

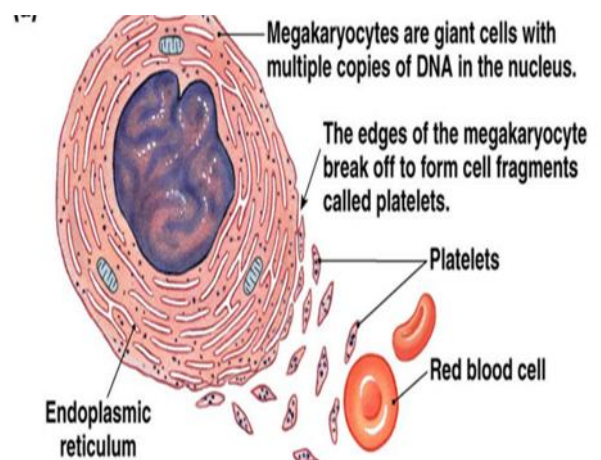
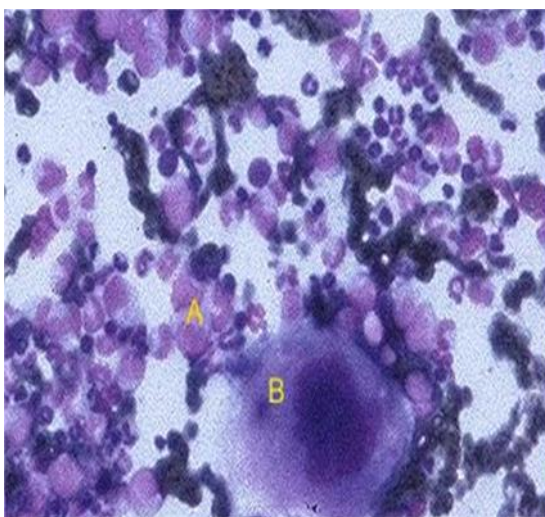
## Platelets (Thrombocytes)

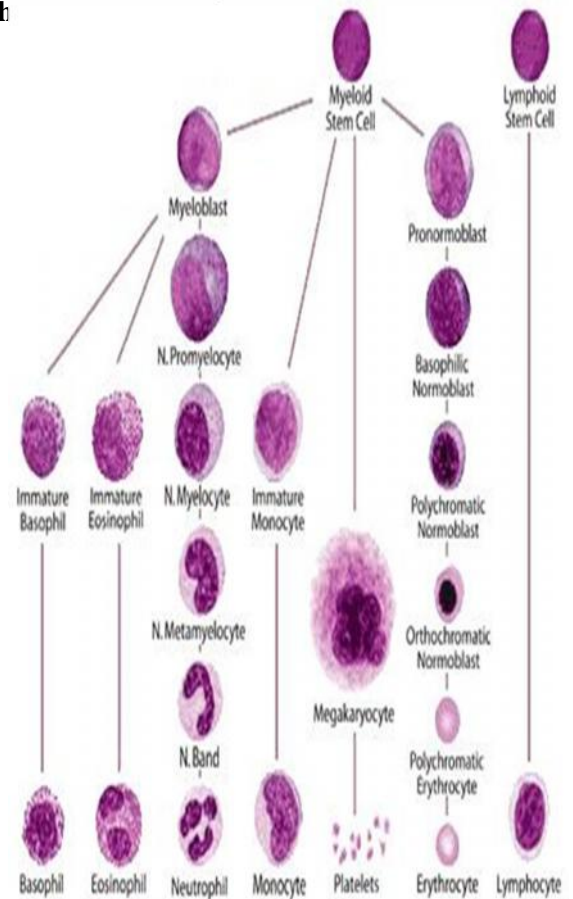
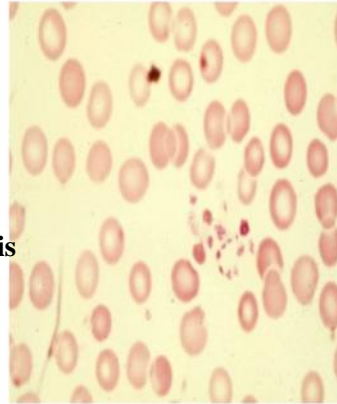
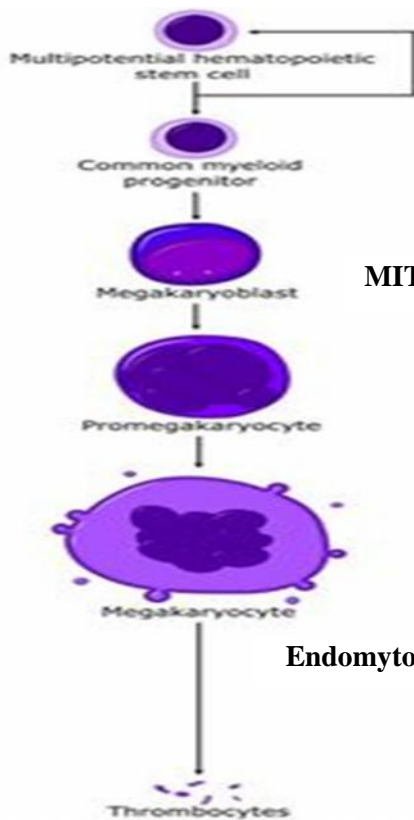
**Definition:** Platelets are cytoplasmic fragments of bone marrow megakaryocytes, they lack a nucleus and are round, oval or spindle-shaped may have multiple cytoplasmic projections. They average 3-5  $\mu\text{m}$  in diameter in most species, feline platelet are most variable in size and may be as large as an RBCs, equine platelets stain faintly with Romanowsky stains. The megakaryocyte is a giant cell and pieces of its cytoplasm and cell membrane bud off to form the thrombocytes. One megakaryocyte can produce up to 6000 thrombocytes.

