Hemostasis

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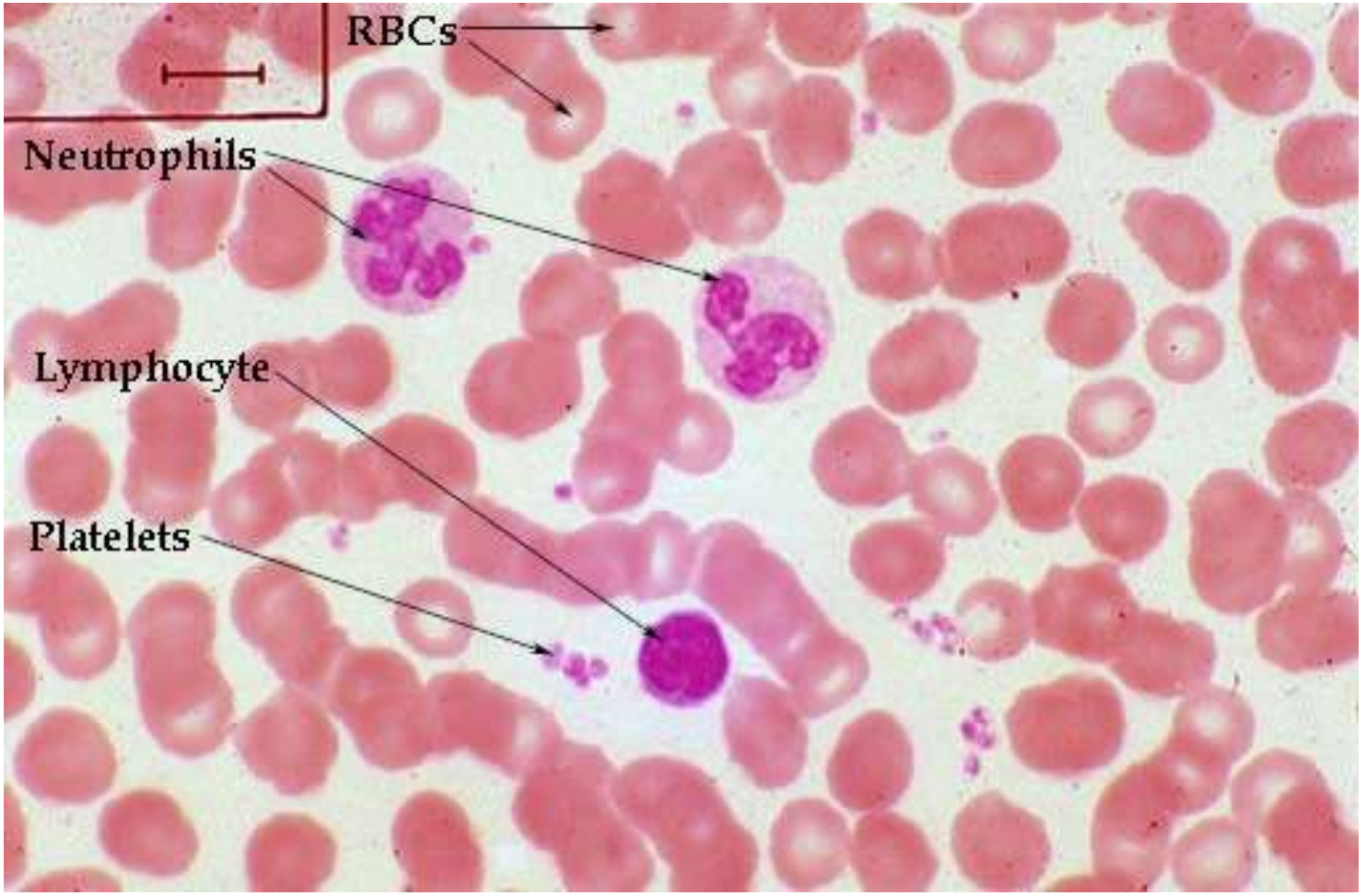
Platelets / Thrombocytes

- These are small, colourless, round or oval discs, 2-4 µ in size. They do not have nucleus.
- Normal count: 1.5-4 lakhs/ mm^3 of blood.
- Life span: 9-12 days.
- Formation: platelets are formed in bone marrow from the fragments of a large multinucleated cell called megakaryocyte.

