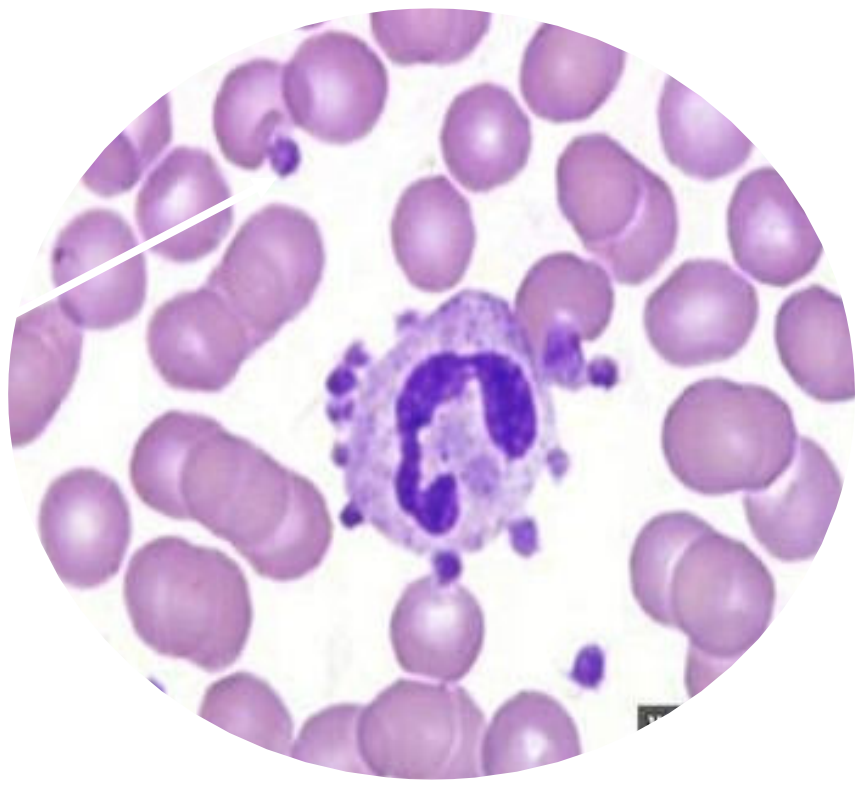
Blood cells – Platelets (Thrombocytes)



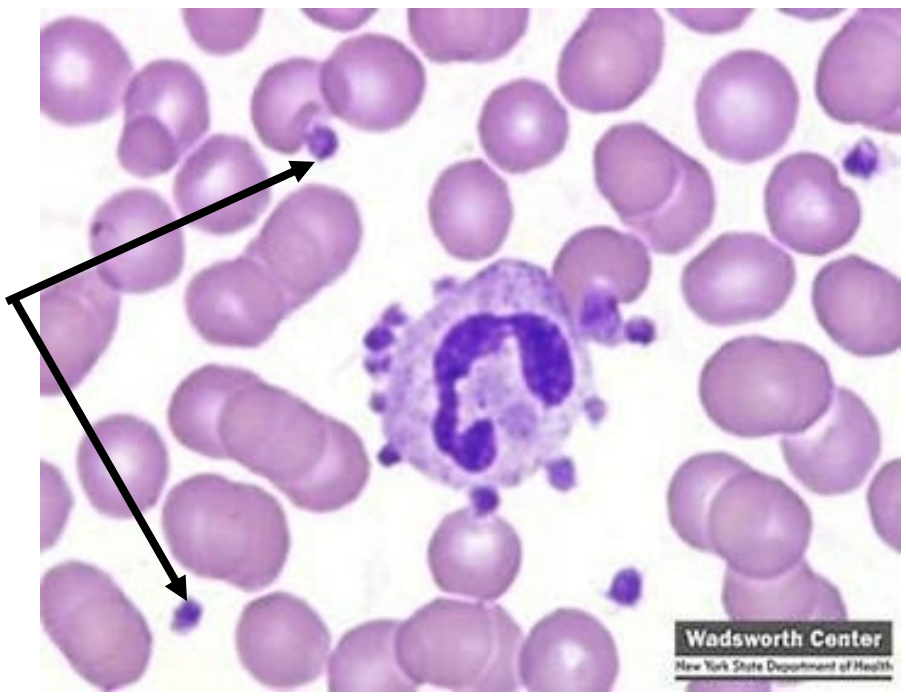
Learning Objectives

Platelets Morphology
Hemostasis
Primary hemostasis
Platelet plug formation
Disorders of primary hemostasis
Secondary hemostasis
Disorders of secondary hemostasis
Tests to assess hemostasis
Anticoagulants

Platelets-Characteristics

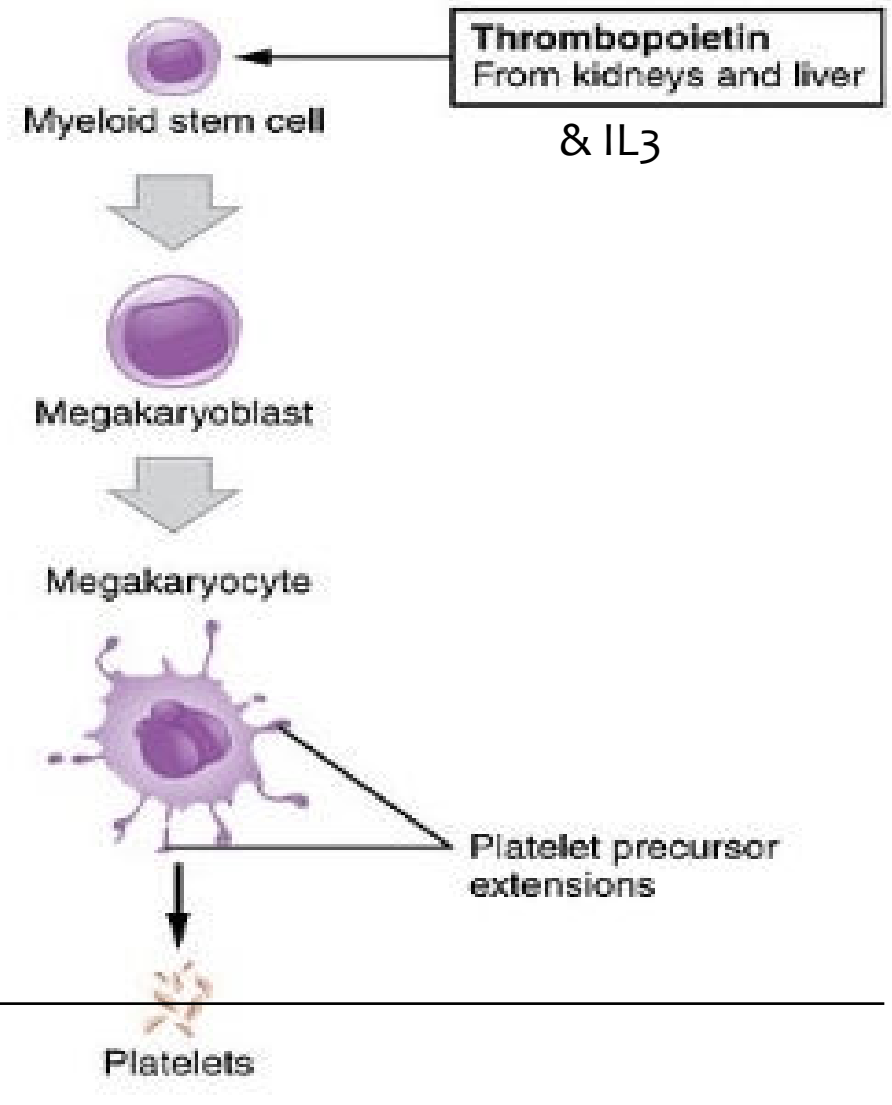
- Small 2-3 µm disc-shaped, anuclear, reddish-purple cells.
- Platelet life span 7-10 days
- Platelets count: 1.5-4 lakh/ mm^3 of blood

