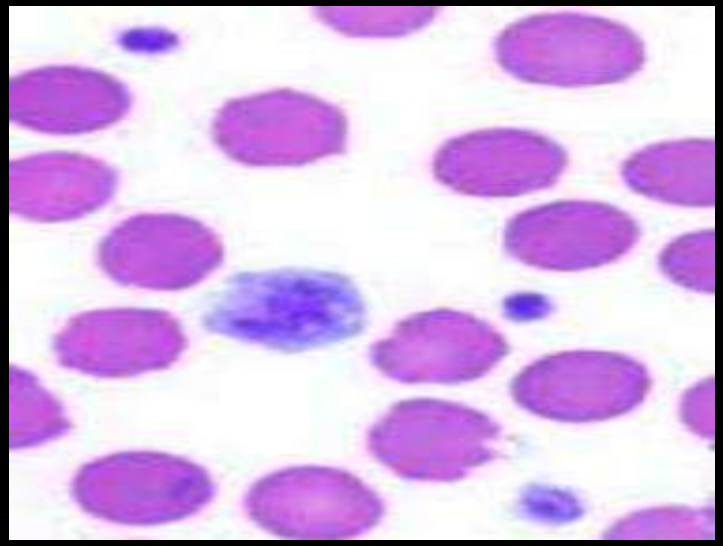
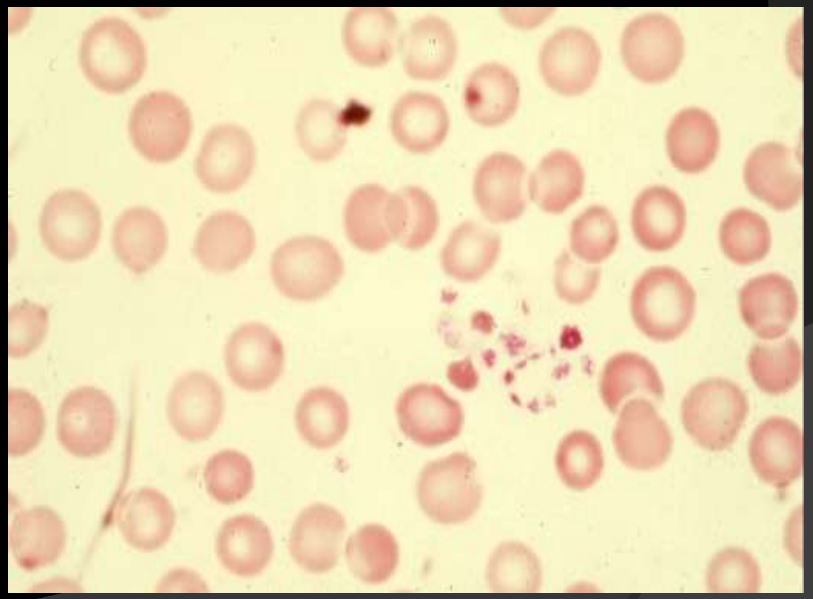
PLATELETS (THROMBOCYTES)





Haemostasis

The function of haemostasis is:

- **to prevent blood loss from severed vessels**
- **to stop bleeding**
- **to prevent thrombosis**

Haemostatic functional modules

Conveniently, it is divided into three components:

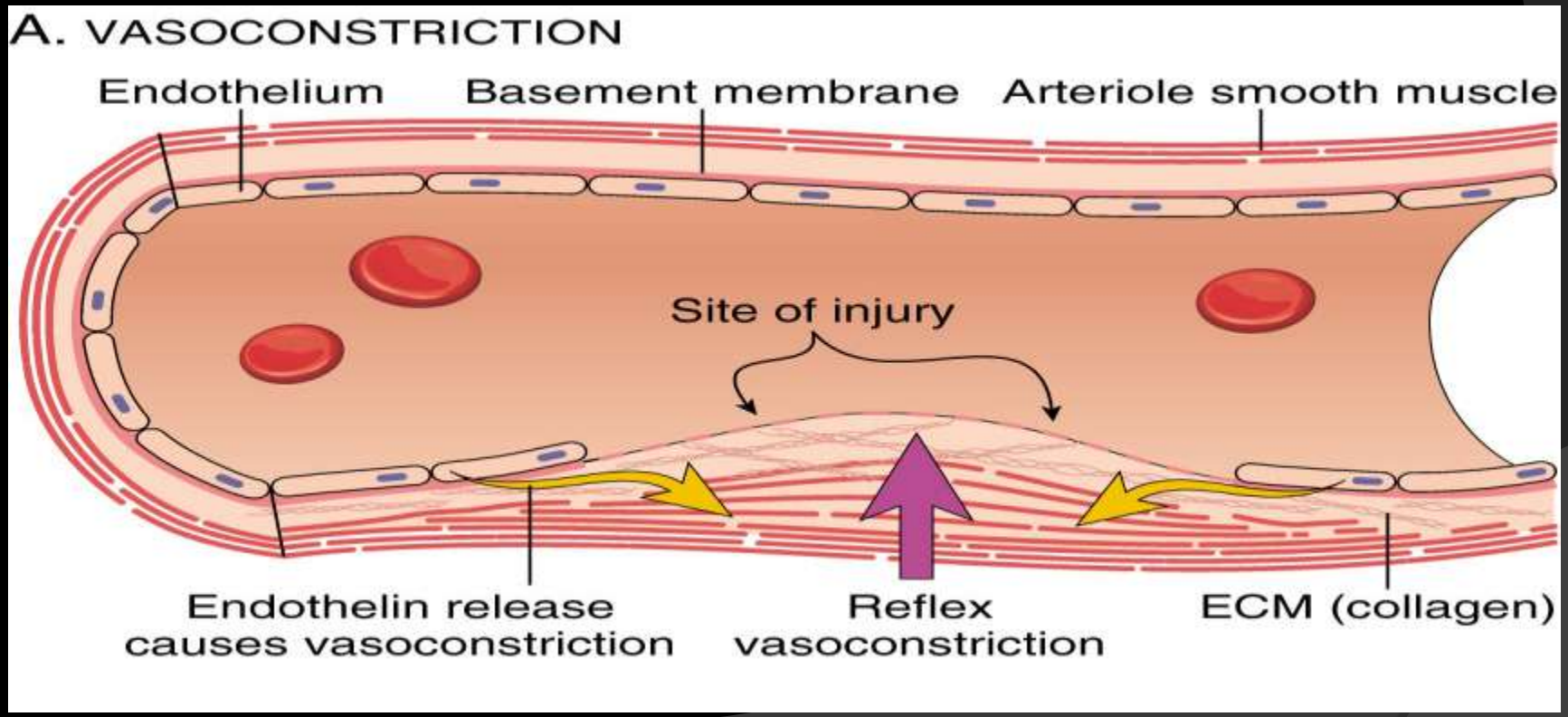
- primary haemostasis
- Coagulation
- fibrinolysis

Components of haemostasis

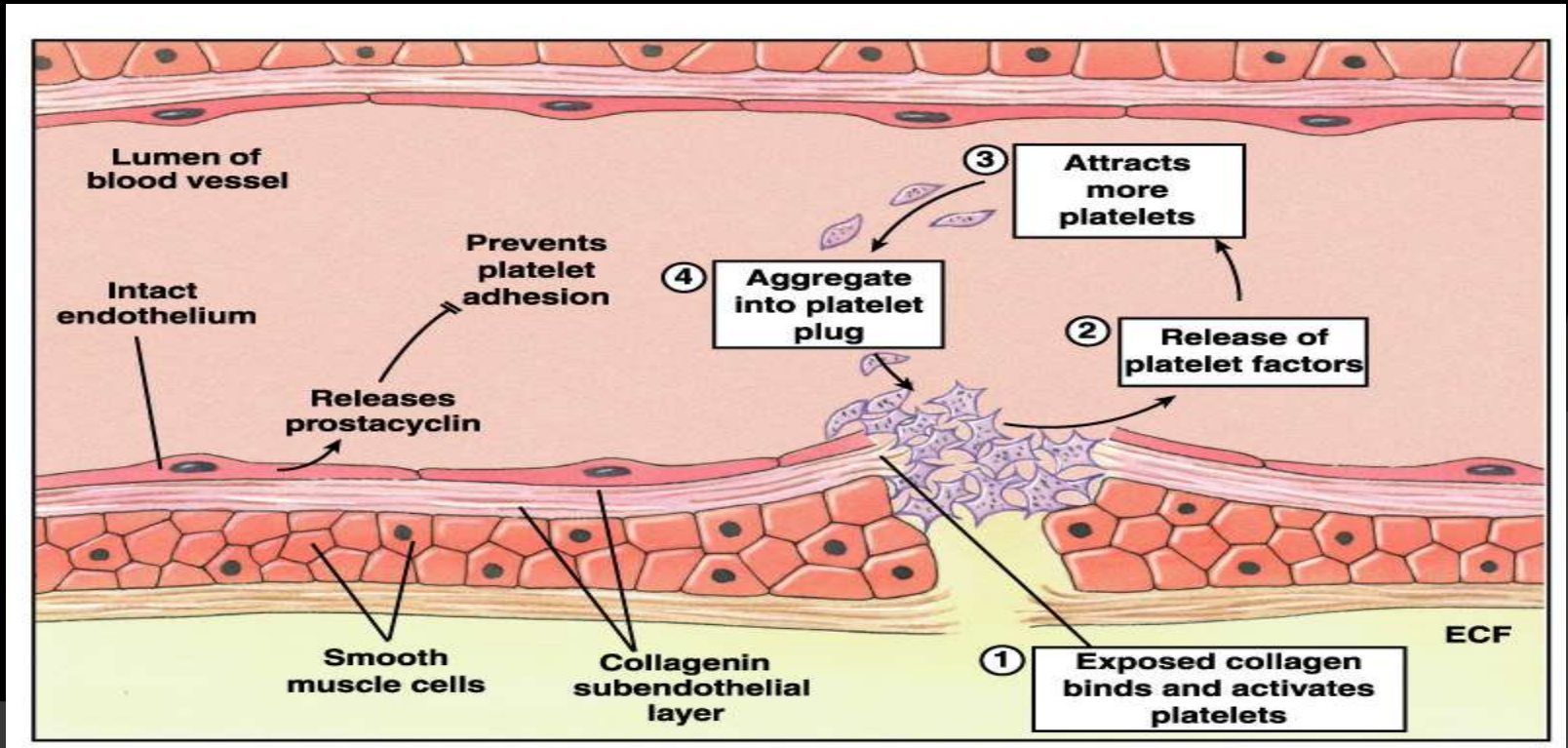
- vasculature
- blood platelets
- proteins of coagulation / fibrinolysis pathways

Steps in haemostasis:

1. Vasoconstriction due to local neural response, and release of endothelin from the endothelium.

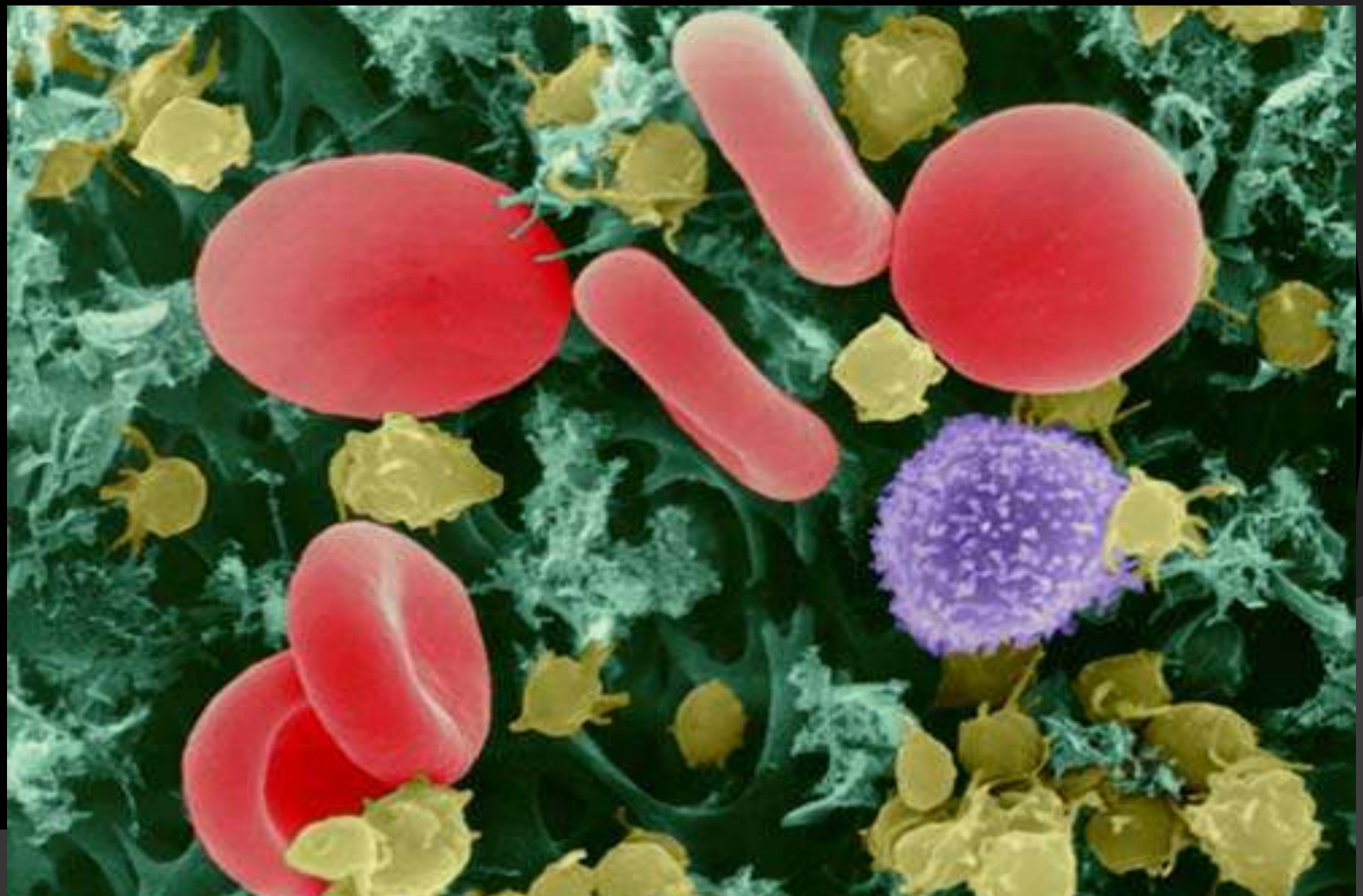


- 2.Primary haemostatic plug: due to platelet adhesion, activation, degranulation (ADP, TXA2) and recruitment of other platelets. **in damaged vessel wall endothelium may be lost and the subendothelium becomes exposed to platelets and coagulation factors**



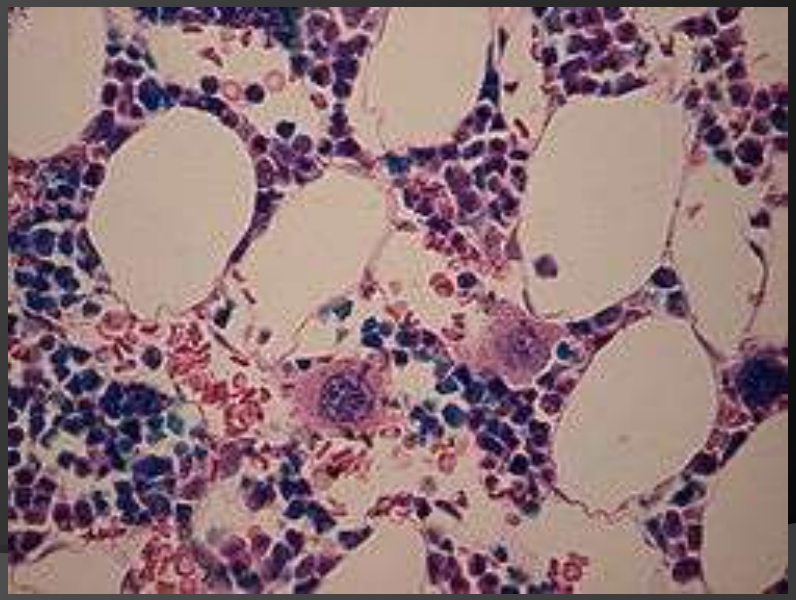
3. Secondary hemostasis due to activation of coagulation cascade by tissue factor and phospholipid via extrinsic pathway- the end result being fibrin which traps the cells in the blood forming a clot.