PBS



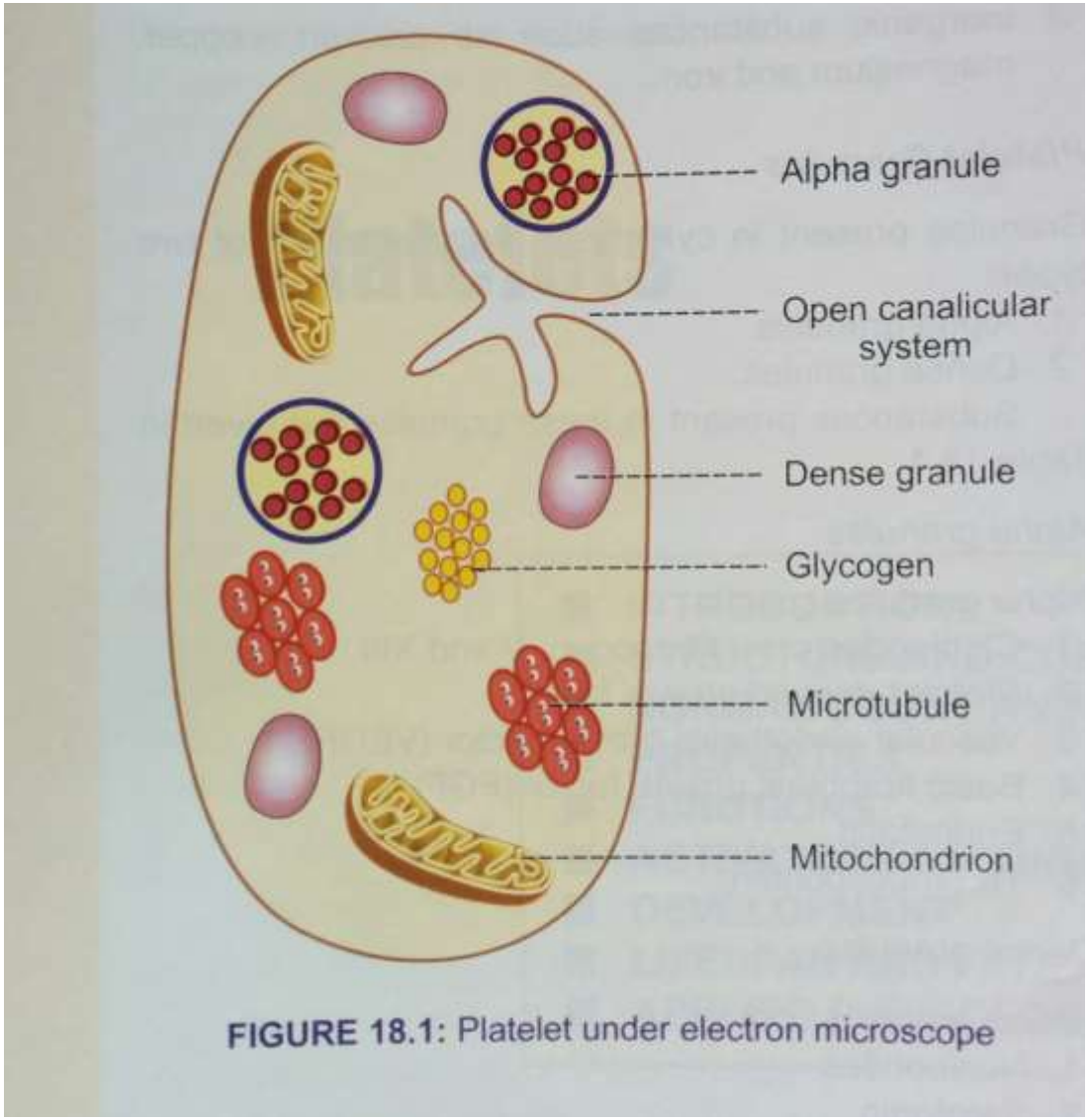


FIGURE 18.1: Platelet under electron microscope

Structure & contents

- Cell membrane:
  - Coating of glycoprotein that prevents adhesion to normal endothelium.
  - Phospholipids
  - Receptors: for von Willebrand factor, collagen, fibrinogen.
- Cytoplasm:
  - contains mitochondria
  - enzymes that form ATP & ADP
  - contractile proteins actin & myosin
  - Three types of granules α, dense & lysosomal enzymes
  - α granules contain platelet derived growth factor, von Willebrand factor, thrombosthenin
  - Dense granules contain nonprotein substances like serotonin, ADP, ATP & Ca^{++}

Functions of platelet

- Hemostatic functions
  - Helps in vasoconstriction by releasing serotonin & thromboxane A2
  - Formation of platelet plug
  - Accelerate process of clotting
  - Clot retraction
- Non-hemostatic functions
  - Involved in phagocytosis
  - Repair of damaged endothelium
  - Storage & transport of serotonin & histamine
  - Role in inflammation

Normal count & variations

- Normal count: 150000 to 400000/ cu mm
- Thrombocytopenia: count < 50000
  - Leads to tendency of bleeding from small capillaries.
  - BT prolonged CT normal
- Causes
  - Viral infections such as dengue fever
  - Bone marrow depression
  - Drug reactions
  - Hypersplenism
- Thrombocytopenic purpura
  - Disease characterized by purplish spots below the skin due to spontaneous bleeding
- Thrombasthenia??
- Treatment:
  - Infusion of fresh platelets
  - Splenectomy in severe cases

