of specific platelet antigens and antigens shared with other formed elements
- **Canalicular system:** numerous invaginations of the platelet surface and, interspersed among these structures, a set of narrower channels termed the dense tubular system
- Dense tubular system : is the major site for storage of Ca^{2+} and the location of cyclooxygenase
- **Cytoskeleton**
- **Microtubules**
- **System of contractile proteins**
- **Granules**

Platelet granules

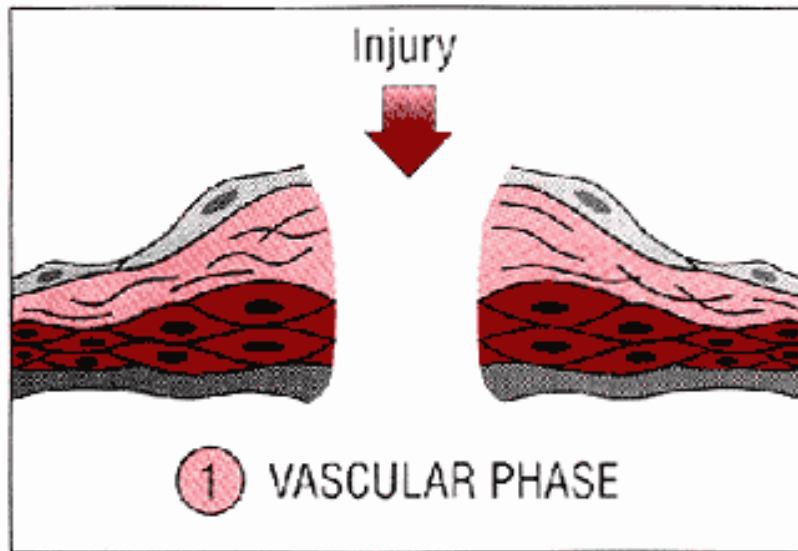
- Dense body: ADP, ATP, GTP, GDP, serotonin, secretable Ca^{++}
- Alpha granules:
 - Platelet specific proteins: PF-4, thromboglobulin, PDGF, thrombospondin
 - Homologs of plasma proteins: fibrinogen, fibronectin, albumin
- Dense tubular system: Prostaglandin converting enzymes, contractile calcium
- Peroxisomes: Catalase
- Lysosomes: Acid hydrolase

Function

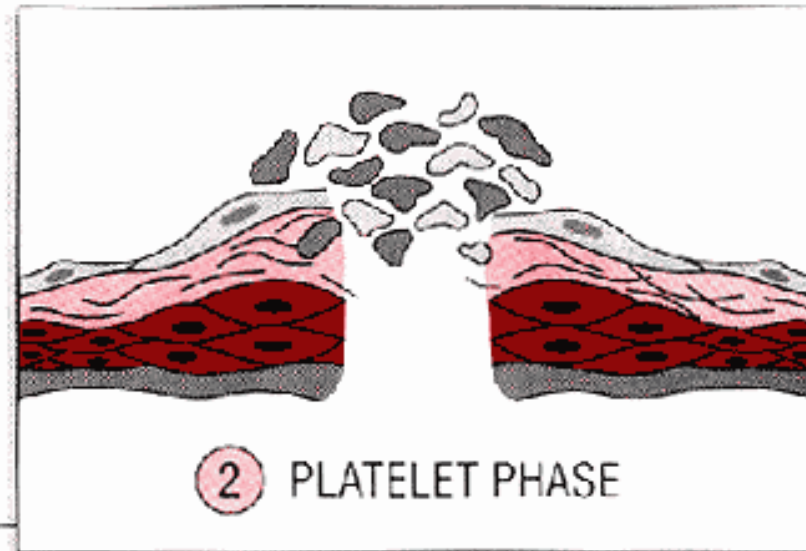
- Hemostasis
- Blood coagulation
- Phagocytosis
- Storage & transport of substances

Events in hemostasis

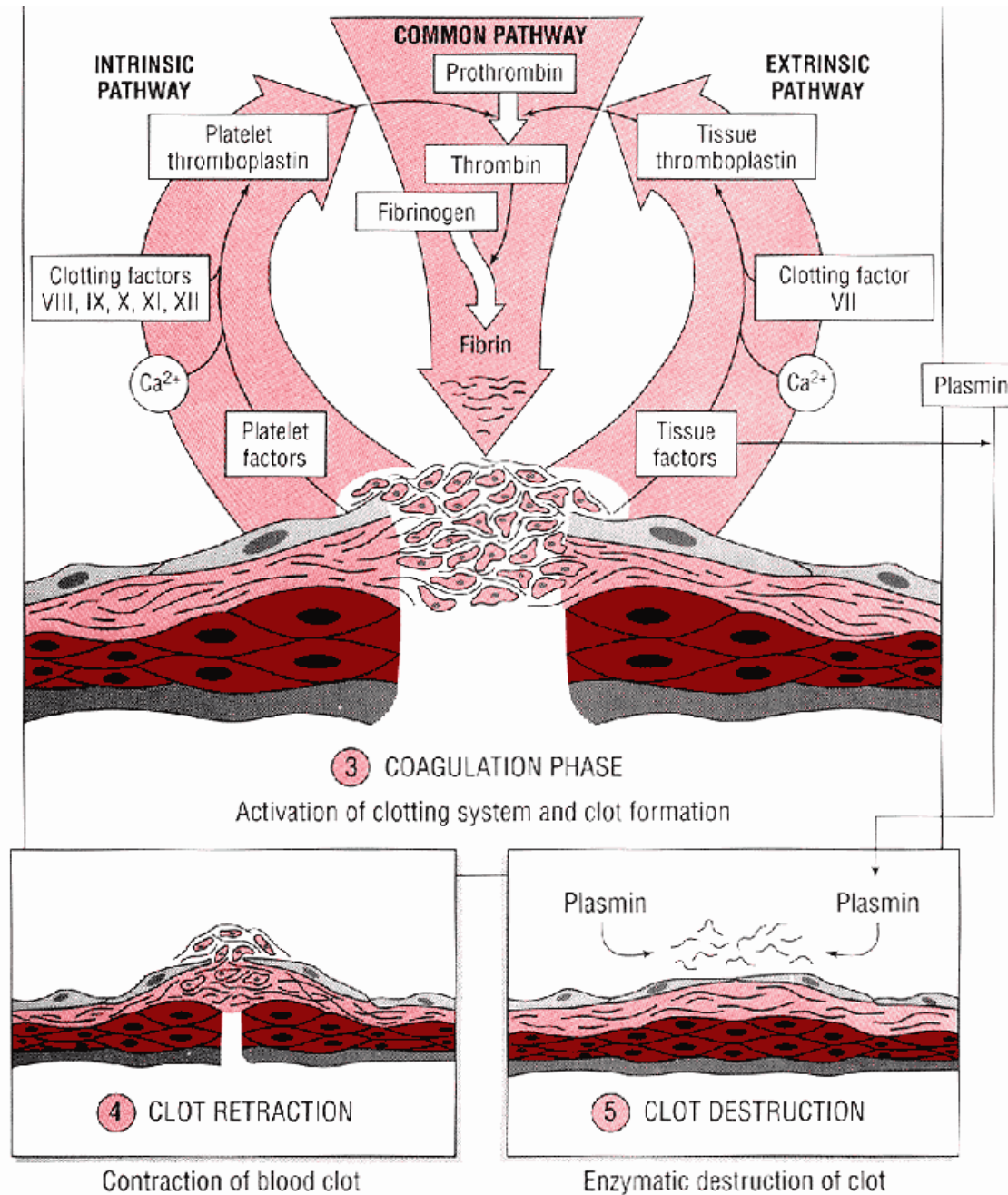
Spasm in damaged smooth muscle



Platelet aggregation and adhesion



Events in hemostasis....



Hemostasis (Arrest of bleeding)

Formation of hemostatic plug due to

- Adhesiveness to damaged lining of blood vessels

Promoted by Ca^{++} ions & ADP

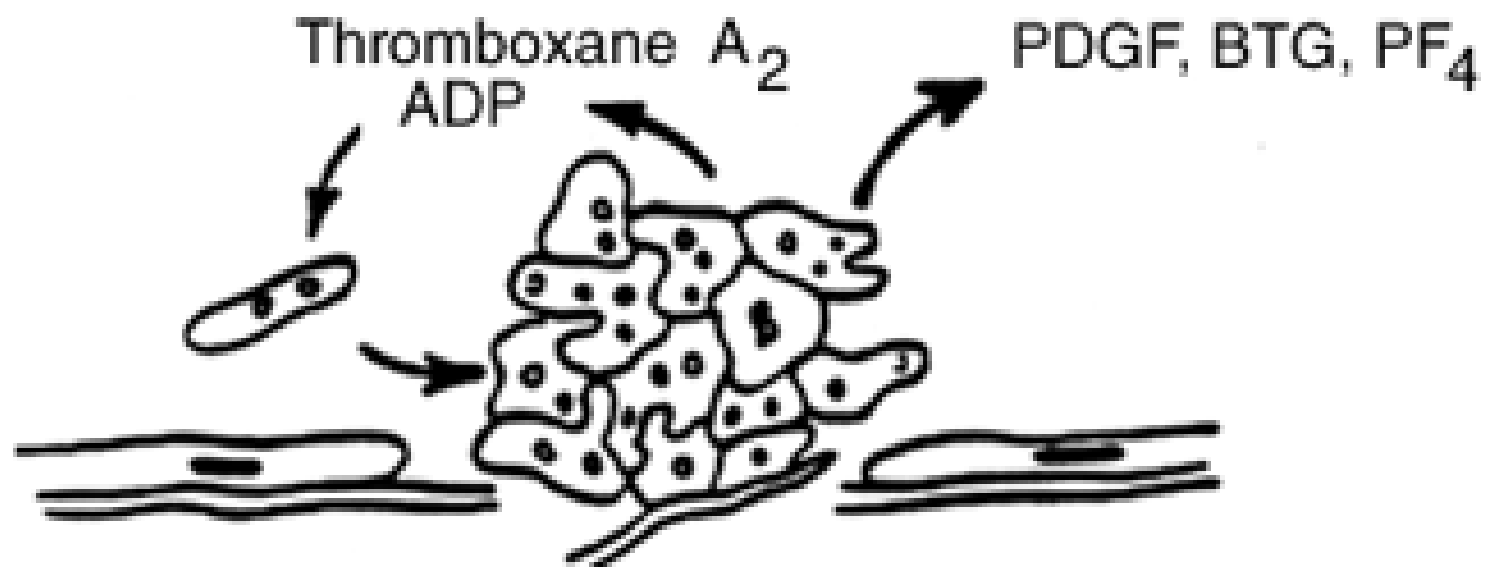
- Aggregation of platelets leads to formation of white bodies or micro thrombi which may grow until they almost fill the lumen of a small vessel

At first the aggregation is reversible

Then it becomes irreversible, platelet granules are discharged, leukocytes begin to adhere to platelets, fibrin deposition occurs

Suggested sequence of events in hemostasis

- Platelet adherence to collagen in damaged vessels wall
- ATP is converted to ADP by ATPase
- ADP is released and promotes aggregation of passing platelets : formation of platelet plug
- Release of tissue factor from damaged vessels & phospholipids from platelets will promote thrombin formation
- Firm clot seals the vessel permanently



Aggregation and release of vasoactive substances and platelet procoagulants

Other reactions in hemostasis

- After the initial dilatation the damaged vessels constrict for about 20 minutes, 5-HT being important in this process
- Prostaglandin forming system is also involved in platelet aggregation
 - Arachadonic acid is released from the platelet cell membrane by phospholipase and then rapidly oxidized by the enzyme cyclo oxygenase to PGG_2 & PGH_2
 - In the platelets: PGG_2 is converted to TX A_2 a highly potent platelet aggregator and constrictor of arterial muscle
 - In the arterial endothelium: PGG_2 & PGH_2 are converted to prostacyclin a potent inhibitor of platelet aggregation and a vasodilator

Blood clotting

- In blood deprived of platelets
 - Clotting time in glass tube is prolonged
 - Activation of prothrombin is incomplete
 - Formed clots do not retract

Platelets are necessary for intrinsic clotting process by releasing Platelet factor 3 which causes conversion of prothrombin to thrombin by factor X and V