(Platelets are released from bone marrow to enter 2/3- peripheral blood and 1/3 seized in spleen.)



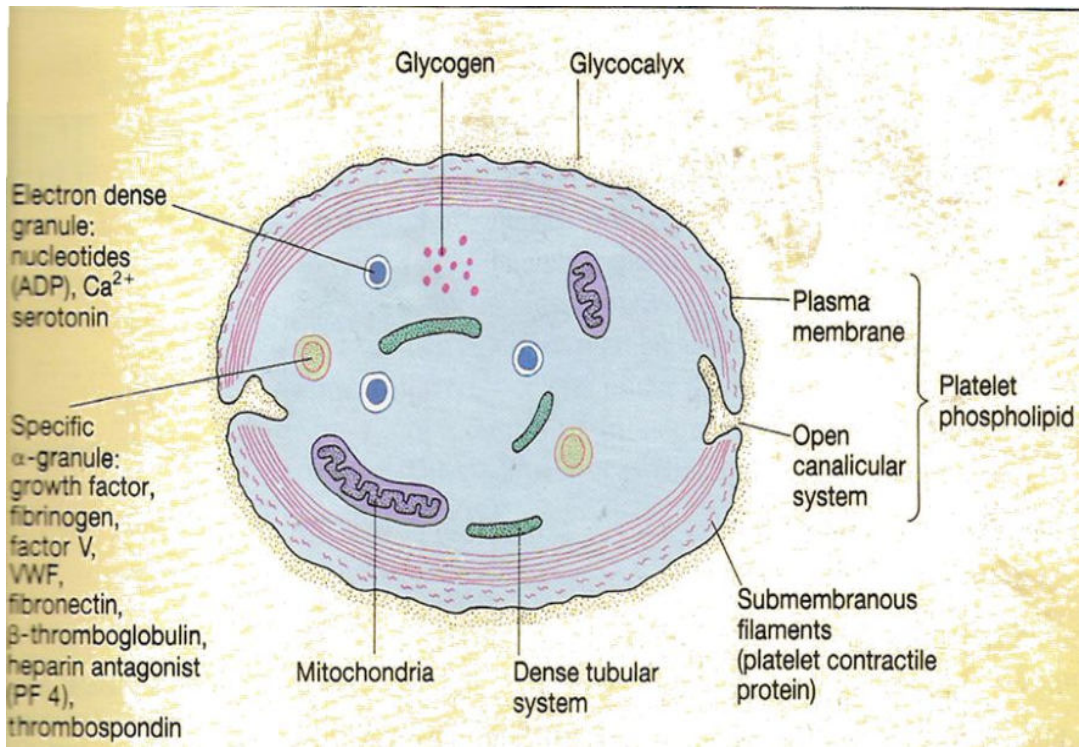
Platelets-Production

Platelets are formed within the cytoplasm of megakaryocytes (1 megakaryocyte produces about 2000 platelets)within bone marrow and released into the circulation.



Platelets structure

Peripheral zone:
Responsible for platelet adhesion and aggregation
Glycocalyx: Fluffy surface coat contains
glycoprotein receptors: GPIa & IIa binds to collagen,
GPIb binds von Willebrand's factor ; GPIIb/IIIa binds fibrinogen
Membrane Zone
Layer called PF3 (platelet factor) surface for interaction of plasma coagulation factors, Initiation of formation of thromboxane A_2
2 systems :Surface-connected open canalicular system (OCS)
Dense tubular system(DTS).
Cytoskeleton
Responsible for platelet retraction and platelet shape
Microtubules/Microfilaments
Binding proteins: Actin & Myosin
Organelle zone
Responsible for storage and platelet release functions
Granules:Dense bodies, alpha granules, lysosomal granules and microperoxisomes. Mitochondria and Glycogen.



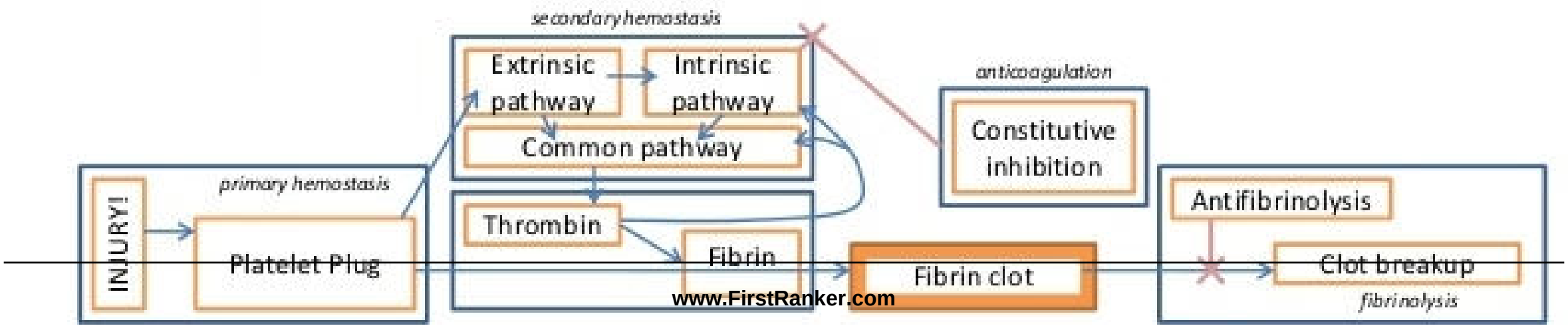
Hemostasis

Hemostasis is the natural process that stops blood loss when an injury occurs. It involves three steps that occur in a rapid sequence:

Primary Hemostasis: (1) vascular spasm, or vasoconstriction, a brief and intense contraction of blood vessels; (2) formation of a platelet plug; and

Secondary Hemostasis: blood clotting or coagulation, which reinforces the platelet plug with fibrin mesh that acts as a glue to hold the clot

fibrinolytic proteins system - Excessive blood clotting is prevented.