Similar to erythropoietin, thrombopoietin (TPO) regulates the production of thrombocytes by stimulating CFU-Meg. to mature into megakaryocytes. TPO is mainly produced in the liver and is regulated by a negative feedback system. It binds to TPO receptors on both the megakaryocytes and platelets; if these are in high concentrations in the blood plasma then the level of TPO is kept low, reducing the maturation.

Cells of the megakaryocytic system are peculiar in that the nucleus undergoes multiple mitotic divisions without cytoplasmic separation (Endomytosis). The multiple nuclei usually remain attached to each other and are often superimposed giving a lobular appearance. The cytoplasm undergoes maturation changes characterized by the development of granules and membranes, culminating in platelet differentiation and liberation.

B- is a megakaryocyte in a bone marrow smear





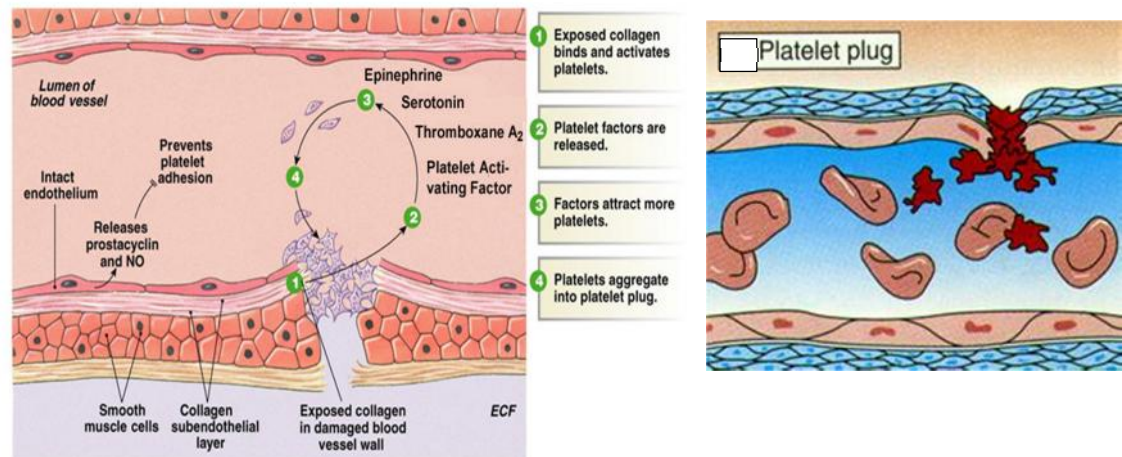
Platelet circulation life span is from 5-9 days in most animals. They stain pale blue with purplish granules, their cytoplasm contain a contractile protein (thrombasthenin) and three types of membrane - bound cytoplasmic granules:

**Function:** Platelets are essential for haemostasis.

1-It helps in vasoconstriction of injured blood vessel through the release of **vasoconstrictors** as **serotonin, ADP and thromboxane A2**.

2-Formation of platelet plug to close the injured blood vessel, 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. **It involves 4 steps:** **a-Platelet adhesion:** It depends on vWF which has two binding sites one to platelets and the other to injured sub-endothelial collagen within seconds. Initial adhesion of some receptors to subendothelial collagen( as gpIa/IIa with collagen

and gpIb/IIIa with vWF) does not need previous activation. However, binding to collagen initiates **platelet activation** which can be produced also by ADP and thrombin. When platelets adhere and activated their shapes transform from discoid to spheres with filapodia, this adherence & change in shape forms the primary haemostatic plug that controls bleeding from minute vascular injuries.



Platelets can be activated by a number of substances including collagen, thromboxanes, adenosine diphosphate and thrombin. One striking feature of activation is the change in platelet morphology from an ovoid disk to an amorphous form with projecting fingers.

**b-Platelet release:** After adhesion contractile protein contracts forcefully and causes the release of granular contents of platelets as fibrinogen, ADP, Ca<sup>++</sup>, vWF, thrombospondin, enzymes are activated and thromboxane-A<sub>2</sub> is generated from membrane phospholipids, it diffuses from platelets to membrane receptors and amplifies platelet activation.

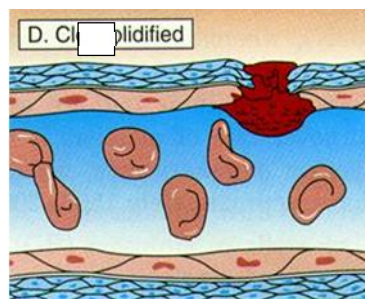
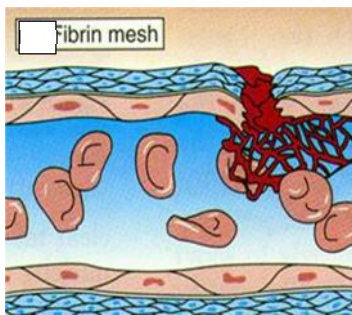
**C-Platelet aggregation:** The ADP and thromboxane A<sub>2</sub> (TxA<sub>2</sub>) released from activated platelets act on nearby platelets and activate them so they become sticky and adhere to the originally activated platelets, release of more ADP and TxA<sub>2</sub> which in turn activate more platelets and thus a vicious cycle of activation and aggregation result in the formation of a platelet plug to close the injured blood vessel (temporary haemostatic plug).



Soon, under the influence of TxA<sub>2</sub> and ADP, platelets contract and a mass of irreversibly aggregated platelets is produced. Aggregated platelets make available PF<sub>3</sub> which is a phospholipid complex that is exposed or activated on platelet surfaces.

**D. Coagulation:** Coagulation is the process by which a blood clot forms to reduce blood loss after damage to a blood vessel.

Every step in the coagulation sequences requires PF<sub>3</sub>, so thrombin will accumulate on platelets surface and acts as a potent stimulator for platelet aggregation.



### Platelets disorders : Abnormal Platelet Morphology:

- A. **Macrothrombocytes** : Platelets that are as large as erythrocytes or larger in diameter are called macrothrombocytes or macroplatelets. Cats have large, variably sized platelets, with some in normal cats as large as erythrocytes (see Fig. 9-1, E).