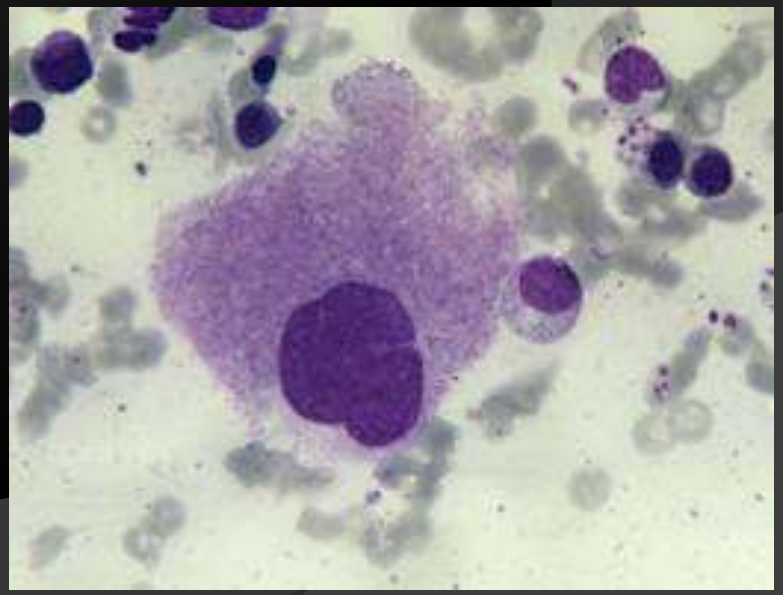
Blood platelets

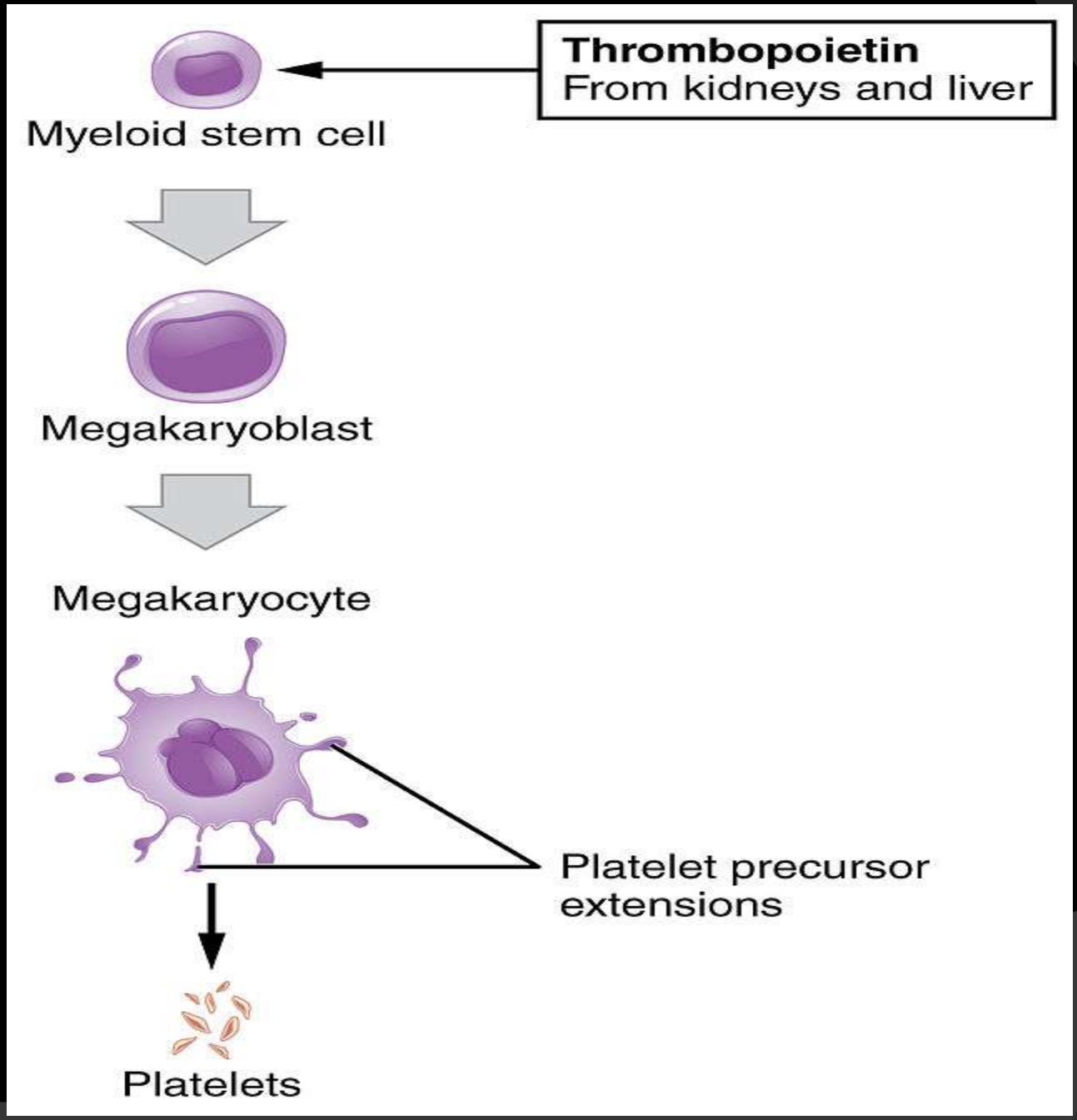


PLATELETS (THROMBOCYTES)

- ✓Cytoplasmic fragments of bone marrow megakaryocytes.
- ✓lack a nucleus, round , oval or spindle-shaped ,may have multiple cytoplasmic projections.

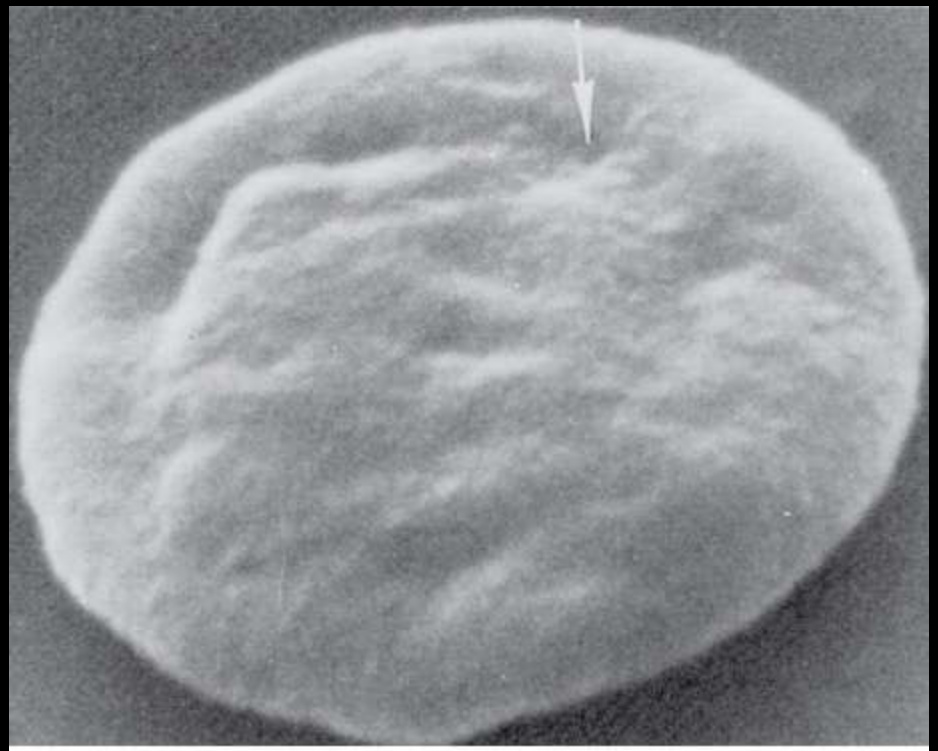





Thrombopoietin
From kidneys and liver
Myeloid stem cell
Megakaryoblast
Megakaryocyte
Platelet precursor extensions
Platelets

Activated platelets become "sticky" and form pseudopodia

Discoid resting inctivate platelet



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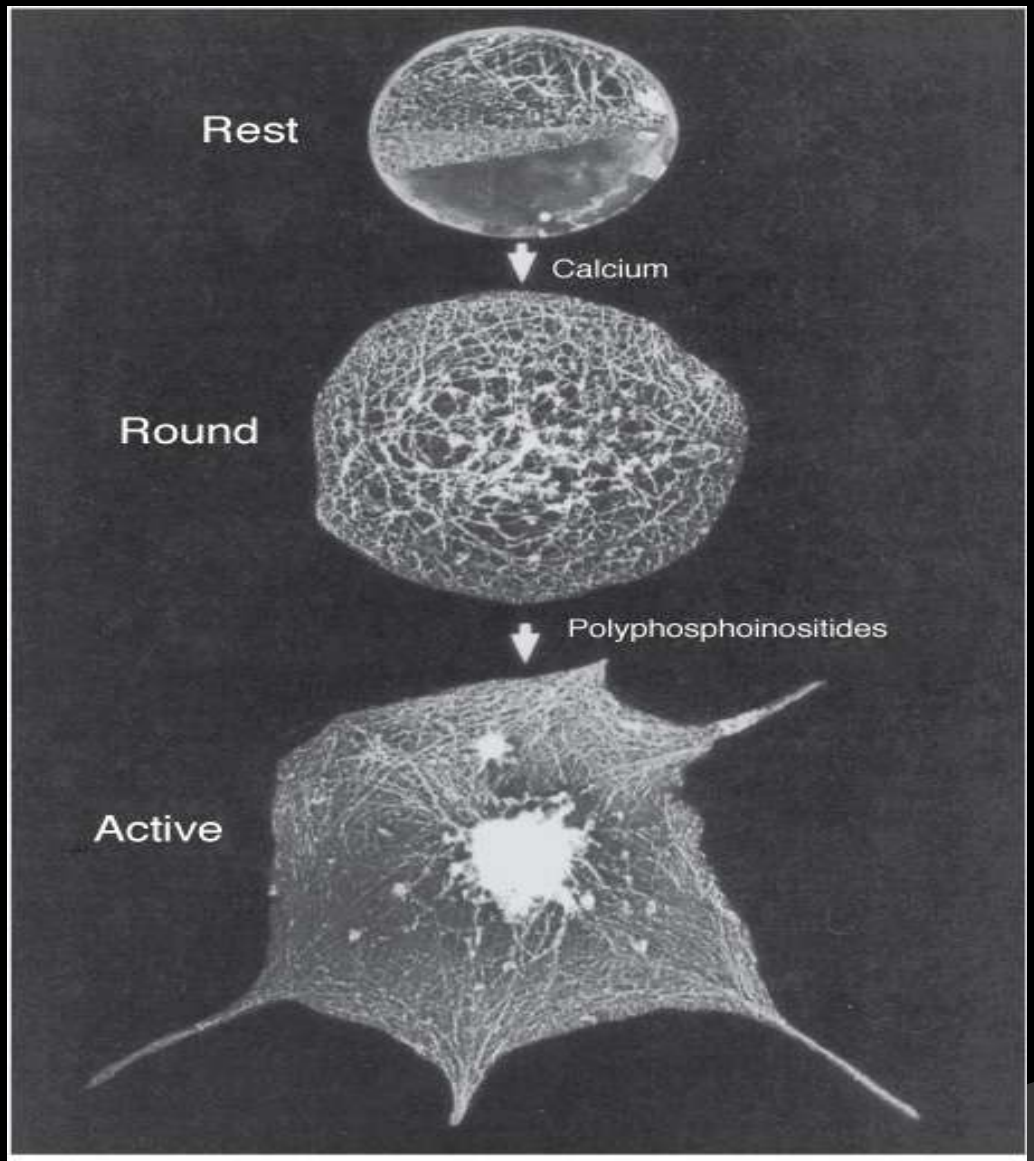
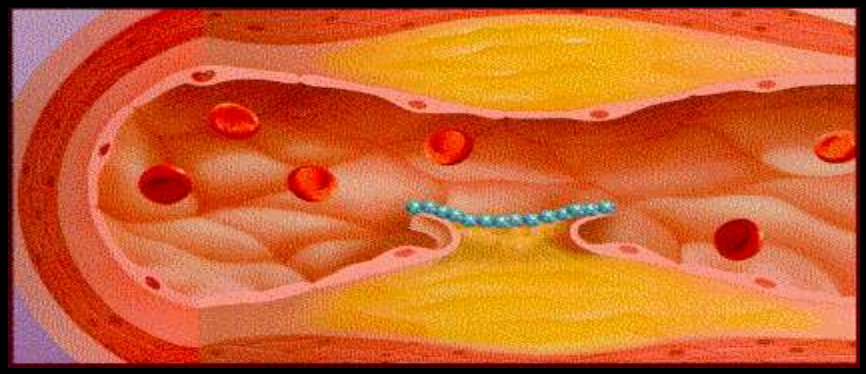
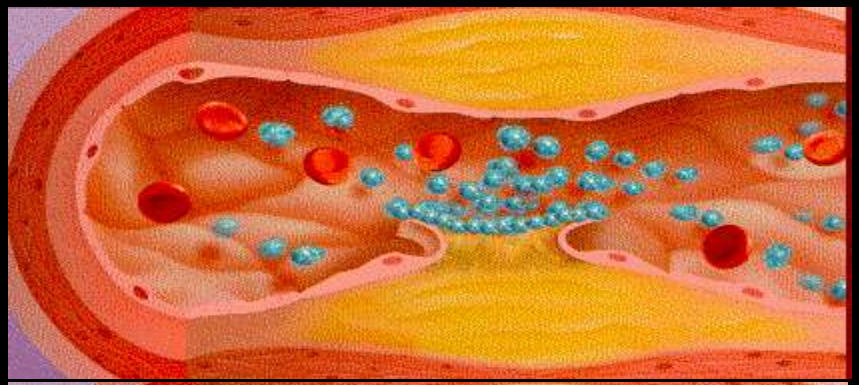


Source: Lichtman MA, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology, 8th Edition*: http://www.accessmedicine.com

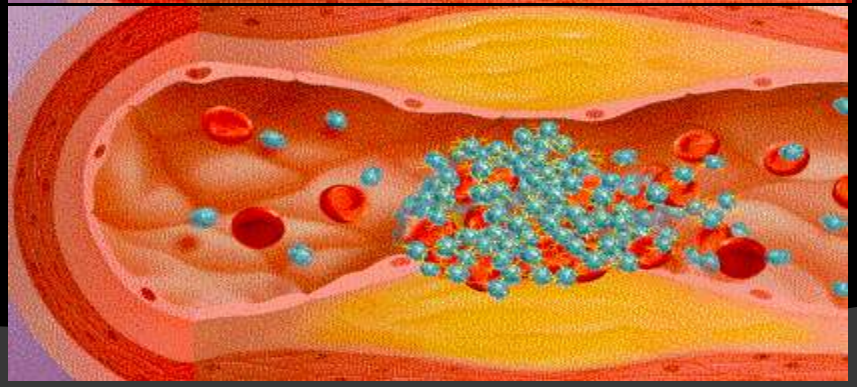
Events Leading to Thrombus Formation



- Adhesion



- Activation



- Aggregation

- Platelets are secretory cells and upon activation, they undergo exocytosis (degranulation), releasing into the surrounding medium vasoactive amines, calcium, ADP, ATP and coagulation factors.

Red blood cells and activated platelets or thrombocytes as a clot begins to form (red clot).

- Cell membrane of platelets composed of ….

Glycopropteins

- **Prevents the adherence of platelets to normal endothelium.**
- **Accelerates the adherence of platelets to collagen and damaged endothelium in ruptured blood vessels.**
- **Forms a receptor for ADP and thrombin.**

Phospholipids

- Accelerate clotting reactions.
- Form precursors for thromboxane A .

Microtubules

The microtubule form a ring around the cytoplasm below the cell membrane.

- The microtubules are made up of polymerized protein called tubulin. The tubules provide structural support for the inactivated platelets to maintain disc shape.