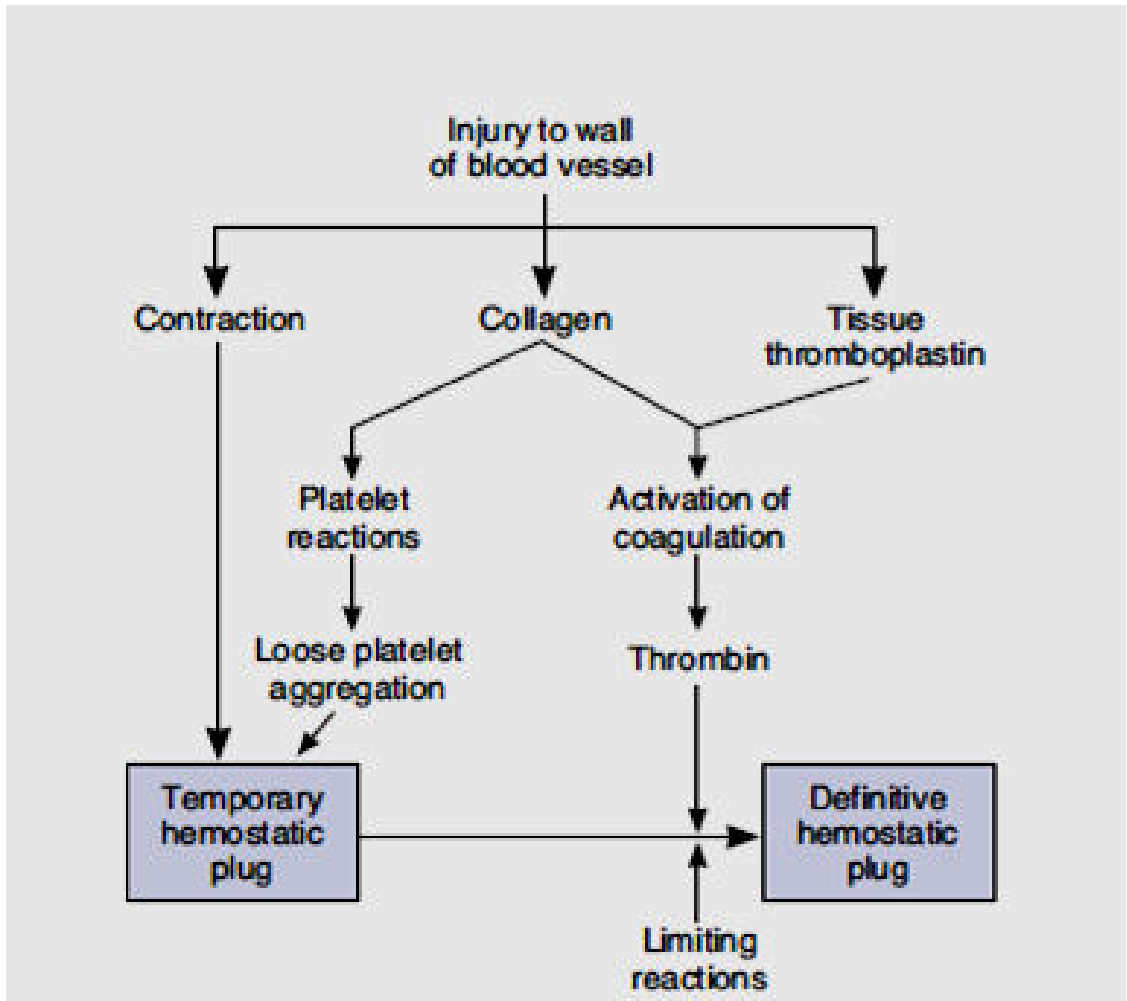


Primary Hemostasis - Vasoconstriction

Vasoconstriction

The vasoconstriction that occurs during hemostasis is a brief reflexive contraction that causes a decrease in blood flow to the area and restrict blood loss.

The vasoconstriction response is triggered by factors such as secretion of serotonin from activated platelets, a direct injury to vascular smooth muscle and nervous system reflexes initiated by local pain receptors.



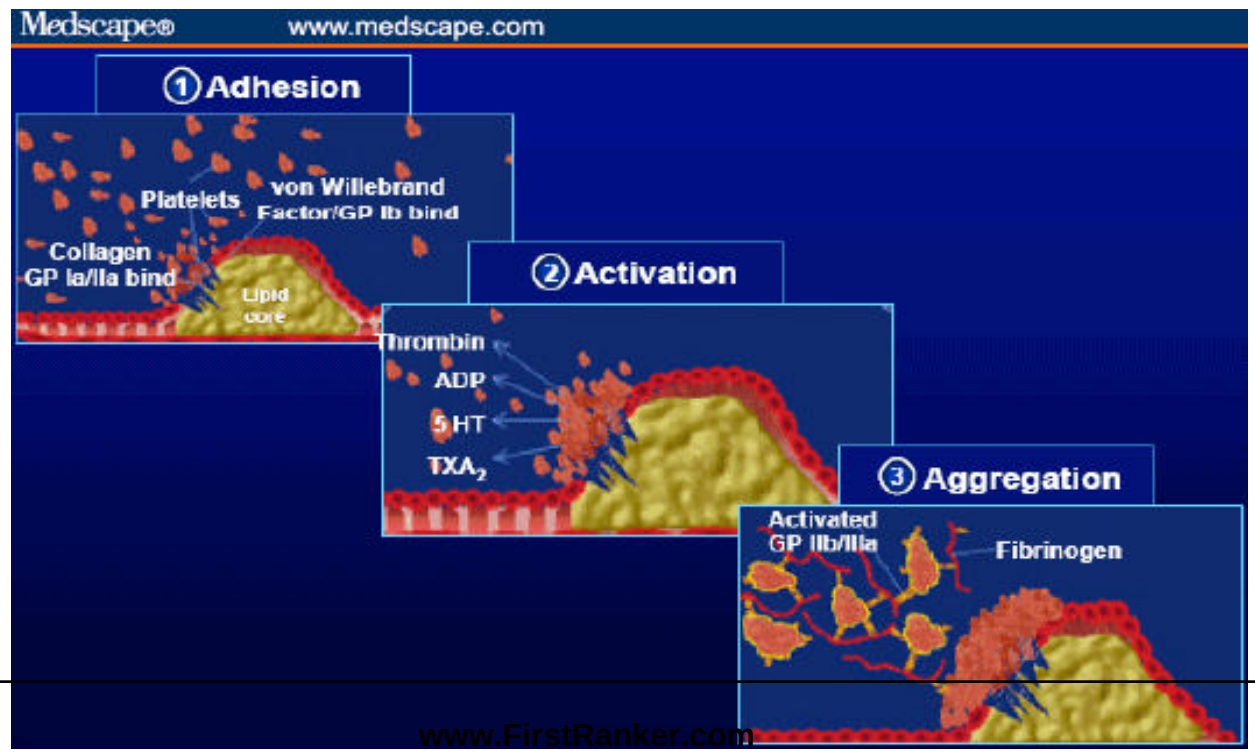
Platelets Function- Pri. Hemostasis - Platelets plug formation