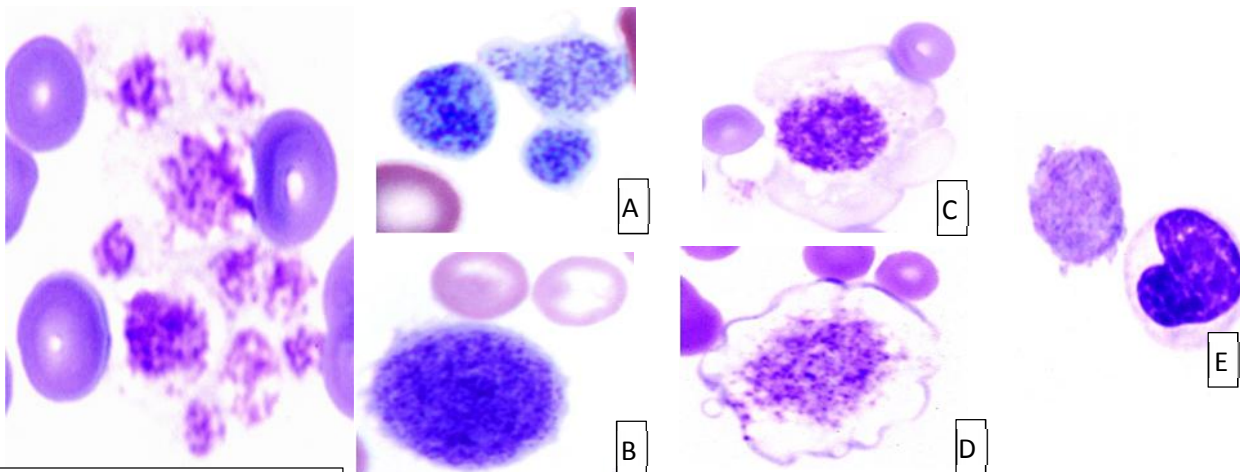


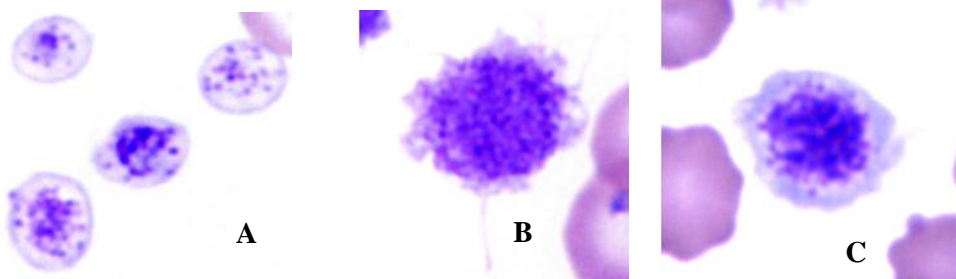
Fig. 9-1, E : Aggregate of platelets in blood from a cat demonstrating the presence of large platelets and the variation in platelet size that is characteristic of this species. Wright-Giemsa stain.

Macrothrombocytes in stained blood films. A, Macrothrombocytes in blood from a dog with a regenerative immune-mediated thrombocytopenia. The intense basophilia suggests that these may be reticulated platelets. B, Macrothrombocyte in blood from a dog with a thrombocytosis associated With chronic iron-deficiency anemia. C, Macrothrombocyte with aggregated granules,,which may be mistaken for a nucleus in blood, from a cat with an abdominal abscess and toxic left shift in, the blood. D, Macrothrombocyte with centrally located granules<sup>4</sup> in blood from a cat with myelodysplastic syndrome. E, Macrothrombocyte (left) and metamyelocyte (right) in blood from a dog with chronic myeloid leukemia. Wright-Giemsa stain.

