CLINICAL PATHOLOGY

Clinical pathology: it means:

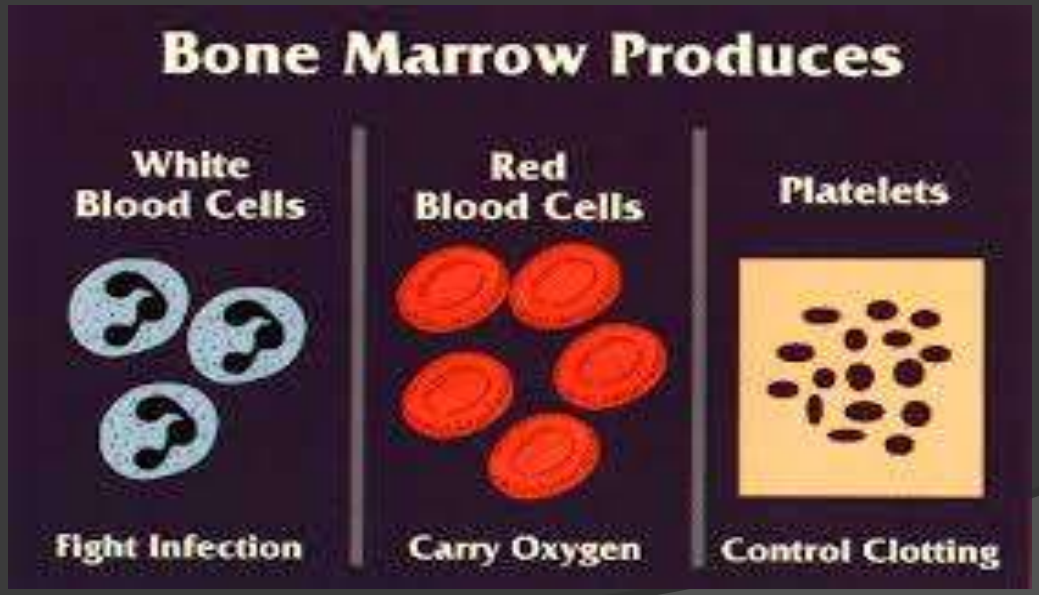
1. Application of different lab. methods on a properly collected sample.
2. To evaluate the health or to study the disease in the living or dead subjects.
3. The subsequent use of the obtained results in making accurate diagnosis and prognosis for the solution of the clinical problem.
4. It makes a link between patient, clinician and lab.

It includes:

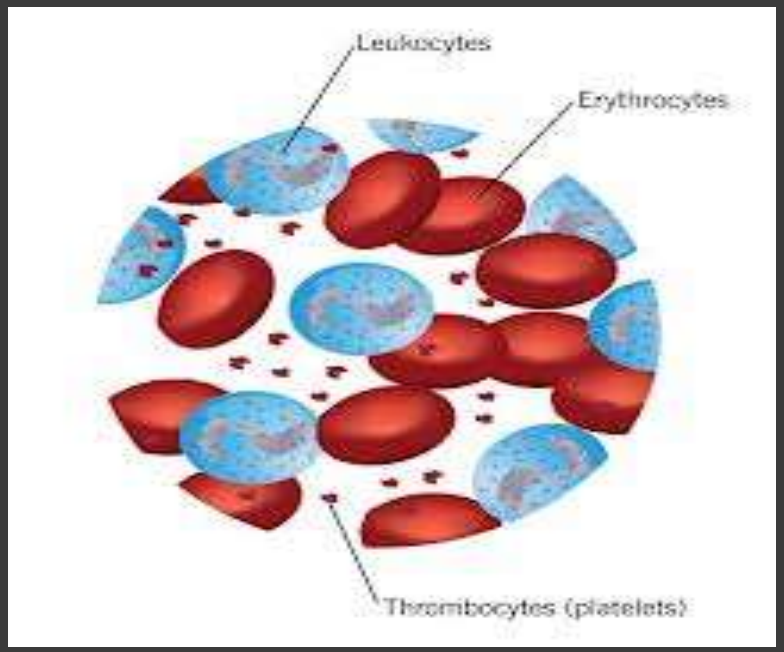
- Clinical haematology.
- Clinical biochemistry.
- Clinical parasitology.
- Clinical microbiology.

CLINICAL HAEMATOLOGY:

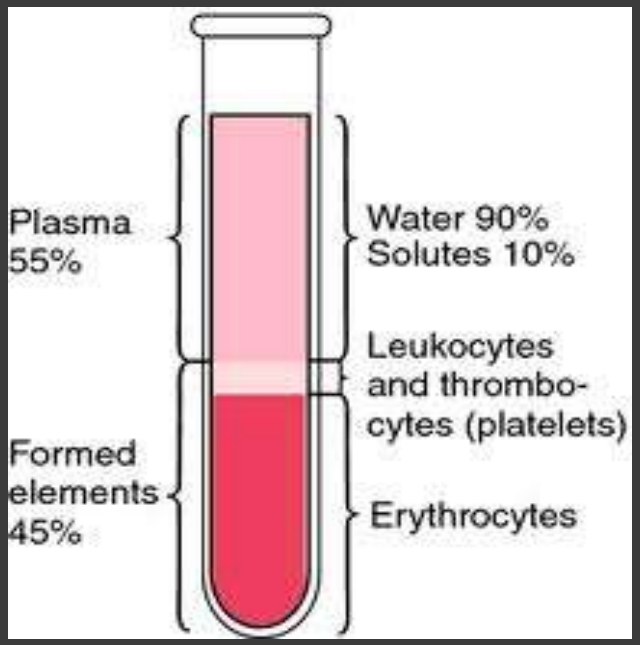
The study of blood and blood forming organs.



•Clinical Haematology Includes the evaluation of cellular components of the blood. which is about (45%) of total blood Volume,



the rest is plasma, the fluid part of the blood in which cellular components are suspended

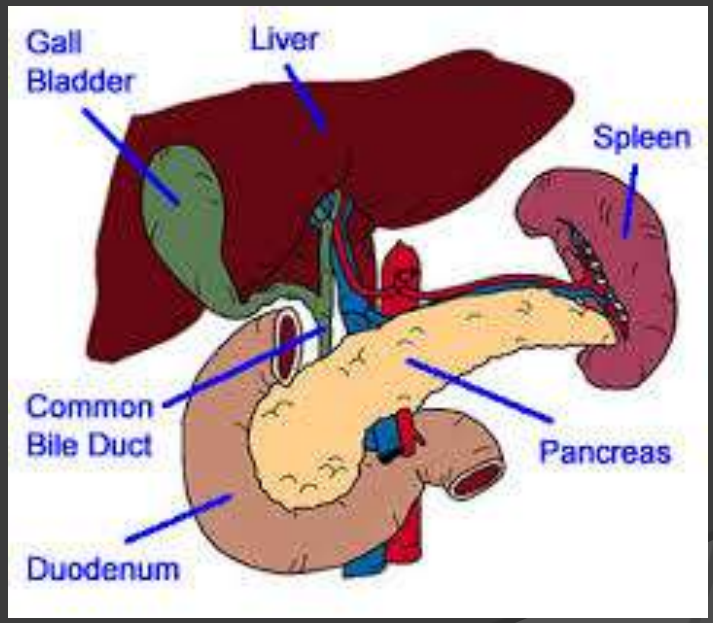


HAEMATOPOIESIS (HAEMOPOIESIS):

It is the process of blood cell production and platelets which continues throughout life, replacing aged cells (which are removed from the circulation).

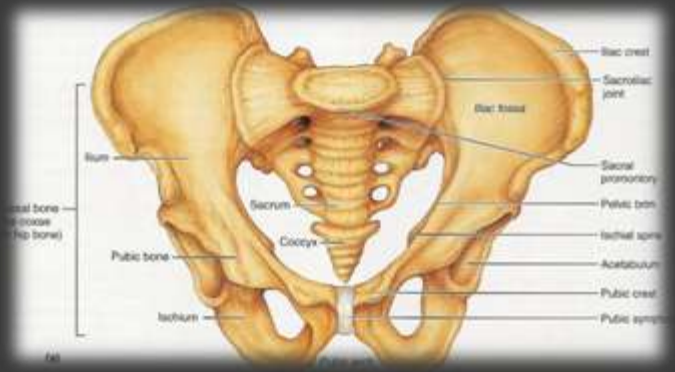
- At the time of birth nearly all blood cells are produced in the bone marrow (MEDULLARY HAEMATOPOIESIS) and haematopoiesis immediately or gradually stops in the liver and spleen .

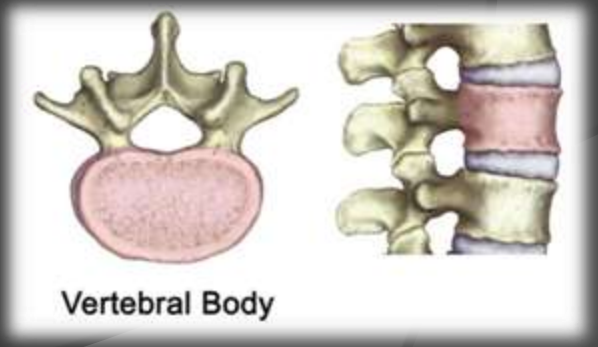
In certain disease conditions, when there is great need for blood cells these two organs (liver and spleen) retain their ability for manufacturing blood cells.



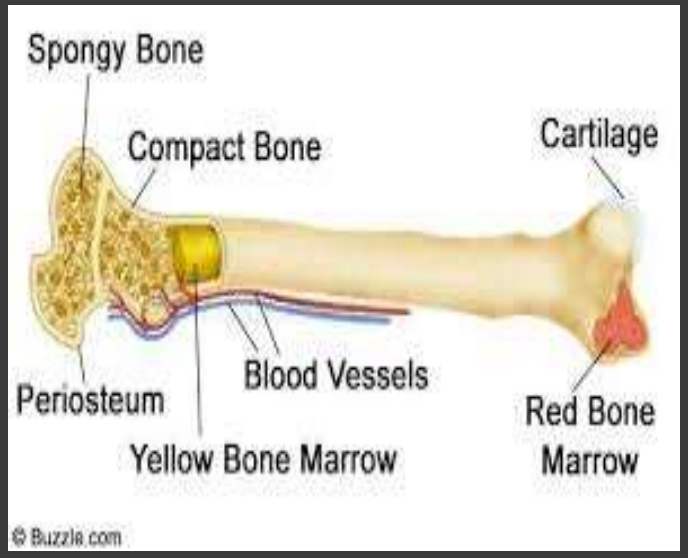
EXTRA MEDULLARY HAEMATOPOIESIS).

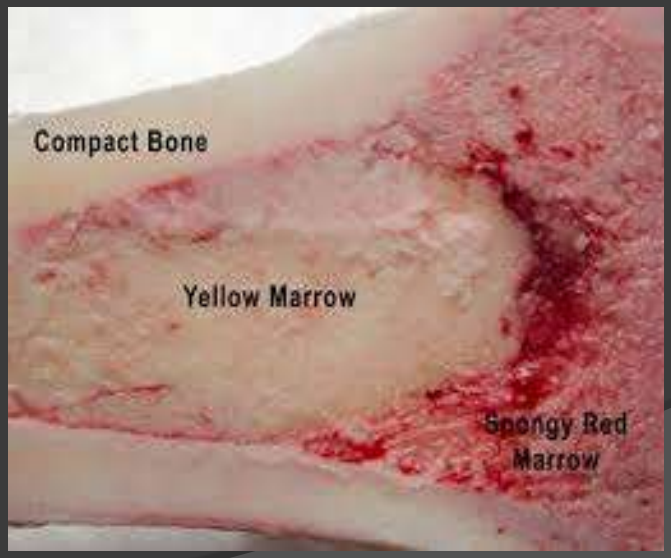
BONE MARROW: It is a large organ about two thirds of the liver size in dogs .In growing animals bone marrow of all bones is haematopoietically active, when growth stops haematopoiesis remains in the *marrow of flat bones (skull, pelvic bones, sternum, vertebrae and ribs) and* epiphysis of long bones it stops in the shaft (diaphysis) .