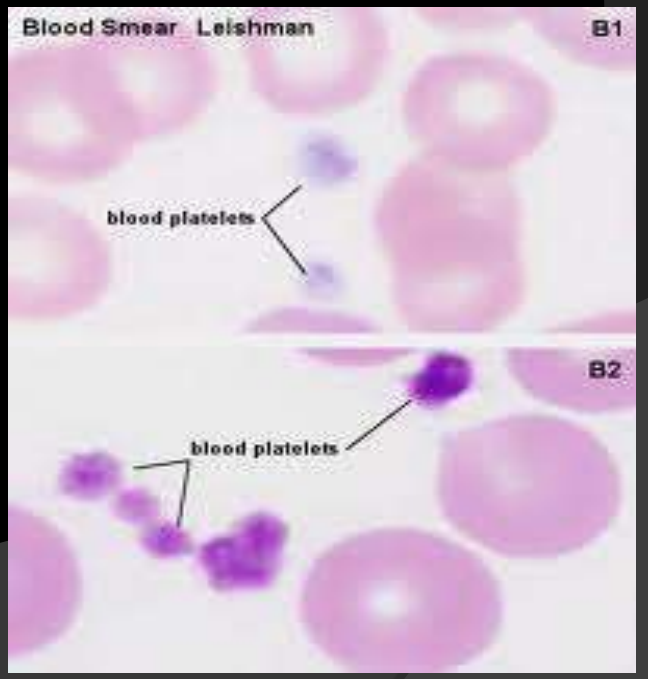
Cytoplasm

The cytoplasm of the platelets include:

- **Golgi apparatus**
- **Endoplasmic reticulum**
- **Mitochondria**
- **Microtubule**
- **Microvessels**
- **Microfilaments**
- **Granules**

- ✓ They stain pale blue with purplish granules,
- ✓ their cytoplasm contain a contractile protein consisted of actin and myosin(thrombosthenin)
- ✓ and three types of

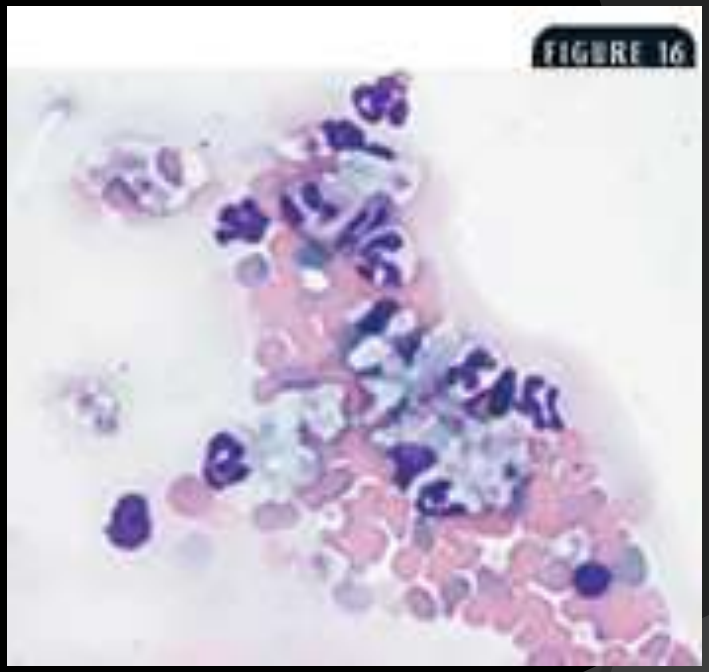
cytoplasmic lysosomes.

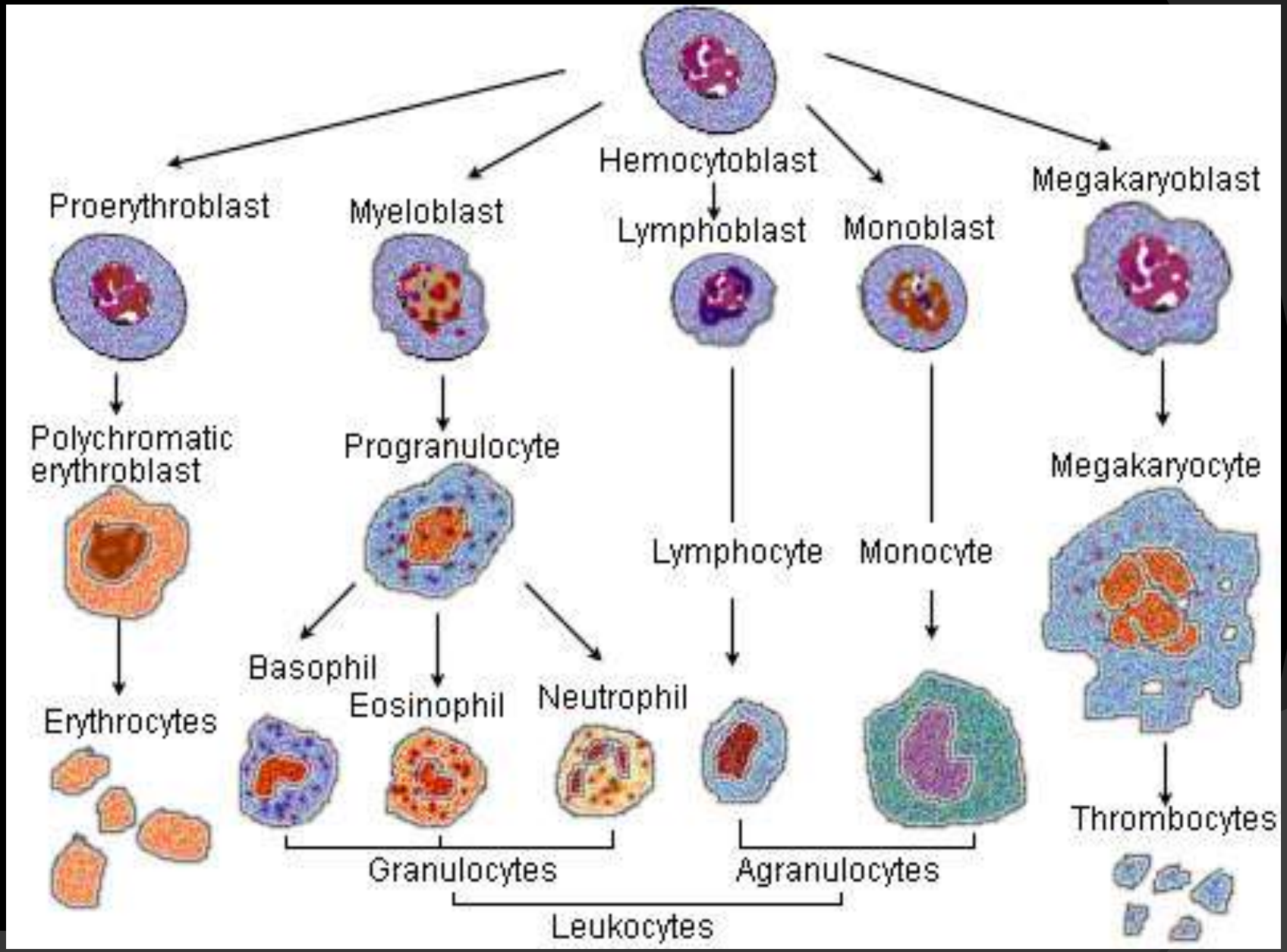


Platelet circulation life span is from 5-9 days in most animals.

feline platelet are most variable in size and may be as large as an RBCs, equine platelets stain faintly with Romanowsky stains.

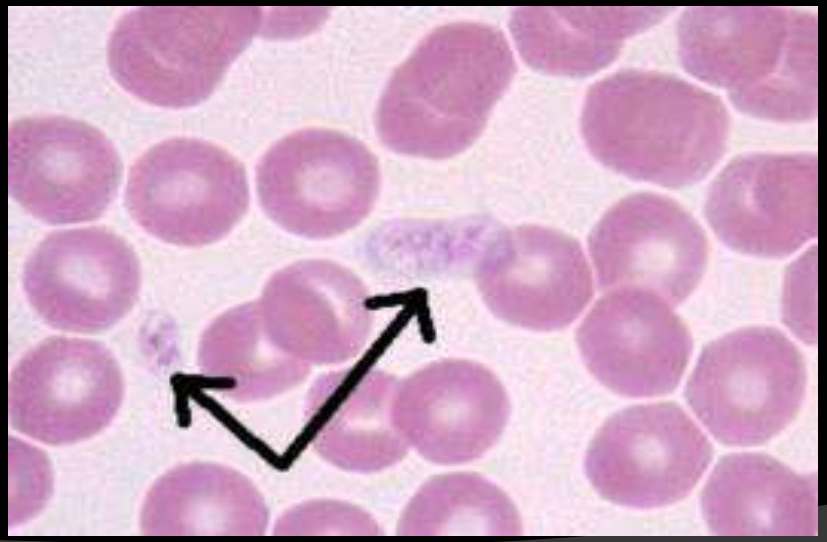
Clumps of platelets in a cat



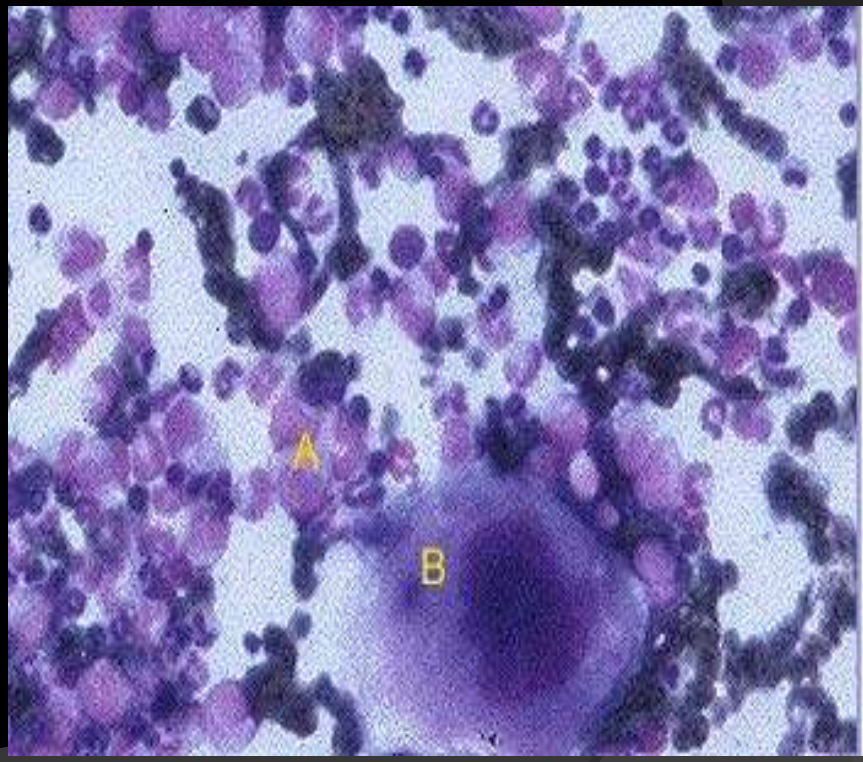

Hemocytoblast
Proerythroblast
Myeloblast
Lymphoblast
Monoblast
Megakaryoblast
Polychromatic erythroblast
Progranulocyte
Megakaryocyte
Lymphocyte
Monocyte
Basophil
Eosinophil
Neutrophil
Erythrocytes
Thrombocytes
Granulocytes
Agranulocytes
Leukocytes

The megakaryocyte is a giant cell and pieces of it's cytoplasm and cell membrane bud off to form the thrombocytes. One megakaryocyte can produce 1000 to 5000 thrombocytes.

canine_platelets



B –megakaryocyte in a bone marrow smear.



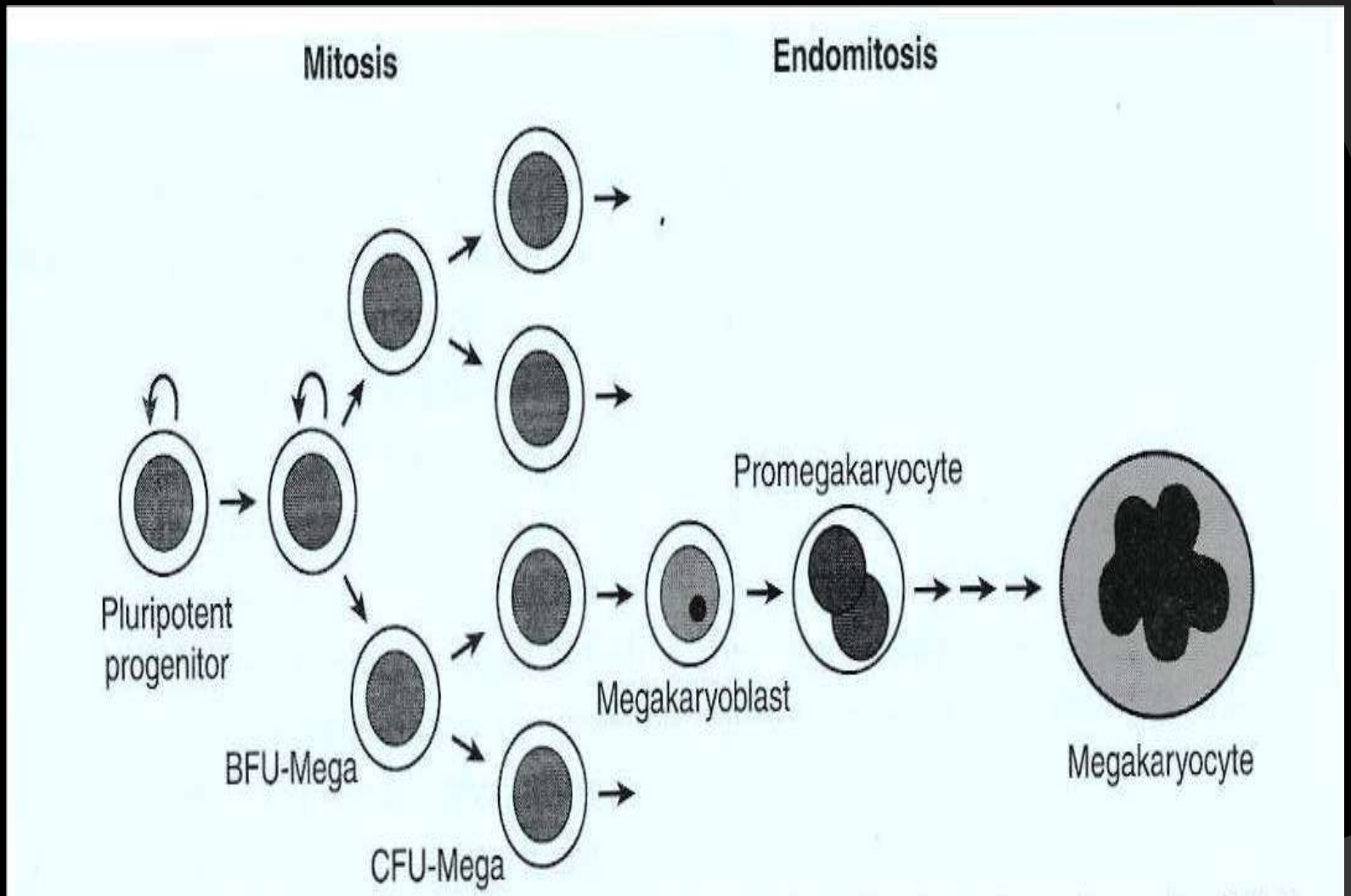
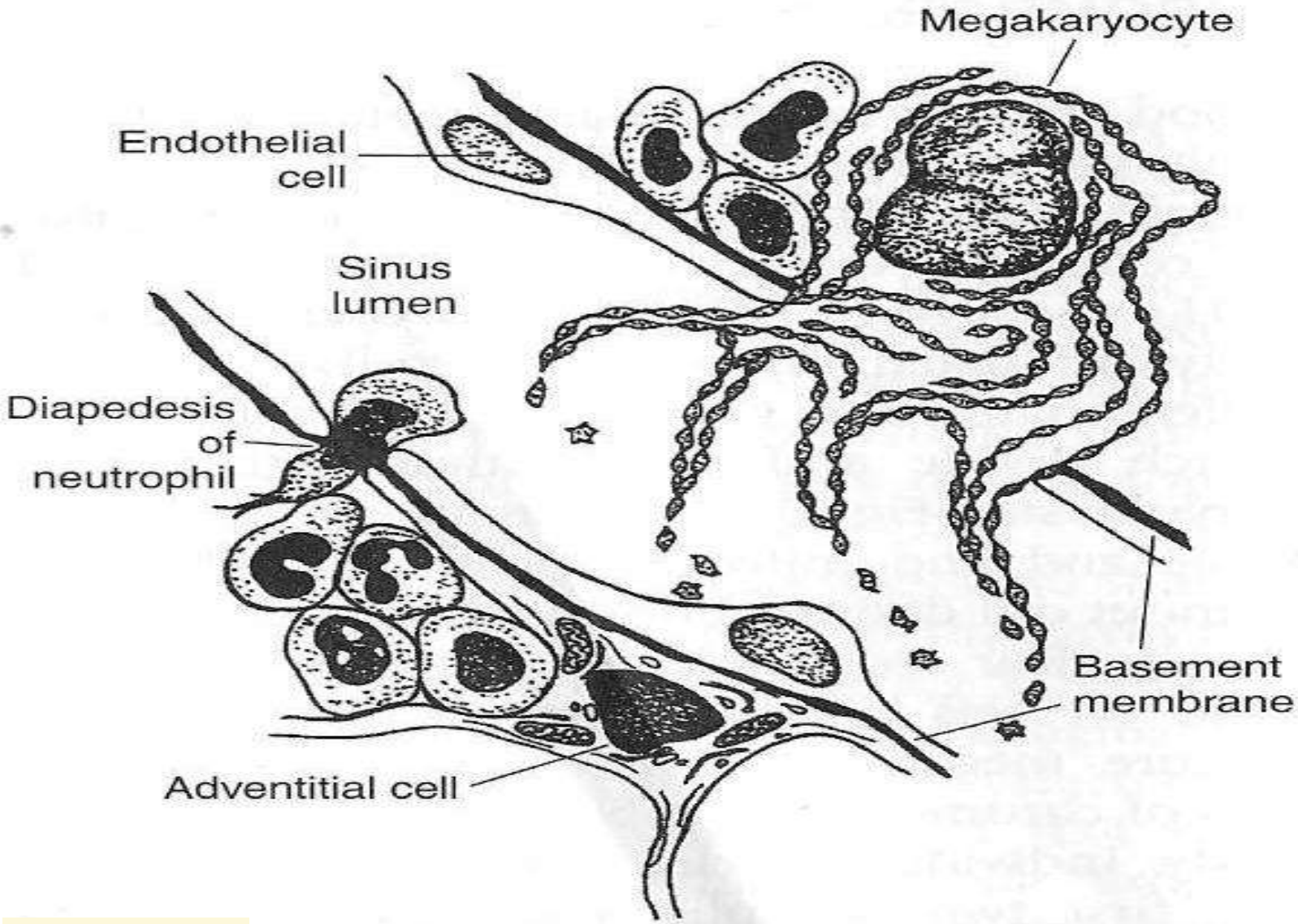
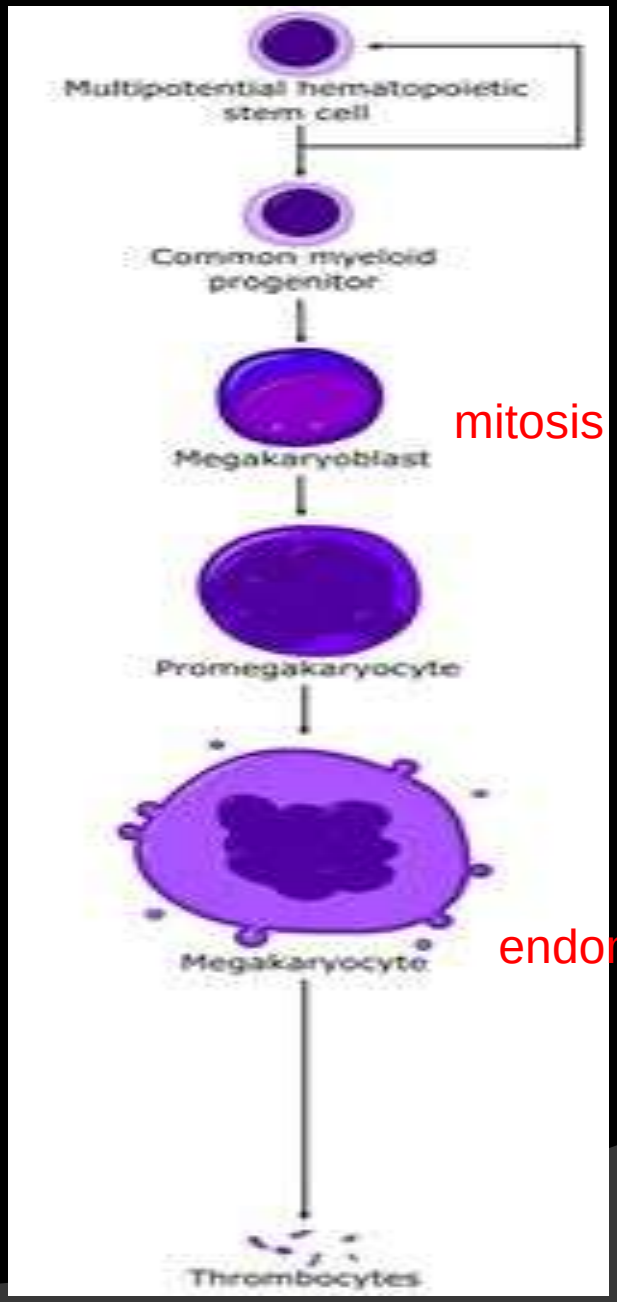