The Macrothrombocytes is present in different cases as

- Thrombocytopenic animal
- Thrombocytopenic animals with myeloid neoplasms.
- Cats with FeLV infections.
- Present in nonthrombocytopenic animals that have recently recovered from thrombocytopenia

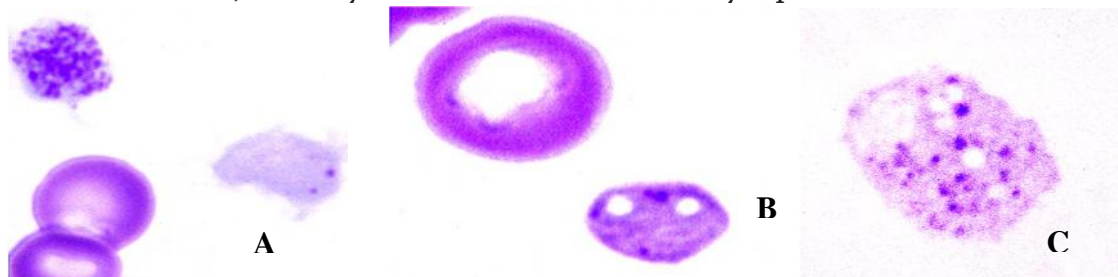
B. Activated Platelets:



**FIGURE 8-(A,B,C)**

Resting versus activated platelet morphology in stained blood films. **A**, Unstimulated platelet morphology in blood from a dog. Platelets appear as round to oval discs without filopodia formation or centralized granules. **B**, Activated platelet in blood from a dog with prominent filopodia formation. **C**, Activated platelet in blood from a cat with centralized granules and filopodia formation.

**C. Hypogranular Platelets:** Hypogranular platelets may result from platelet activation and secretion, but they have also been seen in animals with myeloid neoplasms (Fig. 10 A-C). Hypogranular platelets must be differentiated from cytoplasmic fragments from other cells, as may occur with leukemic lymphomas

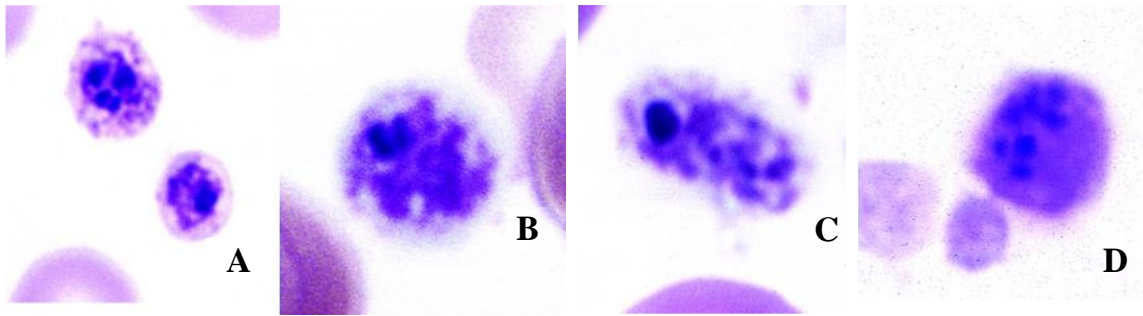


**FIGURE 10 (A,B,C)**

Hypogranular platelets in stained blood films. **A**, Platelet with granules (*top left*) and a hypogranular platelet (*right*) in blood from a dog with chronic myeloid leukemia. **B**, Hypogranular platelet in blood from a dog with erythroleukemia (AML-M6Er). **C**, Hypogranular macrothrombocyte in blood from a dog with chronic myeloid leukemia. Wright Giemsa stain.

**D. Anaplasma platys Infection:** *Anaplasma platys* (previously *Ehrlichia platys*) is a rickettsial parasite that specifically infects platelets and causes infectious cyclic thrombocytopenia in dogs. This agent is unique in *A. platys* organisms appear as blue inclusions in platelets when blood films are stained with Wright-

Giemsa or new methylene blue (Fig. 11, A-D). Similar-appearing inclusions have been seen in platelets from a cat



**FIGURE 11(A,B,C,D)**

*Anaplasma platys* morulae in platelets from dogs. **A-C**, Platelets each containing an *A. platys* morula, which stain dark-blue, in contrast to the normal magenta-staining granules. Wright-Giemsa stain. **D**, Platelet containing an *A. platys* morula, with multiple subunits visible. New methylene blue wet mount preparation.