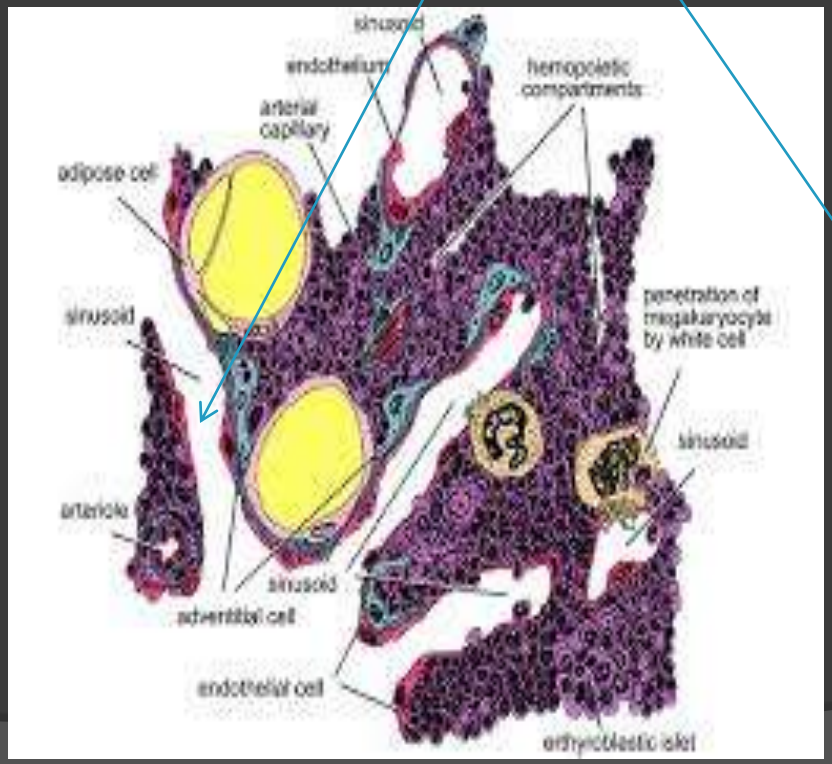


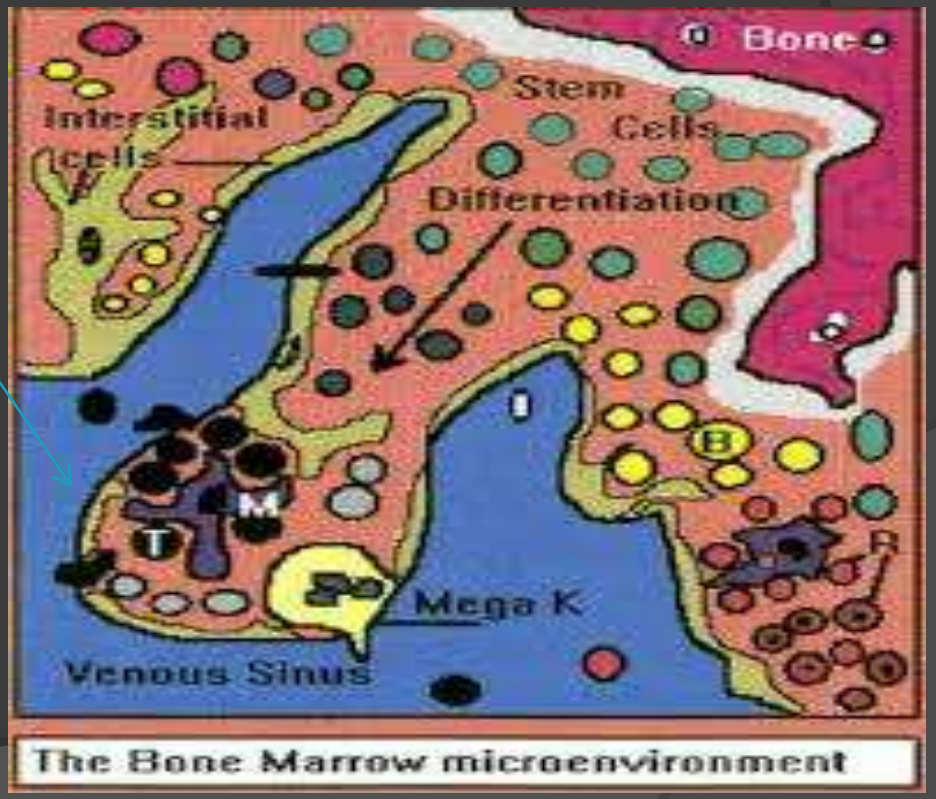
bone marrow, also called myeloid tissue, is a soft, gelatinous tissue that fills the cavities of the bones. Bone marrow is either red or yellow, depending upon the preponderance of hematopoietic (red) or fatty, inactive (yellow) tissue.





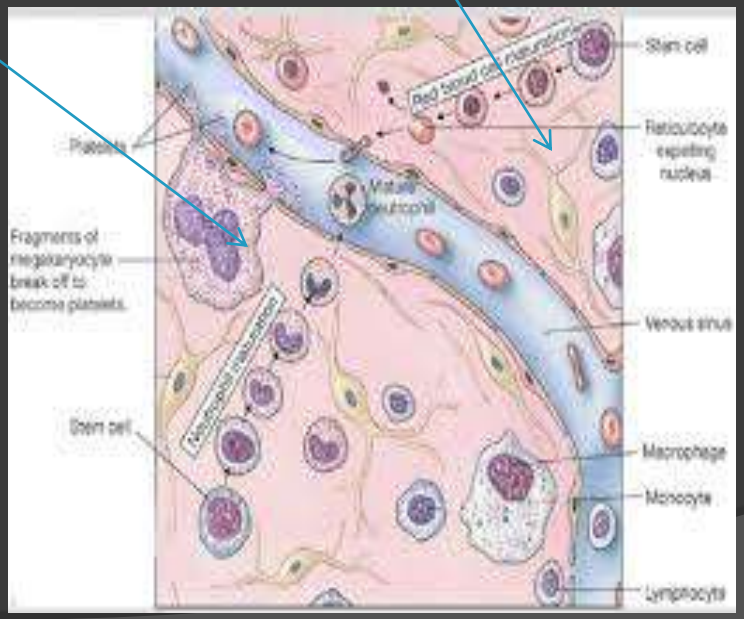
1.The vascular compartment: Composed of blood sinuses lined by special type of endothelial cells and crossed by special type of cells known as the ADVENTITIAL CELL or RETICULAR CELL together with macrophages both have important rules in regulating and maintaining haematopoiesis throughout life.





2.The extra –vascular compartment:

*It is the haemopoietic compartment containing precursors of all blood cells, macrophages, reticular cells(adventitial cells) which have many pseudopods that may completely encircle the developing blood cells.



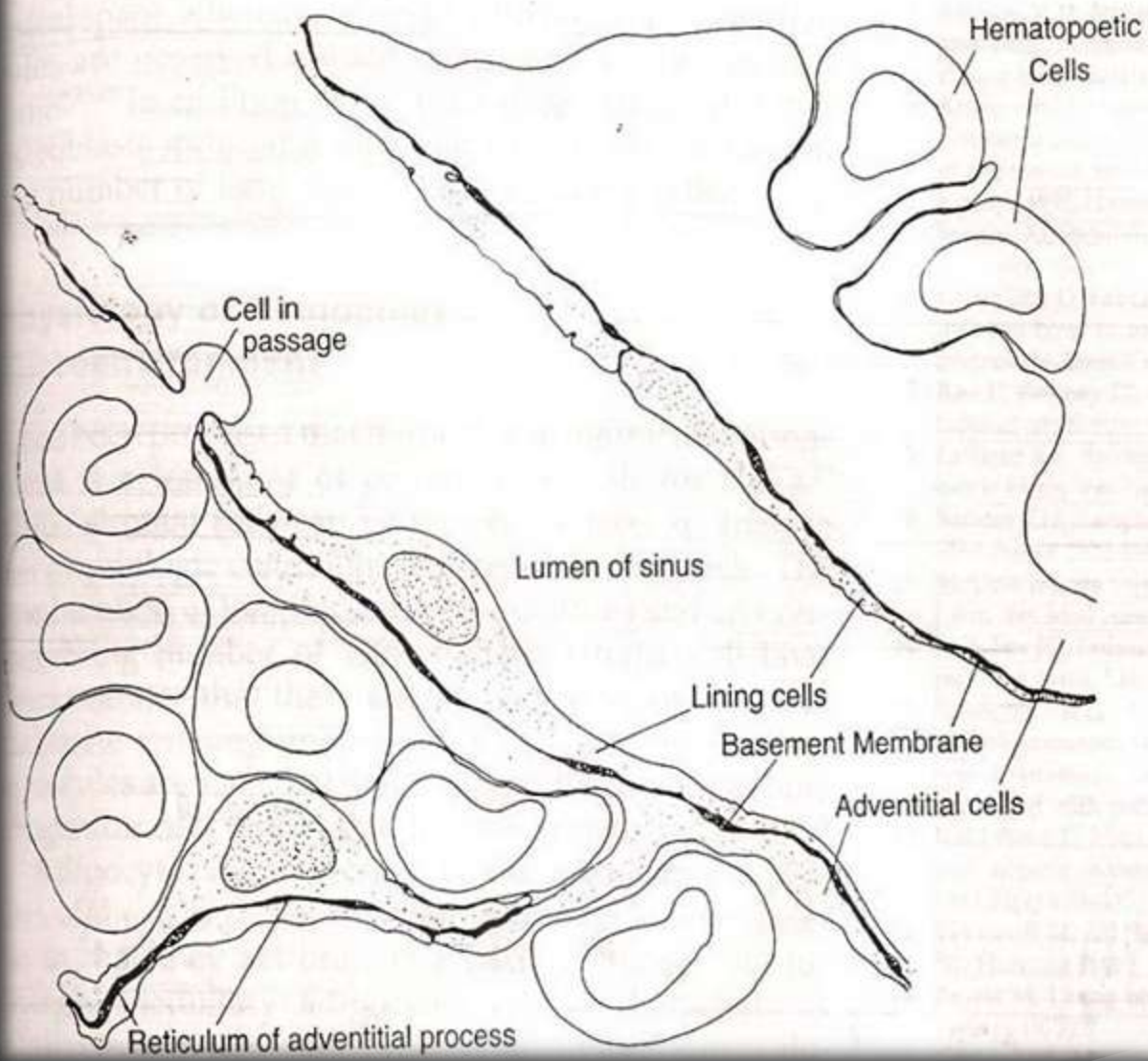


FIGURE 13.4 Segment of the wall of a vascular sinus, including an adventitial process. The sinus wall is trilaminar in most places, consisting of a lining (or endothelial) cell, a basement membrane, and an adventitial cell. In places apertures may occur in the wall, and free cells may pass through such apertures. Elsewhere, the basement membrane or the adventitial layer may be absent, resulting in a wall of one or two layers. The adventitial cells are commonly voluminous and rarefied, extending deeply into the contiguous HS and displacing hemopoietic cells. (The upper labeled adventitial cell exemplifies this.) An adventitial process is of the same structure as the sinus wall.

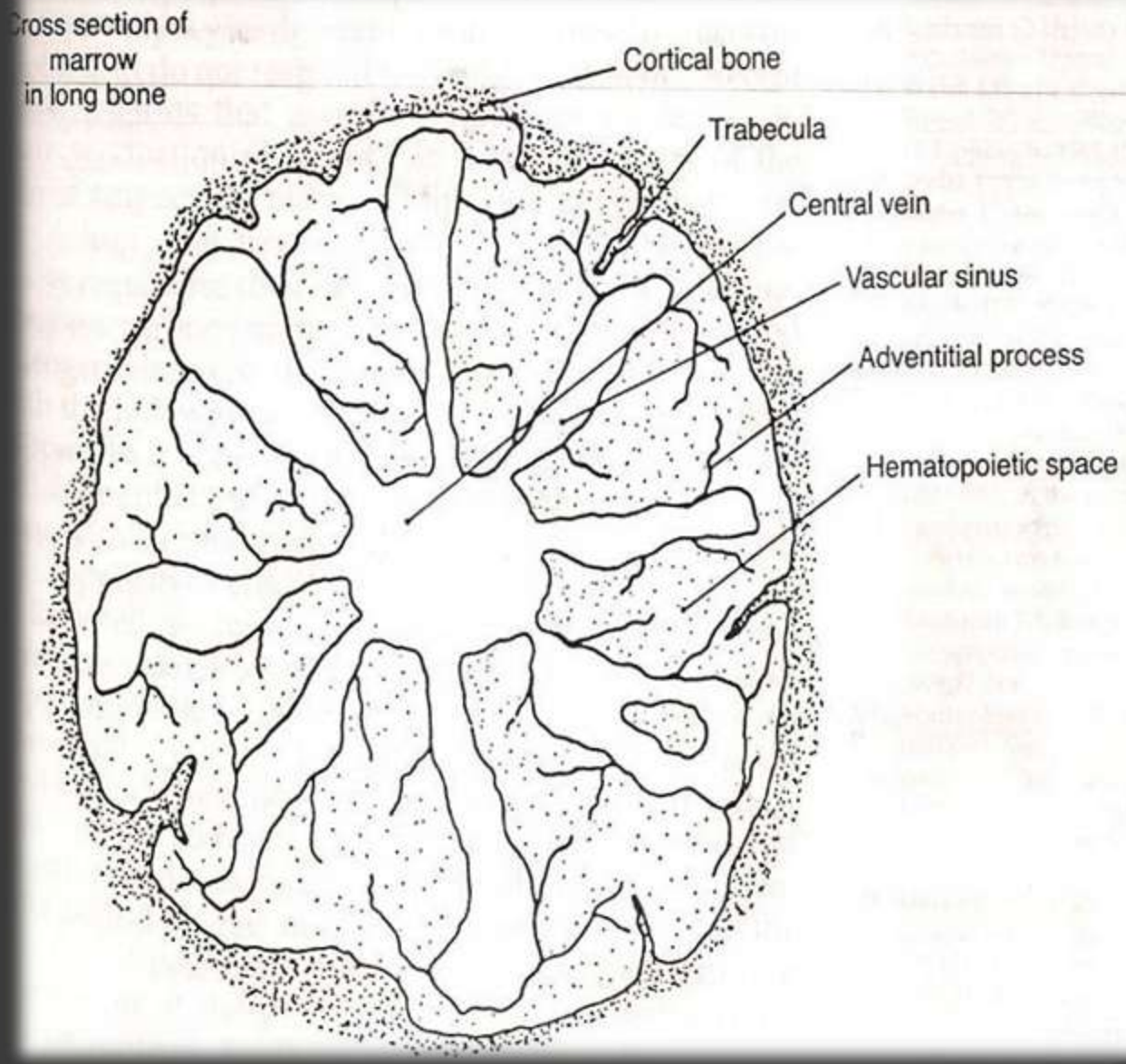
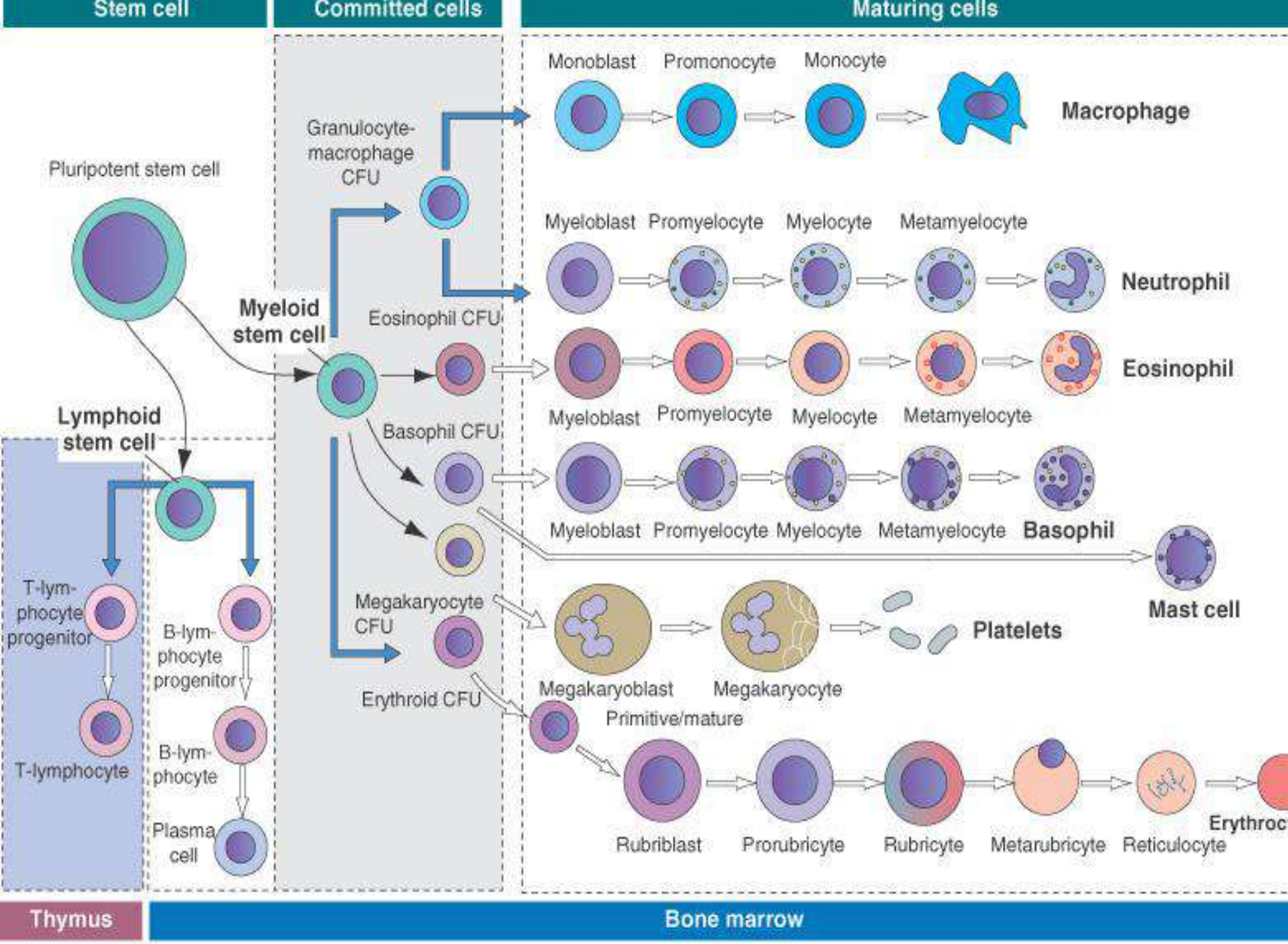