Platelets plug formation

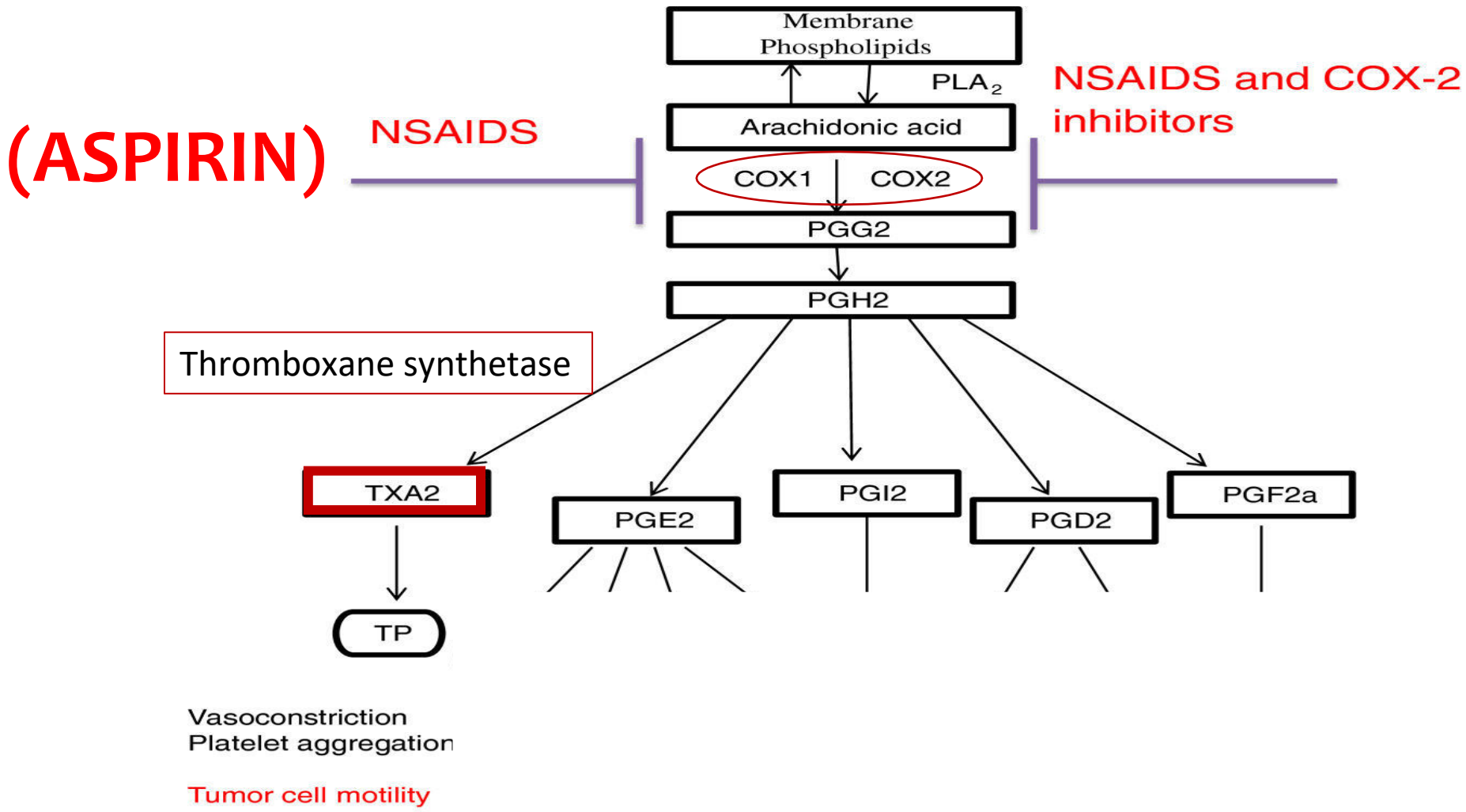
1) Adhesion – exposed collagen (GpIa & GpIIa and vWF-GpIb)

2) Activation –Initiation of formation of thromboxane A_2 and secretion of granule contents specially ADP & Ca++

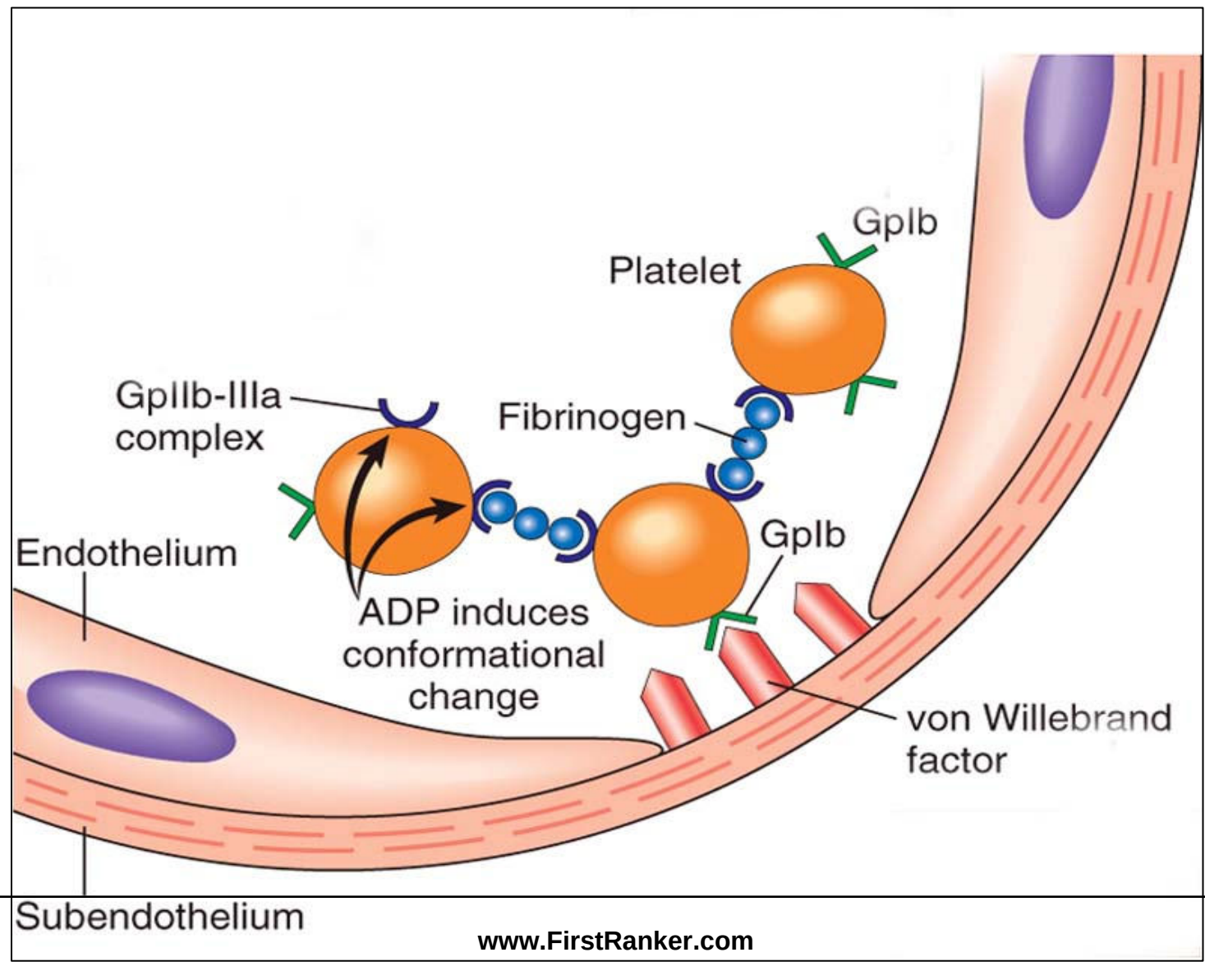
3) Aggregation - Expression of GpIb-IIIa receptors on platelets to glue together