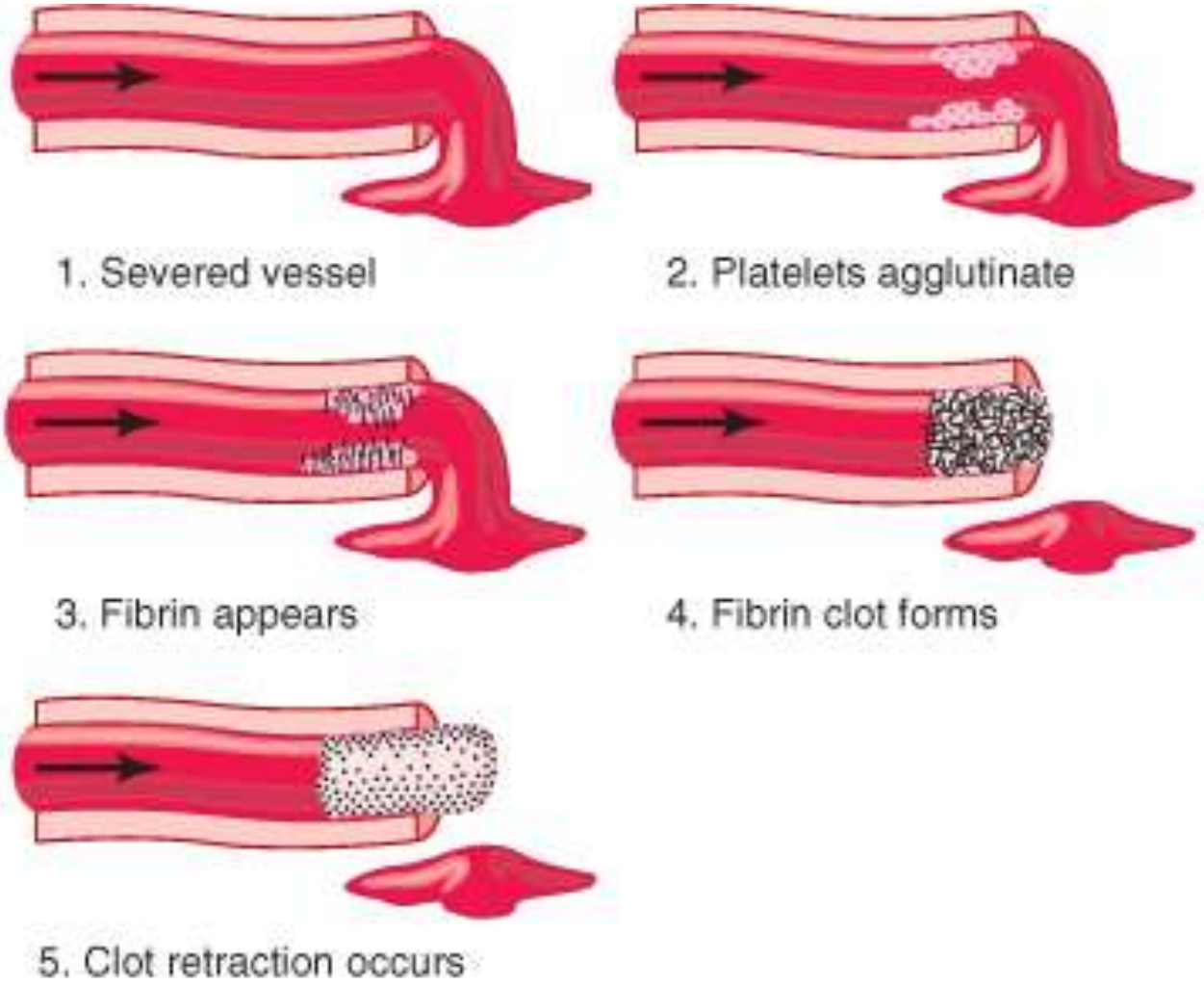
Steps involved in hemostasis

- Hemostasis is defined as arrest or stoppage of bleeding.

- Steps

- Vascular spasm
- Platelet aggregation
- Coagulation

Steps involved in hemostasis



Vascular spasm

- Constriction of smooth muscle in vessel wall: reduces blood flow through vessel
- Prevents further blood loss
- Causes
  - Nervous factors: initiated by pain & other sensory impulses from injured vessel
  - Local myogenic factors
  - Humoral factors – serotonin, thromboxane A_2 by platelets

Platelet plug formation

- Platelet adherence: Platelets become sticky on exposure to collagen. Endothelium secretes von Willebrand's factor which initiates this process.

- Platelet activation: platelets secrete ADP & thromboxane A_2, which activate other platelets to become sticky, the cycle continues.

- Platelet aggregation: Large number of activated platelets stick to each other forming a platelet plug. This is a temporary hemostatic plug which stops the blood loss if the vascular opening is small.