Clot retraction

- Clot retraction occurs by shortening of fibrin fibers produced by contraction of attached platelet pseudopodia, which contain actomyosin like protein.
- Agents which inhibit cell metabolism or enzyme activity also inhibit clot retraction
- Blood allowed to clot in a glass tube at 37⁰ C begins to show clot retraction after 30 minutes

Phagocytosis

- Carbon particles, immune complexes and viruses are phagocytosed by platelets
- A vestigial mechanism of clearing particulate material from the blood

Storage & transport

- Stores of 5-HT and histamine which are released by platelet disintegration
- They also take up 5-HT by active transport
- Epinephrine & potassium

Non hematological functions of platelets

Inflammation

Platelets have 4 features common to all inflammatory cells

- Possess wide range of inflammatory mediators
- Presence of receptors for other inflammatory cells
- Ability to respond to noxious stimuli
- Cooperative with other inflammatory cells

Platelet & growth factors

PDGF, EGF, FGF are released from alpha granules of platelets

Platelets & neurotransmitters

Despite being of different embryological origin it has been shown that platelets behave like serotonergic neurons in CNS. Thus they provide a neuronal model for studying neurons

Platelets & metastasis

Promote tumour survival in blood stream by protecting tumour cells from attack by NK cells

Platelets in asthma

Release of PAF & PF4 cause bronchial hyper reactivity

Hemostasis

- Physiology of coagulation
- Tests for clotting
- Clot retraction
- Fibrinolysis
- Anticoagulants

Hemostasis (Prevention of blood loss)

Mechanisms by which hemostasis is achieved

1. Vascular constriction
 2. Platelet plug formation
 3. Blood clot formation
 4. Clot retraction and fibrinolysis
- Primary hemostasis
- Secondary hemostasis
- Tertiary hemostasis

Primary hemostasis

Vascular phase: Triphasic response

1. Immediate reflex vasoconstriction :An intrinsic response of smooth muscles in small arterioles & pre capillary sphincters
2. Transient vasodilation: Due to PG E₂, Histamine & prostacyclin
3. Sustained vascular contraction:Due to release of local autocooid factors from the traumatized tissue and platelets. Ex. TX A₂, Fibrinopeptide B, epinephrine , nor epinephrine

Primary hemostasis...

Platelet phase of hemostasis:

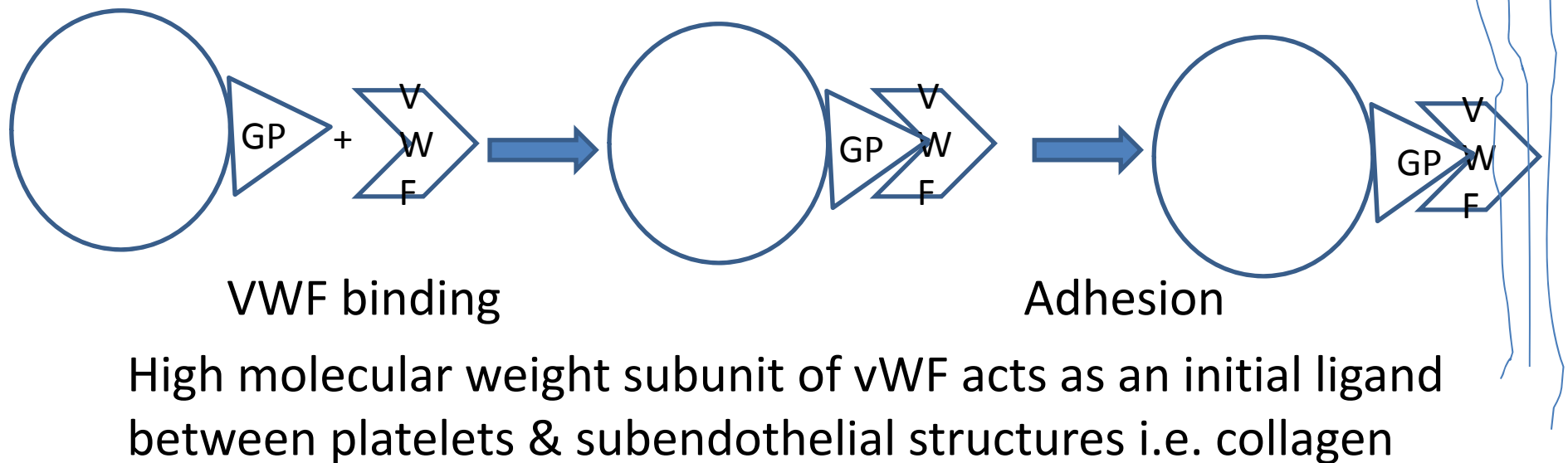
Important in controlling bleeding from capillaries & small venules in erosion of mucosal surfaces

- Adherence to exposed subendothelial structures & activation
- Burst of metabolic activity
- Shape change
- Platelet aggregation/ plugs
- ADP extrusion & other activated substances
- Activation & availability of PF 3 & other procoagulants
- Initiation of blood clotting
- Consolidation of platelet plug by fibrin
- Clot retraction

Adhesion

Defined as the attachment of platelets to non platelet surfaces

The first detectable event after vascular injury



Von Willebrand factor

- A protein synthesised by vascular endothelial cells & megakaryocytes
- Belongs to a class of adhesive proteins .
- Adhesive proteins contain a sequence of amino acids Arg-Gly-Asp(RGD) sequence
- This sequence allows adhesive proteins to bind to integrins (cell surface proteins)
- GP IIb & GP IIIa are two integrins present on platelet surface
- Secreted into plasma and also abluminally into the superficial layers of sub endothelium
- Sub endothelial vWF contributes to platelet adhesion but is insufficient
- After endothelial cell disruption the plasma vWF binds to the subendothelium after which it can bind to surface of unstimulated platelets and link them to the subendothelium

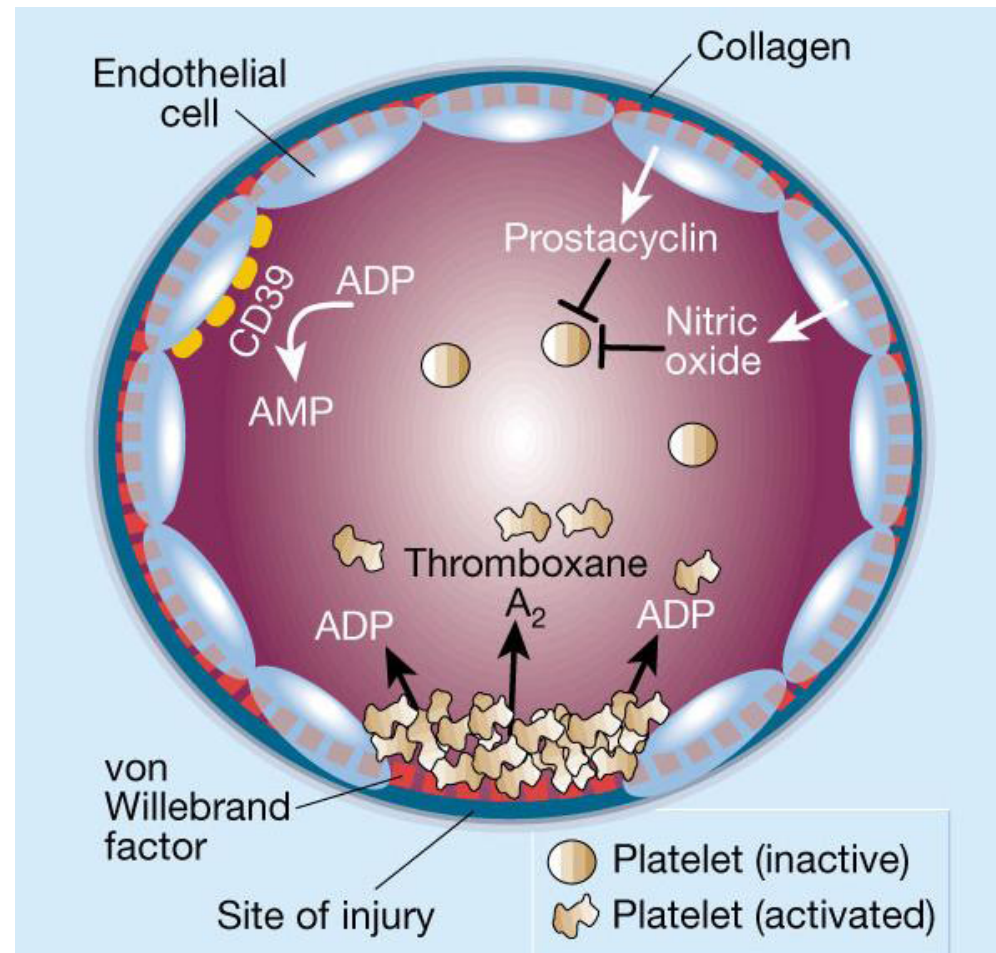
Adhesion...

- Fibronectin

Synthesised by endothelial cells

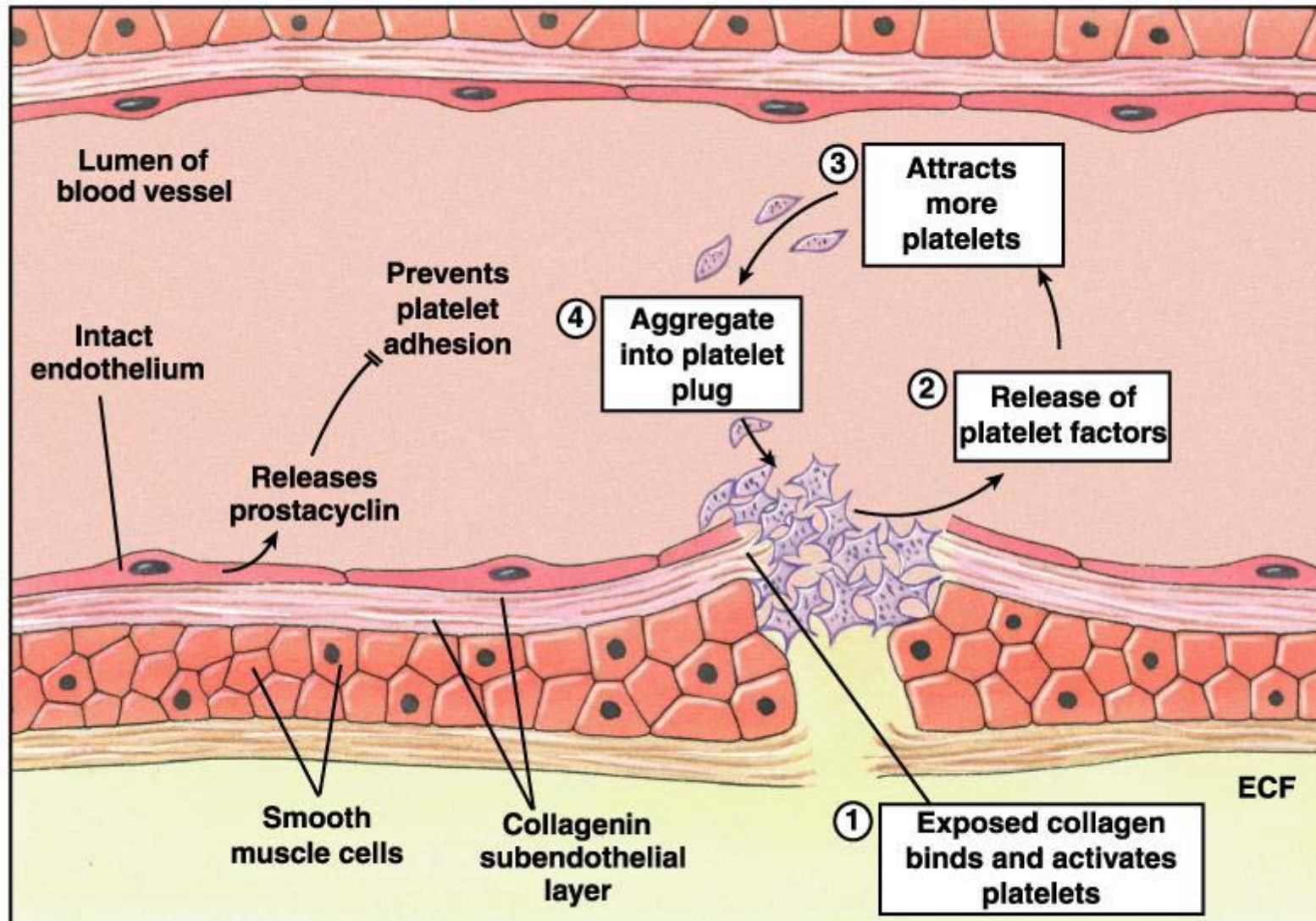
Stored & secreted by platelets

Can bind collagen



Platelet shape change

- Occurs within seconds of exposure to activating agents
- From flattened discs to spheres with multiple projecting pseudopods
- Polymerization of platelet actin
- Microtubules that are normally at the periphery go to the center surrounding the granules that have gone to the center prior to release



Platelet aggregation

Defined as the attachment of activated platelets to each other

- Primary aggregation: ADP & thrombin cause direct aggregation
GP IIb-IIIa is an integrin that recognizes two RGD sequences present on fibrinogen
Fibrinogen thus forms a bridge between two opposing activated platelets
- Secondary aggregation: substances causing aggregation by release of ADP or PG
Thrombospondin from alpha granules leads to irreversible aggregation by binding to fibrinogen & GP IV on platelet surface

Platelet activation...