Formation of thromboxane A_2 and Antiplatelet activity of Aspirin



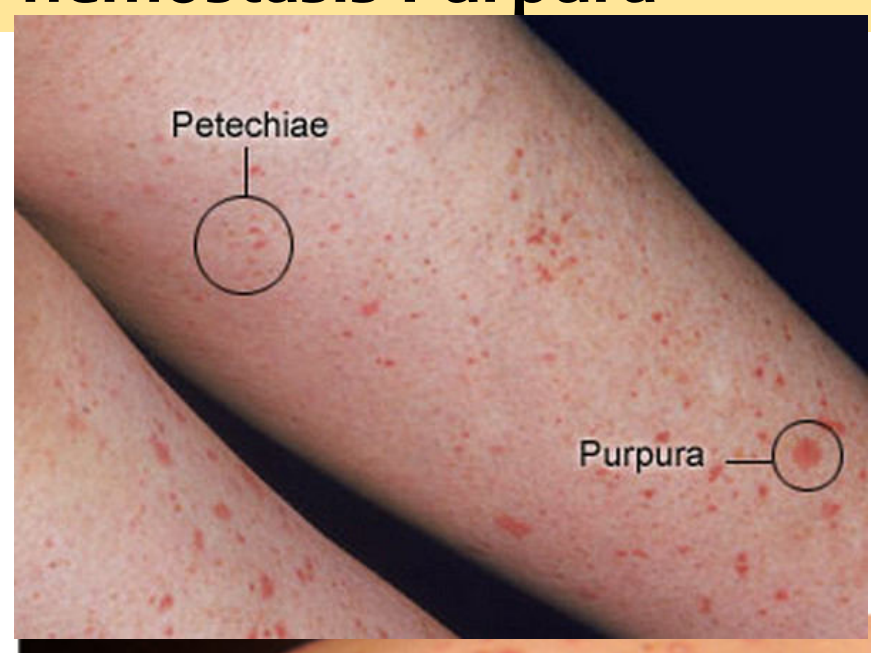
Platelets Function- Pri. Hemostasis - Platelets plug formation

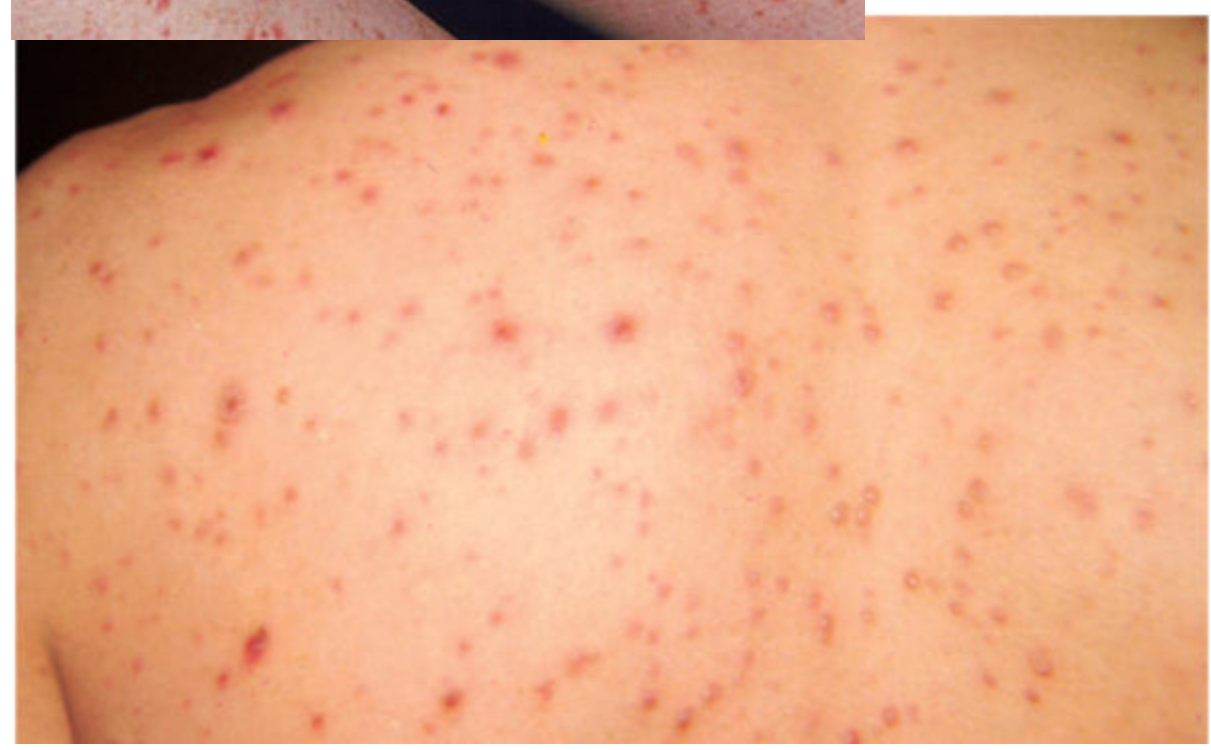


Abnormalities in primary hemostasis-Purpura

Purpura is purple-colored spots and patches that occur on the skin, and in mucus membranes, including the lining of the mouth. Purpura occurs when small blood vessels leak blood under the skin.

Purpura measure between 4 and 10 mm (millimeters) in diameter. When purpura spots are less than 4 mm in diameter, they are called **petechiae.** Purpura spots larger than 1 cm (centimeter) are called **ecchymoses.**





Abnormalities in primary hemostasis

Defects in the blood vessel wall- lead to hemorrhage from mucosal surfaces (epistaxis, gingival bleeding, melaena, haematuria), and into skin and mucosae (petechial or ecchymotic haemorrhages - **Purpura**).

Decreased platelets number (Thrombocytopenia) or function (Thrombosthenia)

Abnormal vWF- Bernard Soulier Syndrome

Primary haemostasis inhibitors - natural inhibitors of platelet function - such as prostacyclin, bradykinin released by endothelial cells.

Induced inhibition of platelet function - more commonly, platelet function is inhibited intentionally by the administration of agents such as **aspirin** for the prevention of thrombosis.