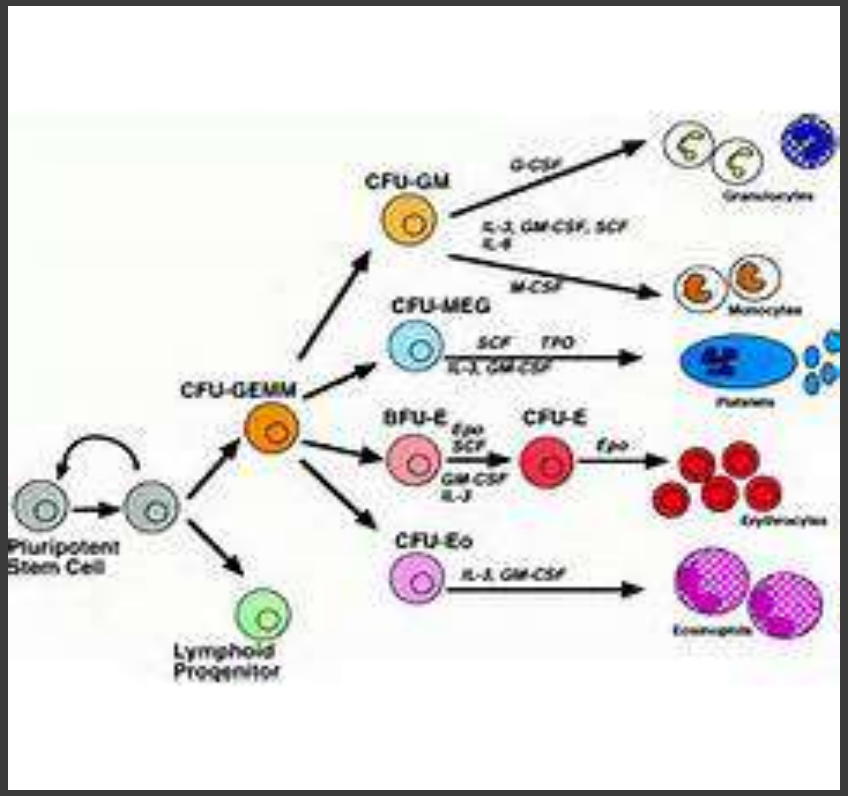
FIGURE 13.5 Organization of the venous vasculature of the marrow of a long bone. Thin-walled vascular sinuses originate at the periphery from termination of transverse branches of the nutrient artery (not shown). The vascular sinuses run transversely toward the center to join the CV. Hemopoiesis takes place in the space between the vascular sinuses. Adventitial processes project into the HS, producing partial compartmentalization.

- Bone marrow examination: The red bone marrow forms all of the blood cells with the exception of the lymphocytes, which are produced in the marrow and reach their mature form in the lymphoid organs.



(From Kierszenbaum AL: Histology and cell biology: an introduction to pathology. St Louis, 2002, Mosby.)

HAEMATOPOIESIS



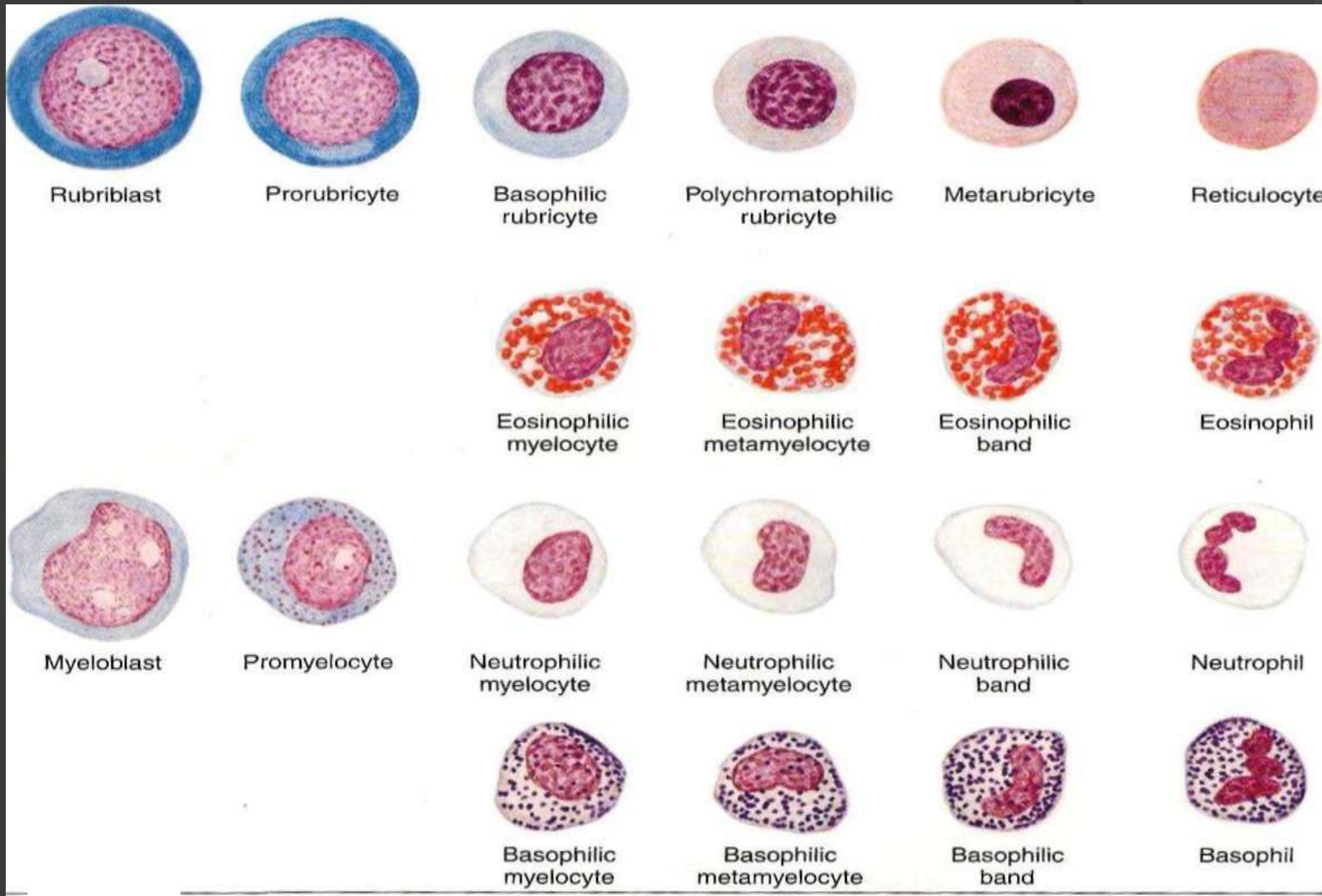


Plate 19 Maturation of canine erythroid and granulocytic cells as they appear in Wright-Giemsa–stained bone marrow aspirate smears. (Drawing by Dr. Perry Bain.)

Clinical evaluation of the hematopoietic system

Some components easily carried:

1. Complete blood count(CBC).
2. Blood smears.
3. Peripheral lymph node aspirates.

Other components require more invasivecomplicated techniques:

4. Bone marrow aspirates and biopsies.
5. Biopsies: lymph nodes, spleen and bone marrow (core).
6. Necropsy: useful for lymphoid organs, less so for marrow.

Bone marrow examination:

It is indicated when peripheral blood abnormalities are detected as:

a. Unexplained continuous decrease in the number of blood cells as:* Persistent neutropenia; *unexplained thrombocytopenia;* poorly regenerative anaemia or a combination of all (Pancytopenia).

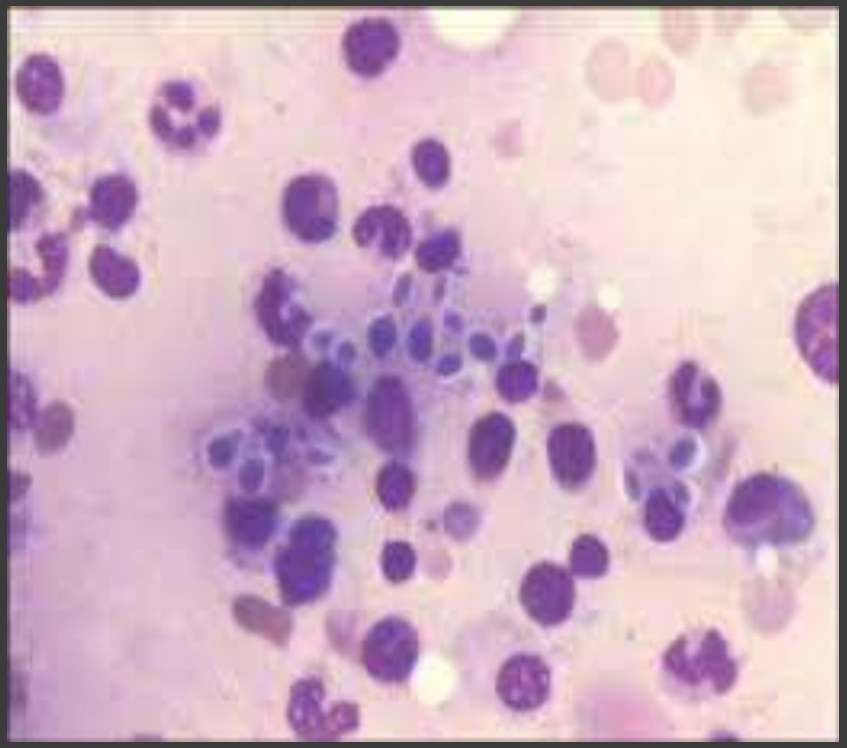
b. Unexplained continuous increase in the number of blood cells or proliferative abnormalities as

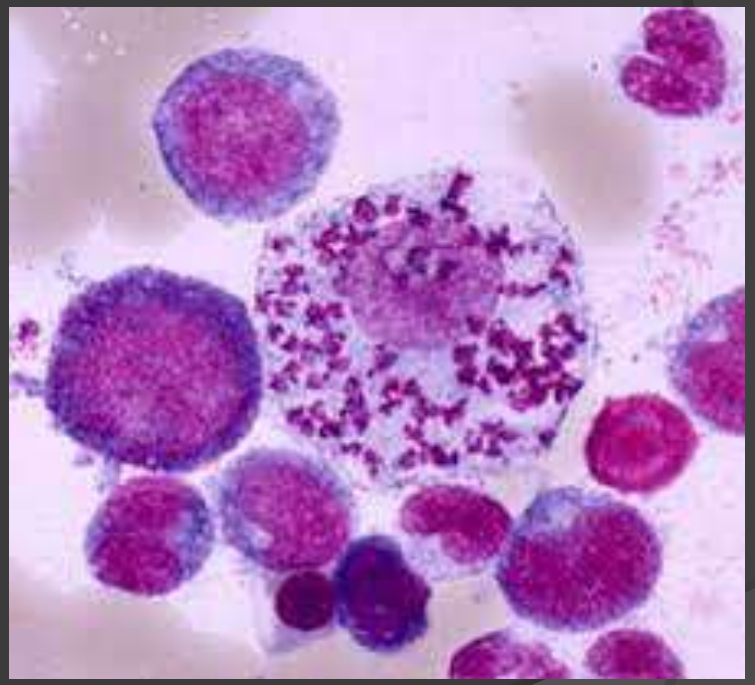
*persistent thrombocytosis; *leukocytosis;*abnormal morphology of RBCs or *unexplained presence of immature cells in the blood.

3. Stage a neoplastic condition as lymphomas , mast cell tumours.
4. Estimate the adequacy of body iron stores.
5. Search for occult disease in animals with fever of unknown origin or unexplained weight loss.