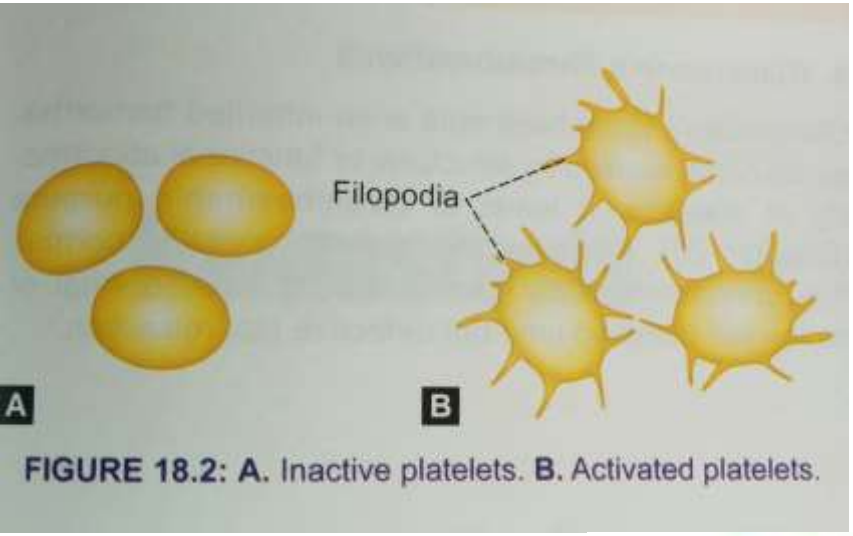
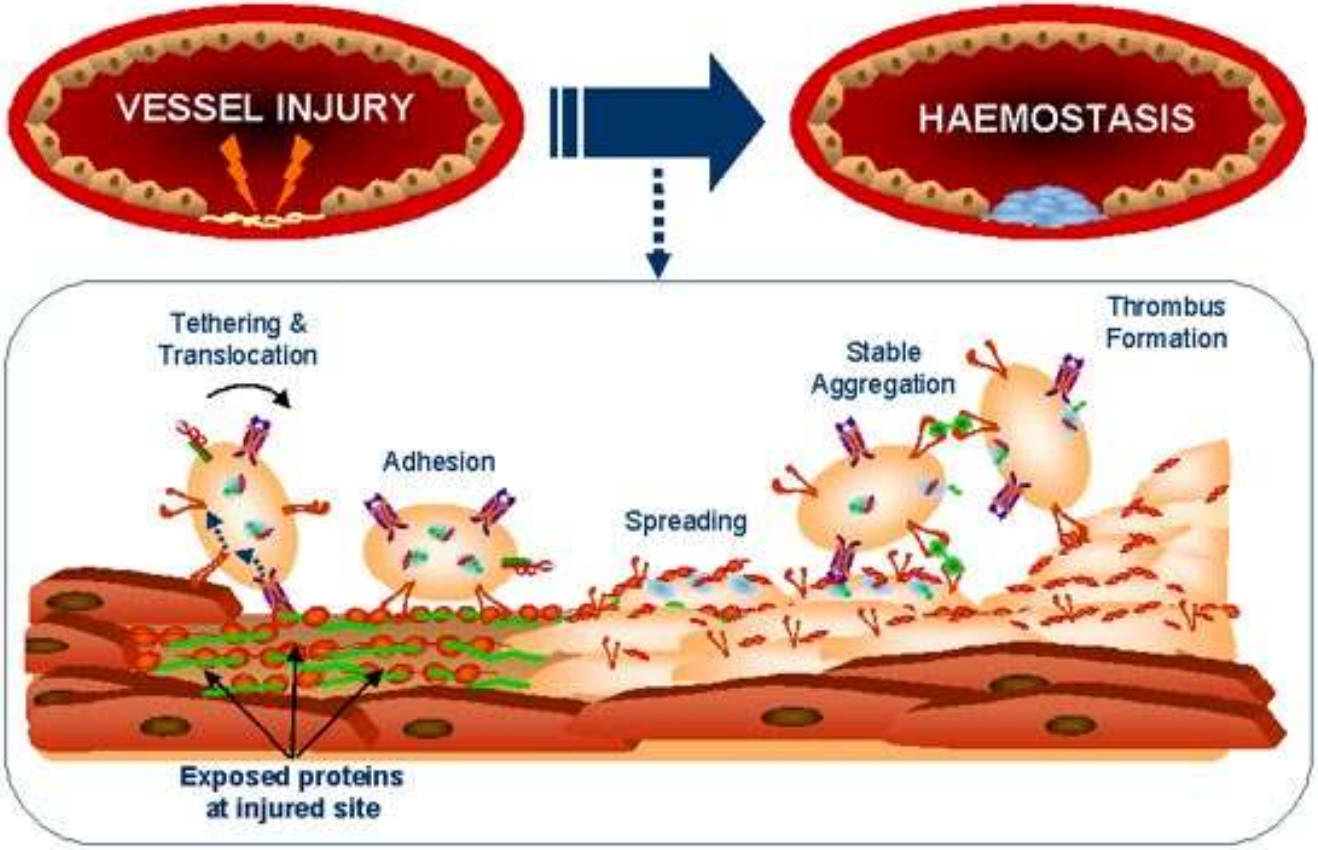


FIGURE 18.2: A. Inactive platelets. **B.** Activated platelets.



Coagulation

- The process in which blood looses its fluidity & becomes a jelly-like mass.
- Extrinsic mechanism
  - Tissue injury-tissue thromboplastin released by injured tissue-acts as proteolytic enzyme

- Intrinsic mechanism
  - Trauma to blood-blood vessel ruptured-endothelium damaged-collagen exposed-factor XII activated.