Series of progressive overlapping events

- Shape change
- Aggregation
- Liberation & oxidation of arachadonic acid
- Secretion of alpha & dense granules
- Reorganization of surface membrane phospholipids
- Oriented centripital contraction of actomyosin

Platelet activation...

Primary agonists triggering platelet activation

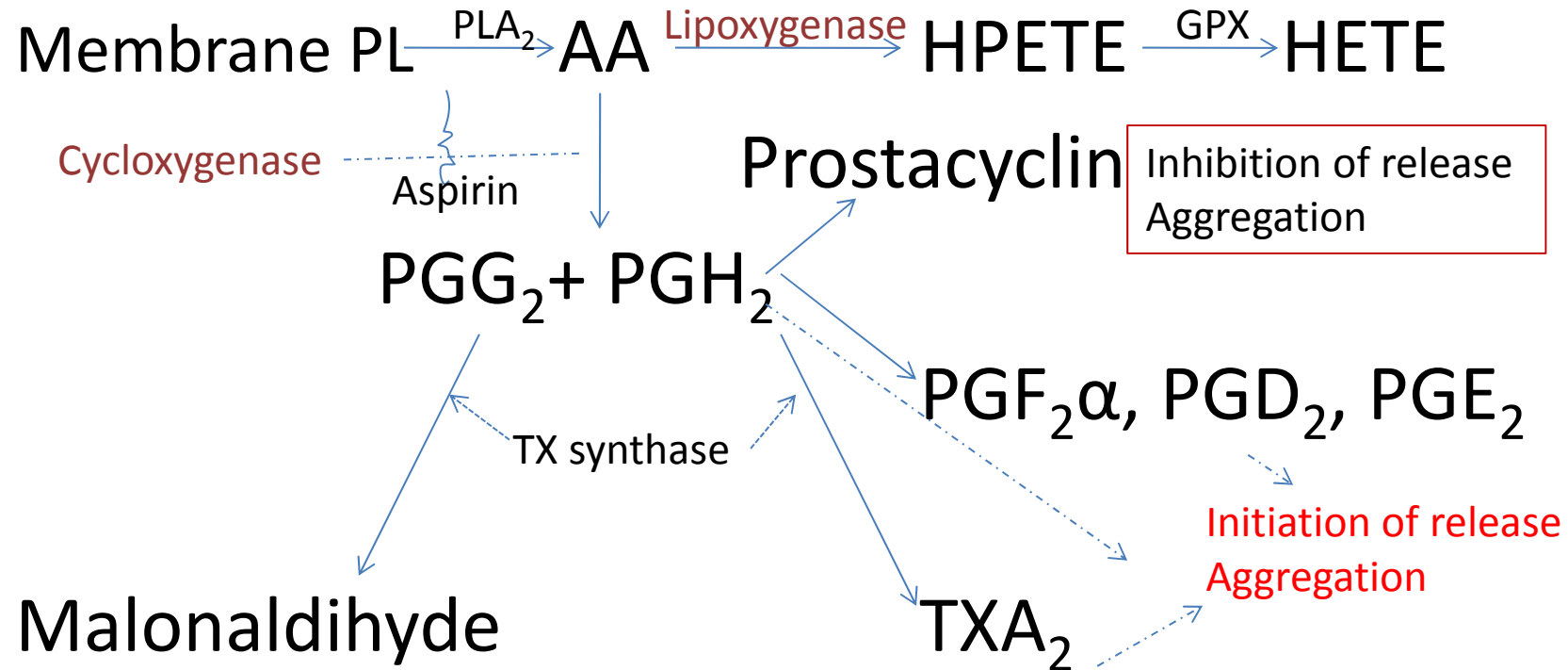
- Thrombin formed at injury site
- Sequence of collagen on subendothelium

Maintenance & amplification of platelet activation

- ADP
- Arachadonic acid oxidation products

- Raised cytosolic Ca^{++} triggers
 - Phosphorylation of myosin light chain kinase. Required for reorientation of cytoskeletal proteins needed for platelet shape change, secretion and contraction
 - Activation of a calcium dependent protease called calpain which through proteolysis activates other platelet enzymes
 - Activation of phospholipase A_2

Prostaglandin thromboxane system



Role of aspirin

- Aspirin irreversibly inactivates platelet cyclooxygenase
- Thus synthesis of PGH_2 & TX A_2 is prevented
- Prolongation of bleeding time may occur
- Therapeutic role in patients of coronary artery disease

- PGH_2 : Acts as a cofactor enhancing collagen's ability to function as a platelet agonist
- TX A_2 : Binds to a specific platelet membrane receptor with resultant activation of phospholipase C & amplification of platelet activation through further generation of DAG & IP_3
- Prostacyclin: Secreted by endothelium helps to keep platelets in an unstimulated state through activation of platelet adenyl cyclase & a resultant rise in platelet cAMP levels
- PGD_2 : Can also activate platelet adenyl cyclase

Phosphoinositol metabolism

Platelet activation



Phosphatidyl inositol bis phosphate(PIP-2)



Diacyl glycerol(DG)+Inositol triphosphate(IP3)

Co factor for
Protein kinase C



Phosphatidic acid(PA)

Mobilises Ca ++ from
intracellular stores



Mobilises Ca ++ from
intracellular stores

Platelet release reaction

α granules

➤ Platelet proteins

Thrombospondin: Platelet aggregation

PF4: Neutralises anticoagulant activity of heparin, competes with antithrombin III for bonding sites on heparan sulphate present on endothelial cells, chemo attractant for WBC, smooth muscle cells & fibroblasts

PDGF: chemo attractant for WBC, smooth muscle cells & fibroblasts

TGF- β : chemo attractant for WBC, smooth muscle cells & fibroblasts

➤ Analogous of plasma proteins:

Albumin, IgG- no known hemostatic function

Fibrinogen, vWF, Factor V- known hemostatic function

Dense granules: ADP, ATP, Calcium, serotonin

Lysosomal granules:

Compaction & stabilization of platelet plug

- The GP IIb-IIIa heterodimer traverses the platelet membrane
- When fibrinogen binds to the external domain of GP IIb-IIIa the cytoplasmic domain is altered and binds to actin filaments
- This orients centripetal contraction

Blood coagulation

Secondary hemostasis

Blood clotting factors

Factor I (Fibrinogen): a soluble plasma protein. In afibrinogenemia clotting does not occur

Factor II (Prothrombin): Inactive precursor of thrombin

Factor III (Tissue factor/ Tissue thromboplastin): converts prothrombin to thrombin in presence of factors V, VII, X, Ca^{++} and phospholipids

Factor IV (Calcium): Essential for clotting.

Factor V (Labile factor/ proaccelerin):

Factor VII (Stable factor/ proconvertin): Deficiency induced by oral anticoagulants

Blood clotting factors...

- **Factor VIII** (Antihemophilic globulin/factor A):
Congenital deficiency causes Classical Hemophilia
- **Factor IX** (Christmas factor/ antihemophilic factor B):
Congenital deficiency leads to a hemorrhagic state resembling Hemophilia (Christmas disease)
- **Factor X** (Stuart-Prower factor)
- **Factor XI** (Plasma thromboplastin antecedent/
Antihemophilic factor C)
- **Factor XII** (Hageman factor, contact factor)
- **Factor XIII** (fibrin stabilizing factor)

Blood clotting factors...

HMW-K: High molecular weight kininogens

Pre-Ka: prekallikrein/ Fletcher factor

Ka: Kallikrein

PL: Platelet phospholipid

- Factors II, VII, IX, X, protein C & protein S are formed in the liver

Vitamin K is necessary for some post translational modifications in these factors

In vitamin K deficiency or

inhibition by oral anticoagulant ,Warfarin

the plasma levels of these factors are low

Basic reactions involved in coagulation

Prothrombin(Factor II)

↓ Ca^{++} , factors derived from damaged tissues,
disintegrating platelets, plasma

Thrombin

Fibrinogen —————→ Fibrin
(Factor I)

Physiology of clotting process

Thrombin-fibrinogen reaction

Formation of fibrin clot is the only visible & measurable part of clotting process

Type of reaction

- Proteolysis

Fibrinogen $\xrightarrow{\text{Thrombin}}$ Fibrin monomers + Peptides

- Polymerization

Fibrin monomers \longrightarrow Fibrin polymers
(Soluble fibrin clot)

- Clotting

Fibrin polymers $\xrightarrow[\text{Ca}^{++}]{\text{Factor XII}}$ Insoluble fibrin clot

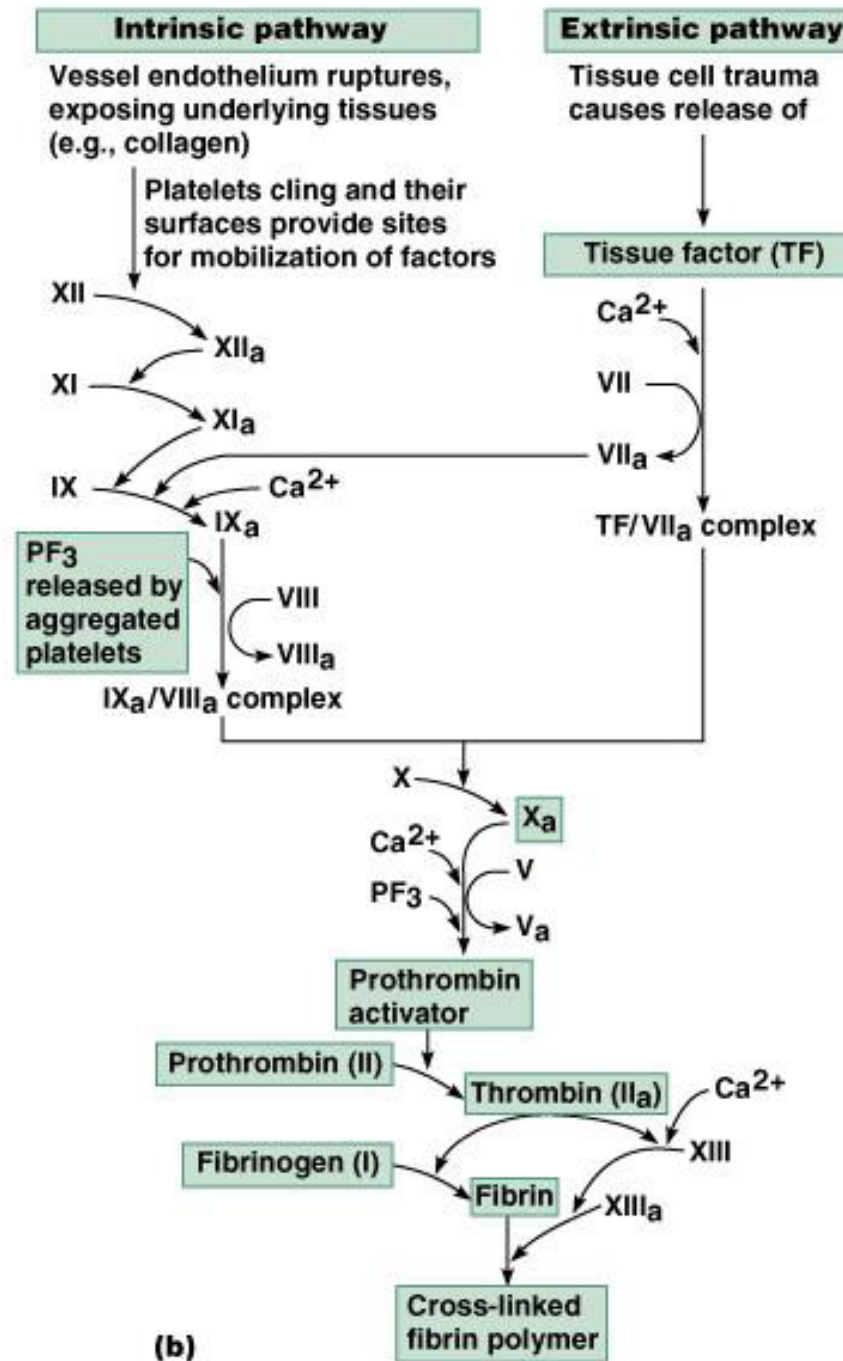
- Conversion of prothrombin to thrombin requires