Fig. 3-10 Stages of megakaryocyte development. *BFU-Mega*, Burst-forming unit-megakaryocyte; *CFU-Mega*, colony-forming unit-megakaryocyte.



Extension of proplatelets into the sinus lumen and the subsequent formation of individual platelets.

Thrombopoiesis



mitosis

endomytosis

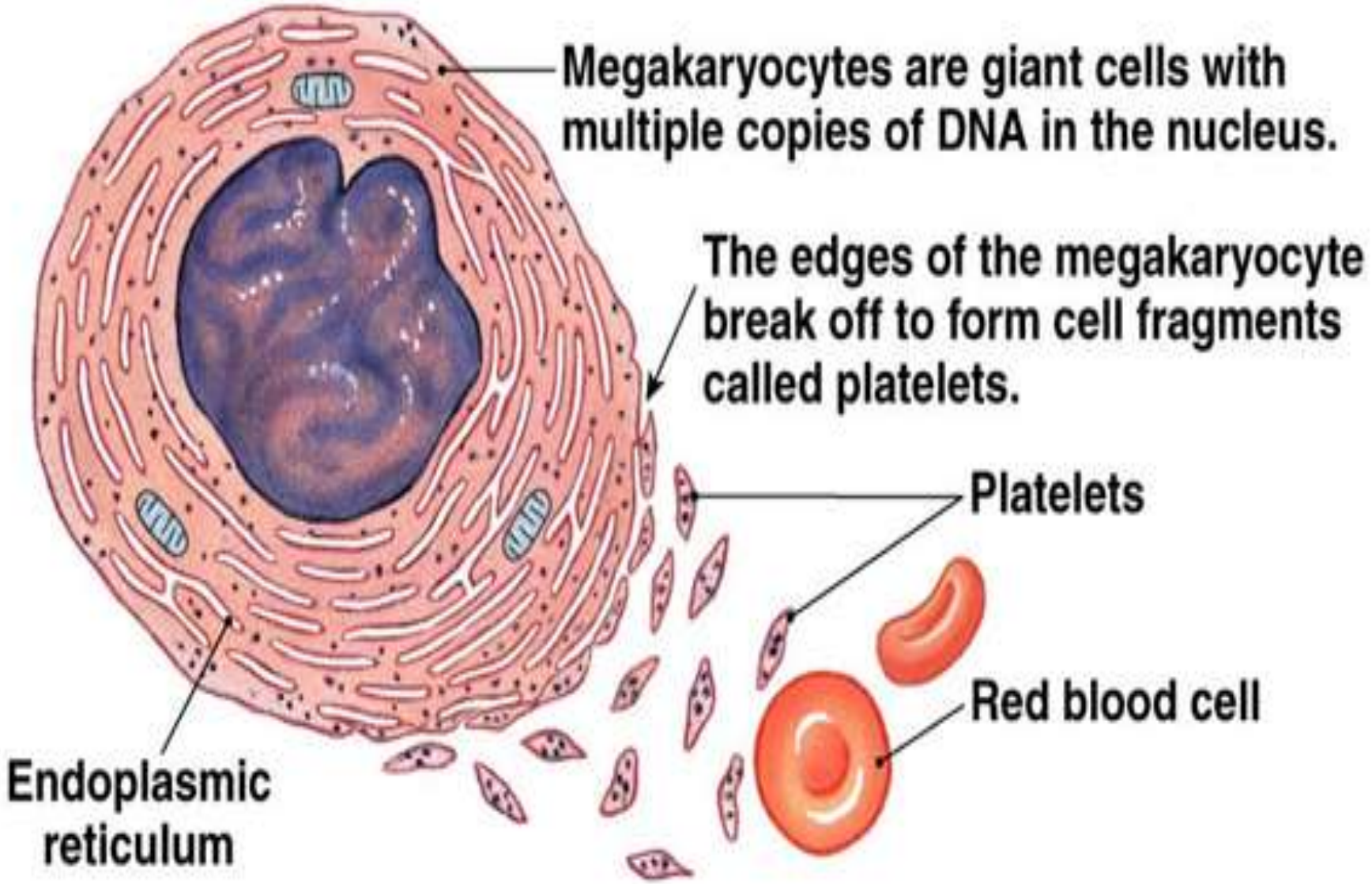


Fig. 16.9

1-The α- granules(specific granules):

Contain

*clotting factors (I, V , VIII :vWF complex).

* heparin-neutralizing polypeptide(platelet factor-4)(PF4).

* platelet- derived growth factor(PDGF) which helps in wound healing.

2. **The delta (∂) granules(dense granules):** which are electron dense storage granules containing non- protein substances that are released in response to platelet activation e.g. ATP, ADP serotonin, Ca++, histamine.

3. γ granules (gamma granules) – similar to lysosomes and contain several hydrolytic enzymes.
4. λ granules (lambda granules) – contents involved in clot resorption during later stages of vessel repair.

Von- Willebrand Factor(vWF):

*Essential plasma cofactor for the normal adhesion of platelets to damaged blood vessels,

*it is produced by megakaryocytes and vascular endothelium,

*vWF is found adsorbed to platelets surfaces, it is essential for platelet adhesion to sub- endothelial collagen and for platelet aggregation.

*In addition to its role as a bridge between platelets and exposed collagen on endothelial surfaces, vWF binds to and stabilizes coagulation factor VIII.

*Binding of factor VIII by vWF is required for normal survival of factor VIII in the circulation.

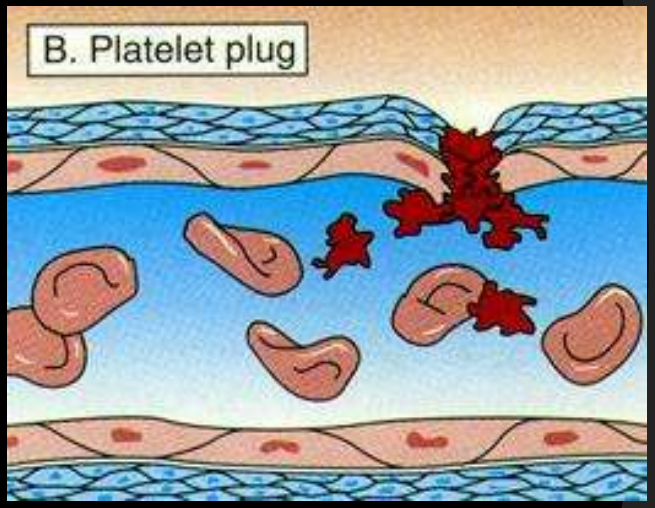
*

Function:

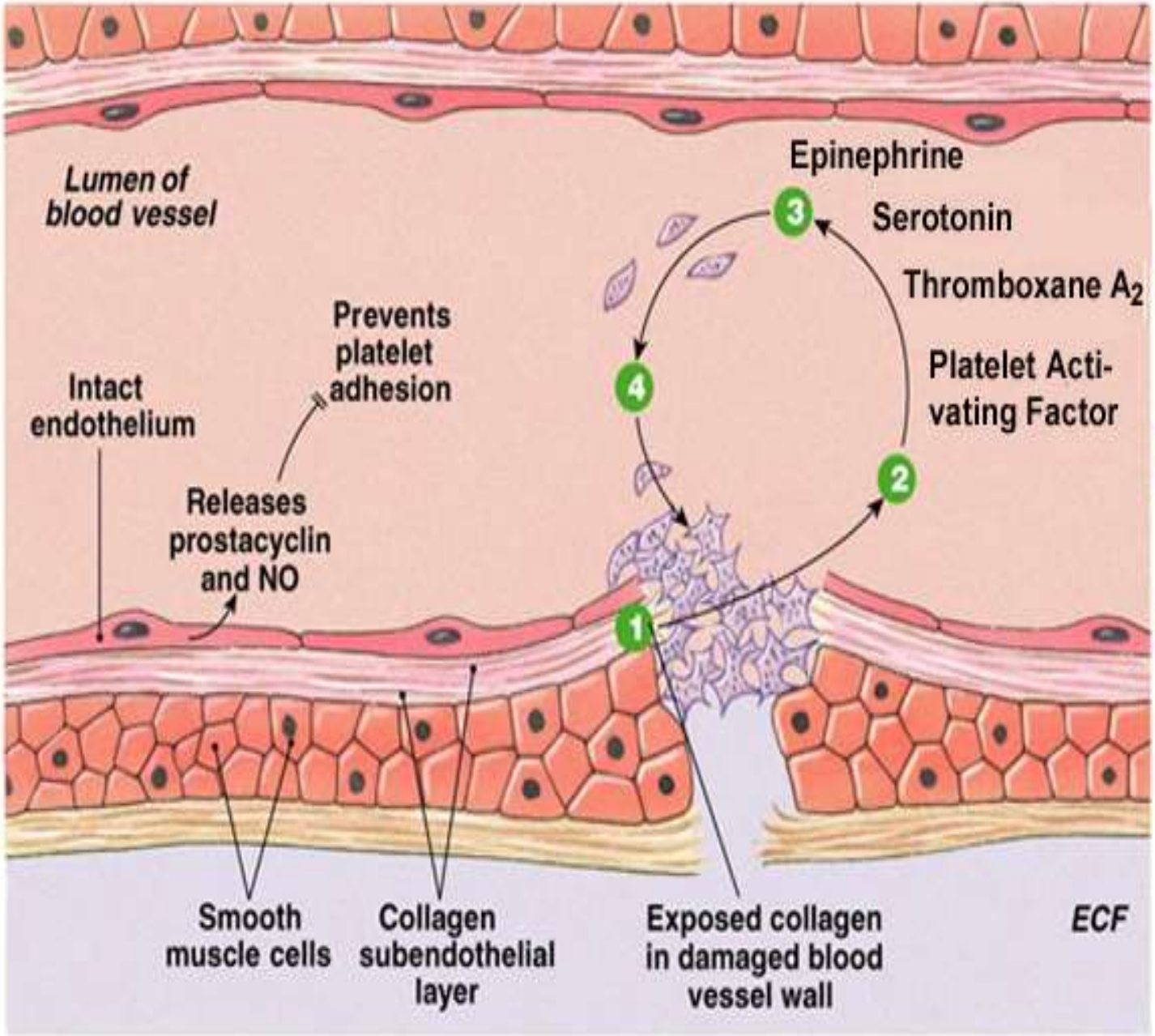
1.Essential for primary (white clot) and secondary haemostasis (red clot).

a. It helps in vasoconstriction of vessel through injured blood he release of vasoconstrictors as Serotonin, ADP and Thromboxane A2.

b - Formation of a platelet plug - platelets aggregate at the point where a vessel ruptures. This occurs because platelets are exposed to collagen (a protein found in the connective tissue located just outside the blood vessel).



Upon exposure to collagen, platelets release ADP (adenosine diphosphate) & thromboxane A2. These substances cause the surfaces of nearby platelets to become sticky and, as 'sticky' platelets accumulate, a 'plug' forms.



1. Exposed collagen binds and activates platelets.
2. Platelet factors are released.
3. Factors attract more platelets.
4. Platelets aggregate into platelet plug.

Figure 16-11

Symptoms of platelet disorders.

1. *Spontaneous and excessive bleeding* . This bleeding can be caused by deficient numbers of platelets, dysfunctional platelets, or very excessive numbers of platelets: over 1.0 million/microliter. (The excessive numbers create a relative vonWillibrand factor deficiency due to sequestration).

2. One can get a clue as to whether bleeding is due to a platelet disorder or a coagulation factor disorder by the;

a. Characteristics and location of the bleeding.