Clotting factors

Factor[a]	Names
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Proaccelerin, labile factor, accelerator globulin
VII	Proconvertin, SPCA, stable factor
VIII	Antihemophilic factor (AHF), antihemophilic factor A, antihemophilic globulin (AHG)
IX	Plasma thromboplastic component (PTC), Christmas factor, antihemophilic factor B
X	Stuart–Prower factor
XI	Plasma thromboplastin antecedent (PTA), antihemophilic factor C
XII	Hageman factor, glass factor
XIII	Fibrin-stabilizing factor, Laki–Lorand factor
HMW-K	High-molecular-weight kininogen, Fitzgerald factor
Pre-K_a	Prekallikrein, Fletcher factor
Ka	Kallikrein
PL	Platelet phospholipid

[a]Factor VI is not a separate entity and has been dropped.

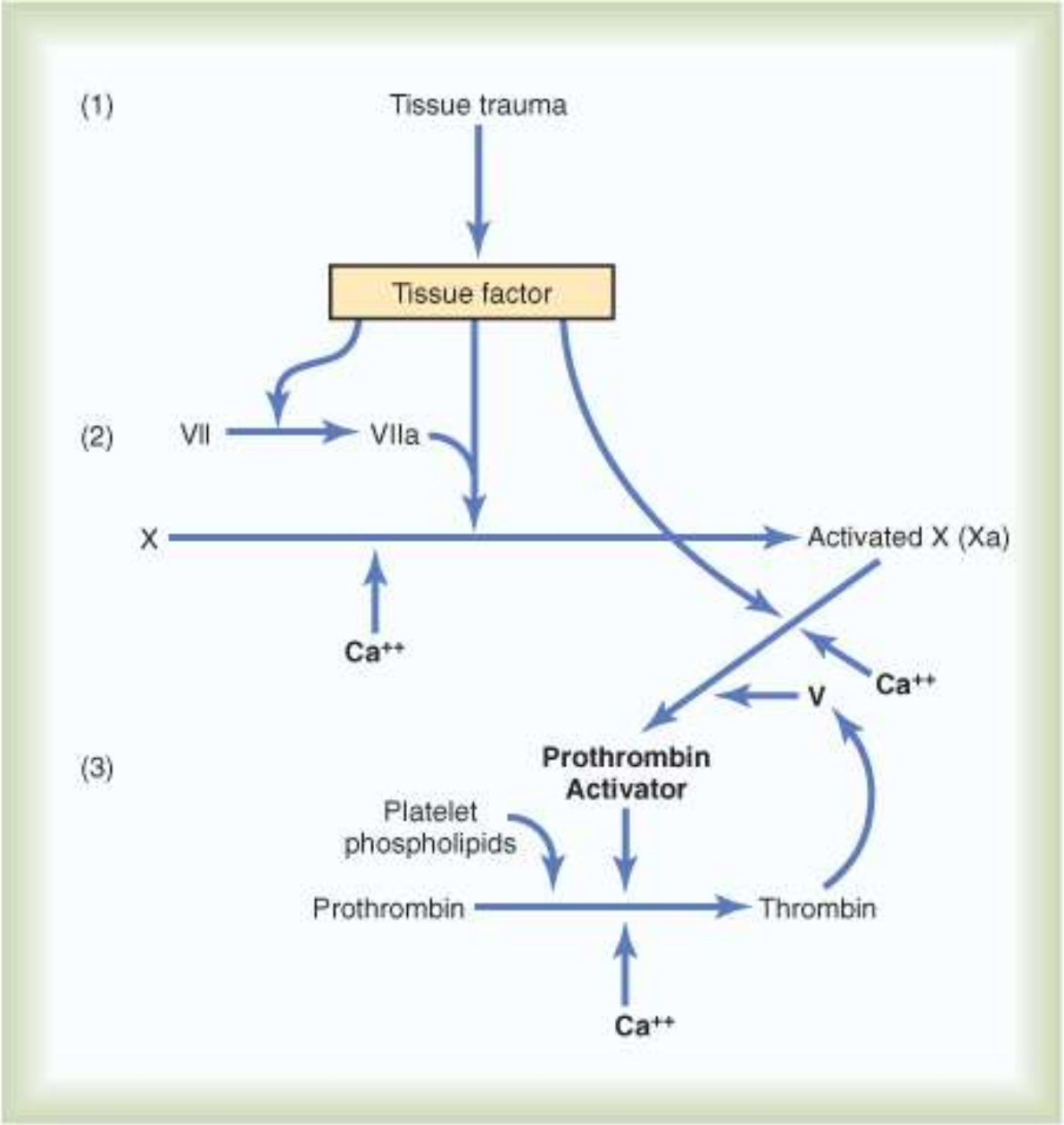
Features of clot formation

- Enzyme cascade theory (waterfall sequence)
- Most of the clotting factors are enzymes
- Inactive form (proenzyme) is converted into active enzyme which activates next proenzyme.
- Cascade: a process that occurs through a series of steps, each step initiating the next, until the final step is reached.