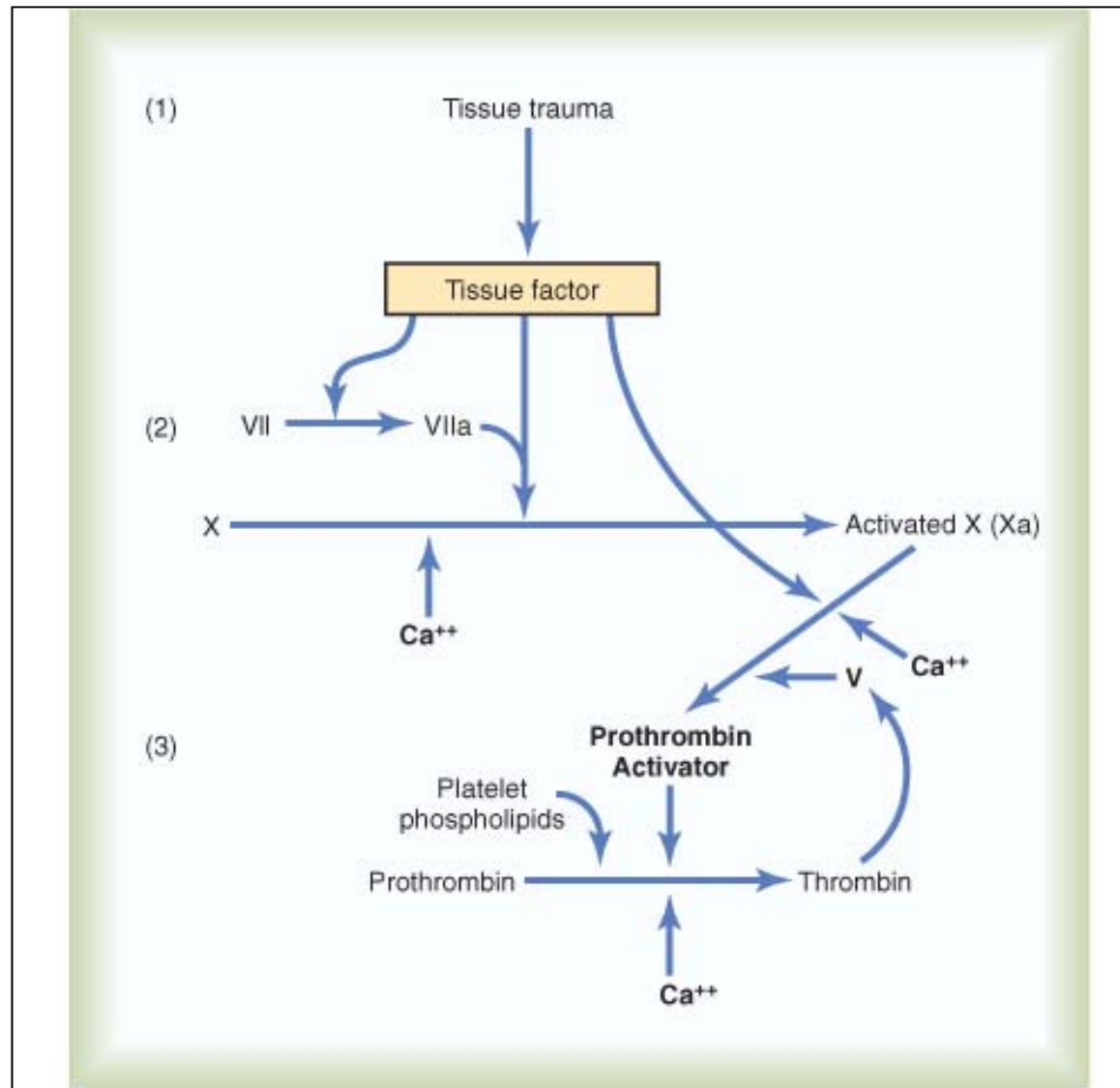
Prothrombin activator or thromboplastin

Prothrombin activator is formed in 2 main ways

- As result of tissue damage (Extrinsic system)
- Activation of blood constituents (Intrinsic system)

Key reaction: Factor X \longrightarrow Factor Xa

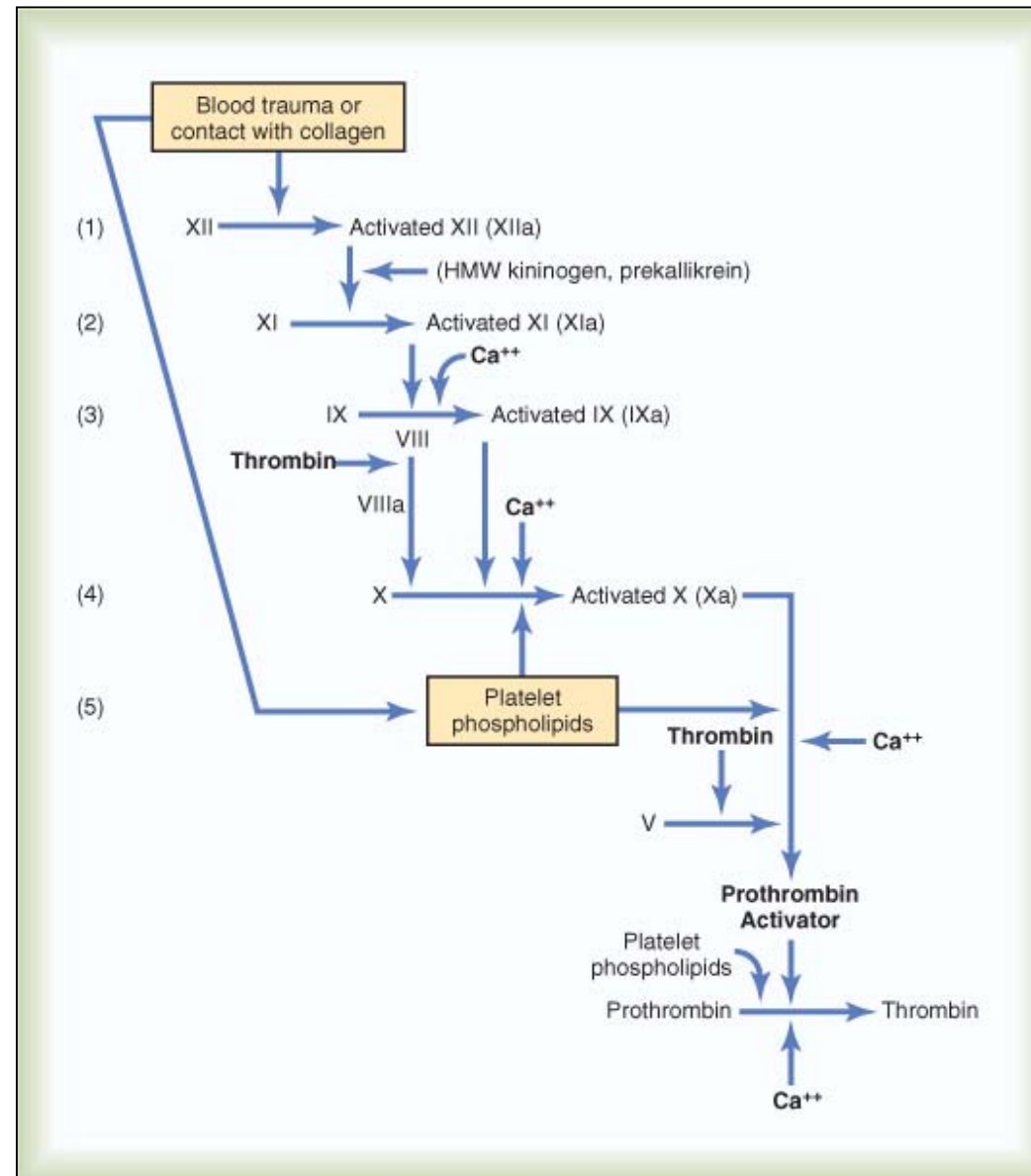




Extrinsic pathway for initiating blood clotting.

Extrinsic system...

- Key event triggering blood coagulation during hemostasis is exposure of blood to tissue factor
- It also acts as a cofactor for activation of factor VII



Intrinsic pathway for initiating blood clotting.

Intrinsic system

- More complicated
- More prolonged
- Fibrinolytic and kinin forming systems are also activated

Enzyme cascade hypothesis(Macfarlane):

Surface contact induces a sequence of changes in which an inactive precursor is converted into an active enzyme which then acts on the next precursor to form the next active enzyme...

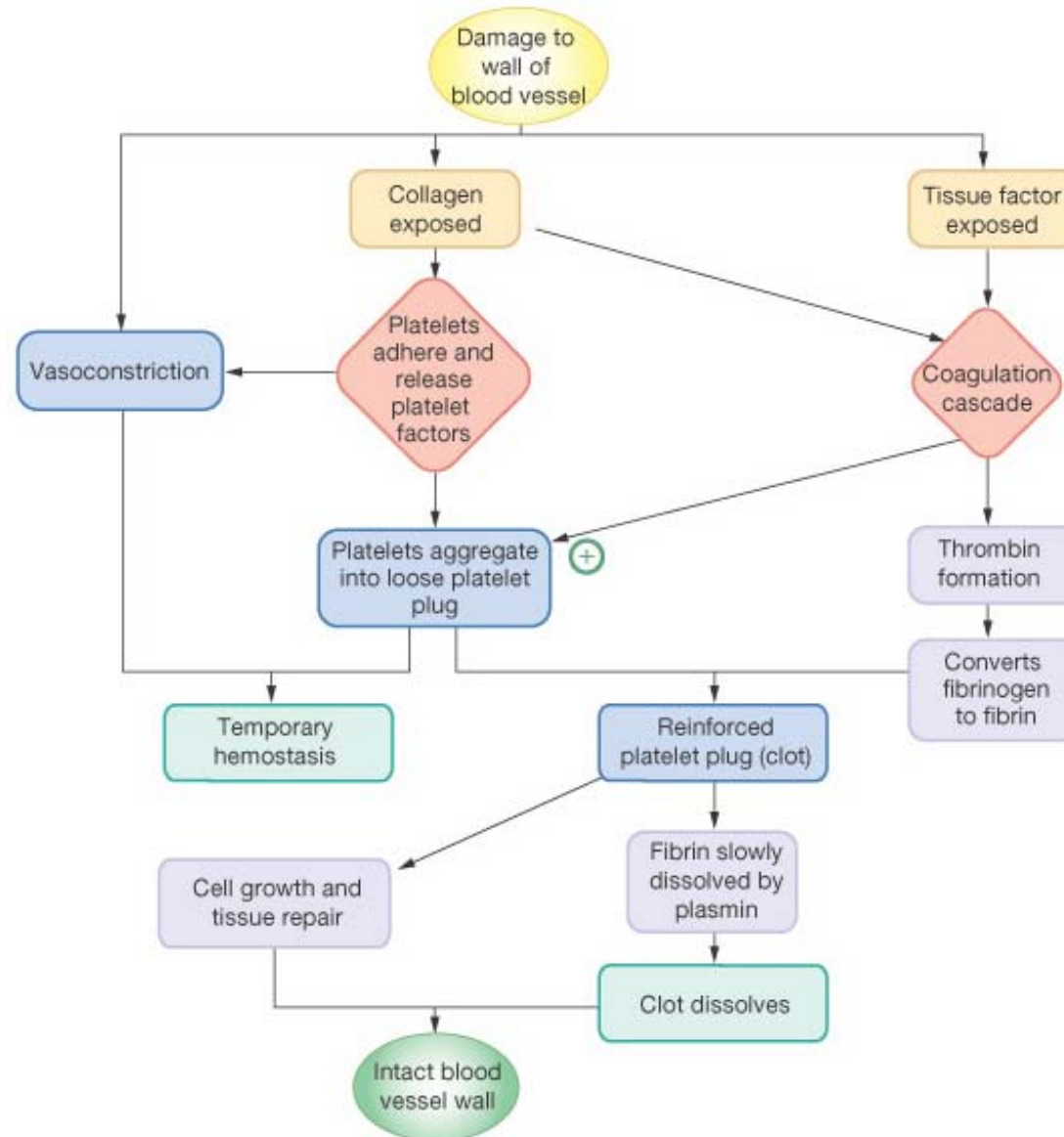
An amplifying system

Intrinsic system...