6. Looking for organisms that cause systemic infection as:

– Histoplasma– Leishmania – Cytauxzoon





7. Looking for occult neoplasia.

Technique for bone marrow biopsy:

Site of collection:

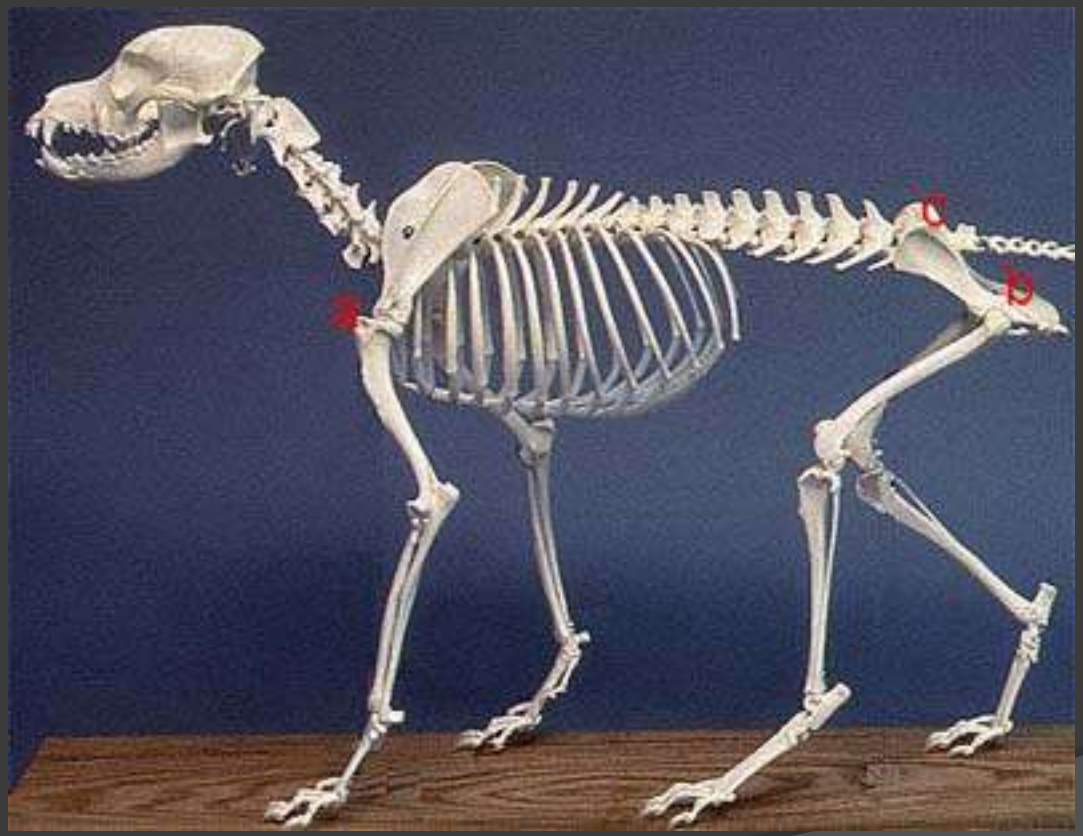
Dogs and cats: (a) the proximal humerus.

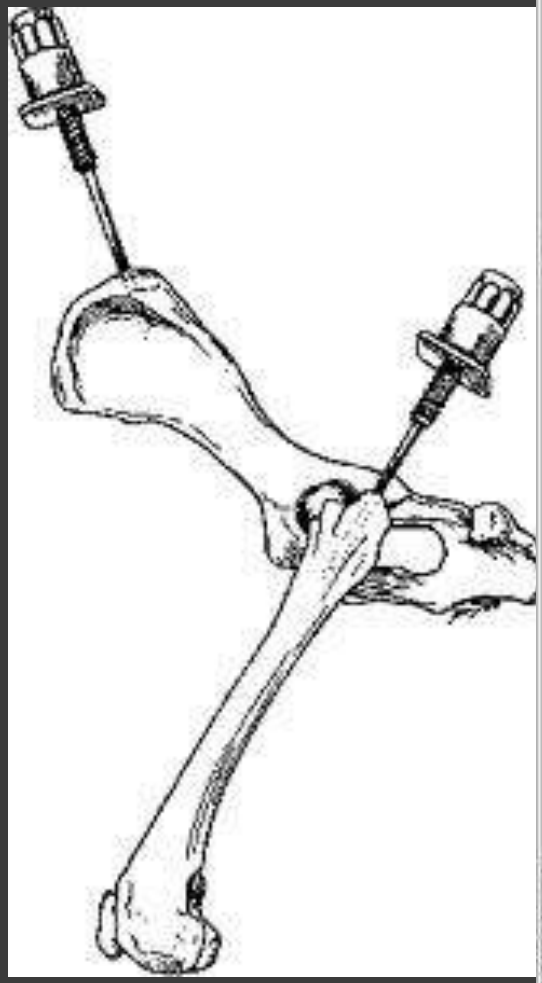
(b) proximal femur.

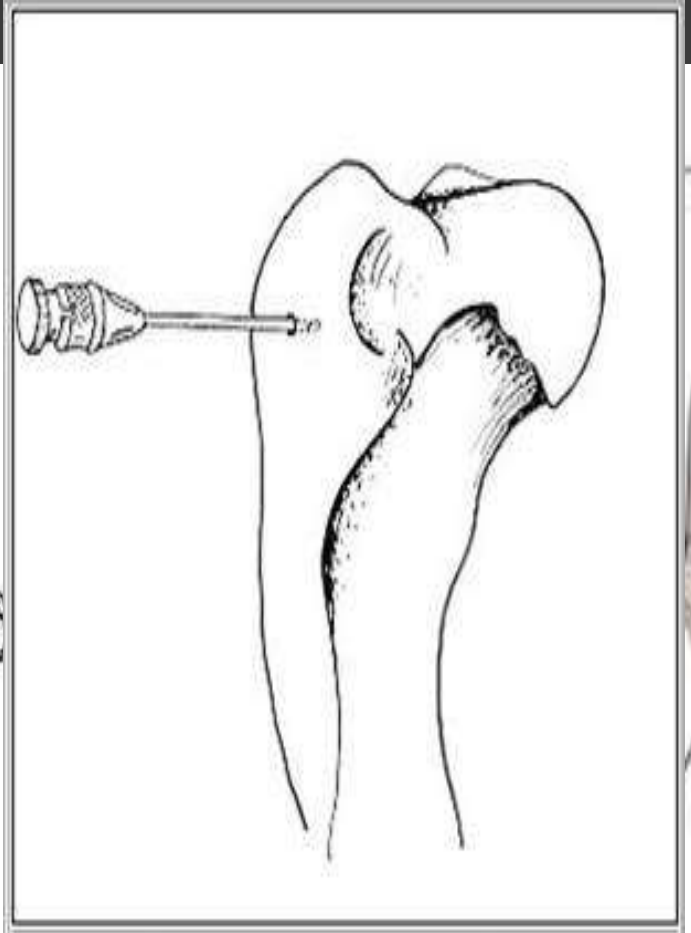
(c) The wing of the ilium.

- The wing of the ilium may be "out of reach" of the bone marrow needle in obese or large dogs. The same applies to the proximal femur.

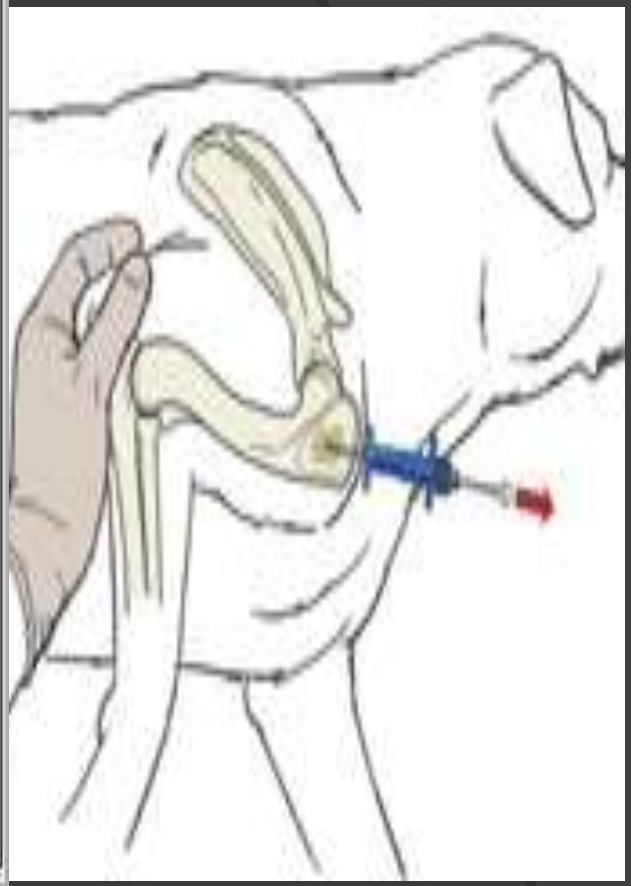
Collection sites. (A) The greater tubercle of the proximal humerus. (B) The trochanteric fossa of the proximal femur.
C) The iliac crest of the pelvis.







Bone marrow aspiration from the proximal humerus. (From Morgan RV: Selected diagnostic and therapeutic procedures. P. 17. In Morgan RV (ed): Handbook of Small Animal Practice. 3rd Ed. WB Saunders, Philadelphia, 1997, with permission.)



Bone marrow aspiration from a rib. (From Morgan RV: Selected diagnostic and therapeutic procedures. P. 17. In Morgan RV (ed): Handbook of Small Animal Practice. 3rd Ed. WB Saunders, Philadelphia, 1997, with permission.)

Jamshidi needle for obtaining a core.

Rosenthal aspiration needle - (16g x 1 - 5/16").

Preparation of Animal

- Most bone marrow aspirates can be obtained with use of a local anesthetic and mild sedation.
- The position of the patient during the procedure depends on the site being aspirated or biopsied.
- Hair is shaved from the site, and the skin is scrubbed as for a sterile procedure. A local anesthetic, such as lidocaine, is injected into the area to be aspirated

With the stylet in place, align the needle along the long axis of the humerus and perpendicular to the cortical surface of the greater tubercle. To penetrate the cortex, apply forward pressure while rotating the needle clockwise and counterclockwise.

Description of Technique

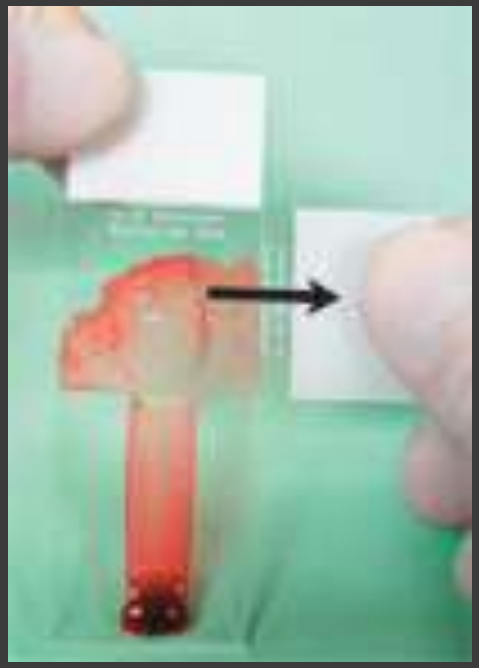
*A small incision is made in the skin. The biopsy needle is advanced through this incision,

*The biopsy needle is inserted and, with firm pressure and a clockwise-counterclockwise motion, inserted through the cortical bone. The stylet is removed and a syringe applied (2-20ml) Negative pressure is applied to the syringe until blood appears in the tip of the syringe.

- A sample of marrow is aspirated into the syringe attached to the bone marrow needle and submitted for analysis, few drops of EDTA are added to the syringe to prevent clotting of the bone marrow aspirate.

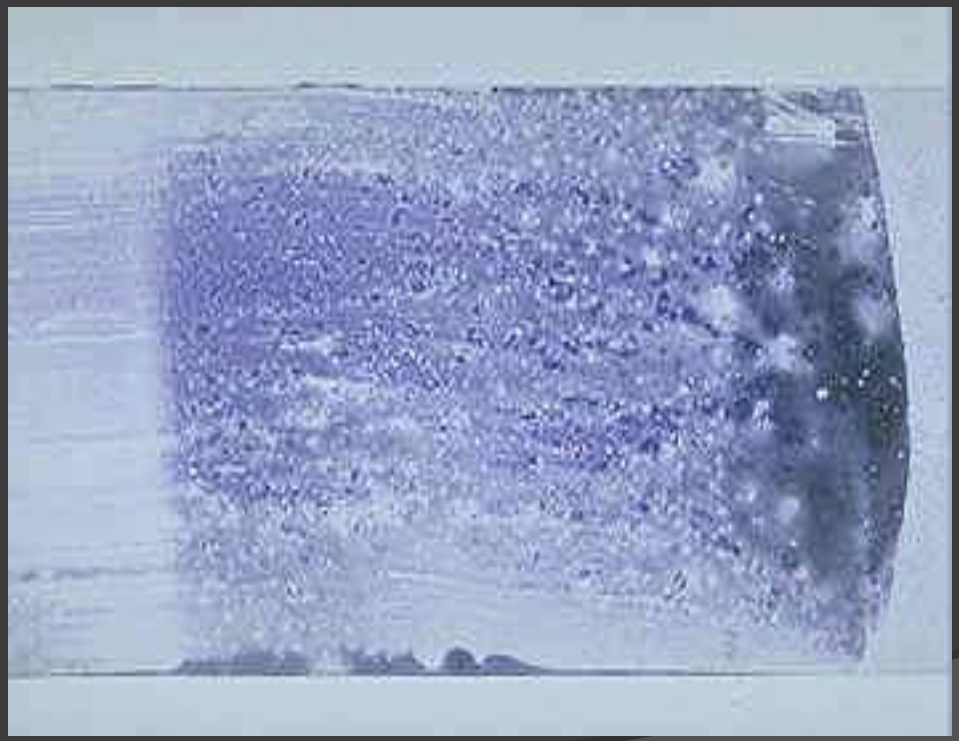
- Once a good sample has been obtained, the needle is removed, and the small incision in the skin is sutured, glued together, or left to heal by itself.
- The biopsy needle used to aspirate bone marrow must have a removable stylet that remains in place until bone marrow cavity is entered to prevent obstruction of the needle lumen with bony tissues.