## **Quantitative platelet function defect:**

**Thrombocytopenia:** It means decrease in the number of circulating platelets below the lower normal level for the particular species. It can be caused by:

### **1-Increase in platelet destruction:**

**a-Immune-mediated** : An agent sensitize the platelets, attached to it as a hapten and abs are formed against the platelet- agent complex, it may be primary or idiopathic of unknown primary cause, or secondary to other diseases like autoimmune diseases , drugs, lymphoproliferative disorders, viral infections or toxic agents.

**b-Non- immunological:** Haemolytic diseases of newborns, malignancies, drugs(heparin), splenomegaly (sequestration in enlarged spleen).

### **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.

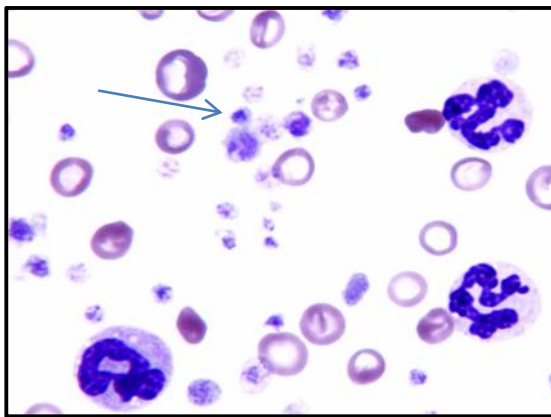
**c-Drugs** like estrogen, thiazide (diuretic) and myelosuppressive drugs.

**d- Viral and rickettsial infections** as last stages of ehrlichiosis, FeLV, FIV, EIA, BVD and others.

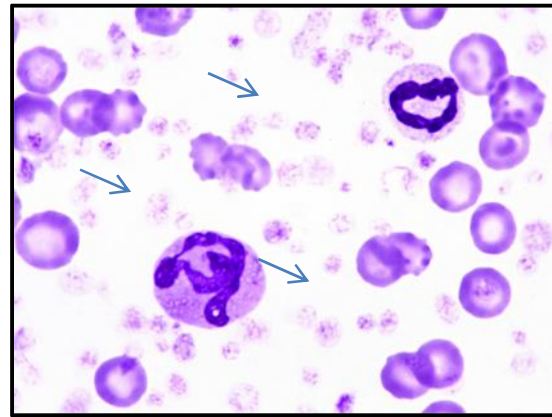
**3-Sequestration:** Of platelets in spleen, lung or liver caused by endotoxaemia or in hypersplenism, together with wbc's and rbc's.

**4-Loss of platelets:** As in massive haemorrhage.

**Thrombocytosis:** Increase in the number of circulating platelets above the maximum normal range for the particular species. It may be caused by:



Thrombocytosis in blood from a dog with chronic iron-deficiency anemia. Smaller hypochromic erythrocytes and several larger polychromatophilic erythrocytes are present. A monocyte (bottom) and two neutrophils are also present. Wright-Giemsa stain.



Marked thrombocytosis in blood from a dog diagnosed with essential thrombocythemia. A basophil (bottom) and neutrophil (top right) are also present. Wright stain.

**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-Increased 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). hemorrhage, splenectomy, excitement, fractures, high circulating glucocorticoid concentrations, myelofibrosis, and iron deficiency anemia.

## **Qualitative platelet function defect:**

**1. Congenital extrinsic platelet dysfunction:** An important example is Von Willebrand Disease (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 Vwf synthesis will lead to bleeding disorders like mucosal haemorrhages (epistaxis, bleeding from GIT, Haematuria, prolonged bleeding from wounds and increased cutaneous bruising, petechiae are not seen. **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 diluted coat colour and may experience prolonged bleeding and haematoma formation at venipuncture.

**b. Thrombasthenia (Glanzmann's ):** It is a defect in or deficiency of certain glycoproteins in the receptors on platelets surface (IIb, IIIa) 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.