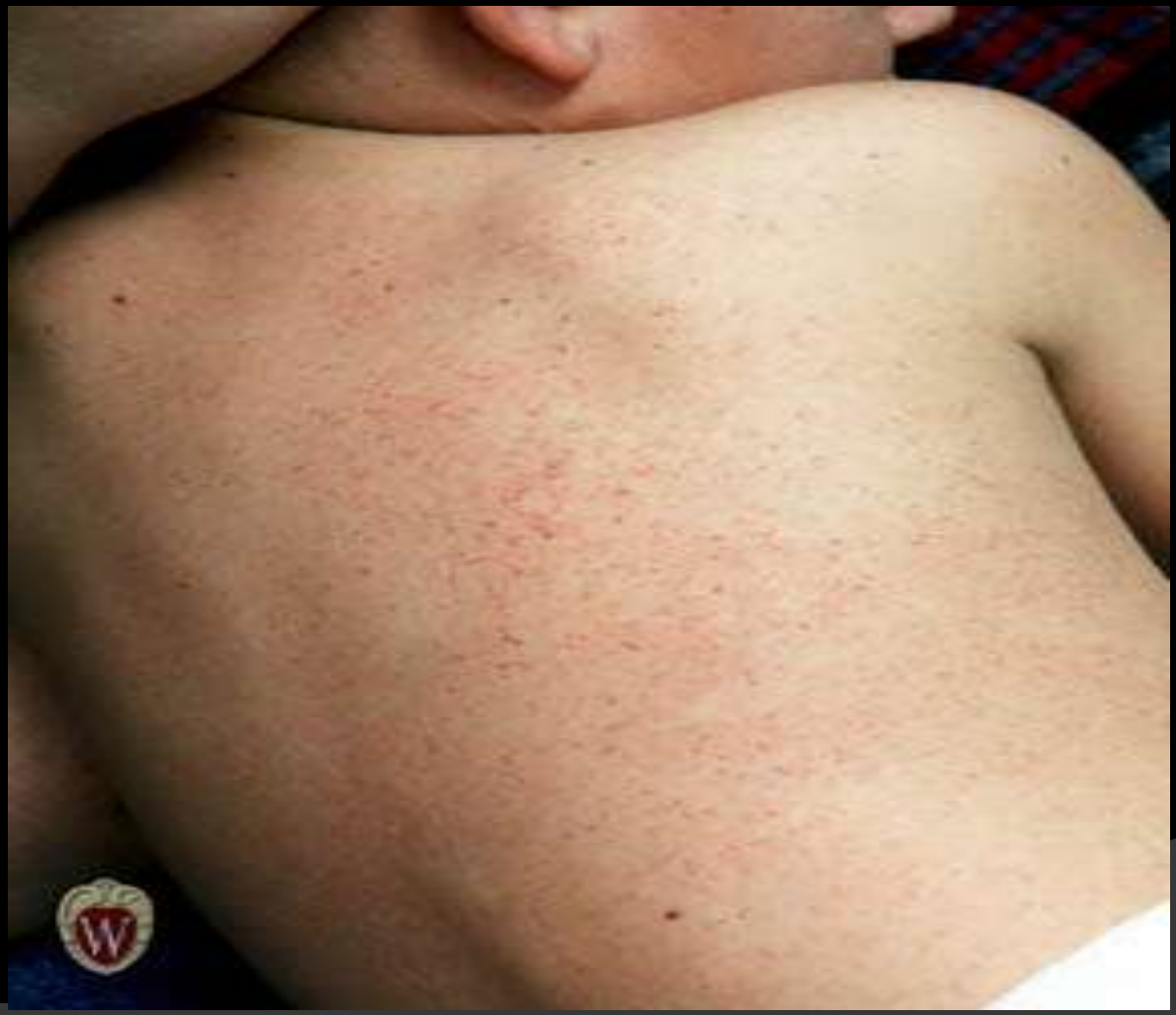
All of the following suggest platelet bleeding, not coagulation bleeding:

- Bleeding from a skin cut such as a razor nick is prompt and excessive, but can be controlled by pressure;
- Spontaneous bleeding into the skin which causes a purplish stain named by its size: petechiae, purpura, ecchymoses;
- Bleeding into mucous membranes causing bleeding gums, nose bleed, and gastrointestinal bleeding; menorrhagia; and intraretinal and intracranial bleeding.

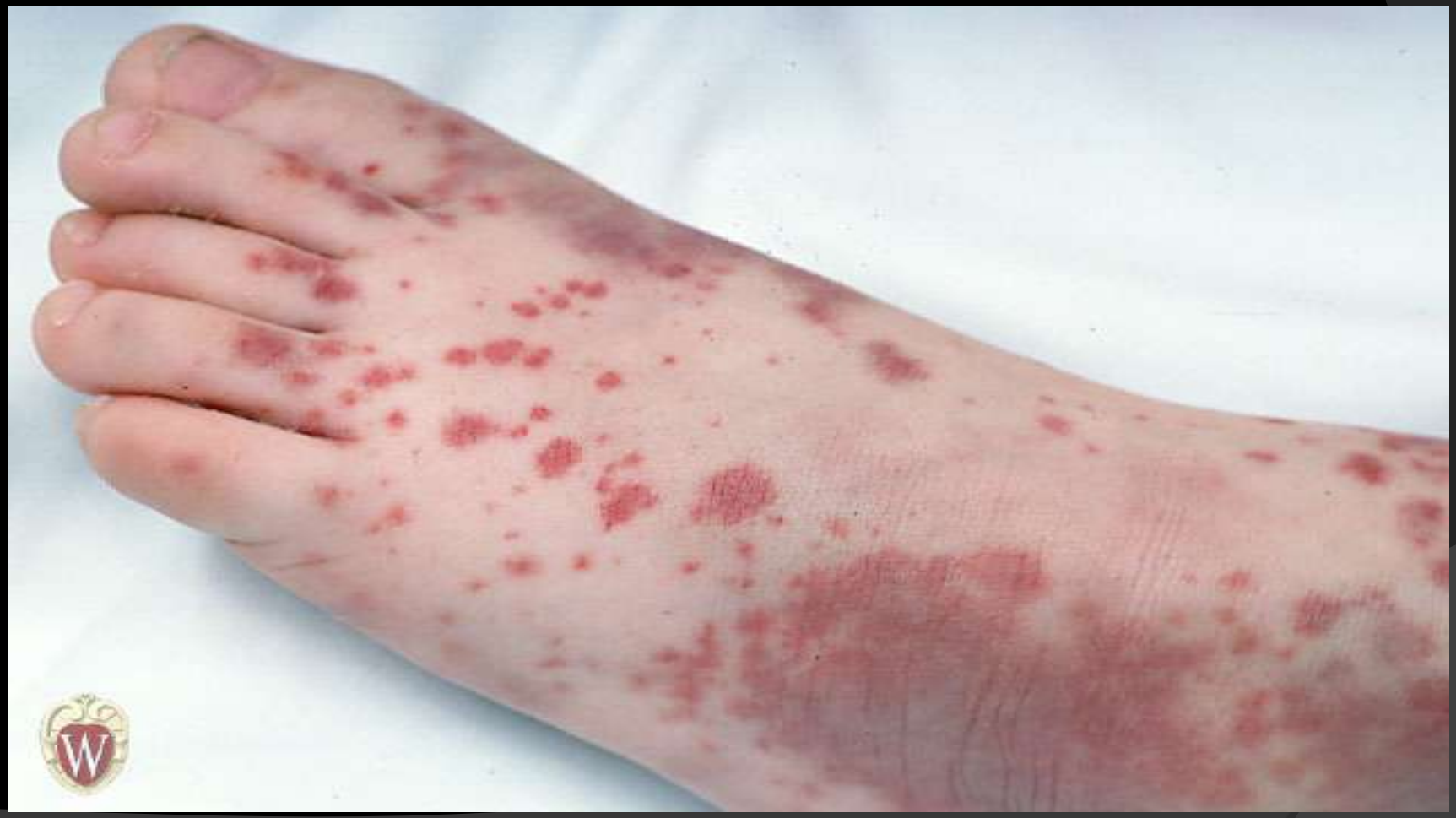
Three terms that refer to bleeding that occurs in the skin are petechiae, purpura, and ecchymoses. Generally, the term "petechiae" refers to smaller lesions.

"Purpura" and "ecchymoses" are terms that refer to larger lesions. In certain situations purpura may be palpable. In all situations, petechiae, ecchymoses, and purpura do not blanch when pressed.

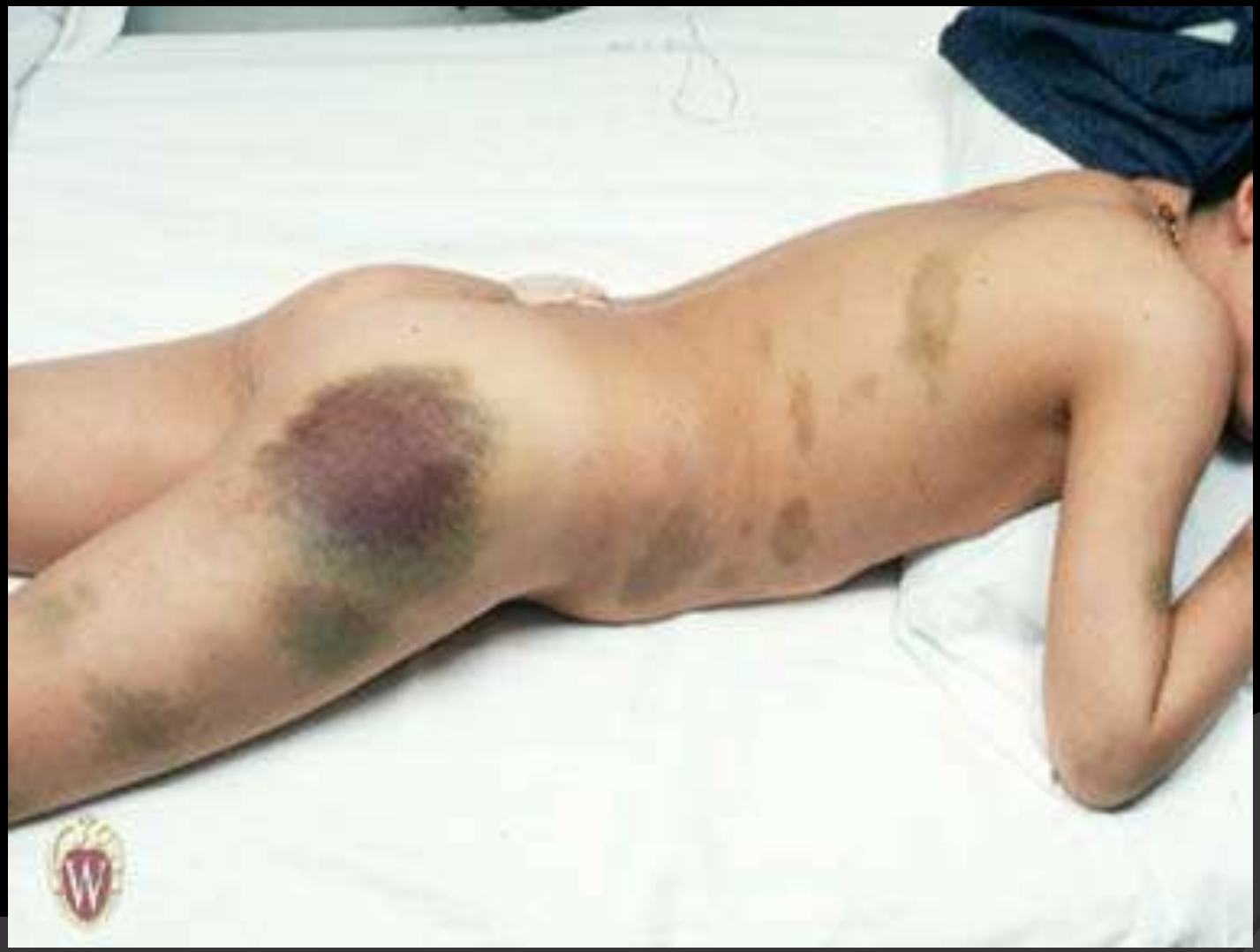
This 7-year-old boy has petechiae from thrombocytopenia secondary to chemotherapy



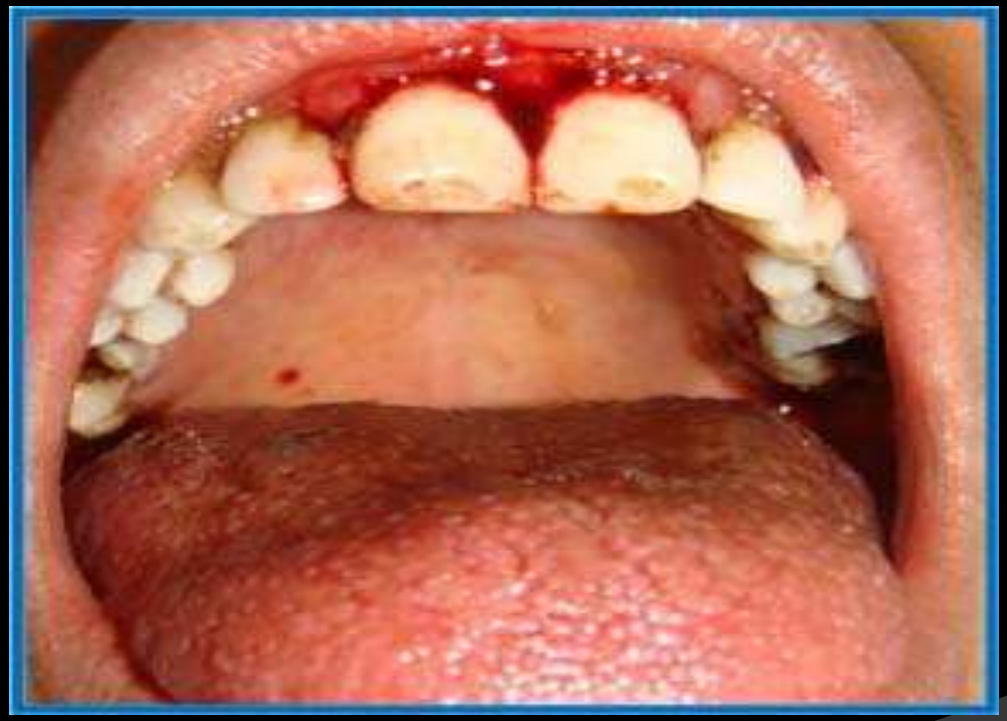
The palpable purpura on the foot of this nearly 3-year-old boy .



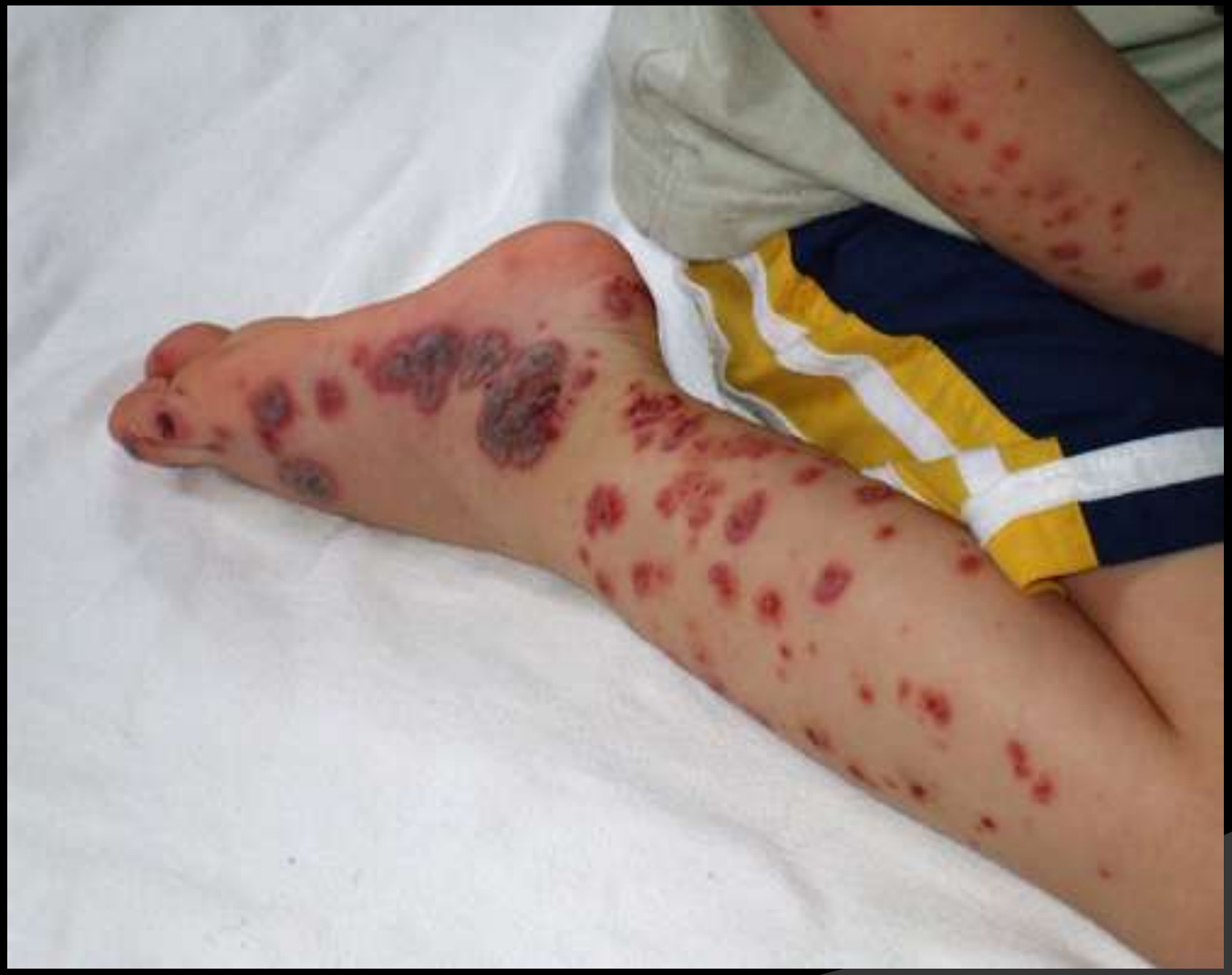
The purpura and ecchymosis on the skin of this 12-year-old boy were the presenting symptoms of his acute myelogenous leukemia.