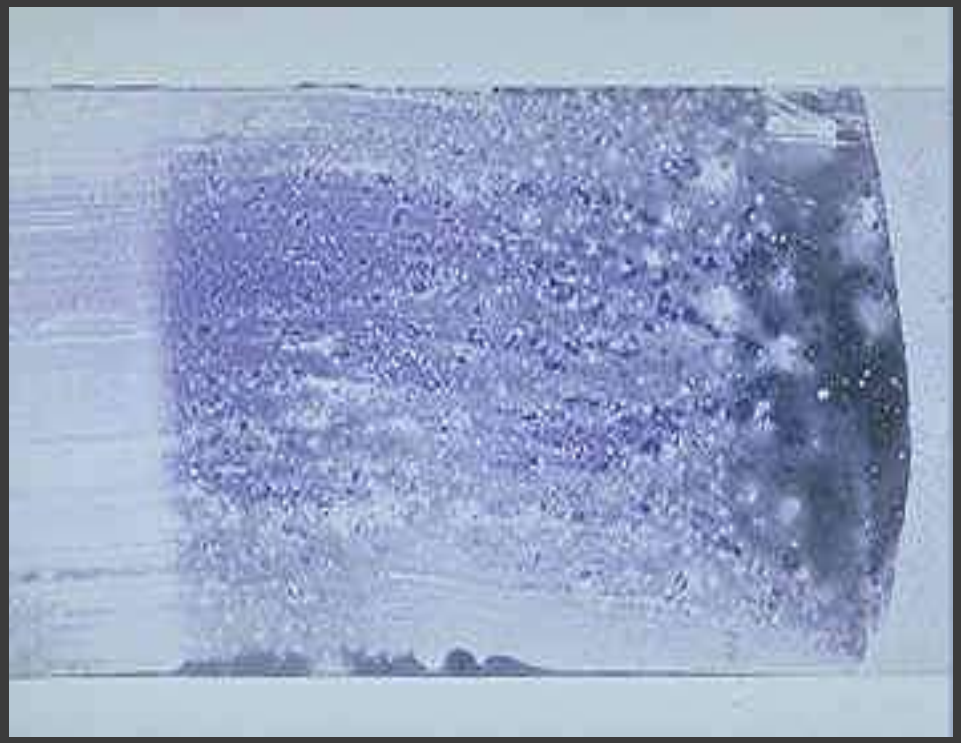
- a- Express a small amount of marrow onto a slide, and tilt the slide to allow excessive blood to flow away from the spicules.



b -Place a second slide on top of the spicules perpendicular to the first slide, and gently squash the spicules as you pull the second slide across the first, and use it to gently squash the marrow particles or spicules. Gently pull apart the two slides, spreading the marrow spicules across the slides

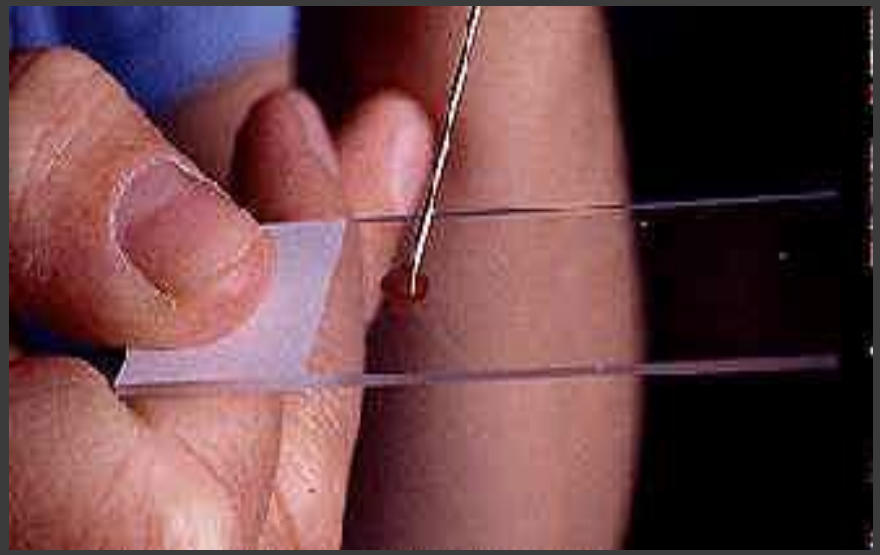
c- Allow the smears to dry before fixing with methyl alcohol and staining with appropriate stain.



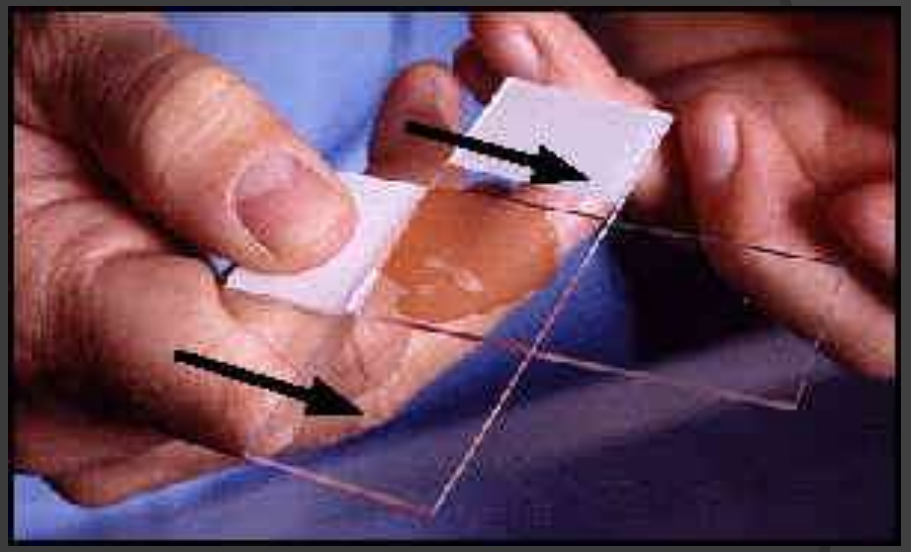


smear of an aspirate of bone marrow

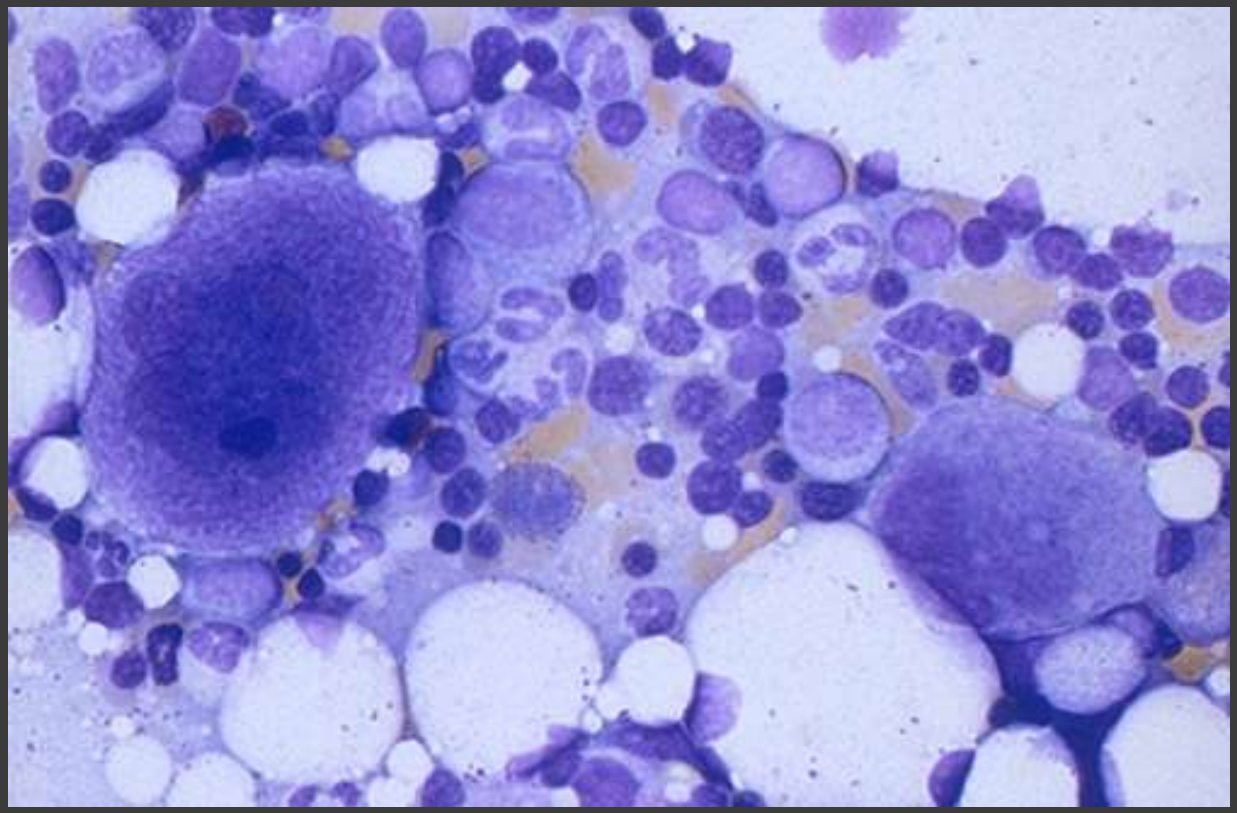
a core sample of bone marrow



Place a small drop of marrow on each slide.



The top slide is drawn in this direction.

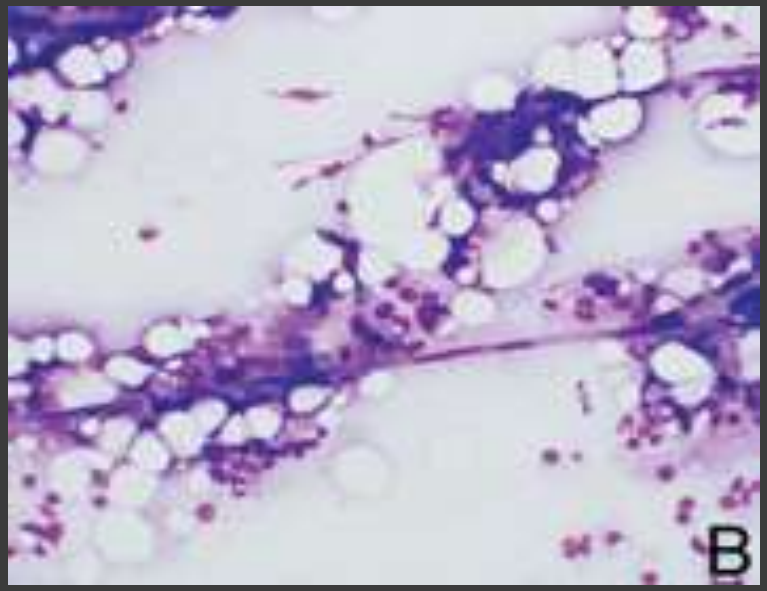


Bone marrow: Microscopic evaluation

Bone marrow aspirate/smears:

Important for:

- Cellularity and Cellular morphology
- Erythroid to myeloid ratio
- Primary or metastatic neoplasia



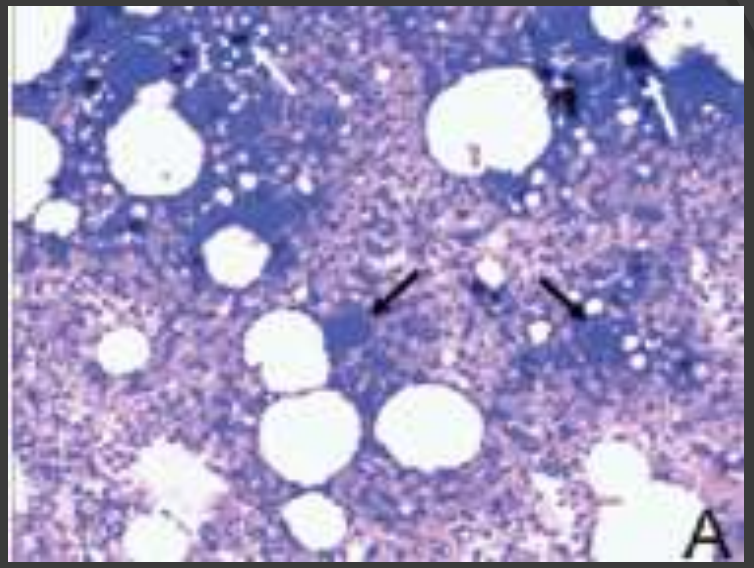
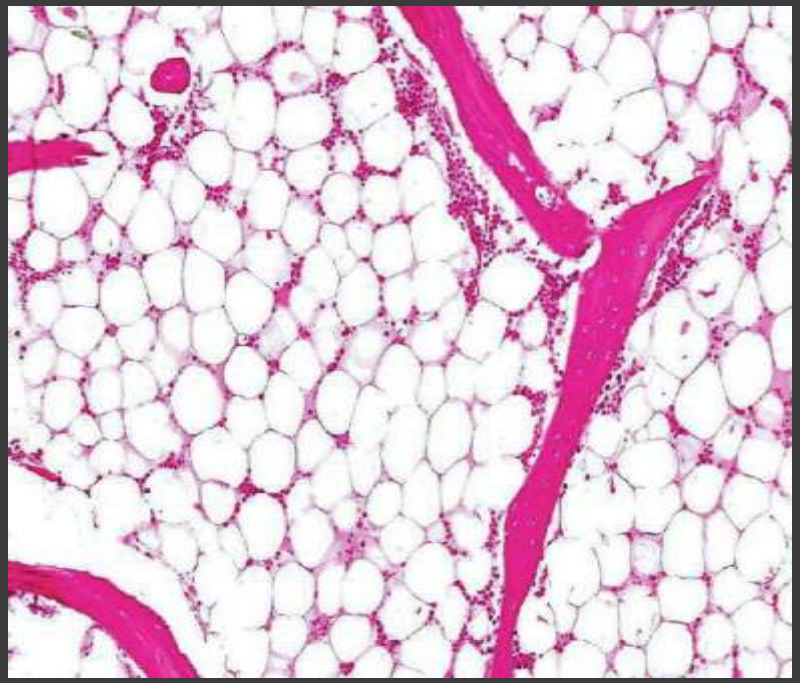
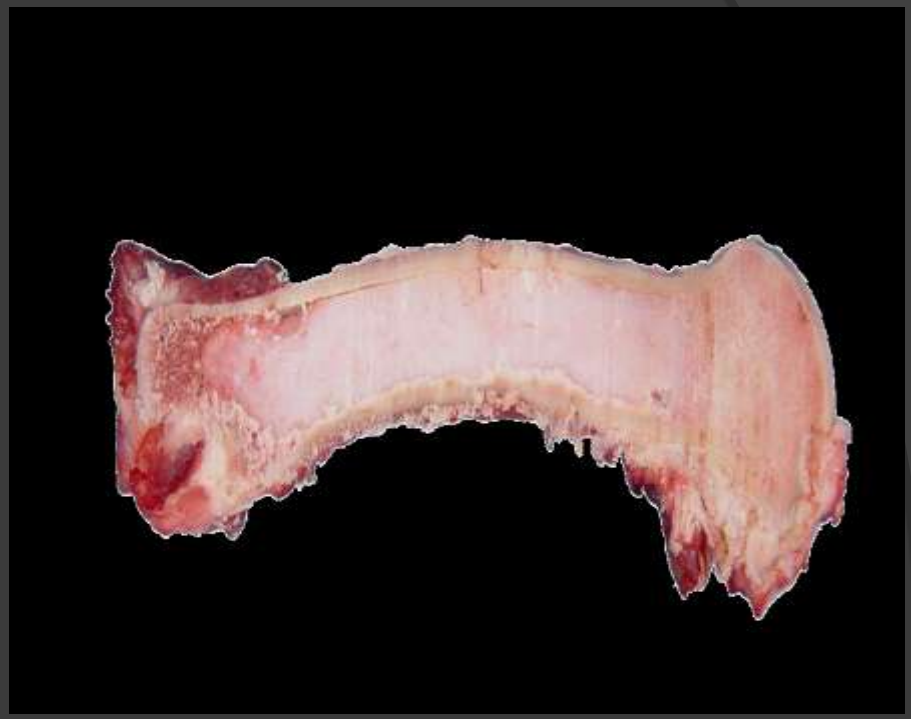