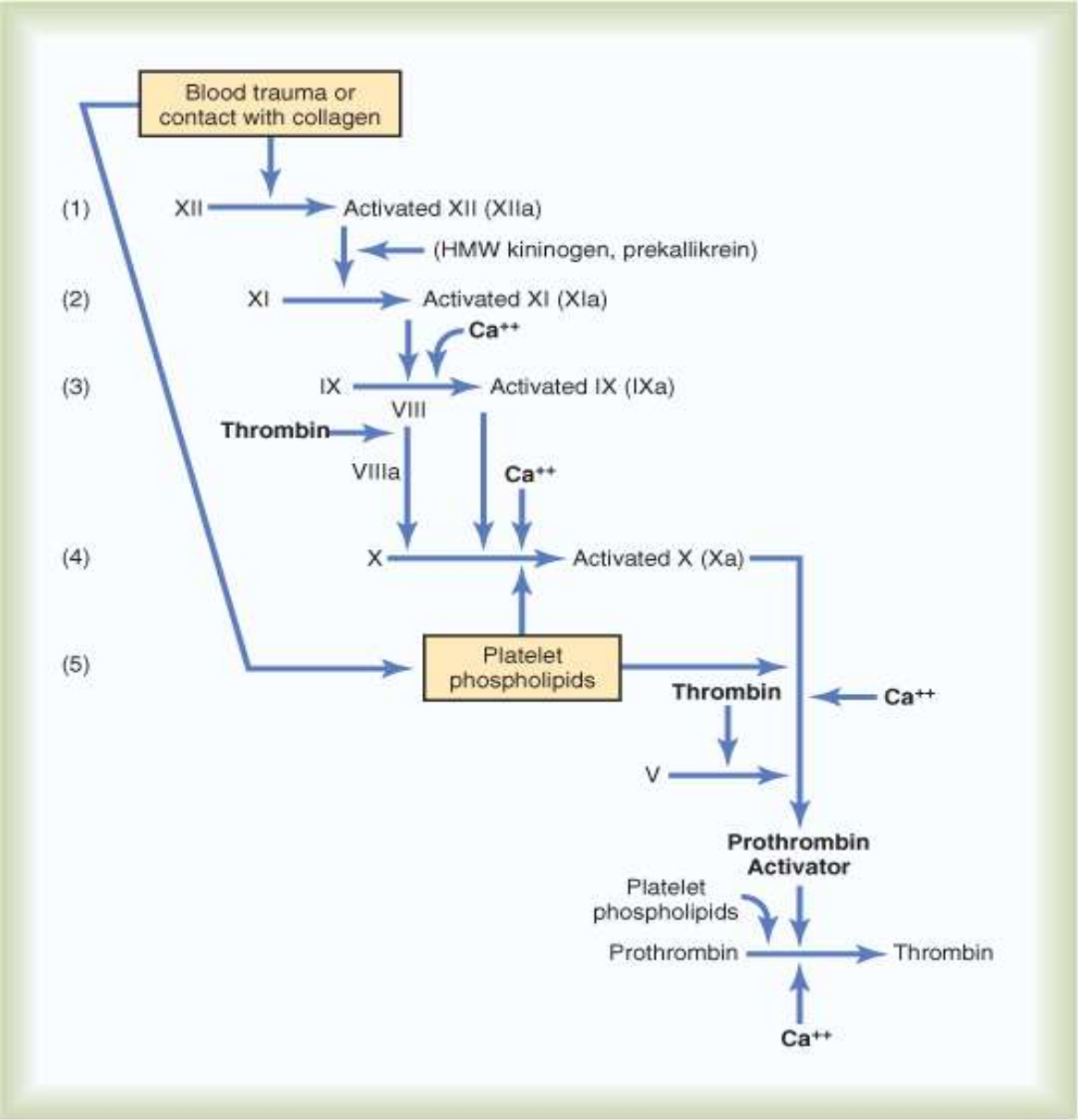
Principal steps of clot formation

- Formation of protrombin activator
  - By intrinsic mechanism
  - By extrinsic mechanism
- Conversion of prothrombin into thrombin
- Conversion of fibrinogen into fibrin