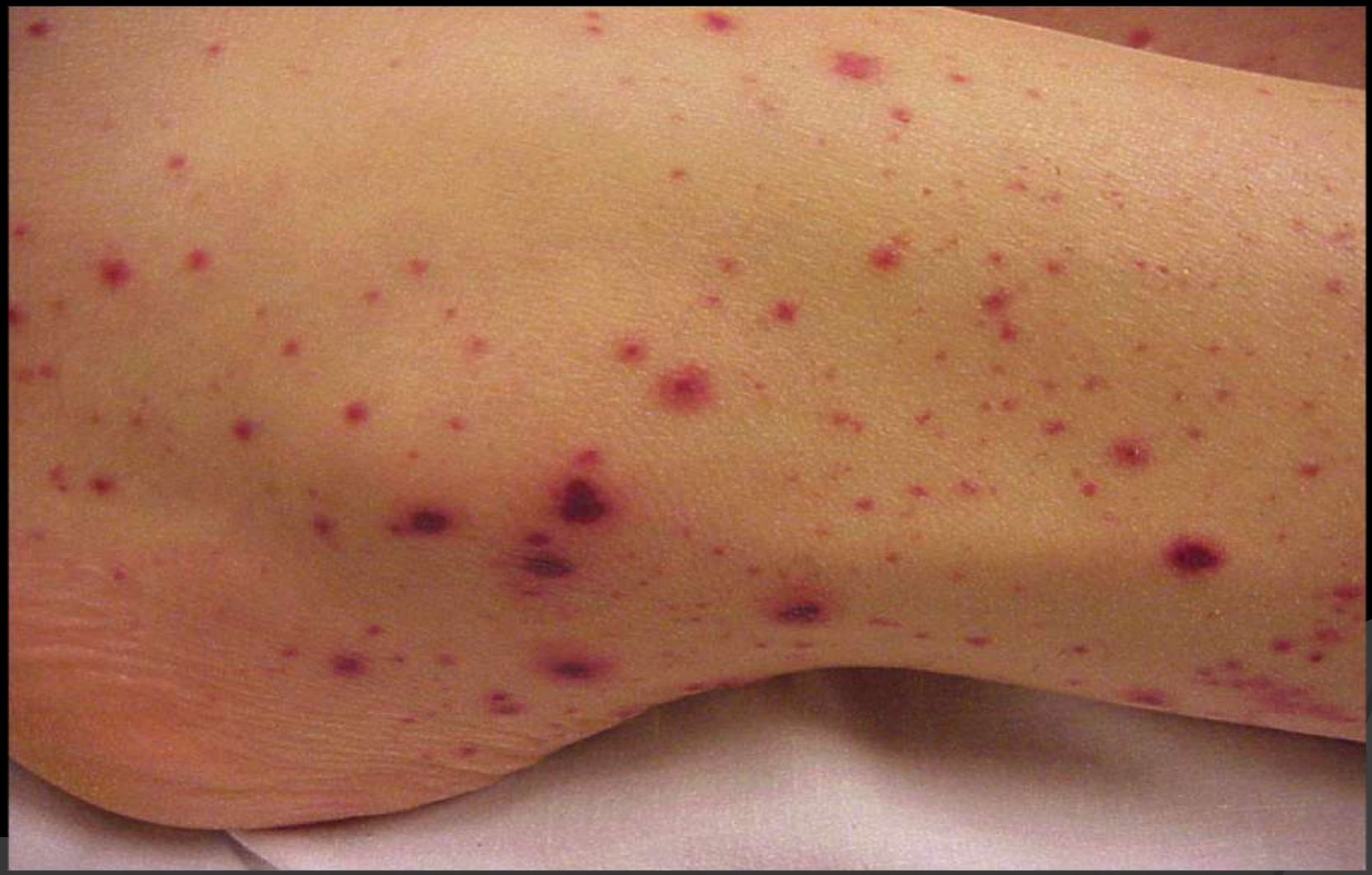
- Petechiae on soft palate & bleeding from gingiva. Diagnosis: Immune Thrombocytopenic Purpura



petechiae and purpura ITP purpura



ITP



- You send off a CBC and note that the white blood cell count is 10.2, the hemoglobin/hematocrit 12.7/38.1 and the platelets 27
- Based on the lab results and his reassuring appearance you make the diagnosis of ITP. Idiopathic thrombocytopenic purpura (ITP) is an IgG-mediated antibody reaction versus platelets leading to their premature destruction.

Quantitative platelet function defect:

Thrombocytopenia:

1. Increase in platelet destruction:

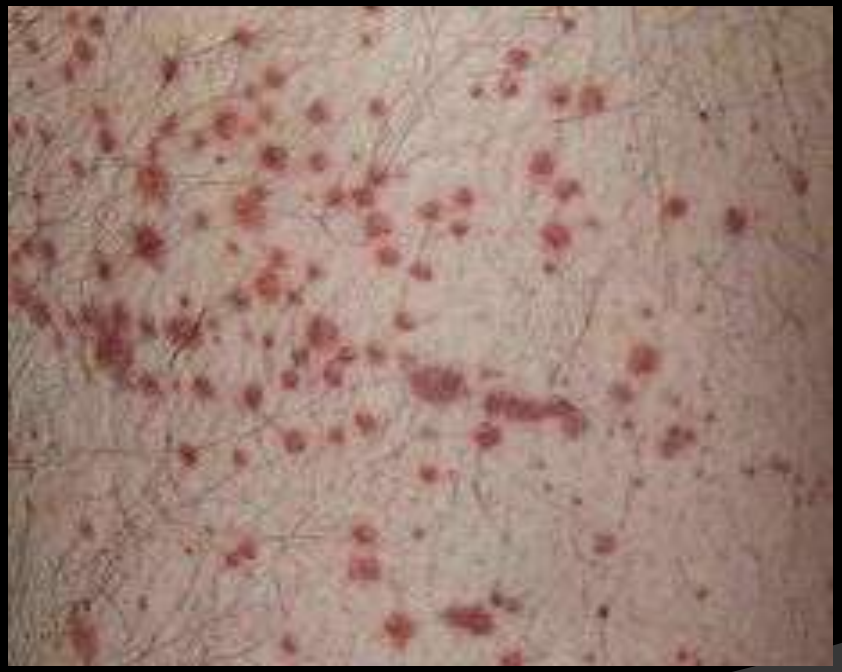
a. Immune-mediated : An agent sensitizes the platelets, attached to it as a hapten and abs are formed against the platelet- agent complex,

it may be

A- Primary or idiopathic of unknown primary cause.

also known as primary autoimmune thrombocytopenic purpura and (Idiopathic thrombocytopenic purpura-ITP) , is defined as isolated low platelet count (thrombocytopenia) with normal bone marrow and the absence of other causes of thrombocytopenia. It causes a characteristic purpuric rash and an increased tendency to bleed.

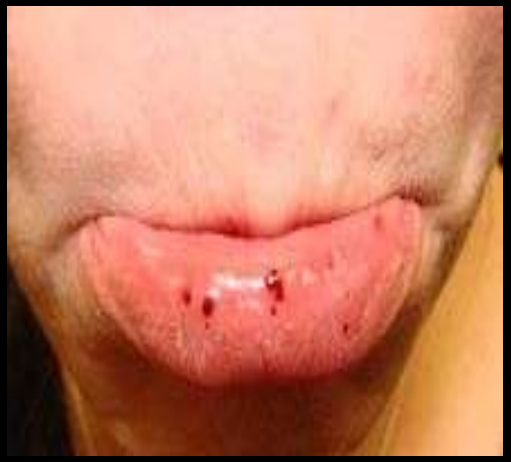
- Petechiae, or small bruise-like markings, may occur in ITP



B- Secondary immune mediated, as a sequel to other diseases like autoimmune diseases , drugs, lymphoproliferative disorders, viral infections or toxic agents.

b. Non- immunological: Haemolytic diseases of newborns, malignancies, drugs(heparin- HIT is caused by the formation of abnormal antibodies that activate platelets), Splenomegaly (sequestration in enlarged spleen).

- Oral petechiae /purpura – lower lip



2. Decrease in production:

a. Aplastic anaemia associated with leukopenia and anaemia , it is due to toxic effect on bone marrow e.g. Braken-fern poisoning ,ionizing radiation.

b. Bone marrow infiltration with abnormal cells e.g. leukemia, systemic fungal infection or metastatic neoplastic masses.

c. Drugs like estrogen, thiazide(diuretic) and myelosuppressive drugs .

d. Viral and richettsial infections..

As last stages of ehrlichiosis, FeLV, FIV, EIA, BVD and others.

3. Sequestration of platelets in spleen lung or liver caused by endotoxaemia or in hypersplenism, together with wbcs and rbcs.

4. Loss of platelets as in massive haemorrhage.

Thrombocytosis: May be caused by:

a. Increased production as in

*myeloproliferative disorders e.g. polycythemia Vera ,chronic leukemia, thrombocythemia (abnormal proliferation of megakaryocytes leads to increase in platelet production).

*Chronic inflammatory diseases,

*malignancies,

*acute haemorrhage,

*iron deficiency.

b. Increase release from tissue stores; as a response to exercise or adrenaline – induced splenic contraction(in health, spleen contain about 30-40% of all circulating platelets).

Qualitative platelet function defect:

1. Congenital extrinsic platelet dysfunction:

An important example is;

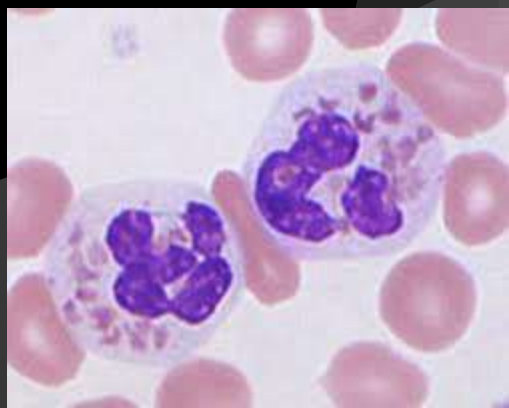
Von Willebrand (vWD) disease, vWF is an extrinsic factor essential for platelet function, its deficiency will lead to abnormality in platelet function and instability of clotting factor VIII. This congenital defect in vWD synthesis will lead to bleeding disorders like mucosal haemorrhages(epistaxis, bleeding from GIT, haematuria, prolonged bleeding from wounds and increased cutaneous bruising. It is common in man, dog, rare in cats, horse and cow.

2.Congenital intrinsic platelets disorders: These are disorders within the platelets including membrane protein defects or deficiencies, and abnormalities in granule contents or structure:

.

a. Chediak- Higashi syndrome

Characterized by lack of dense granules and insufficient stores of ADP and serotonin. Affected Persian cat mostly have a dilu coat coloure and may experier prolonged bleeding and haematoma formation at venipuncture. Blue smoke Persians are predisposed to Chediak-Higashi syndrome



b.Thrombasthenia- (Glanzman,s d.):

It is a defect in or deficiency of certain glycoproteins in the receptors on platelets surface; it is an autosomal recessive disorder in one of two genes,

platelets are weak , it is characterized by normal morphology and normal number of platelets with defect in aggregation, with inability or deficiency of fibrinogen binding. All leads to severe bleeding diathesis which is purpuric in nature.

Symptoms

Common symptoms include:
Excessive bleeding after skin damage (dentist, surgery, accidents).
Spontaneuos bleeding within the joints.
Spontaneuos bleeding within mucosal tissues.

3. Acquired qualitative functional disorders:

a. Hyporesponsive platelets: It is caused my different agents as drugs (Aspirin, NSAIDs, Penicillin and others), uraemia, liver diseases, infection(FelV, *Ehrlichia canis*), paraproteinaemia of plasma cell myeloma and some snake venoms.

b. Enhanced platelet function(hyperactivity)

This may be observed in

- nephrotic syndrome due to (hypoalbuminaemia),
- Erythropoietin dministration(increase in immature platelet which are hyperactive).
- some infectious agents as FIP virus in feline and heart worm in dogs.

Tests for evaluation of platelet function:

1.Platelet count: Direct and indirect method using a well – stained and prepared blood film.

*Less than 3 thrombocytes/ most oil immersion fields indicate thrombocytopenia

*plt count must be above 100,000 plts/UL.

Why is EDTA unacceptable for coagulation testing?

- inactivates factor VIII (8)
- inhibits the conversion of fibrinogen to fibrin
- bind reagent calcium when calcium is added to initiate clot-based tests

Why is sodium citrate the anticoagulant of choice for coagulation testing?

- binds calcium ions to prevent coagulation
- buffer stabilizes pH
- will not invalidate hemostasis test results

2. Clot retraction test:

a. Take1-2ml of blood from a normal animal and the patient without anticoagulant.

b. Incubate for 2-4 hours in 37c(4-8 hours in 25c).

c. Compare the amount of clot retraction between normal and diseased animal.

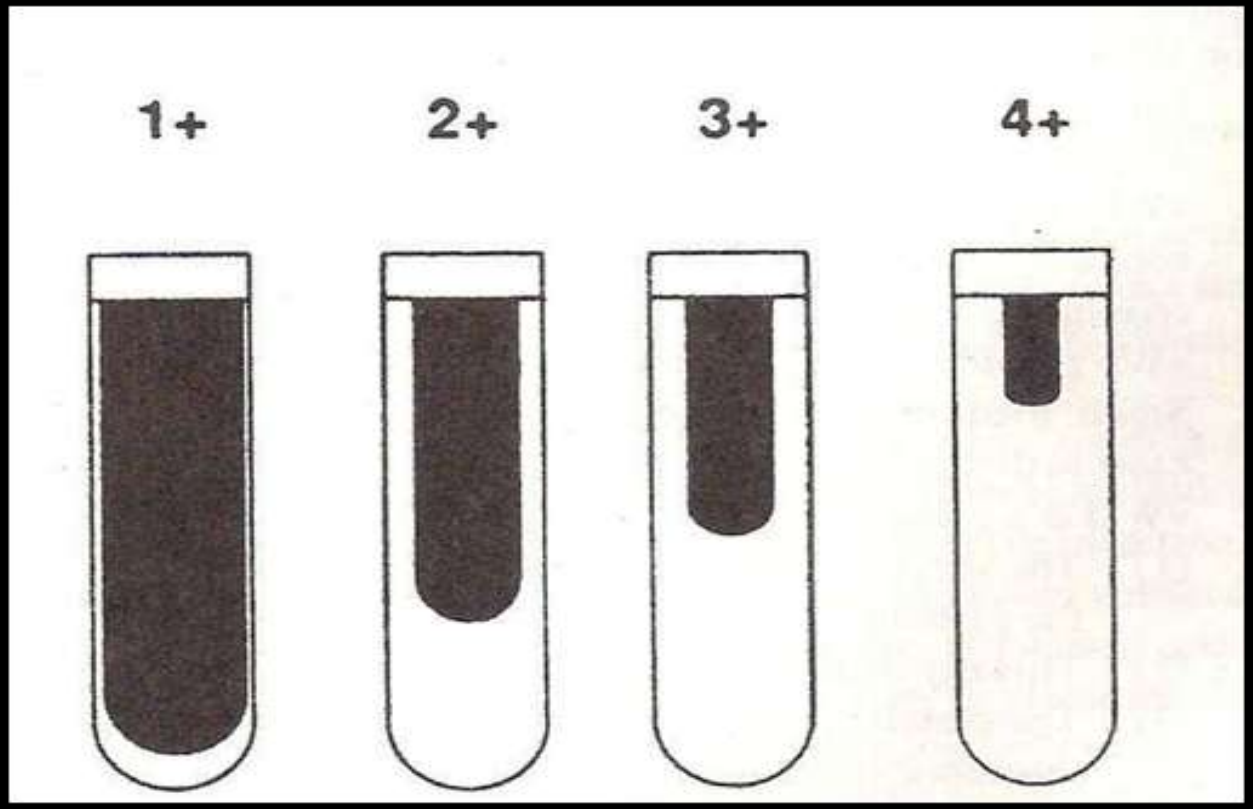
- Normal: clot retraction begins within 30 minutes, complete at 2-4 hours
  - Poor: retraction after 4 hours, within 24 hours
  - None: no retraction occurs after 24 hours