Figure A. Note the highly cellular spicule with abundant iron (white arrows), several large megakaryocytes (black arrows), a heterogeneous population of hematopoietic cells, and large, lipid vacuoles.

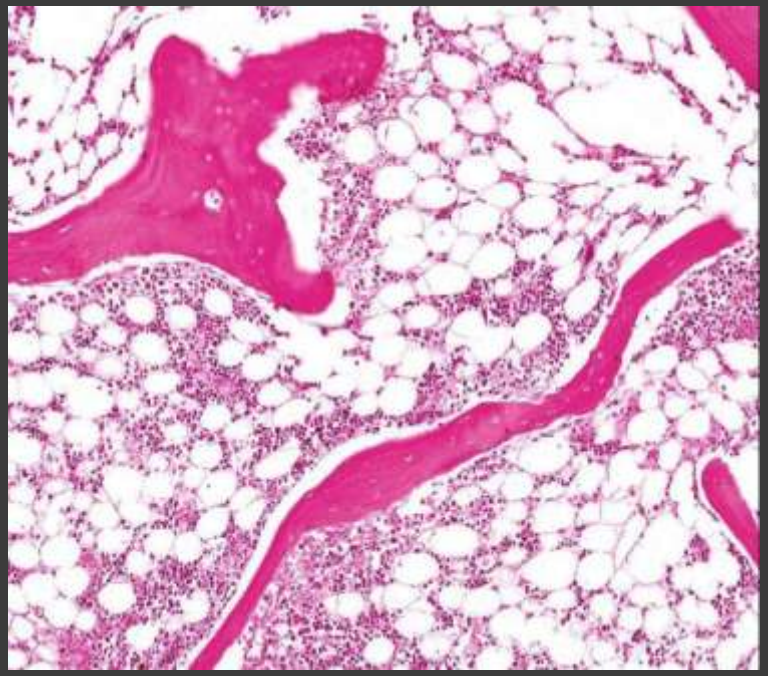
Fig. B-The microscopic appearance of a poorly cellular spicule, which may be secondary to poor sampling or reflect true hypocellularity.



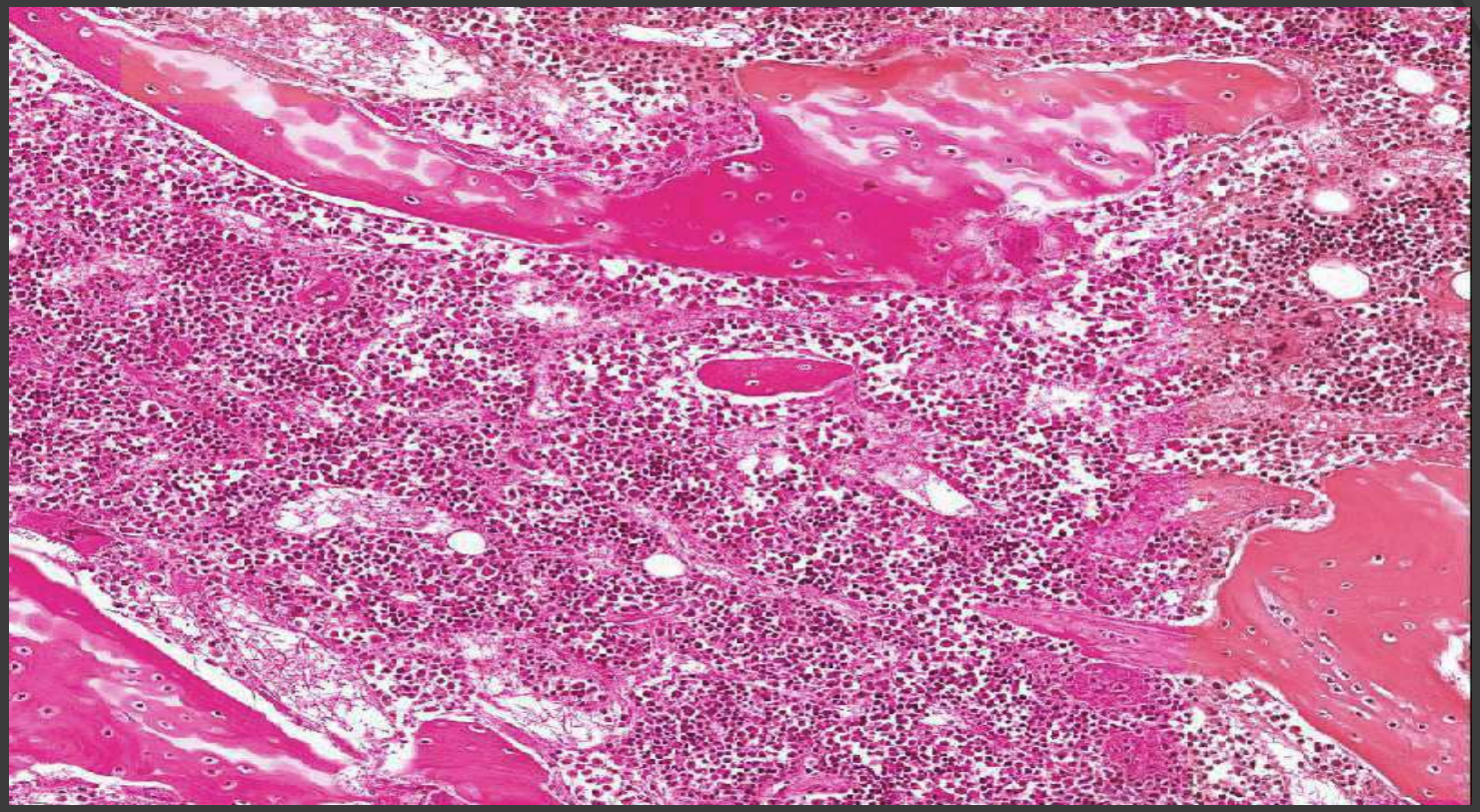


Gross → Increased yellow marrow (Hypoplastic bone marrow)

Histo → Increased ratio of fat to hematopoietic cells



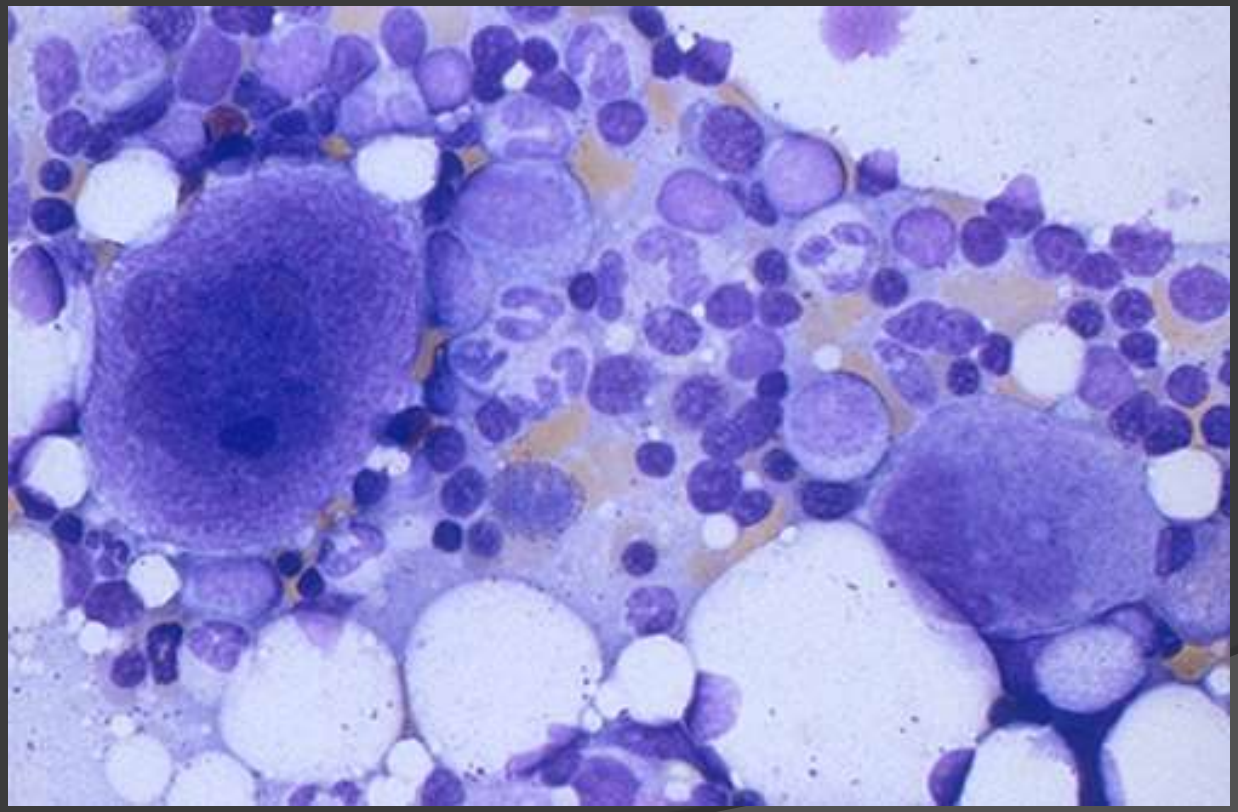
Normal bone marrow- cellularity ~ 50/50



Hyperplastic bone marrow

Increased cellularity (decreased ratio of fat to hematopoietic cells)

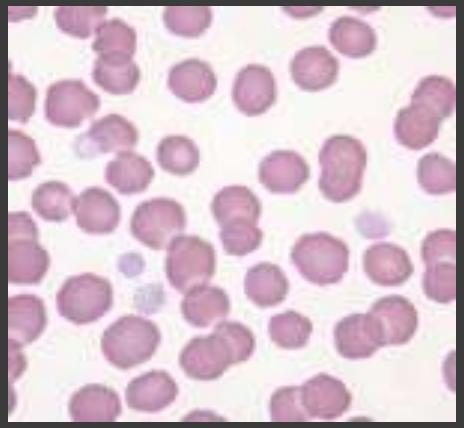
*Smears should be examined using the low power to evaluate cellularity and adequacy of megakaryocytes.



* A myeloid to erythroid (M: E) ratio is calculated by examining 500 cells and determining the ratio of granulocytic cells (including mature granulocytes) to nucleated erythroid cells. Normal M: E ratio:

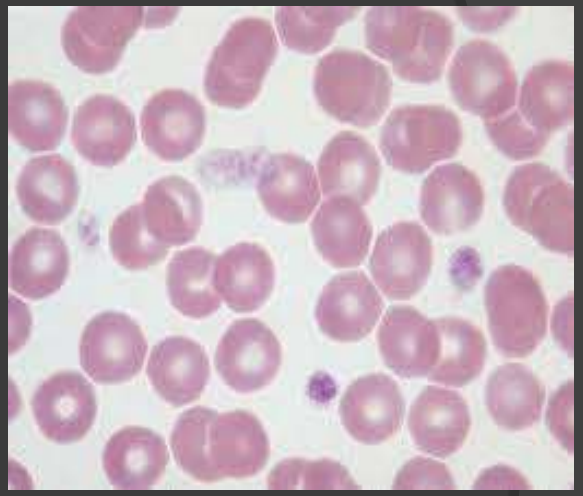
Dog: 0.75-2.5, cat: 1-3, horse: 0.5-1.5

1. RED BLOOD CELLS, ERYTHROCYTES (RBC)

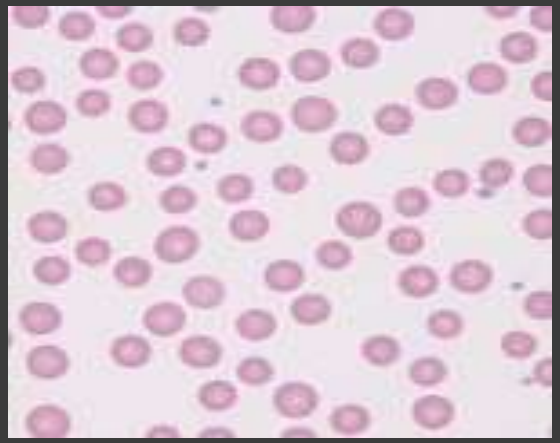


Equine erythrocytes are of similar size as feline erythrocytes and also lack central pallor

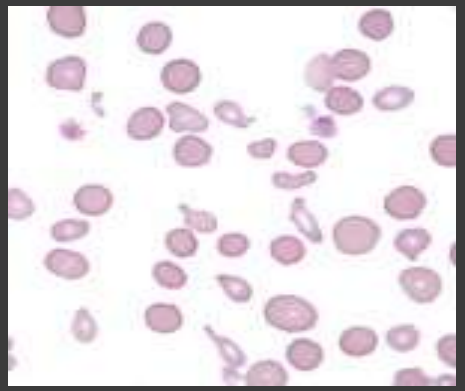
Feline erythrocytes are smaller and more variable in size and shape than those of dogs. With little to no central pallor1.