- Factor XII, prekallikrein, Factor XI & High molecular weight kininogen are known as the contact activation factors
- Factor XII, prekallikrein & HMWK are essential for triggering blood coagulation in a glass test tube
- However they play no role in normal hemostasis since patients with isolated deficiencies of each of these do not bleed abnormally

- Once Xa is formed clotting occurs within seconds
- Contact with a foreign substance i.e. glass or urate crystal produces clotting only after 4-8 minutes

Overview of clotting



Effects of thrombin

- Positive feedback effect on factors concerned with formation of prothrombin activator
- Activates factor VIII
- Increases activity of factor V & XIII
- Promotes aggregation of platelets
- Increases the amount of available phospholipids
- After activating these mechanisms soon inactivates them

Regulation of blood coagulation

- Adsorption of thrombin onto fibrin
- Neutralization of thrombin by plasma proteinase inhibitors, Antithrombin III, α_2 macroglobulin & heparin cofactor II
- Antithrombin III also inhibits the activity of key intermediate enzymes, Factor IXa & Xa
- Activated protein C inhibits the cofactors for Factor VIIIa & Va
- Extrinsic pathway inhibitor (EPI) & factor Xa neutralize the catalytic activity of Factor VIIa/tissue factor complex

Clot retraction

Platelet contractile proteins contract.

- Squeezes fluid (serum) out of the clot.
- Draws the edges of the torn blood vessel together.
- Sets the stage for repair.

–PDGF

–VEGF

Clot retraction

- Freshly formed fibrin threads are extremely sticky and adhere to each other, blood cells, tissues & foreign substances
- Freshly shed blood sets in a soft jelly –like mass
- Gradually it contracts down(retracts) to 40% of its original volume, squeezing out serum
- Final clot is more tougher and solid
- Clot retraction is impaired if platelets are removed from blood

Fibrinolysis (Dissolution of clot)

Plasminogen $\xrightarrow[\text{activators}]{\text{Extrinsic / Intrinsic Plasminogen}}$ Plasmin

Fibrin $\xrightarrow[\text{(Fibrin degradation products)}]{\text{Plasmin}}$ Small peptides

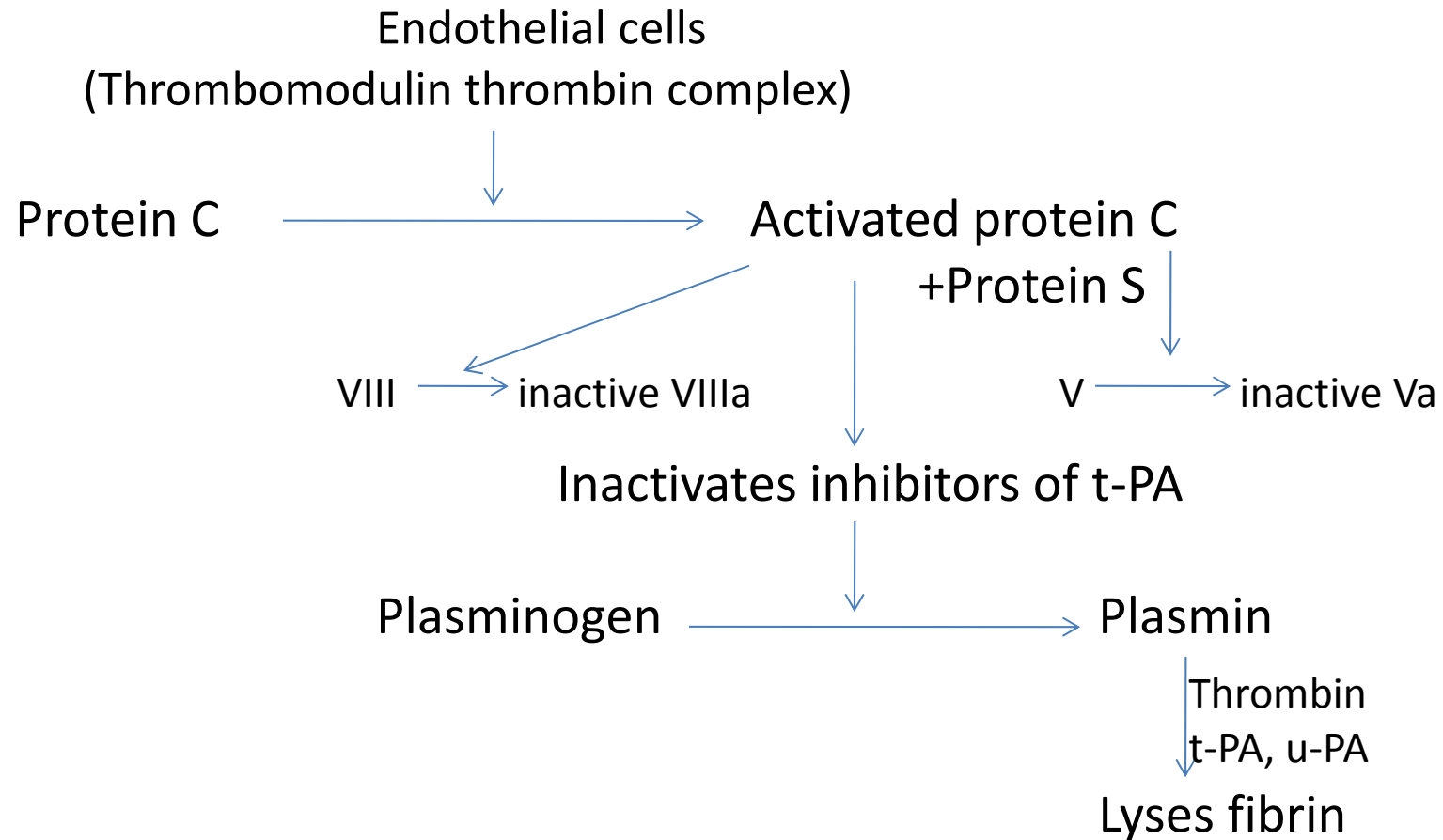
Intrinsic plasminogen activators get activated by:

Body or mental stress, operation, violent exercise, adrenalin injection

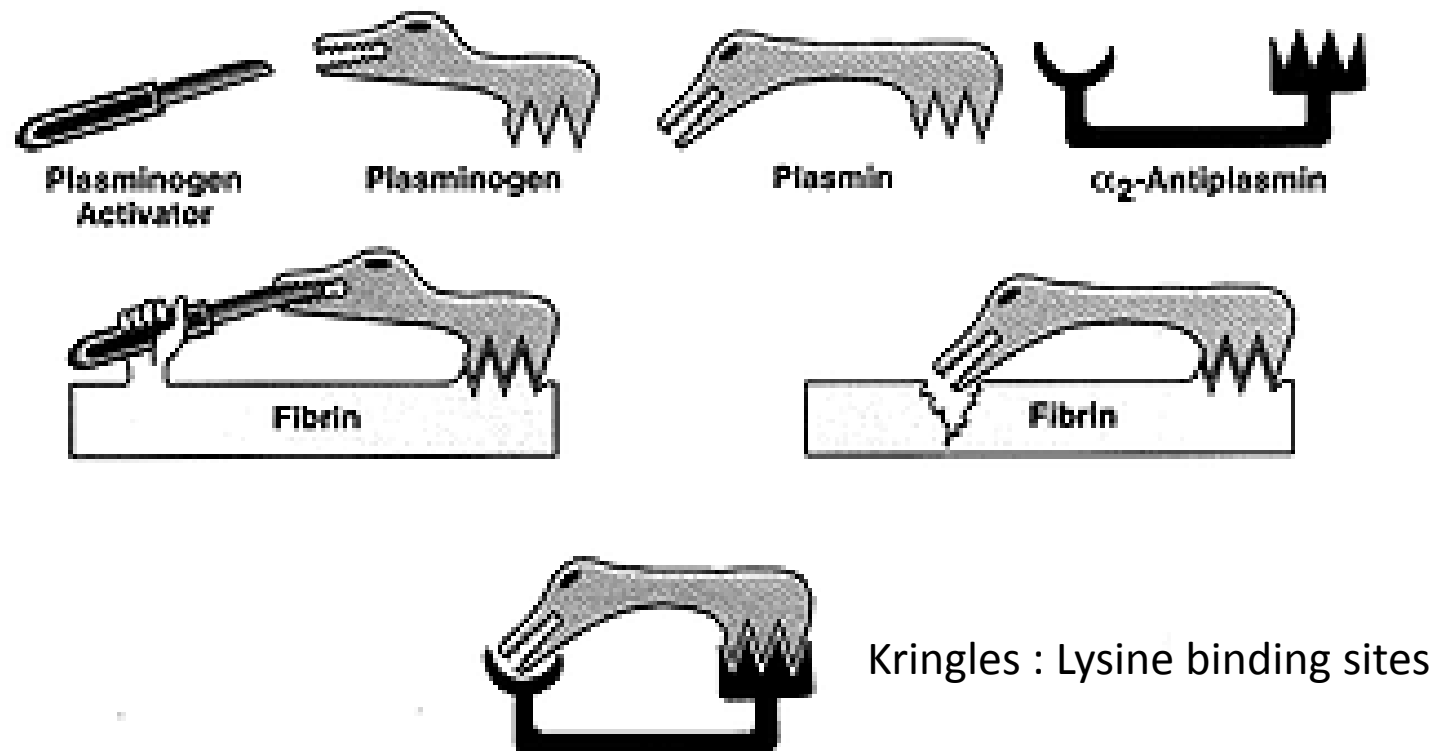
Extrinsic activators: Widely distributed throughout the cell & body fluids