**c. Cyclic haematopoiesis of grey collies** is associated with fluctuation of platelet count on a 12-14 days cycle(with neutrophils) and qualitative defects in the function of platelet . Platelet count is seldom below the reference range.

## **3. Acquired qualitative functional disorders:**

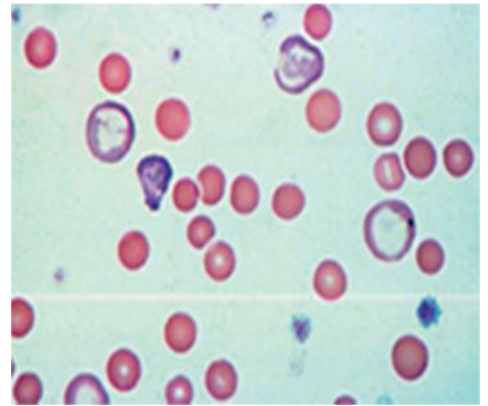
**a. Hyporesponsive platelets:** It is caused by different agents as drugs (Aspirin, NSAIDs, Penicillin and others), uraemia, liver diseases, infection(FelV, *Ehrlichia canis*), paraproteinaemia of plasma cell myeloma and some snake venoms.



**b. Enhanced platelet function(hyperactivity):** This may be observed in nephrotic syndrome due to (hypoalbuminaemia), erythropoietin administration(increase in immature platelet which are hyperactive) and some infectious agents as FIP virus in feline and heart worm in dogs.

The presence of enlarged platelets which correlates with increased mean platelet volume (MPV), is supportive of an increased rate of thrombopoiesis in the bone marrow in response to a peripheral demand. This interpretation can be used with most species; however, in cats, enlarged platelets is a normal finding.

A blood film from a dog with immune-mediated hemolytic anemia. Note the enlarged platelets Also note the polychromasia, spherocytosis, ( (modified Wright's )stain X 100.

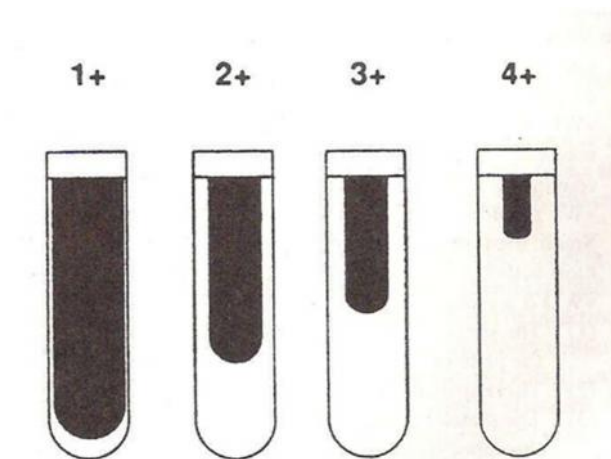


### Tests for evaluation of platelet function:

**1-Platelet count:** Direct and indirect method.

**2-Clot retraction test:**

- Take 1-2ml of blood from a normal animal and the patient without anticoagulant.
- Incubate for 2-4 hours in 37c (4-8 hours in 25c).
- Compare the amount of clot retraction between normal and diseased animal.



### 3-Bleeding time (Buccal mucosal bleeding time):

- a- Make a small and deep puncture in the buccal mucosa of the patient with a lancet.
- b- Record the time when first blood appear , remove drops of blood with filter paper being careful not to touch the skin.
- c- Record the time when no longer blood appear from the puncture site .Normal value ranges from 1-5 minutes in most domestic animals. Prolonged bleeding time indicates either platelet defect, von- WF disease or vascular lesions.



A: 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.

B: The simple device is triggered 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.

**Normal Range:** Two to four minutes.

**Interpretation:** Lip bleeding time is expected to be prolonged in patients with severe acquired or inherited platelet dysfunction or severe von Willebrand disease. 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

#### **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 presence of enlarged platelets which correlates with increased mean platelet volume (MPV), is supportive of an increased rate of thrombopoiesis in the bone marrow in response to a peripheral demand. This interpretation can be used with most species; 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.

**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.