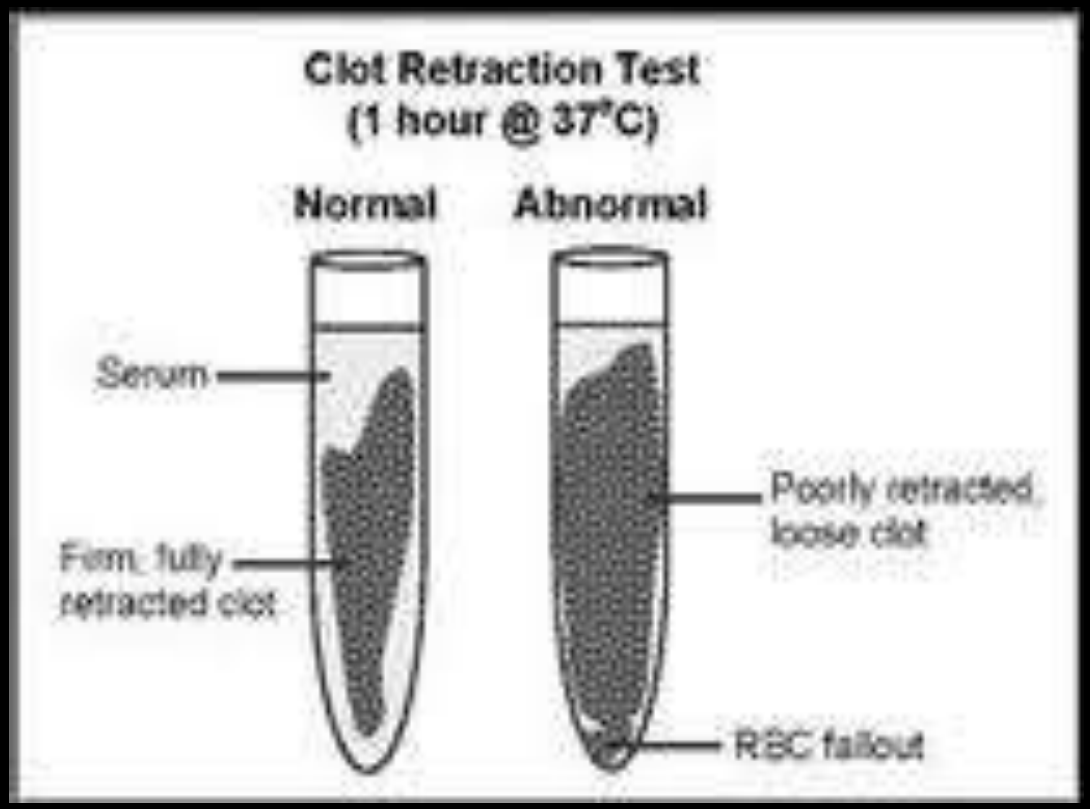
Bedside Clot Retraction Tes(CT)

- It simply tests the clotting time - a test of decreased fibrinogen
- 2 ml blood in test tube - no clot formed but if occurs it is prolonged, soft and not retracted after half an hour, leaving a clot volume more than serum volume.

  (the clot doesn't retract)

Clot retraction test




Clot Retraction Test
(1 hour @ 37°C)
Normal
Abnormal
Serum
Firm, fully retracted clot
Poorly retracted, loose clot
RBC fallout

3. Bleeding time(Buccal mucosal bleeding time): I t is a laboratory test used to evaluate primary haemostatic system

- ***Platelet function.**

c.

- *** Vascular integrity.**

Normal Range: Two to four minutes .

Interpretation: Lip bleeding time is expected to be prolonged in patients with:

*severe acquired or inherited platelet dysfunction.

* Thrombocytopenia(probably < 90,000 platelets/μl)

*Thrombopathy (uremia and aspirin therapy).

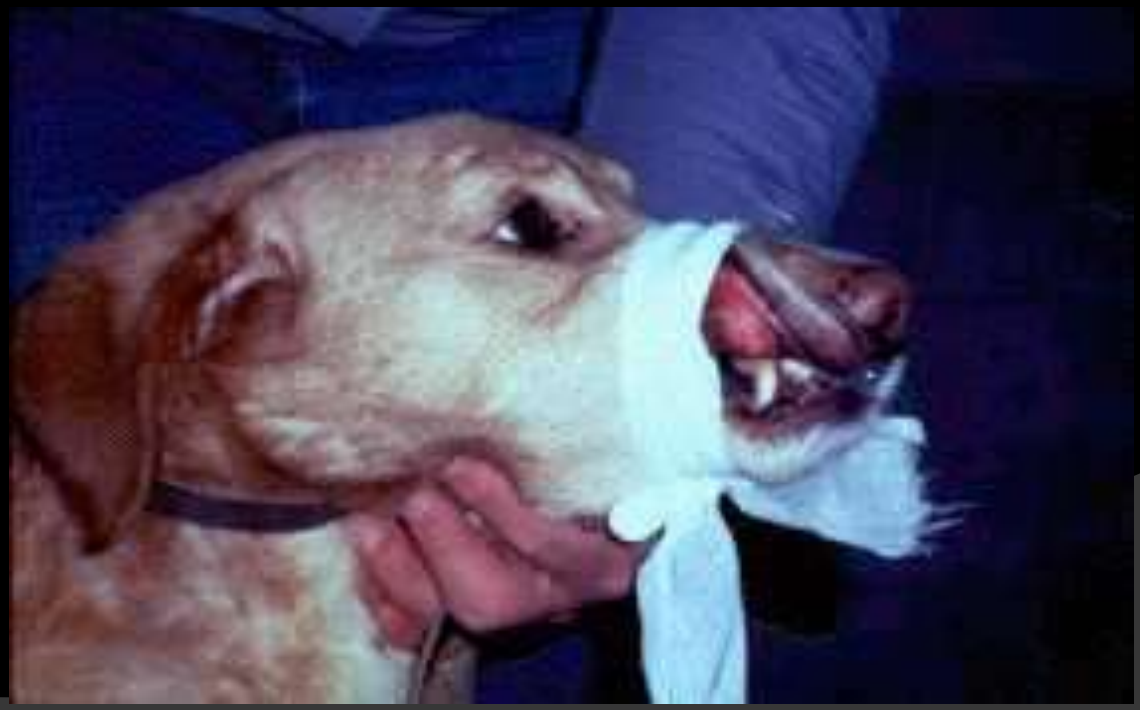
* Severe von Willebrand disease. Chediak-Higashi syndrome in cats

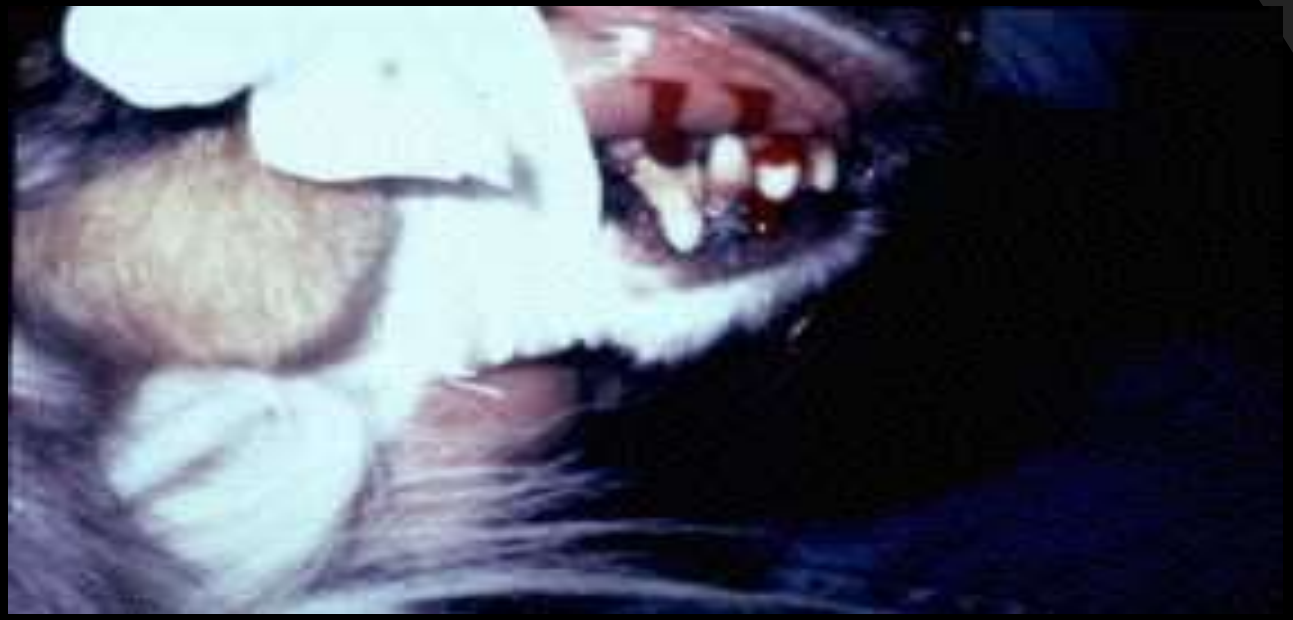
* There is a variable response in dogs with DIC or mild forms of vWD.

*Dogs with even severe coagulation factor deficiencies usually have normal lip bleeding time.

- Surgicuttt Vet H enables the veterinarian to make a standardised incision (1.0mm deep and 5.0mm long) in 1/1000 seconds

The upper lip is everted and held in place with muzzle gauze that encircles the upper and lower jaw. The gauze must be tied snugly. The buccal bleeding time is not inherently painful, but dogs must remain quiet and in position for up to 10 to 12 minutes. Sedation may be required for adequate restraint.





Using a special device make two incisions in the buccal mucosa parallel to the lip margin. Blood flowing from the wounds is then gently blotted below the incisions. Do not wipe or disturb the wounds. The time from incision to cessation of blood flow is recorded as the buccal mucosal bleeding time.

4. Coagulation time(capillary tube method):

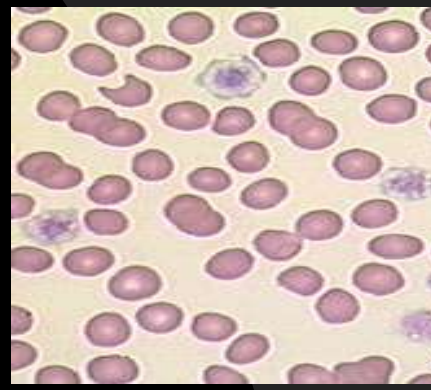
a. A skin puncture is made , whip the first drop of blood.

b. Fill a capillary tube with blood noting when first blood appear.

c. Gently break the tube every 30 seconds , until a strand of fibrin is seen extending across the gap between the two ends of the tube. **Coagulation time** is the interval between the appearance of blood and appearance of fibrin strands.

5. Bone marrow examination: For the number, size and morphology of megakaryocytes.

6.Platelet morphology:



*Giant platelets(shift platelet, macroplatelet: The a platelet is considered giant when it's diameter is equal or more than that of a mature erythrocyte which correlates with increased mean platelet volume (MPV), . They could be observed in the normal animal. Their presence has also been associated with myeloproliferative disease as well as with regenerative response to thrombocytopenia.

However, in cats, enlarged platelets is a normal finding.

- **Platelet fragments(microplatelets):* It may indicate;
- iron deficiency associated with thrombocytosis,
- immune- mediated thrombocytopenia.
- or as artifact associated with in vitro storage and aging in EDTA for more than 24 hours.

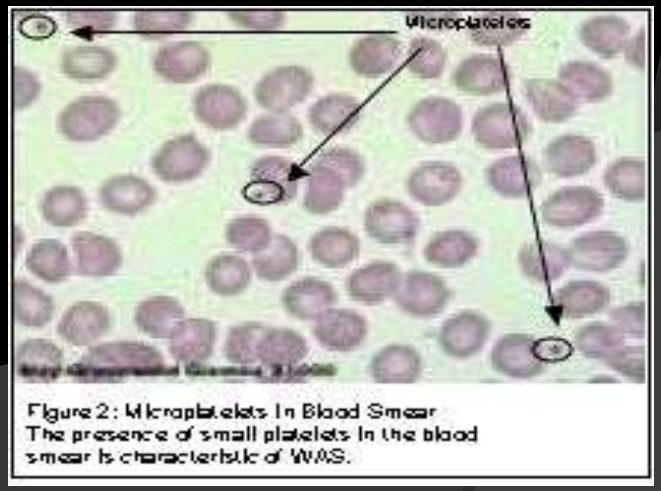
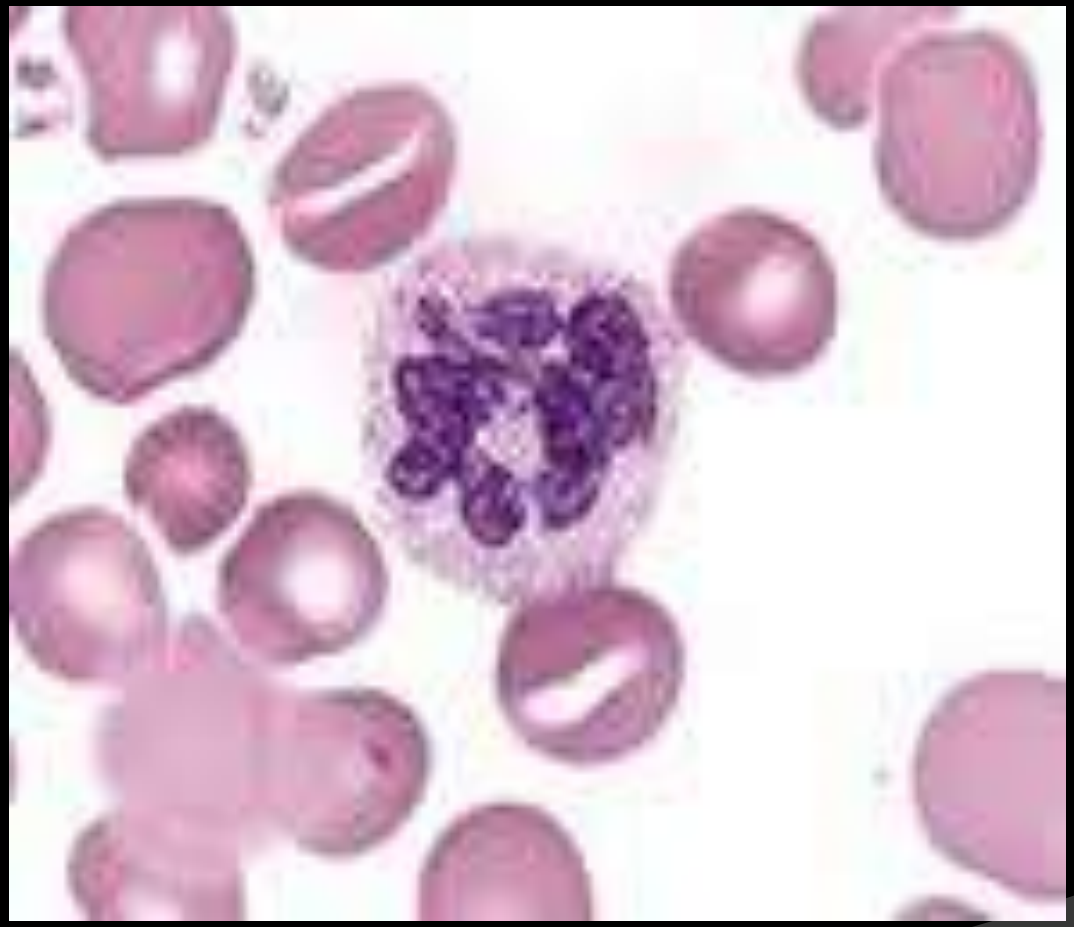


Figure 2: Microplatelets In Blood Smear
The presence of small platelets in the blood smear is characteristic of WAS.