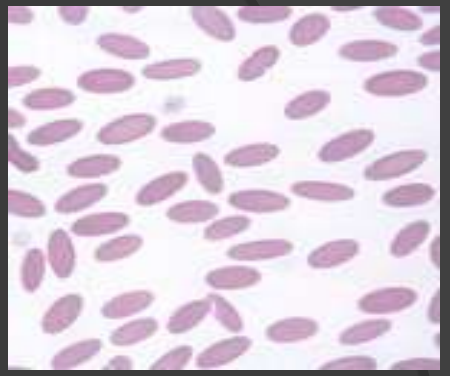
The canine erythrocyte in health is a relatively large, uniform, biconcave disc



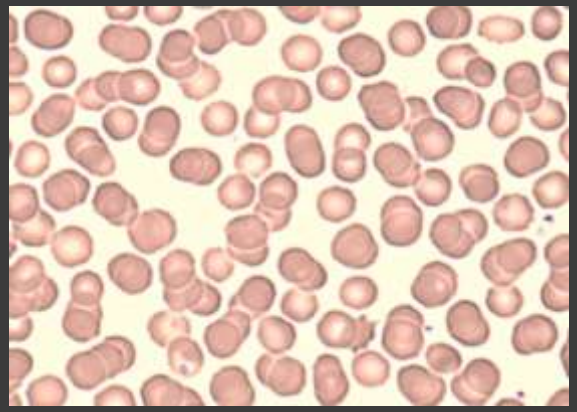
Bovine RBC similar in size to horse RBC, have a small amount of central pallor. Some degree of anisocytosis



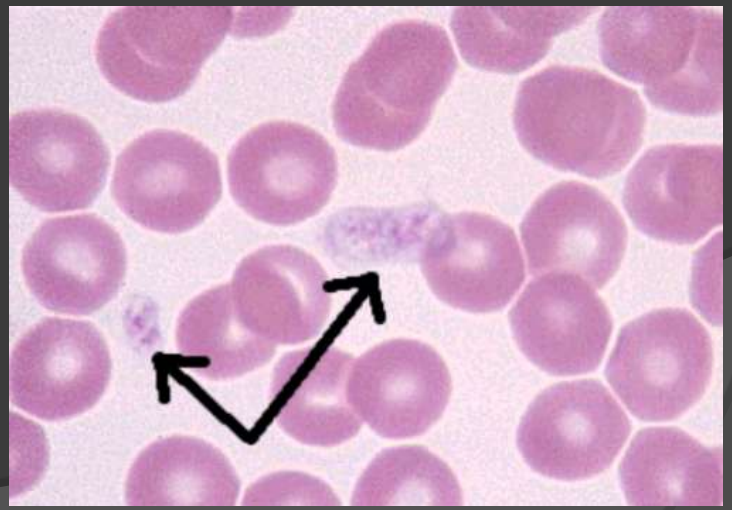
Caprine RBC, the smallest with normal poikilocytosis in some breeds



Camelidae rbcs, small , oval (elliptical) and flat



RBC of man looks like that of dog



RBCs OF A DOG

3. Platelets or thrombocytes, they are not true cells but cytoplasmic fragments of the giant BM megakaryocytes.

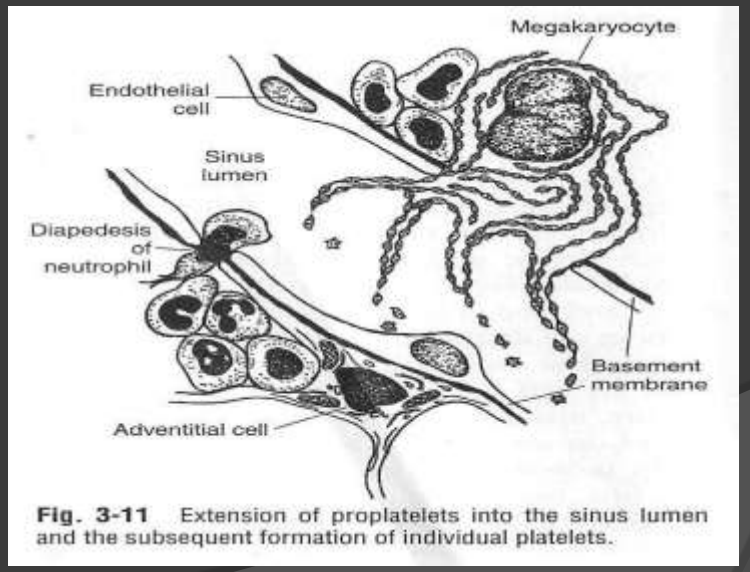
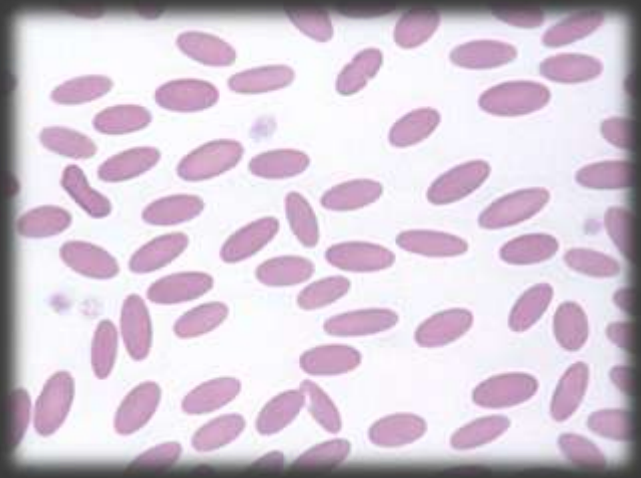
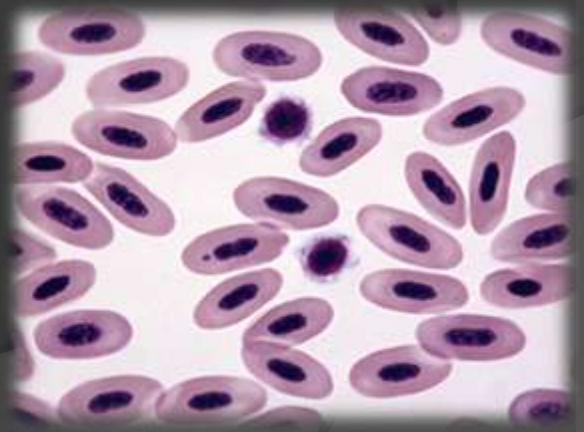


Fig. 3-11 Extension of proplatelets into the sinus lumen and the subsequent formation of individual platelets.

THE ERYTHRON

It is a term applied to the *circulating RBC mass,* RBCs precursors in the bone marrow and *erythropoiesis regulating factors.





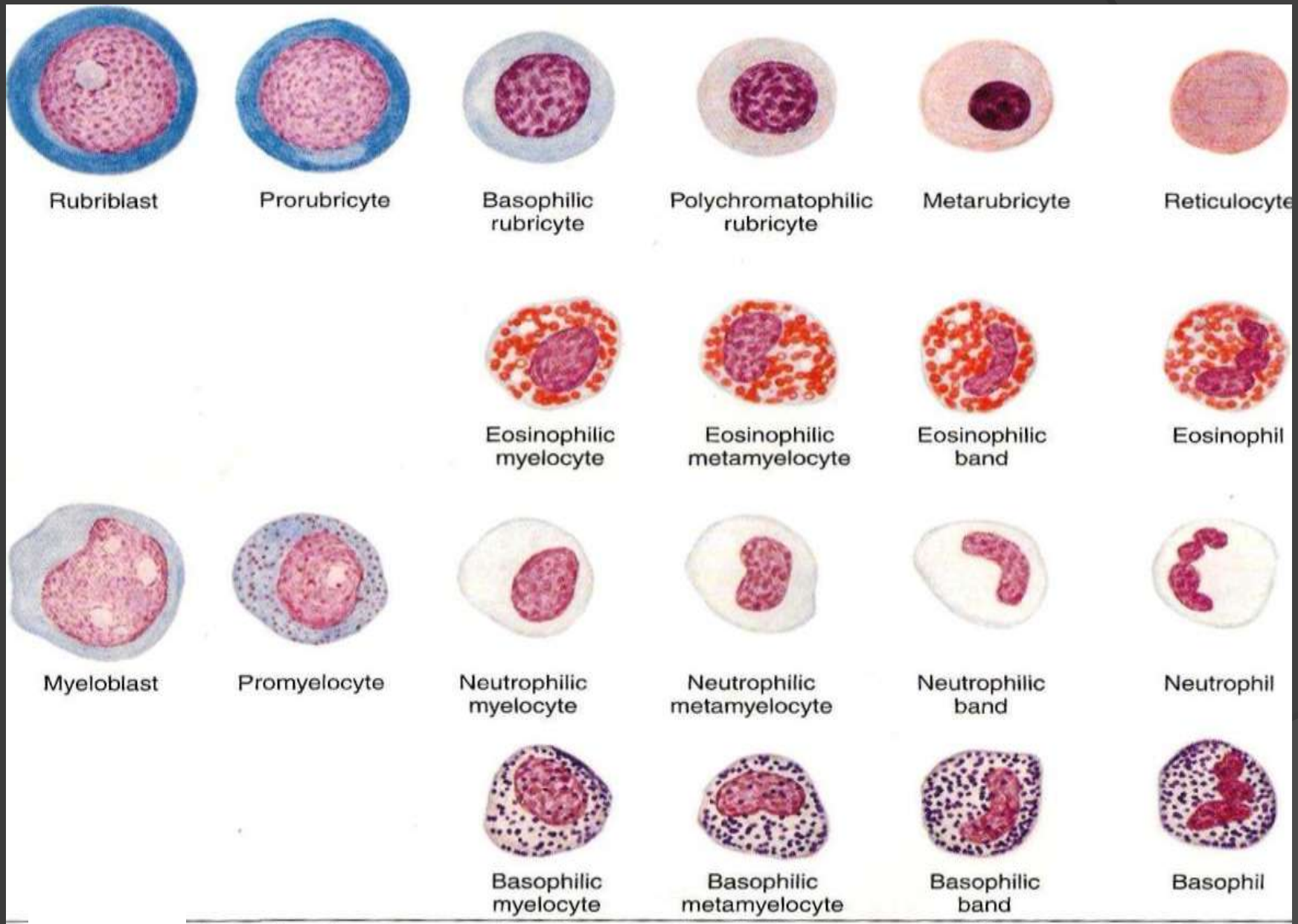
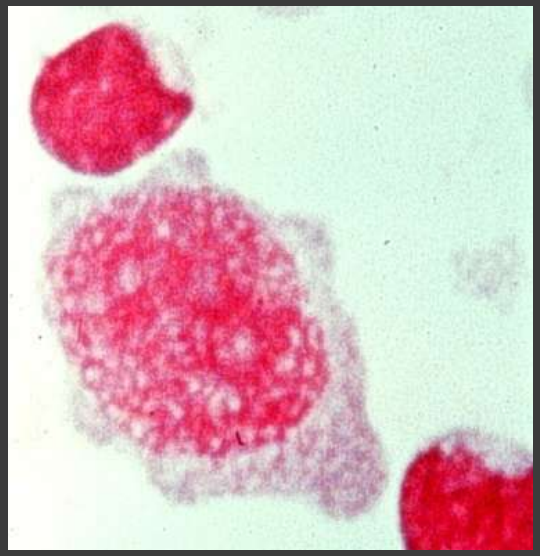
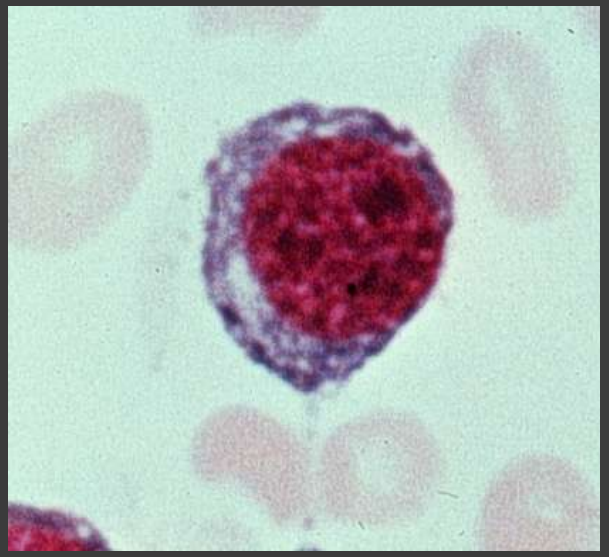
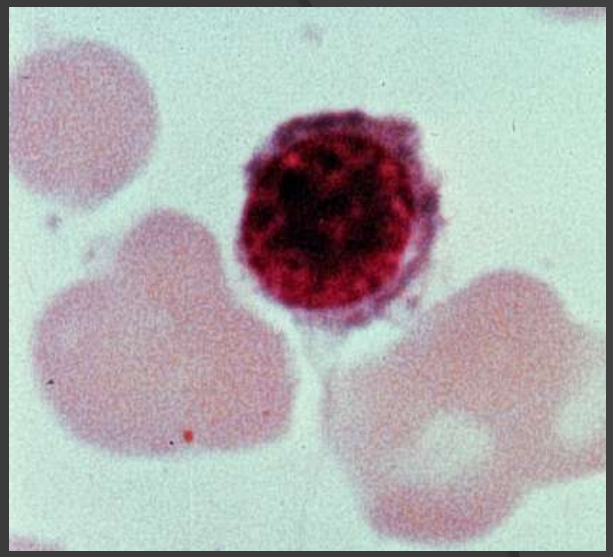
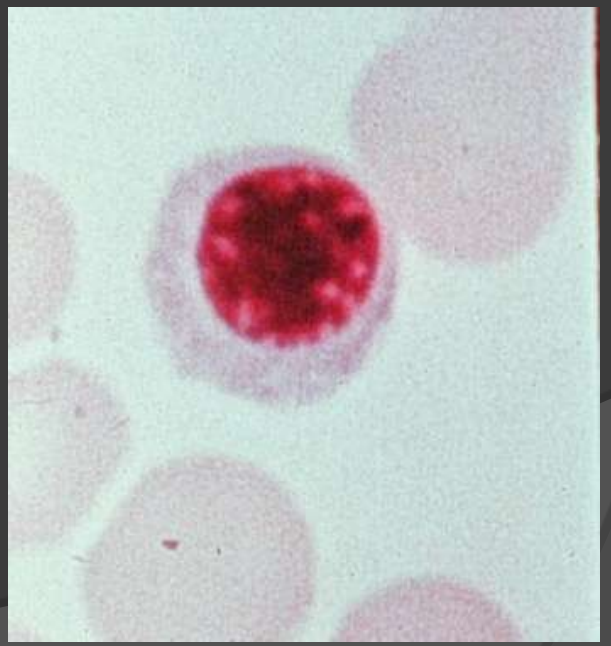