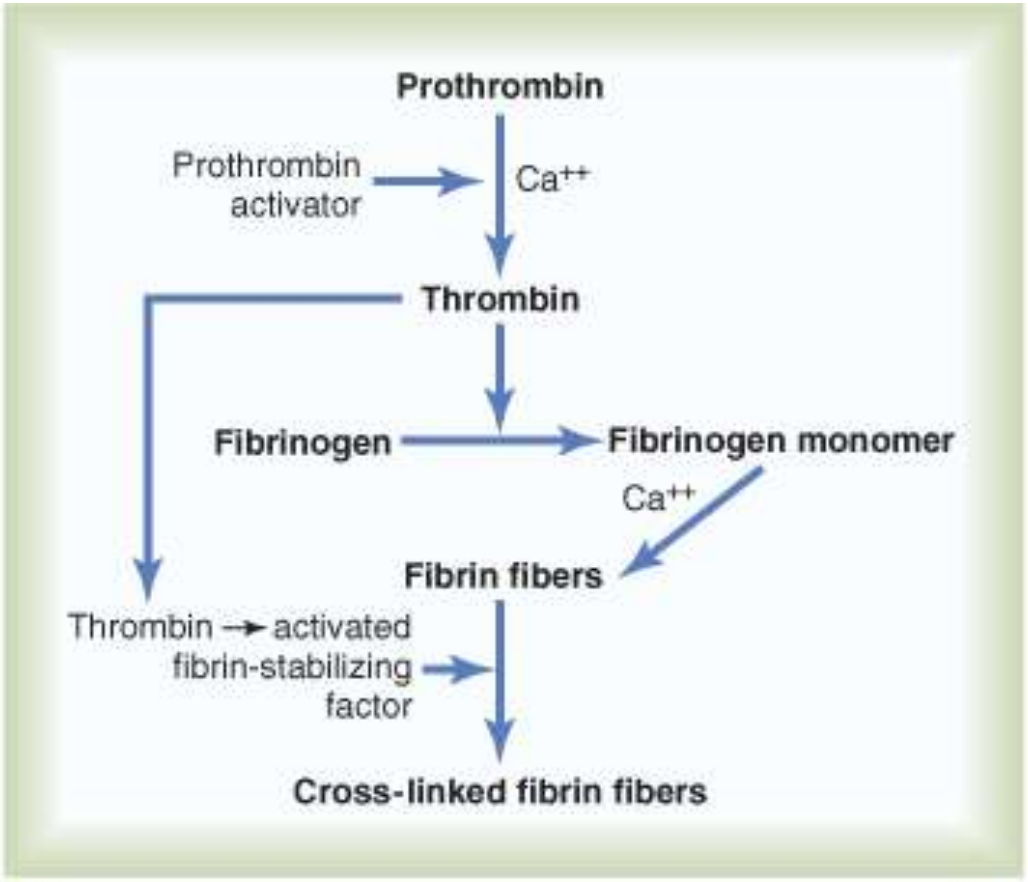
Extrinsic mechanism



Intrinsic mechanism



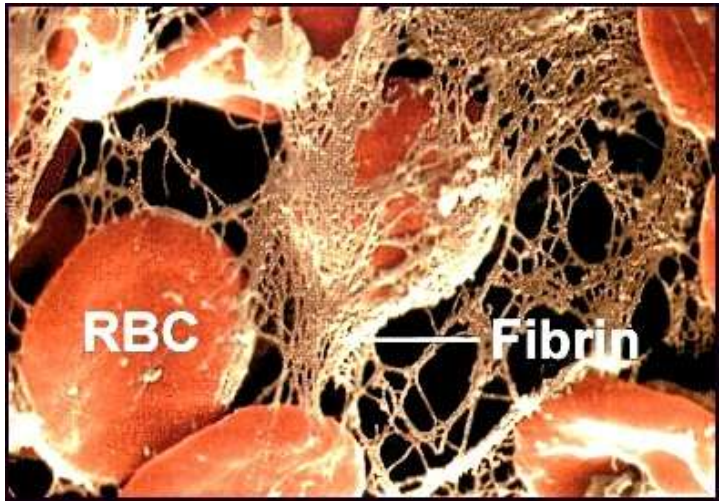
Steps of clot formation



Clot

- Structure
  - It's a meshwork of fibrin threads along with the entrapped blood cells.
- Clot retraction
- The process involving contraction of clot & oozing out of serum. Begins within 30 -60 min.
- Function of platelets – actin-myosin & thrombosthenin

- Fate of clot
  - Dissolution (fibrinolysis)
  - Fibrosis



Role of various factors in coagulation

- Calcium:
  - Required for almost all steps of coagulation. Removal of calcium prevents coagulation in vitro.
  - Ex. Oxalates & citrates used in blood banks.
- Vitamin K:
  - Necessary for synthesis of clotting factors II, VII, IX & X in liver.
  - Source of vit K – intestinal bacterial flora.
- Liver:
  - Synthesis of clotting factors, synthesis of anticoagulants, removal of activated procoagulants from blood.
  - Liver failure can cause bleeding disorders or uncontrolled clotting.
- Blood vessels:
  - Endothelium secretes von Willebrand's factor, tissue factor for extrinsic mechanism
  - Sub endothelial collagen initiates intrinsic mechanism.