Tissue activators occur in microsomes, urokinase in urine

Fibrinolytic system & its regulation by protein C

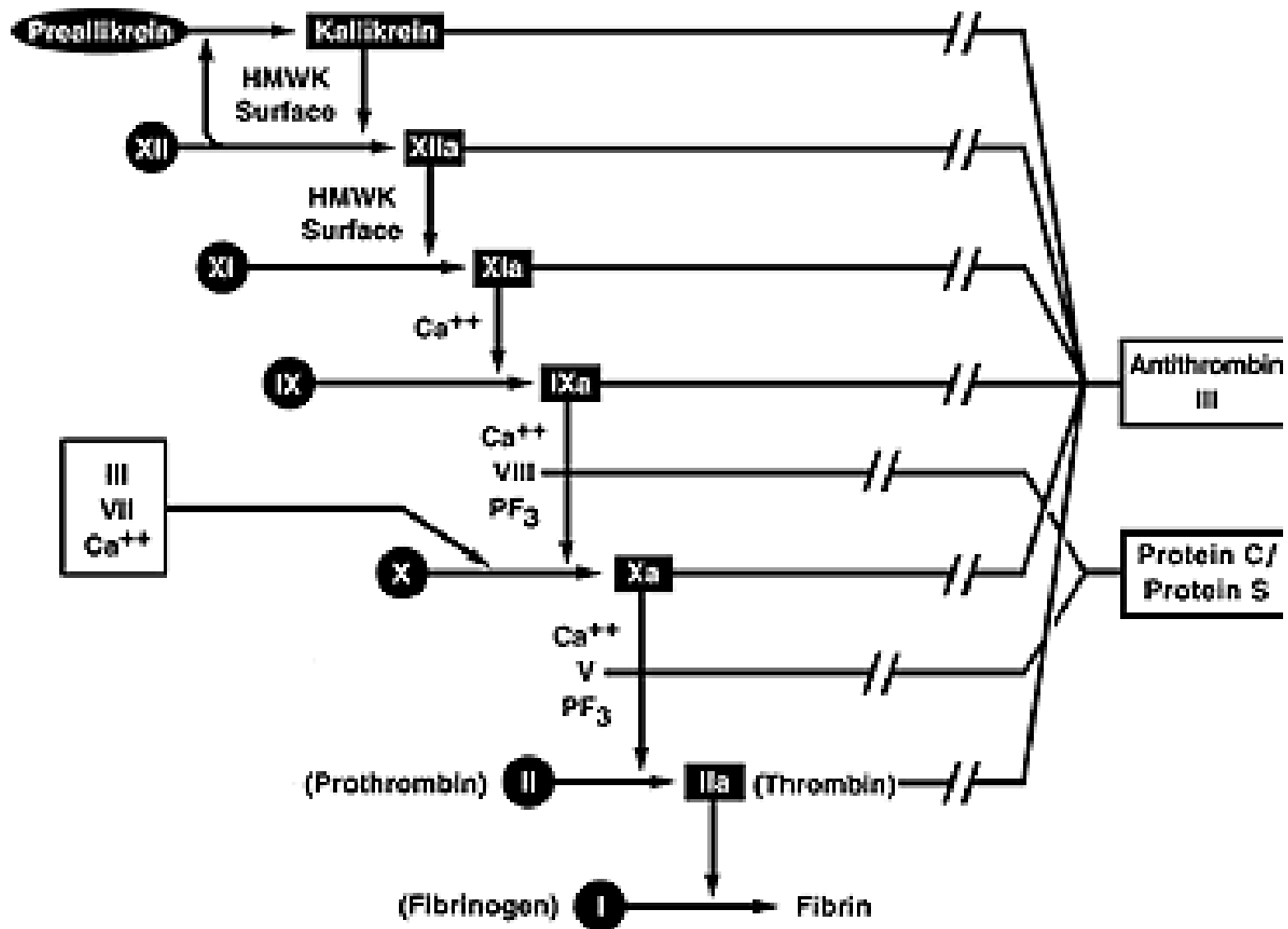


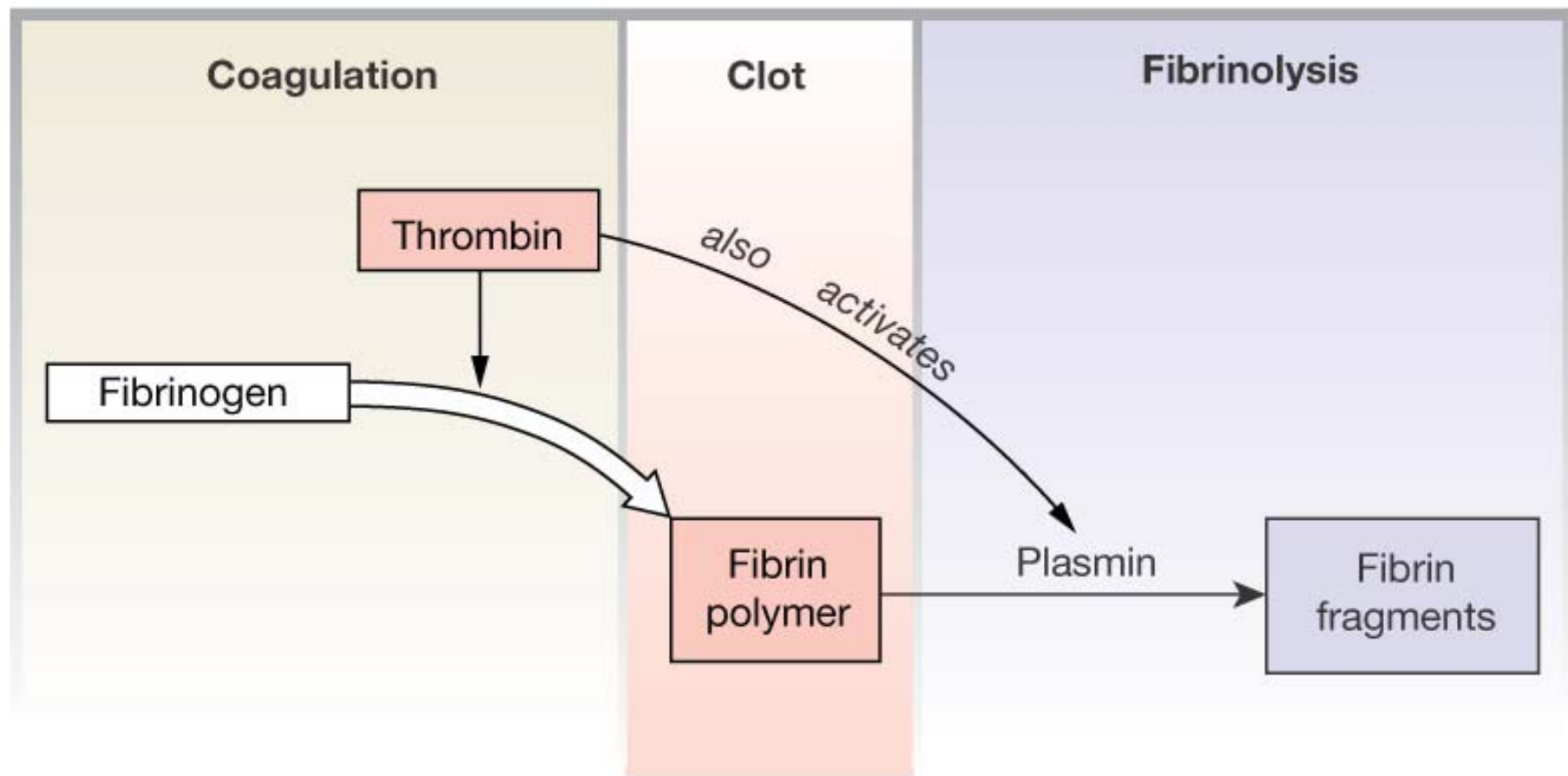
Plasminogen (fibrinolytic) system



t-PA: Produced by recombinant DNA technology
Used in myocardial infarction and stroke

INHIBITORS OF COAGULATION SYSTEM





Tests for defects in blood clotting

Screening for adequacy of hemostatic plug formation

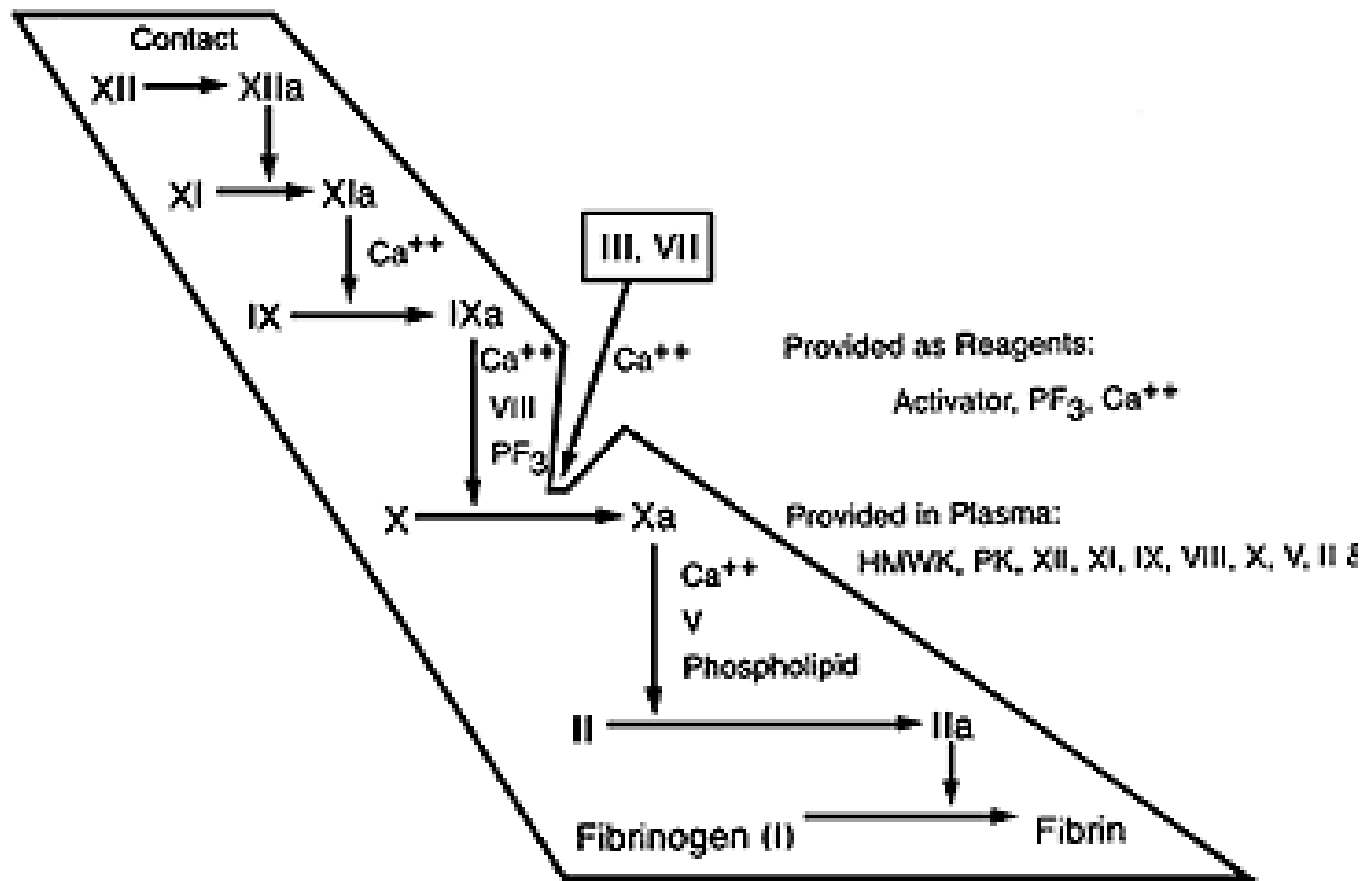
- Platelet count
- Bleeding time: for conditions other than thrombocytopenia that can impair the formation of hemostatic plug

Tests for defects in blood clotting...

Screening for the adequacy of blood coagulation

- Prothrombin time: Measures the adequacy of reactions that clot plasma when a very high concentration of tissue factor is present.
Tests the adequacy of extrinsic system
- Activated partial thromboplastin time: Measures the adequacy of clotting reactions that clot plasma when a reagent optimizing the contact activation reactions & providing procoagulant phospholipid is present
Tests adequacy of intrinsic system

Activated partial thromboplastin time



Disorders affecting formation of platelet plug

Thrombocytopenia

- Physiological: New born, Menstruation
- Pathological:
 - Decreased marrow production: Tumours, fibrosis, aplasia, drugs(thiazide diuretics)
 - Splenic sequestration: Splenomegaly
 - Increased destruction: Auto antibody formation, aspirin, insecticides, cardiac valves, Sepsis, vasculitis

Disorders affecting formation of platelet plug...