Causes of Thrombocytopenia

Bone Marrow Dysfunction –Many conditions and factors can damage stem cells
Cancer- leukemia or lymphoma. Cancer treatments, such as radiation and chemotherapy.
Toxic Chemicals- pesticides, arsenic, and benzene
Medicines-diuretics and chloramphenicol (aspirin or ibuprofen, also can affect platelets functioning)
Alcohol-especially if they're eating foods that are low in iron, vitamin B12, or folate.
Viruses-Chickenpox, mumps, rubella, Epstein-Barr virus, HIV and dengue fever virus etc often develop thrombocytopenia.
Genetic Conditions-Wiskott-Aldrich syndromes.

The Body Destroys Platelets at a higher rate
ITP (Idiopathic thrombocytopenic purpura)
Autoimmune Diseases-immune thrombocytopenia . An autoimmune response is thought to cause most cases of ITP eg SLE and RA
Margination in congestive splenomegaly
Medicines-quinine; antibiotics that contain sulfa; dilantin, vancomycin, and rifampin.
Infection-a widespread bacterial infection septicemia, mononucleosis or cytomegalovirus
Surgery-Prosthetic heart valves, blood vessel grafts, or machines and tubing used - blood transfusions or bypass surgery.
Pregnancy-About 5 percent of pregnant women develop mild thrombocytopenia when they're close to delivery.

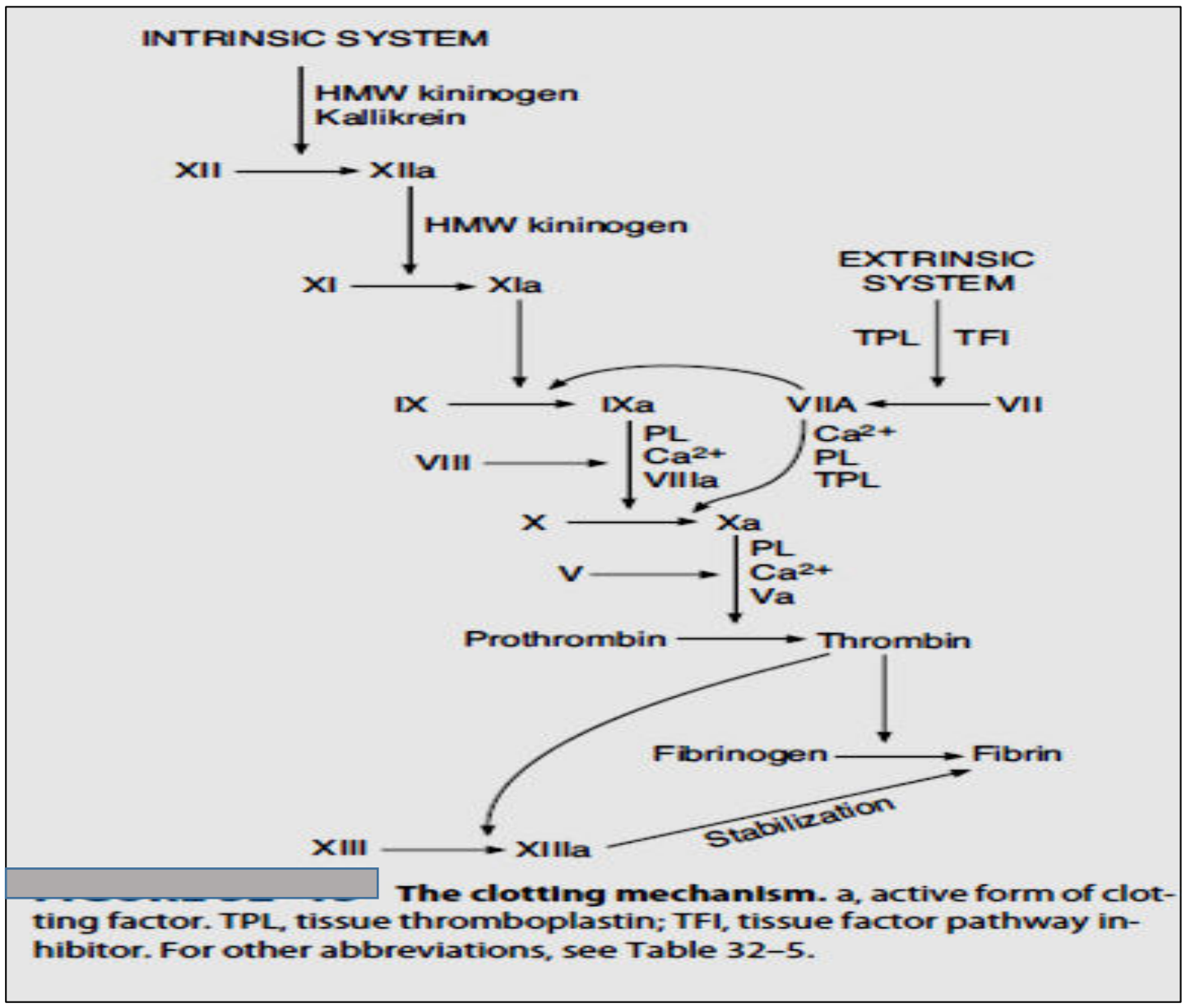
Process of secondary hemostasis-Blood Coagulation-Clotting Factors

Table 17.3 Blood Clotting Factors (Procoagulants)

FACTOR NUMBER	FACTOR NAME	NATURE	SOURCE	PATHWAY; FUNCTION
I	Fibrinogen	Plasma protein	Liver	Common pathway; converted to fibrin (insoluble weblike substance of clot)
II	Prothrombin	Plasma protein	Liver*	Common pathway; converted to thrombin (converts fibrinogen to fibrin)
III	Tissue factor (TF)	Plasma membrane glycoprotein	Tissue cells	Activates extrinsic pathway
IV	Calcium ions (Ca^{2+})	Inorganic ion	Plasma	Needed for essentially all stages of coagulation process; always present
V	Proaccelerin	Plasma protein	Liver, platelets	Common pathway
VI†				
VII	Proconvertin	Plasma protein	Liver*	Both extrinsic and intrinsic pathways
VIII	Antihemophilic factor (AHF)	Plasma protein	Liver, lung capillaries	Intrinsic pathway; deficiency results in hemophilia A
IX	Plasma thromboplastin component (PTC)	Plasma protein	Liver*	Intrinsic pathway; deficiency results in hemophilia B
X	Stuart factor	Plasma protein	Liver*	Common pathway
XI	Plasma thromboplastin antecedent (PTA)	Plasma protein	Liver	Intrinsic pathway; deficiency results in hemophilia C
XII	Hageman factor	Plasma protein; activated by negatively charged surfaces (e.g., glass)	Liver	Intrinsic pathway; activates plasmin; initiates clotting in vitro; activation initiates inflammation
XIII	Fibrin stabilizing factor (FSF)	Plasma protein	Liver, bone marrow	Cross-links fibrin, forming a strong, stable clot

*Synthesis requires vitamin K
†Number no longer used; substance now believed to be same as factor V

Process of secondary hemostasis-Blood Coagulation



The clotting mechanism. a, active form of clotting factor. TPL, tissue thromboplastin; TFI, tissue factor pathway inhibitor. For other abbreviations, see Table 32–5.