Fibrinolysis

- Lysis of clot inside the blood vessel
- Requires plasmin or fibrinolysin

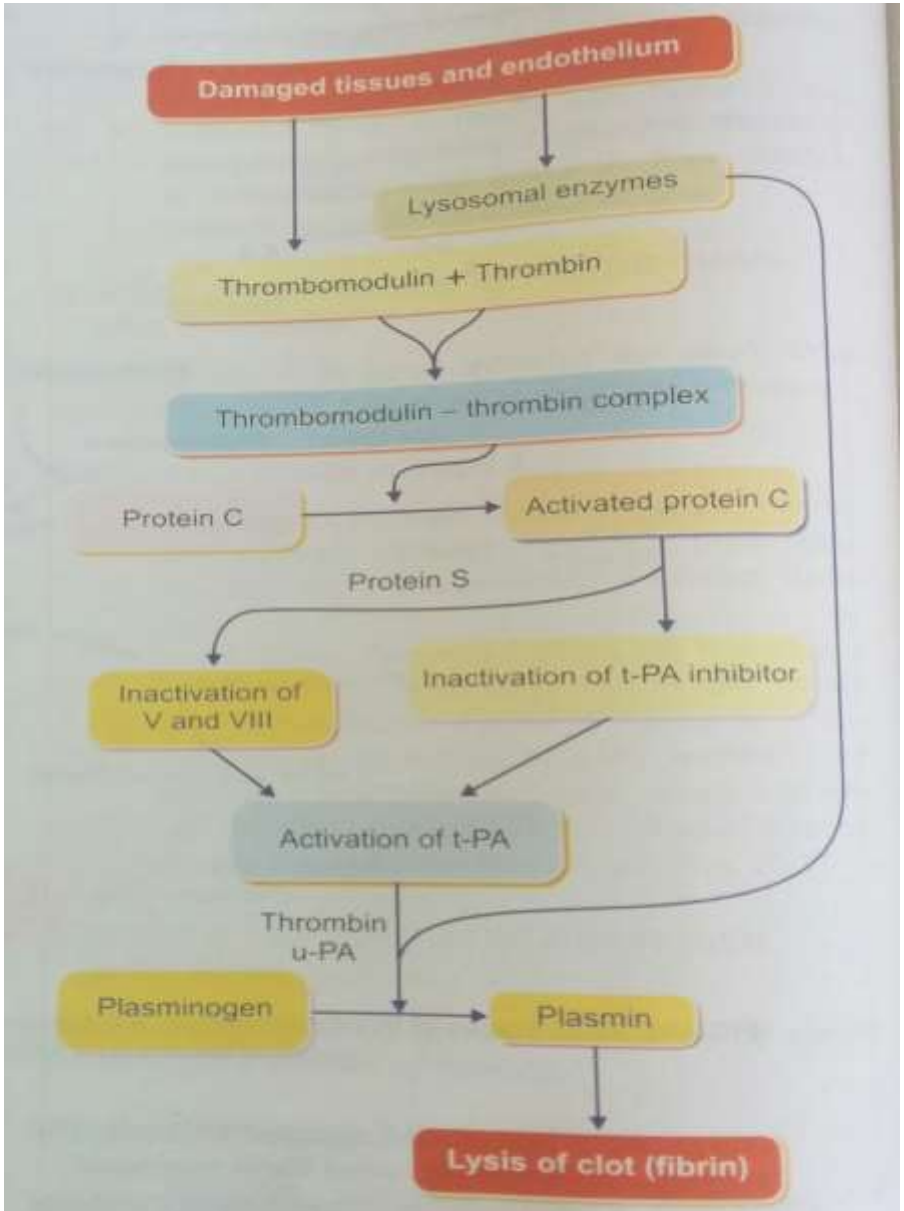


FIGURE 20.2: Fibrinolysis. t-PA = Tissue plasminogen activator, u-PA = Urokinase plasminogen activator.

Anti-clotting mechanisms of the body

- Physical factors
  - Continuous circulation of blood
  - Smooth endothelial lining

- Chemical factors
  - Natural anticoagulant heparin
  - Thrombomodulin by endothelial cells

Anticoagulants

- Heparin
  - Produced by mast cells & basophils
  - Mechanism of action: anti-thrombin activity
  - Uses:
  - In vivo IV injection during surgery, dialysis etc
  - In vitro for blood collection

- Coumarin derivatives: Warfarin & dicoumoral
  - Inhibit action of vit K, essential for formation of clotting factors like II, VII, IX & X.
  - Oral anticoagulants to prevent myocardial infarction, stroke, thrombosis etc.

Applied physiology

- Bleeding time & clotting time
- Bleeding disorders
- Thrombosis: intravascular blood clotting, may lead to myocardial infarction/ stroke
- Treatment of thrombosis: streptokinase/ urokinase activate fibrinolysis

Bleeding disorders

- Platelet disorders
  - BT increased, CT normal
  - Thrombocytopenic purpura
- Coagulation disorders
  - CT increased, BT normal
  - Deficiency of clotting factors e.g. haemophilia
  - Vitamin K deficiency
  - Anticoagulant overdose
  - Disseminated Intravascular Clotting
- Vascular disorders
  - Vascular purpura

Hemophilia

- Sex linked inherited coagulation disorder, males affected, females carrier
- Hemophilia A (83%): Deficiency of factor VIII
- Hemophilia B (15%): Deficiency of factor IX
- Hemophilia C (2%): Deficiency of factor XI
- Symptoms (bleeding tendencies) start early in life.
- CT increased, BT normal
- Treatment: transfusion of factor VIII