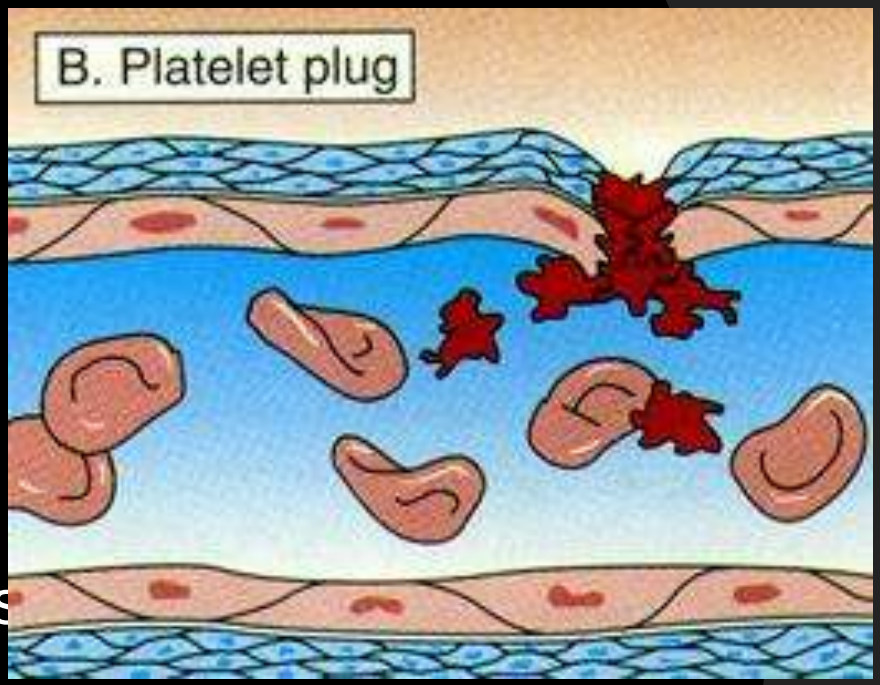
7. Platelet function assays are available in some human medicine institutions but they are not adapted to commercial or diagnostic animal laboratories, major drawback is their technically demanding nature.

Haemostasis

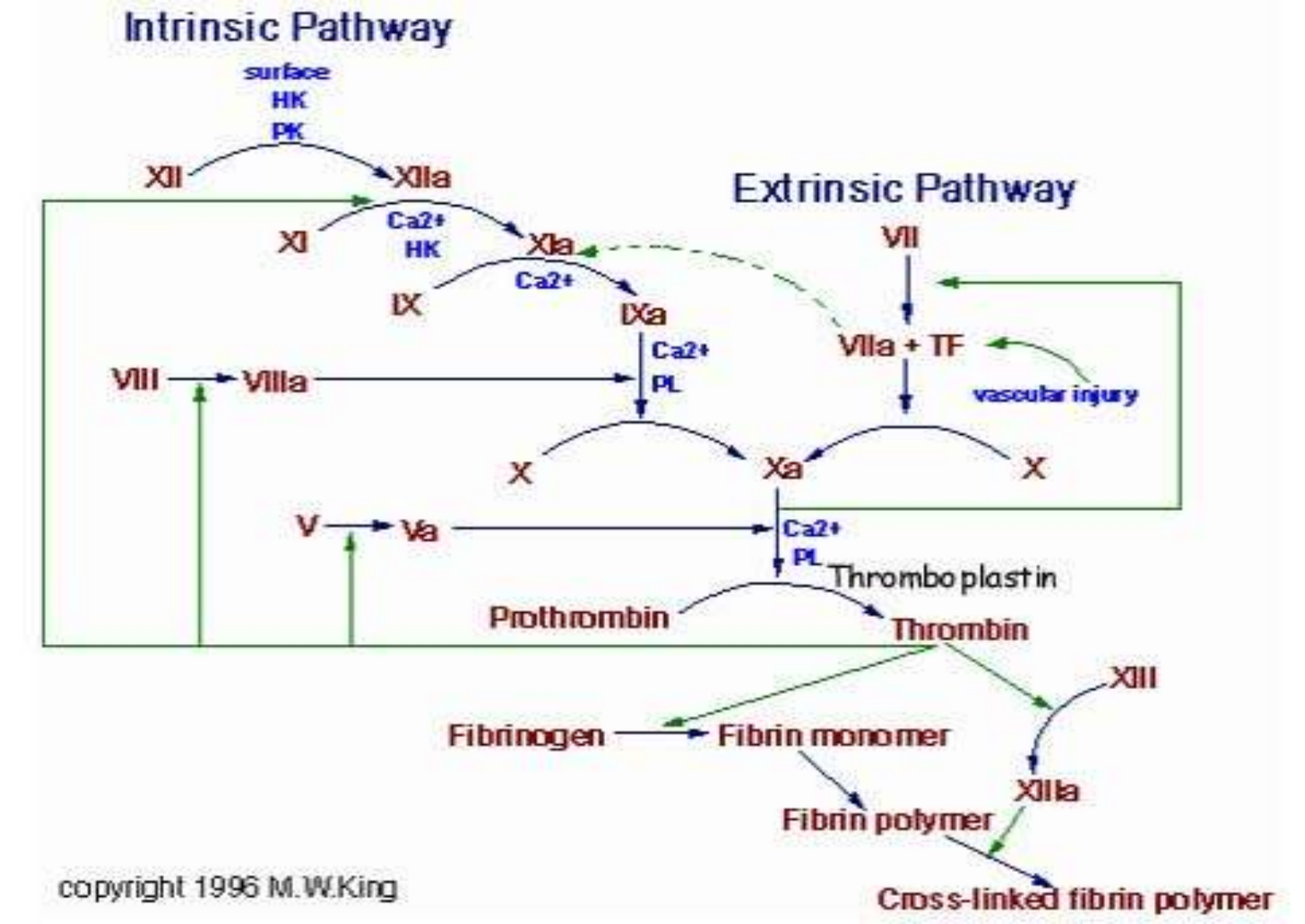
prevention of blood loss from broken vessel :

1 - Vascular spasm - vasoconstriction of injured vessel due to contraction of smooth muscle in the wall of the vessel. This 'spasm' may reduce blood flow & blood loss but will not stop blood loss.

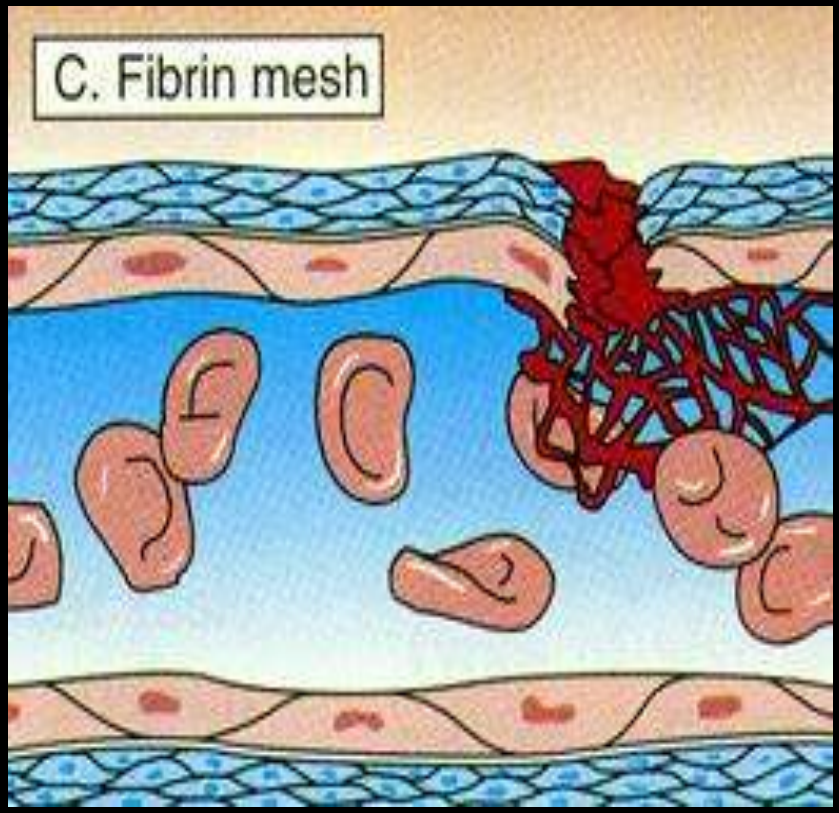
2 - Formation of a platelet plug - platelets aggregate at the point where a vessel ruptures. This occurs because platelets are exposed to collagen (a protein found in the connective tissue located just outside the blood vessel). Upon exposure to collagen, platelets release ADP (adenosine diphosphate) & thromboxane. These substances cause the surfaces of nearby platelets to become sticky and, as 'sticky' platelets accumulate, a 'plug' forms.

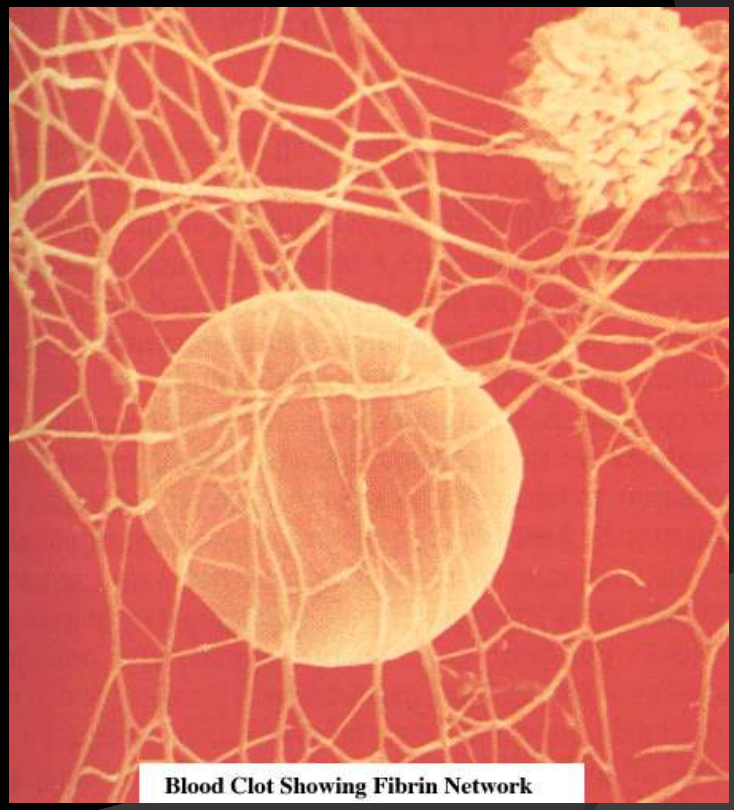


3-Blood coagulation cascade:



The result of all of this is a clot formed primarily of fibrin threads (or polymers), but also including blood cells & platelets.
Blood clots in the right places prevent the loss of blood from ruptured vessels, but in the wrong place can cause problems such as a stroke (see below under inappropriate clotting).




Blood Clot Showing Fibrin Network

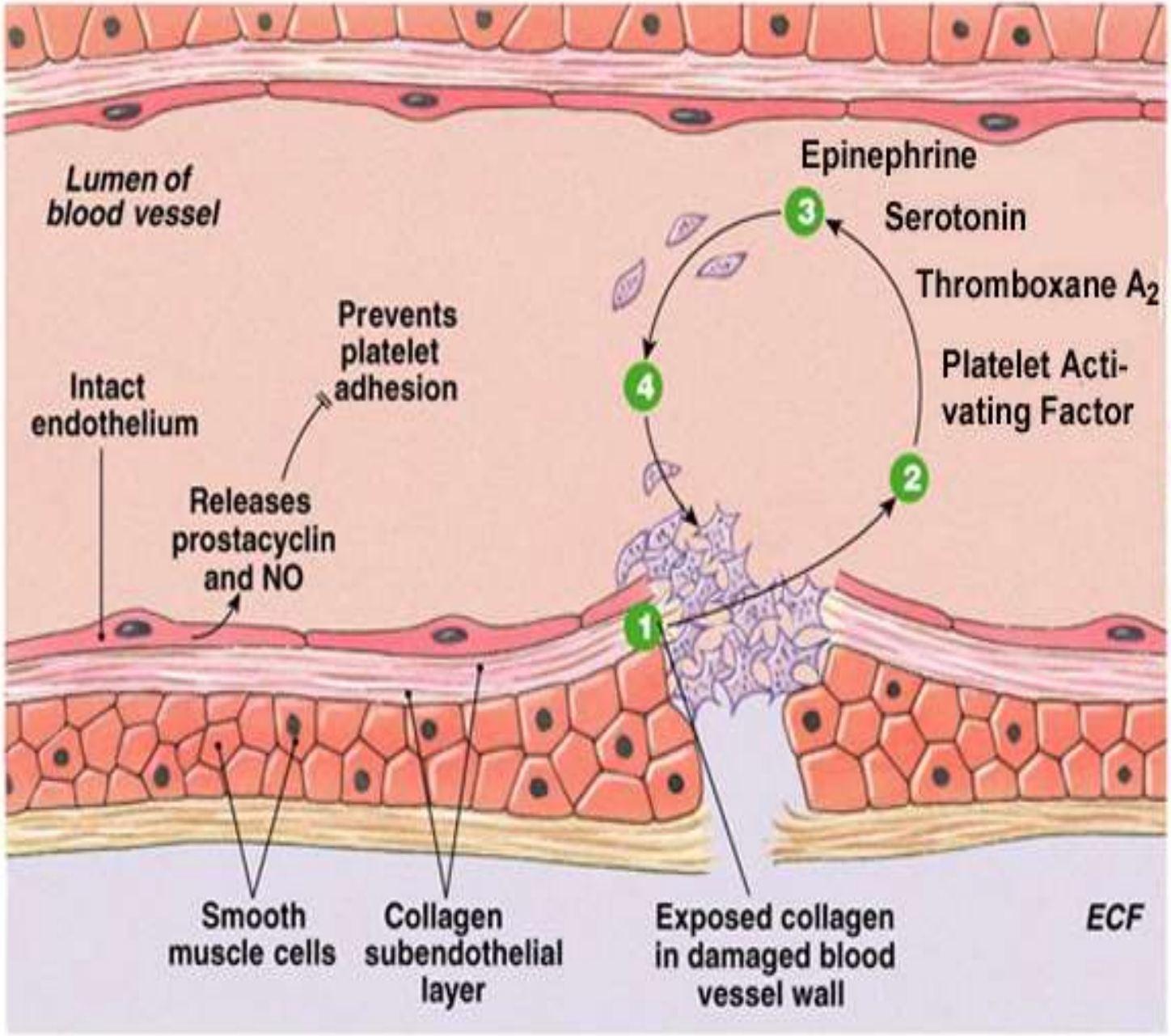
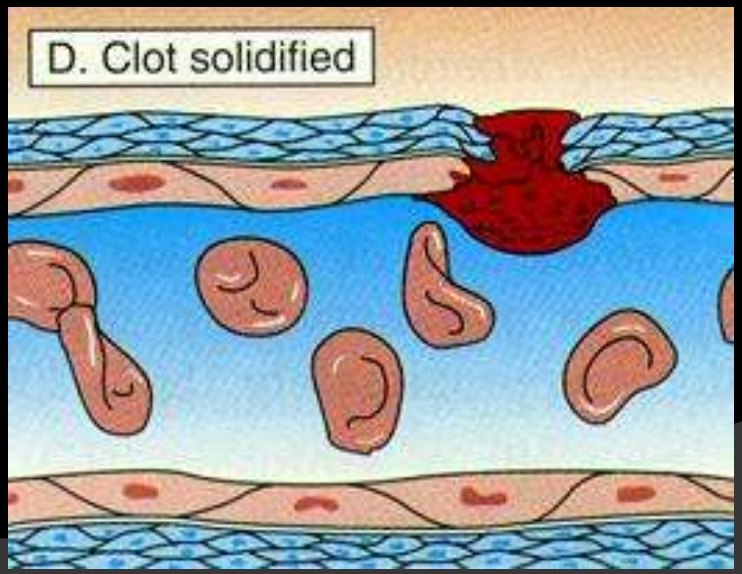


Figure 16-11

D-Clot retraction:

tightening" of clot ,contraction of platelets trapped within clot shrinks fibrin meshwork, pulling edges of damaged vessel closer together

Over time (with the amount of time depending on the amount of damage), the clot is dissolved and replaced with normal tissue



E-Fibrinolysis:

dissolution of clot

mechanism = plasminogen (a plasma protein) is activated by many factors & becomes PLASMIN. Plasmin then breaks down fibrin meshwork & phagocytic WBCs remove products of clot dissolution.