Disorders of Blood Coagulation- Coagulopathy

Coagulopathy is a condition in that the blood's ability to coagulate is impaired. This condition can cause a tendency toward prolonged or excessive bleeding (bleeding diathesis), which may occur spontaneously or following an injury

Disorders of Blood Coagulation- Hypocoagulopathy -CAUSES

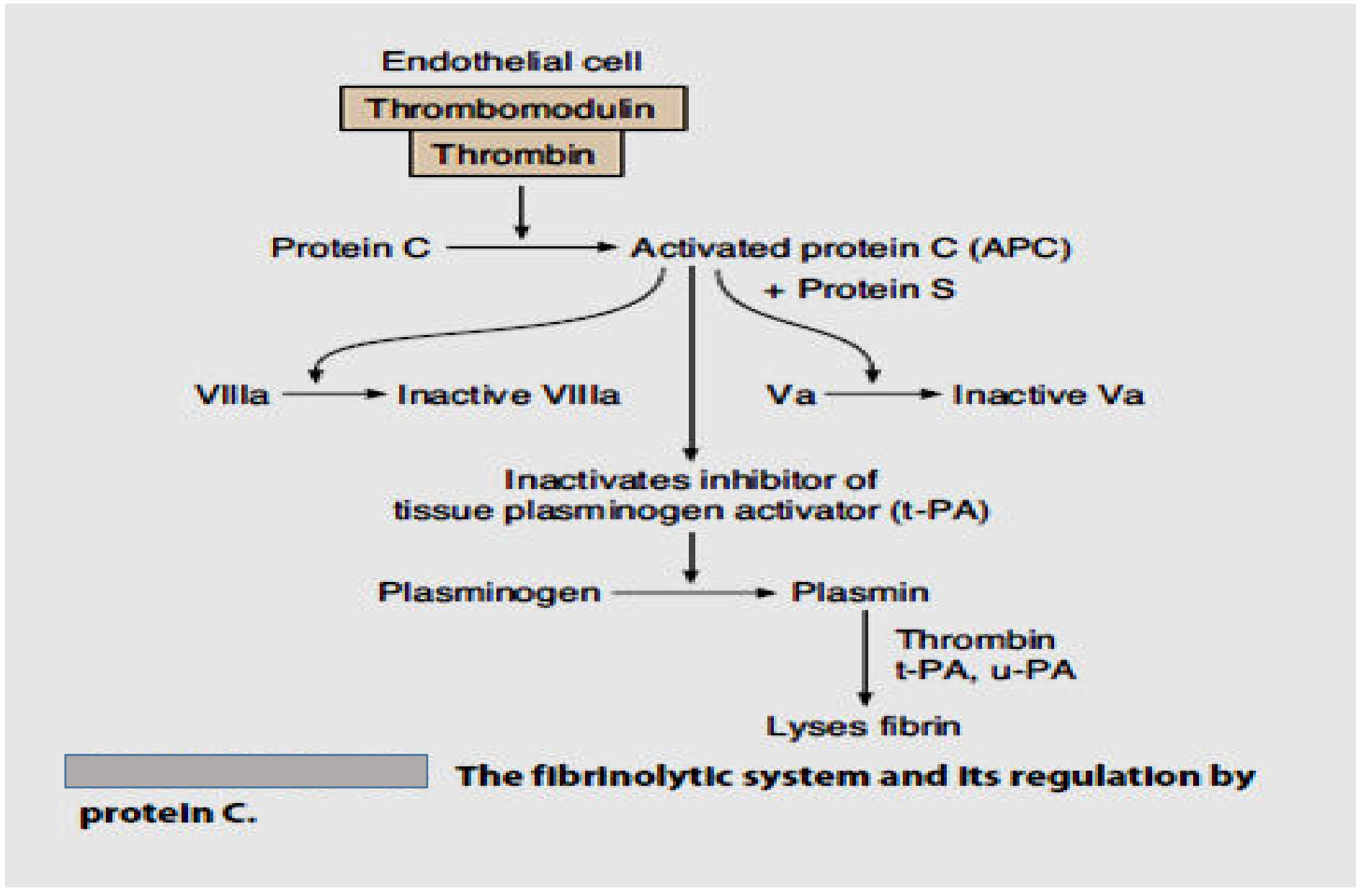
Major causes of coagulation disorders resulting in bleeding include:

- Hemophilia-Factor VIII deficiency (X chromosome linked disorder)
- Von Willebrand disease
- Other clotting factor deficiencies (Christmas disease)
- Disseminated intravascular coagulation
- Liver Disease
- Vit K deficiency

Disorders of Blood Coagulation- Hyper-coagulopathy - CAUSES

- **Conditions increasing the risk of a blood clot:**
- Inherited (genetic) abnormalities that cause an increased tendency to clot
- A surgical procedure
- Sitting or lying down for long periods (more than 4 hours)- for example, after surgery or on long plane or car rides. This lack of movement reduces blood flow in the legs by 50%.
- Major injury
- Increasing age
- Cancer
- Heart failure
- Pregnancy
- Use of hormone therapy such as birth control pills or hormone replacement therapy
- Having a history of DVT
- Tobacco

Fibrinolytic System



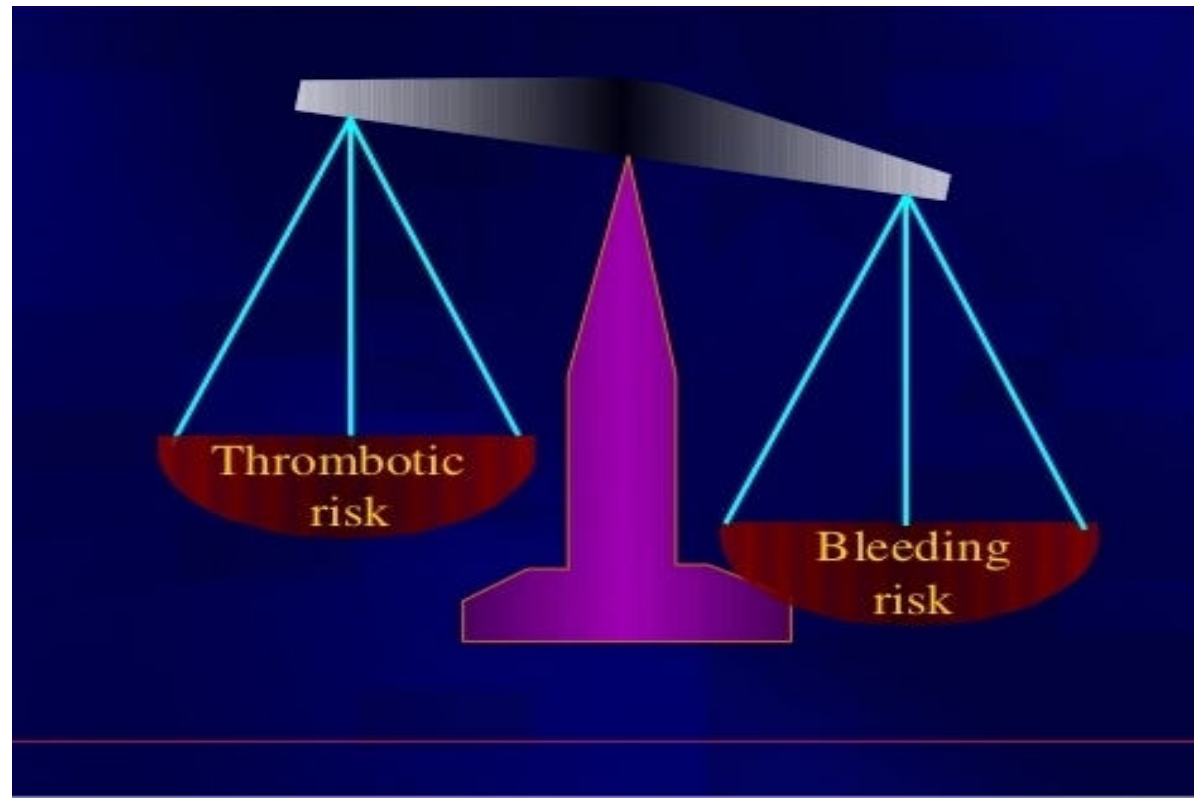
The fibrinolytic system and its regulation by protein C.