Functional platelet defects

- Disordered adhesion
Von Willebrand's disease: ↓ vWF, ↓ Factor VIII activity, ↑ BT
Most common hereditary hemostatic disorder
- Disordered aggregation
Thrombasthenia (Glanzmann's syndrome)
Afibrinogenemia
- Disordered granule release
Chediak Higashi syndrome
Cardiopulmonary bypass
Myeloproliferative disorders
Aspirin & other NSAIDS

- Platelet count
- >1 lakh: Asymptomatic, Normal bleeding time
- 50000-1 lakh: Bleeding occurs after severe trauma, Bleeding time slightly prolonged
- <50000: Easy bruising manifested by skin purpura after minor trauma
- <20000: Spontaneous bleeding, usually have petechiae, intracranial/ spontaneous bleeding

Disorders affecting blood coagulation

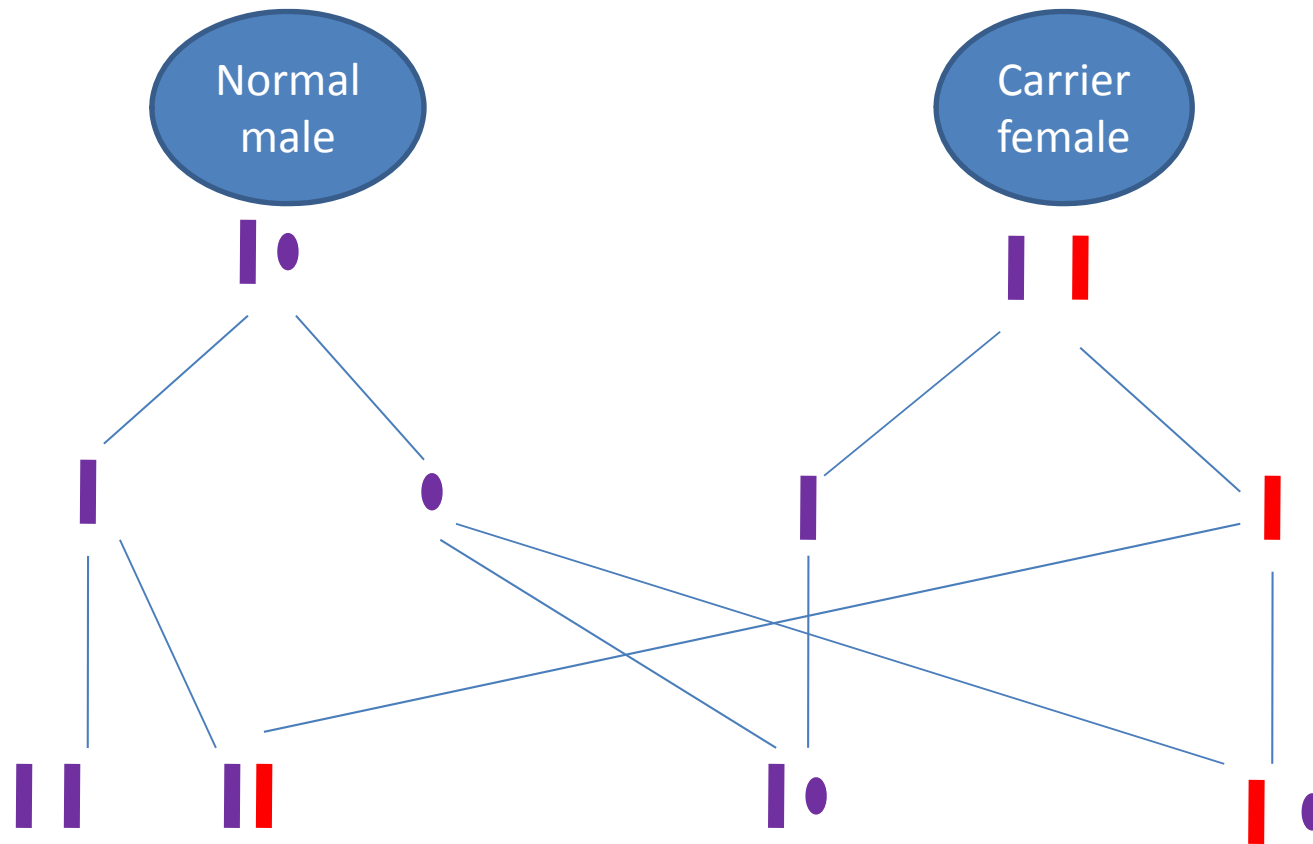
Hereditary disorders

- Factor VIII deficiency(Hemophilia A)
 - Factor IX deficiency(Hemophilia B)
 - Factor XI deficiency: Autosomal recessive
- } X linked
recessive disorders

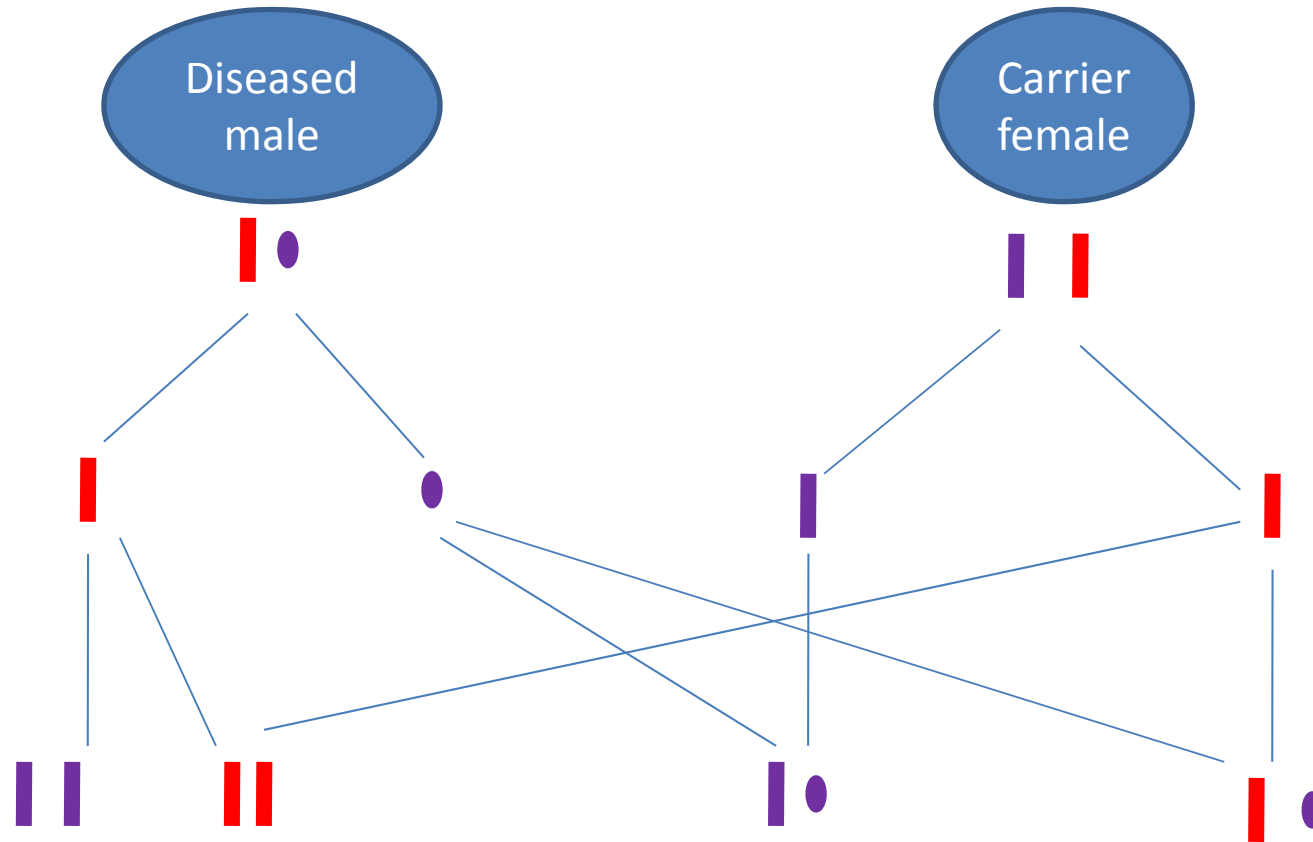
Acquired disorders

- Vitamin K deficiency
- Liver disorders: Fall in all factors except factor VIII
- Disseminated intravascular coagulation
- Acquired antibodies against clotting factors

Transmission of Hemophilia



Transmission of Hemophilia



Anticoagulants in vivo

- Antithrombin III: Serine protease inhibitor
- Heparin
- Thrombomodulin: Thrombin binding protein
- Thrombomodulin-thrombin complex: Activates protein C
- Activated protein C and S
- Plasmin
- Dicumarol & Warfarin: Inhibit action of vitamin K

Anticoagulants in vitro

- **EDTA**
- **Oxalate**
- **Heparin**
- **Sodium Citrate**
- **Sodium Fluoride/Potassium Oxalate**

Characteristics

- It must not alter the size of the red cells
- It must not cause hemolysis
- It must minimize platelet aggregation
- It must minimize disruption of the staining and morphology of leukocytes
- It must be readily soluble in blood

EDTA

(Ethylenediaminetetraacetic acid)

- Best meets the above requirements, and is used most frequently.
- The tripotassium salt (**K₃EDTA**), and the disodium salt (**Na₂EDTA**).
- **Calcium EDTA is not used as an anticoagulant, but in the treatment of lead poisoning**
- Mode of action: It forms insoluble calcium salts by chelation
- Uses: Making a blood smear for cell morphology studies, test for microfilaria, perform cell counts, BUN, plasma protein, fibrinogen, glucose, determination of Coomb's Test
- EDTA preserves the staining and morphological characteristics of leukocytes
- Disadvantages
 - Excessive concentrations of EDTA will cause shrinkage of RBC's and erroneous PCV, MCV and MCHC results

Oxalate

- A mixture of dry ammonium oxalate and potassium oxalate in the ratio of 3:2 is used
- Mode of action: It combines with calcium to form insoluble Ca oxalate.
- Potassium oxalate alone causes red cells to shrink; ammonium oxalate alone causes red cells to swell. Used together, little cellular distortion occurs in the first hour after collection
- Uses: The oxalate mixture may be used for hematological sedimentation studies. Potassium oxalate alone is valid for immediate glucose determinations. Ammonium oxalate is used as a diluent in some methods for manually counting WBCs and platelets
- Disadvantages
 - 1) It does not prevent platelet aggregation *in vitro* as effectively as EDTA.
 - 2) It is poisonous and should not be used for blood transfusion.

Heparin

- Chemical structure: A polysaccharide derived from glucosamine and glucuronic acid. Contains many sulphate groups.
- Molecular weight averages 15000-18000
- Anticoagulant action due to its strong electronegative charge due its sulphuric acid group
- Antagonists: Toludene blue & protamine by antagonizing the negative charge of heparin

Heparin...

- First isolated from Liver
- Subsequently demonstrated in extract of many other organs
- Inhibits blood coagulation both in vitro & in vivo
- Mechanism of action:
 - Prevents activation of prothrombin to thrombin
 - Neutralizes action of thrombin on fibrinogen
 - Facilitates the action of antithrombin III

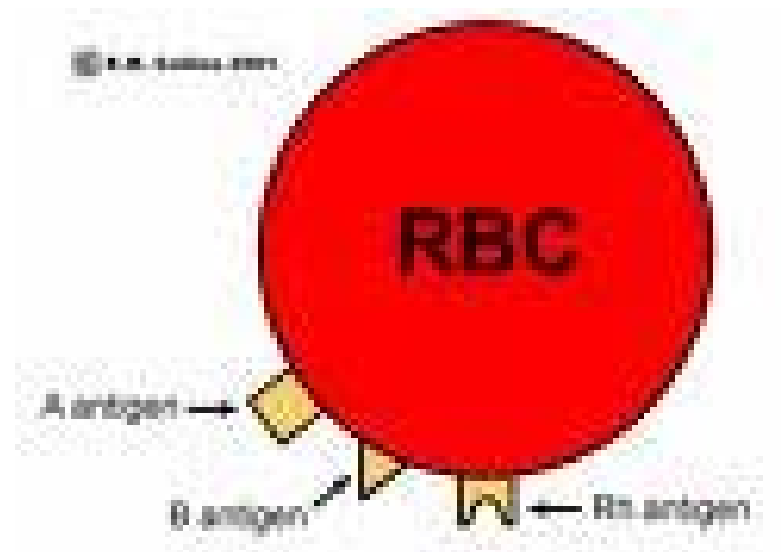
Heparin...

- Heparin is the anticoagulant of choice for blood pH and blood gas analysis for acid-base balance.
- Disadvantages
 - 1) It causes clumping of leukocytes.
 - 2) It interferes with the staining of leukocytes.
 - 3) It is the most expensive of the anticoagulants.
 - 4) Blood will clot in 8-12 hours because clotting is only delayed and not prevented.
 - 5) It is not suitable for agglutination tests, coagulation studies (prothrombin time tests) or plasma fibrinogen determination

Sodium citrate

- Acid citrate dextrose (ACD) is prepared from disodium hydrogen citrate and is the anticoagulant of choice for blood transfusions. used in the ratio of 1 part ACD to 4 parts of blood
- Mode of action: It combines with calcium to form an insoluble salt of calcium citrate.
- Sodium citrate is the anticoagulant of choice for studies of platelet function and morphology
- e. Disadvantages:
 - 1) It interferes with many chemical tests.
 - 2) Used alone it preserves blood for only a few hours.
 - 3) It has a tendency to shrink cells.
 - 4) Because of a 10% dilution of blood, Na-citrate is generally not used for CBC.