Process of Thrombosis & Bleeding (fibrinolysis) should be balanced

Clotting factor deficiencies may cause the development of a significant bleeding tendency.

Formation of clots inside blood vessels is called **thrombosis due to hyper coagulability of blood** Thromboses are a major medical problem.

They frequently occlude the arterial supply to the organs in which they form, and bits of thrombus **(emboli)** sometimes break off and travel in the bloodstream to distant sites, damaging other organs. An example is obstruction of the pulmonary artery or its branches by thrombi from the leg veins **(pulmonary embolism).**



Tests of Hemostasis-Laboratory Investigations

Capillary Fragility Test • The test is defined by the WHO as one of the necessary requisites for diagnosis of Dengue fever. • A blood pressure cuff is applied and inflated to a point between the systolic and diastolic blood pressures for five minutes. • The test is positive if there are 10 or more petechiae per square inch. • In DHF the test usually gives a definite positive result with 20 petechiae or more. Indicative of hemorrhagic tendency of a person

Platelet Count • Platelet Count can be determined by improved Neubauer's counting chamber with RBC pipette & 1% ammonium oxalate. • They can be seen as tiny diameter, well seprated, highly refractile rounded bodies with silvery appearance. • N : 1.5 – 4 lacs / cumm. • Leishman stain : in clumps, blue cytoplasm, reddish purple granules, no nucleus. • Surgical bleeding usually does not occur until the platelet count is less than 50,000/mm3. • Spontaneous bleeding does not occur until the platelet count is less than 10,000-20,000/mm3. ↓Platelet Count • THOMBOCYTOPENIA : • Bone marrow depression – Aplastic anemia • Hypersplenism • Viral infections • Drugs : Aspirin, Heparin, Chemotherapy • ITP • TTP • DIC

Bleeding Time • It is the time taken from the puncture of the blood vessel to the stoppage of bleeding. N : 1-6 minutes
• The bleeding time test is a useful tool to test for platelet plug formation and capillary integrity.
• BT is more imp. than CT.
• CT concerns the blood only i.e. how firm the clot is formed, whereas BT involves the interaction of blood with injured tissues.
• People with von Willebrand disease usually experience increased bleeding time. • Von Willebrand factor is a platelet agglutination protein, but this is not considered an effective diagnostic test for this condition

Tests of Hemostasis-Laboratory Investigations

Clotting Time It is the time taken from the puncture of the blood vessel to the formation of a fibrin thread. • A. Capillary Glass Tube Method : Here the blood is collected in capillary tube & total time is noted to form FIBRIN THREADS on breaking tube every 30 seconds. N : 3-8 minutes • Mechanism Involved is INTRINSIC Pathway. • CT depends on presence of all clotting factors. • It gets prolonged in : - 1. Deficiency of clotting factors – Hemophilia. - 2. Vitamin K Deficiency – Factor II, VII, IX & X. - 3. Anticoagulant overdose. • BT & CT is measured before surgery & liver or bone marrow biopsy. • PURPURA : BT increased, CT normal. • HEMOPHILIA : BT normal, CT increased.

Prothombin Time • The prothrombin time (PT) and its derived measures of prothrombin ratio (PR) and international normalised ratio (INR) are measures of the extrinsic pathway of coagulation. • They are used to determine the clotting tendency of blood, in the measure of warfarin dosage, liver damage and vitamin K status. • PT measures factors I, II, V, VII and X. • The reference range for prothrombin time is usually around 11-16 seconds; the normal range for the INR is 0.8–1.2.

APTT • The partial thromboplastin time (PTT) or activated partial thromboplastin time (aPTT or APTT) is a performance indicator measuring the efficacy of both the intrinsic and the common coagulation pathways. • Apart from detecting abnormalities in blood clotting, it is also used to monitor the treatment effects with heparin, a major anticoagulant. • Normal PTT times require the presence of the following coagulation factors: I, II, V, VIII, IX, X, XI & XII. • Deficiencies in factors VII or XIII will not be detected with the PTT test.

ANTICOAGULANTS

●Heparin is a naturally occurring anticoagulant
that facilitates the action of anti-thrombin III. Low-molecular weight fragments with an average molecular weight of 5000 have been produced from unfractionated heparin, and these low-molecular-weight heparins are seeing increased clinical use because they have a longer half-life and produce a more predictable anticoagulant response than unfractionated heparin.

● In vivo, a plasma Ca^{2+} level low enough to interfere with blood clotting is incompatible with life, but clotting can be prevented in vitro if Ca^{2+} is removed from the blood by the addition of substances such as oxalates, which form insoluble salts with Ca^{2+}, or **chelating agents,** which bind Ca^{2+}. Used in vitro only.

● Coumarin derivatives such as **dicumarol** and **warfarin** are also effective anticoagulants. They inhibit the action of vitamin K, which is a necessary cofactor for the enzyme that catalyzes the conversion of glutamic acid residues to γ-carboxyglutamic acid residues. Six of the proteins involved in clotting require conversion of a number of glutamic acid residues to γ-carboxyglutamic acid residues before being released into the circulation, and hence all six are vitamin K-dependent. These proteins are factors II (prothrombin),VII, IX, and X, protein C, and protein S.

Self Assessment

- Megakaryocyte produces about ……..platelets.
- Platelet life span …….. Days.
- ……receptors causes gluing of platelets together.
- Spontaneous bleeding does not occur until the platelet count is less than ……../mm3.
- Surgical bleeding does not occur until the platelet count is less than ……../mm3.
- Secondary Hemostasis includes ……processes.
- Liver Disease results in hypocoagulability of blood because……

Thank you