Blood groups



Blood groups

- Agglutinogen : The antigen present on RBC surface
- Major O-A-B blood types

Blood group A: A antigen A present on surface

Blood group B: B antigen present on surface

Blood group AB: A & B antigen present on surf.

Blood group O: No antigen present on surface

Genetic determination of agglutinogens

- Genes that determine the A& B phenotypes are found on chromosome 9p
- They are expressed in a mendelian co-dominant manner
- The gene products are glycosyl transferases, A or B, which confer the enzymatic ability of attaching the specific antigenic carbohydrate groups

Genetic determination of agglutinogens...

- Glycosyl transferase A directs the formation of antigen A on RBC surface
- Glycosyl transferase B directs the formation of antigen B on RBC surface
- When A & B transferases are absent then no antigen is present on RBC surface and blood group is said to be O

Agglutinins

- Antibodies directed against RBC antigen may result from “natural exposure” particularly to carbohydrate that mimic some blood group antigens

Blood types with their genotypes

Genotypes	Blood types	Agglutinogen	Agglutinin
OO	O	-	Anti a & Anti b
OA or AA	A	A	Anti b
OB or BB	B	B	Anti a
AB	AB	A & B	-

Relative frequencies of different blood groups

	Western countries	India
O	47%	32%
A	41%	20%
B	9%	40%
AB	3%	8%

Landsteiner's law

States that if an agglutininogen is present in the RBCs of an individual the corresponding agglutinin must be absent in the plasma

Or conversely if an agglutininogen is absent in the RBCs of an individual the corresponding agglutinin must be present in the plasma

Rh system of blood grouping

- RBC of rhesus monkey when injected into a rabbit, the rabbit developed antibodies to the rhesus RBC
- Further it was discovered that rabbit serum containing anti rhesus antibodies could agglutinate not only rhesus RBC but also human RBC in 85% cases
- The RBCs of these humans have on their surface an antigen identical to or similar to rhesus antigen
- Thus humans having this antigen were termed as Rh positive

Rh blood group

- Three Rh genes are present
- Rh genes are located on chromosome 1
- Types of Rh antigens/ factor: C, D, E
- D is most antigenic
- The term Rh positive is generally used to mean that the individual has agglutinogen D
- 85% Caucasians are Rh positive
- 99% Asians are Rh positive

Difference between ABO and Rh groups

- ABO antigens are present on cells other than RBCs whereas the Rh antigen is not present on any other cell except RBCs
- In the ABO system the plasma antibodies responsible for transfusion reaction develop spontaneously whereas in the Rh system antibodies only develop in Rh negative persons when they have been previously sensitized

- Knowledge of the mode of inheritance of blood groups is useful in cases of disputed paternity
- The negative verdict is definitive: It rules out the possibility of a given man being the father

Child's blood group	Child's genotype	Mother's blood group	Possible contribution of mother	Possible contribution of father	Father might have been	Father could not have been
A	AA AO	A or AB B	A O	A A	A or AB A or AB	O or B O or B
B	BB BO	B or AB O	B O	B B	B or AB B or AB	O or A O or A
AB	AB AB	A B	A B	B A	B A or AB	O or A O or B
O	OO OO	A B	O O	O O	A or B or O	AB AB

Rh incompatibility

- If a Rh negative person is transfused with Rh positive blood for the first time: No immediate adverse reaction
- If the same individual receives a second transfusion later anti Rh antibodies are synthesized promptly in large amounts

Rh incompatibility in pregnancy...

- Rh negative female
- Rh positive male
- Fetus is Rh positive

Chances of an apparent abnormality resulting from Rh incompatibility are

Negligible in first pregnancy

3% during second pregnancy

10% during third pregnancy

Rh incompatibility in pregnancy...

Erythroblastosis foetalis/ Hemolytic disease of newborn

Fetal RBCs undergo massive hemolysis

Compensatory increase in erythropoietic activity leading to immature cells in circulation

Enlargement of extramedullary sites of hematopoiesis leads to hepatomegaly & splenomegaly

Child is anemic, Jaundice (Hemolytic) is present, edema

Hydrops fetalis: Death in utero

Kernicterus: Deposition of unconjugated bilirubin in the basal ganglia

If bilirubin > 20 mg % then brain damage

Erythroblastosis fetalis...

Preventive treatment: Giving anti Rh antibodies to Rh negative pregnant mother

Mechanism by which anti Rh antibody prevent the complication of Rh incompatibility

- They destroy fetal red cells which cross over to the maternal circulation. Thus antigenic stimulus is reduced
- High antibody levels inhibit production of the same antibody molecules

Definitive treatment: Exchange transfusion with Rh negative blood

Other blood group systems

- Lewis system
- I System
- P system
- MNSSU sysetm
- Kell system: Immunogenicity is third behind the ABO & Rh systems
- Duffy system
- Kidd system

Blood transfusion: Compatibility testing

- **ABO-Rh typing:** Antibodies are directed against those antigens that lack in the individual's own blood
- **Cross matching:** A trial transfusion within a test tube in which donor RBC are mixed with recipient serum to detect a potential for serious transfusion
- **Antibody screen:** A trial transfusion between the recipient's serum & commercially supplied RBC's that are specifically selected to contain optimal number of RBC antigens

Storage of blood

- CPDA-1

Citrate : Anticoagulant

Phosphate : Buffer

Dextrose: energy source

Adenine: For resynthesis of ATP so as to extend the storage time from 21 to 35 days

AS-1: (Adsol): Adenine, glucose, mannitol & NaCl

Increases shelf life to 42 days

AS-3: (Nutricel): Contains adenine, glucose, citrate, phosphate, NaCl

Storage of blood

Duration of storage

Set by the US federal regulation

At least 70% of the transfused RBC's remain in circulation for 24 hours after infusion

Since RBC's that survive 24 hours after transfusion disappear from circulation at the normal rate

Blood transfusion

Storage of blood

Three problems

- Anticoagulation
- Preservation of cell viability
- Preservation of cell function

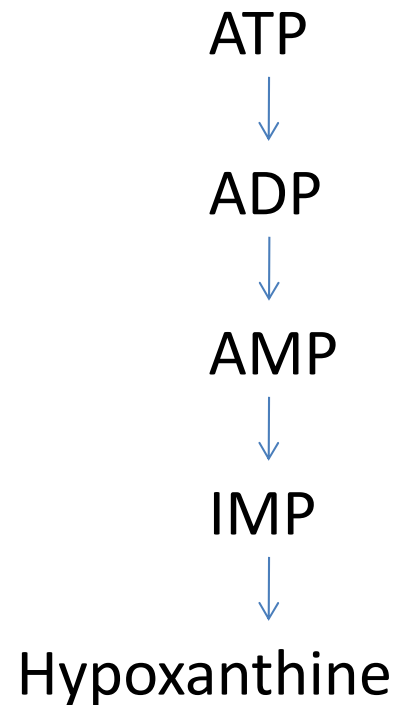
Criteria for assessing the adequacy of banked blood

70% survival at 24 hours

RBC storage lesion

Stored RBCs undergo a series of biochemical & structural changes

- RBC nucleotide depletion



RBC storage lesion...

- RBC membrane changes

Normal disc shaped



Spherical with surface projections

Loss of membrane proteins

Loss of deformability

RBC storage lesion...

- 2,3 DPG depletion

Earliest detectable change

➤ With acid citrate dextran(ACD)

DPG drops to < 50% within 48 hours

➤ With citrate phosphate dextran(CPD)

DPG falls significantly after 2 weeks

RBC storage lesion...

- Decreased DPG
- Increased affinity of Hb for oxygen
- Increased Hb but no proportionate increase in oxygen availability

RBC storage lesion...

- Accumulation of lactate
- Accumulation of H^+ ions
- Decrease in pH
- Loss of K^+ and gain of Na^+
- Increased osmotic fragility
- Some cells undergo lysis

Use of anticoagulant

- Citrate

Most important anticoagulant

Mode of action

After transfusion it is readily metabolized by the recipient

Preserves RBC's in a viable state for 1 week only

- ACD(Acid citrate dextran)
Glucose is present
RBC storage for 3 weeks
- CPD(Citrate phosphate dextran): Modified ACD with added NaH_2PO_4
Viability after first 24 hours is better
At 4°C blood may be stored for 21 days
- CPDA-1: CPD supplemented with adenine and with 25% more glucose than CPD(Standard anticoagulant preservative)
RBC storage up to 35 days

2,3 DPG preservation

- In ACD, CPD, CPDA-1, 2, 3 DPG disappears by 2 weeks
- Chemical agents capable of maintaining normal levels during storage
dihydroxyacetone, pyruvate, ascorbic acid

Indications of blood transfusion

- Hypovolemia: Blood loss < 20 % of total blood volume does not require RBC transfusion
fluid replacement is sufficient
- Surgery
- Anemia: Hb levels < 6 gm % require transfusion

Pre transfusion testing

Pre transfusion testing of a potential recipient consists of the following

- Forward type: Determines the ABO & Rh phenotype of the recipient
- Reverse type: Detects isoagglutinins in the patients serum and should correlate with the forward type testing.

The alloantibody screen identifies antibodies directed against other RBC antigens

- Cross matching: Donor RBC's are mixed with recipient plasma on a slide and checked for agglutination

Adverse effects of transfusion

- Immune mediated reactions

Acute hemolytic transfusion reactions

Occur due to pre formed antibodies that bind & lyse donor RBC

ABO isoagglutinins are responsible for the majority of these reactions

Presentation: Hypotension, tachypnea, tachycardia, fever, chills, hemoglobinemia, hemoglobinuria, chest/flank pain, discomfort at catheter infusion site

Adverse effects of transfusion...

- Immune mediated reactions...

Delayed hemolytic & serological transfusion reaction:

Occurs in patients previously sensitized to RBC alloantigen who have a negative alloantibody screen due to low antibody levels.

When transfused with antigen positive blood an anamnestic response results in the early production of alloantibody that binds to donor RBC

Adverse effects of transfusion...

- Immune mediated reactions...

Febrile non hemolytic transfusion reaction

Most frequent reaction

S& S; Chills & rigor & 1⁰C or more rise in temperature

A diagnosis of exclusion

Antibodies against donor WBC's & HLA antigens

Adverse effects of transfusion...

- Immune mediated reactions...

Allergic reactions

Urticarial reactions characterized by pruritic rash, edema, headache & dizziness

Related to plasma proteins found in transfused components

Adverse effects of transfusion...

- Immune mediated reactions...

Anaphylactic reactions

S& S: difficulty in breathing, coughing, nausea & vomiting, hypotension, bronchospasm, respiratory arrest, shock & loss of consciousness

Treatment; Epinephrine, glucocorticoids

Adverse effects of transfusion...

- Immune mediated reactions...

Graft versus host disease

Mediated by donor T lymphocytes that recognize host HLA as foreign and mount an immune response

S&S: fever, cutaneous eruptions, diarrhoea & liver abnormalities

Clinical manifestations occur 8-10 days post transfusion

Adverse effects of transfusion...

- Immune mediated reactions...

Transfusion related acute lung injury

Uncommon reaction

Due to transfusion of donor plasma that contain high titre anti HLA antibody that bind corresponding antigen on recipient leukocytes.

These leukocytes then aggregate in pulmonary vasculature & release mediators causing an increase in capillary permeability

Adverse effects of transfusion...

- Immune mediated reactions...

Post transfusion purpura

Presents as thrombocytopenia 7-10 days after platelet transfusion

Due to production of antibodies that react to both donor & recipient platelets

Adverse effects of transfusion...

- Non immune reactions

Fluid overload

Hypothermia

Electrolyte toxicity

Iron overload

Adverse effects of transfusion...

- Infectious complications

Viral: HCV(Most common)

HBV, HIV, CMV, HTLV(I), Parvovirus B19

Bacterial: Pseudomonas & Yersenia(Can grow at 1-6°C)

Parasitic: Malaria, Babesiosis, Chaga's disease

Adverse effects of transfusion...

- Low factor V & VIII