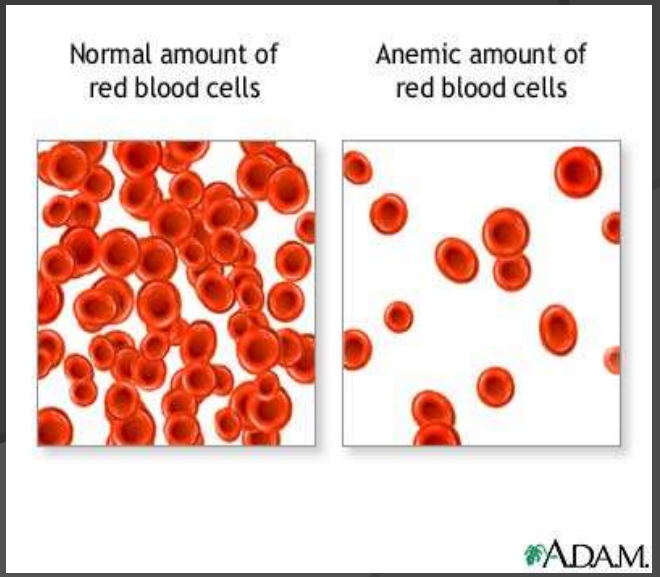
Plate 19 Maturation of canine erythroid and granulocytic cells as they appear in Wright-Giemsa–stained bone marrow aspirate smears. (Drawing by Dr. Perry Bain.)



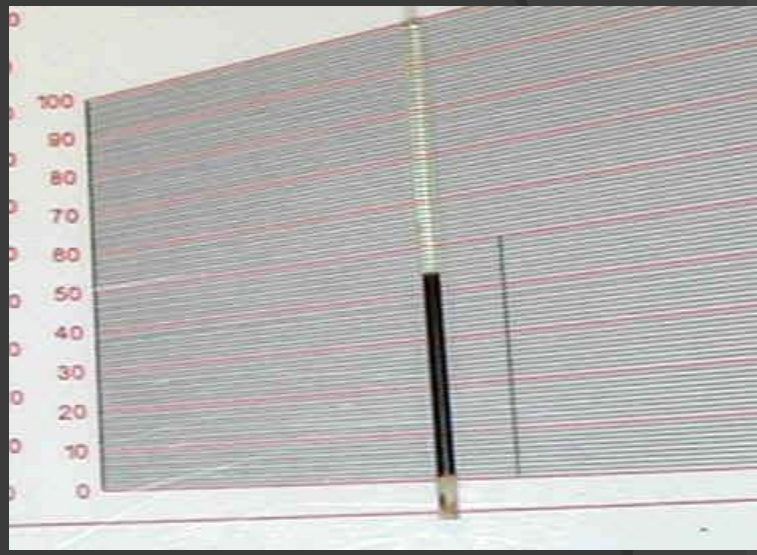
Evaluation of Erythron

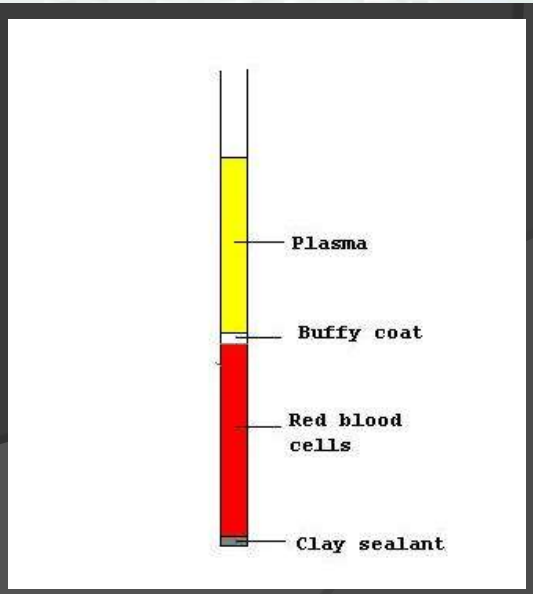
1. RBC count/µl of blood ; decrease in RBCs means anaemia while increase in RBCs means polycythemia.



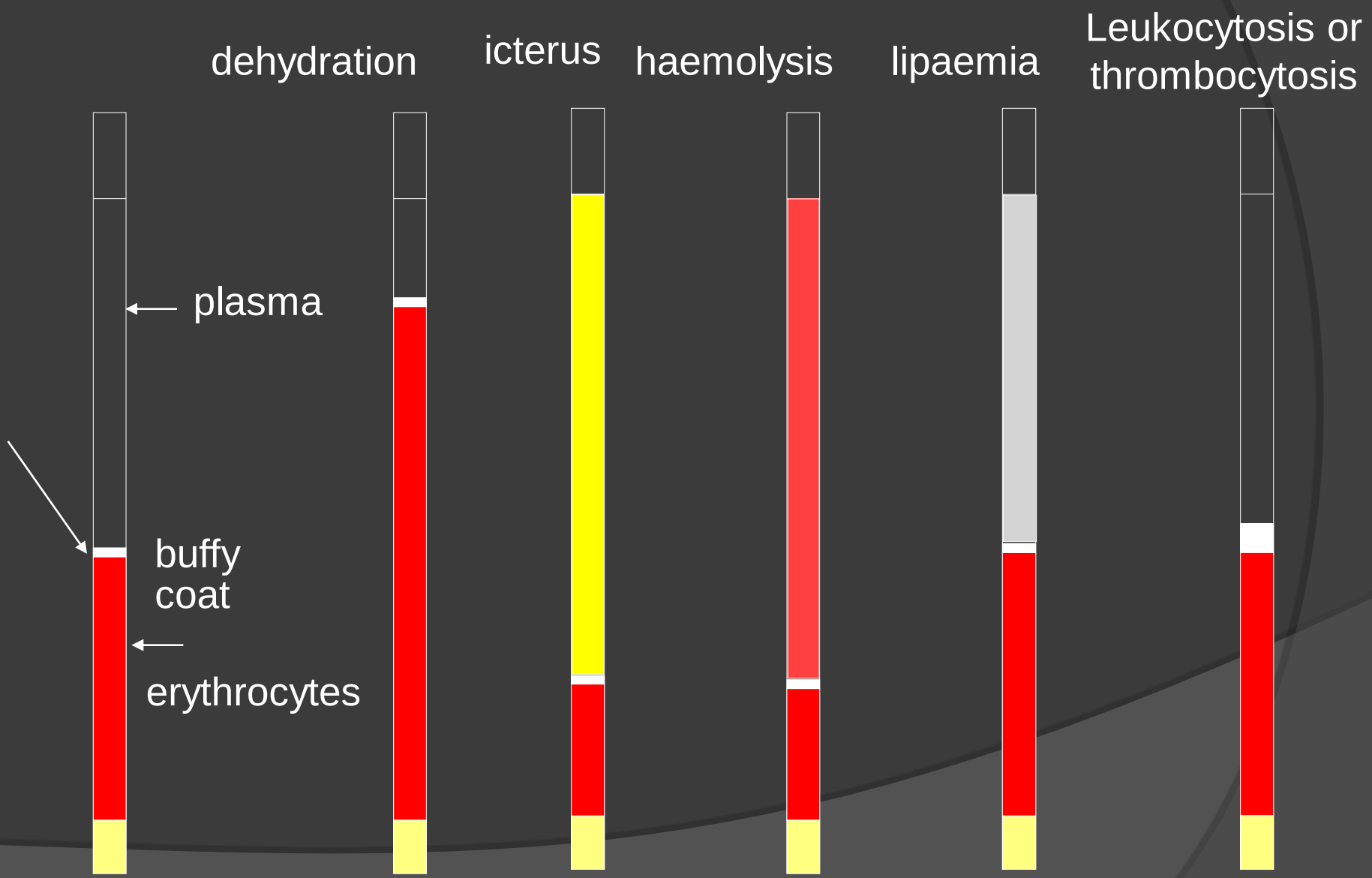
2-Packed cell volume (PCV%)

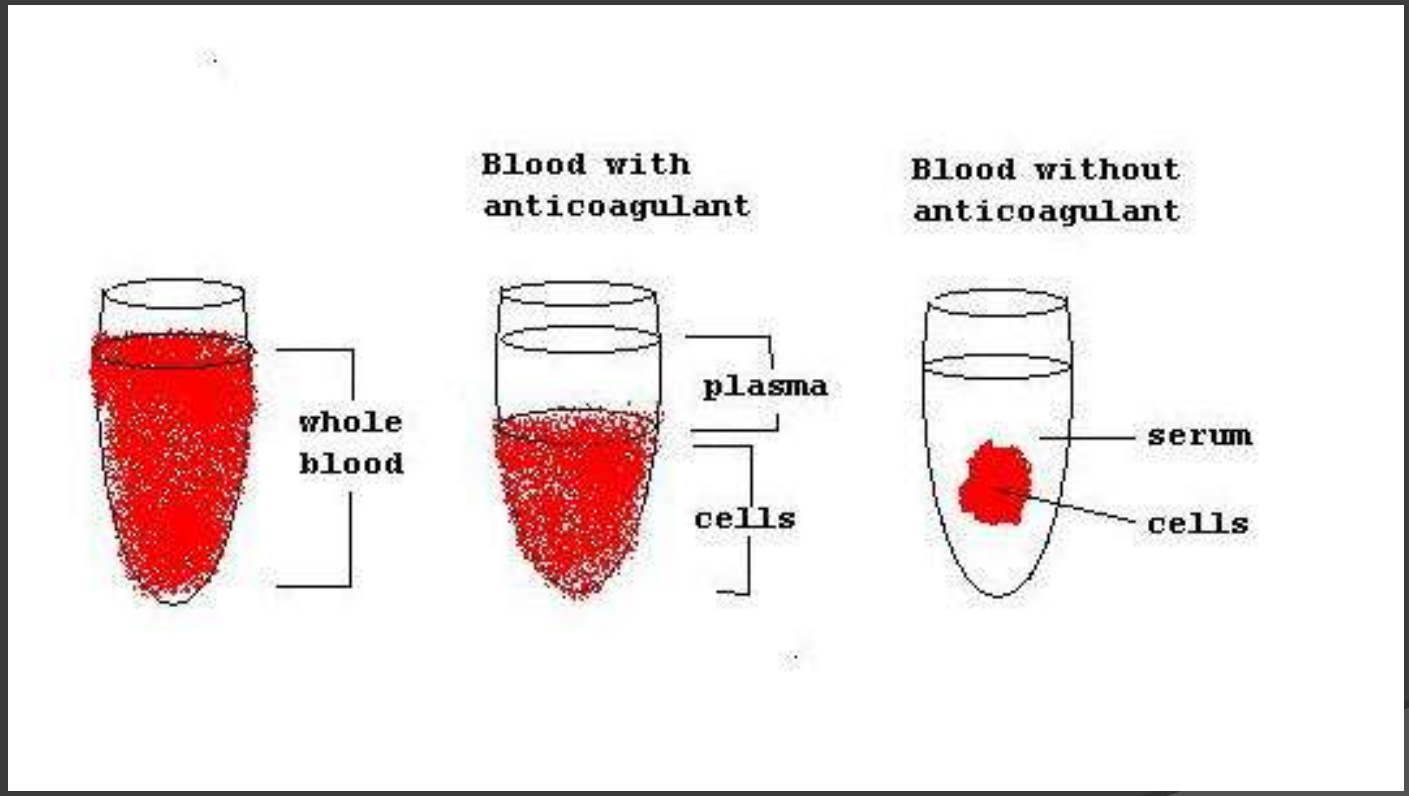
Also known as haematocrit value (Hct %) , it is the most efficient & common blood test performed in a veterinary clinic for evaluating the Erythron. **PCV is the percentage of RBC in the blood.**





CAPILLARY TUBE SECTIONS AND PLASMA COLOURS




Blood with anticoagulant
Blood without anticoagulant
whole blood
plasma
cells
serum
cells

3-Haemoglobin concentration (Hb g/dl).
`11a

4. Erythrocyte indicies used for typing of anaemia, it is a calculation obtained from rbc count, PCV and Hb values .It includes:

a. Mean corpuscular (cell) volume(MCV)

$= \frac{\text{pcv } \% \times 10}{rbc \text{ count}}$,measured in Femtoliter (fl), increase in MCV indicates macrocytic type of anaemia due to increase in size of rbcs as in responsive anaemia which is characterized by increase in the number of large- sized immature rbcs in the peripheral circulation.

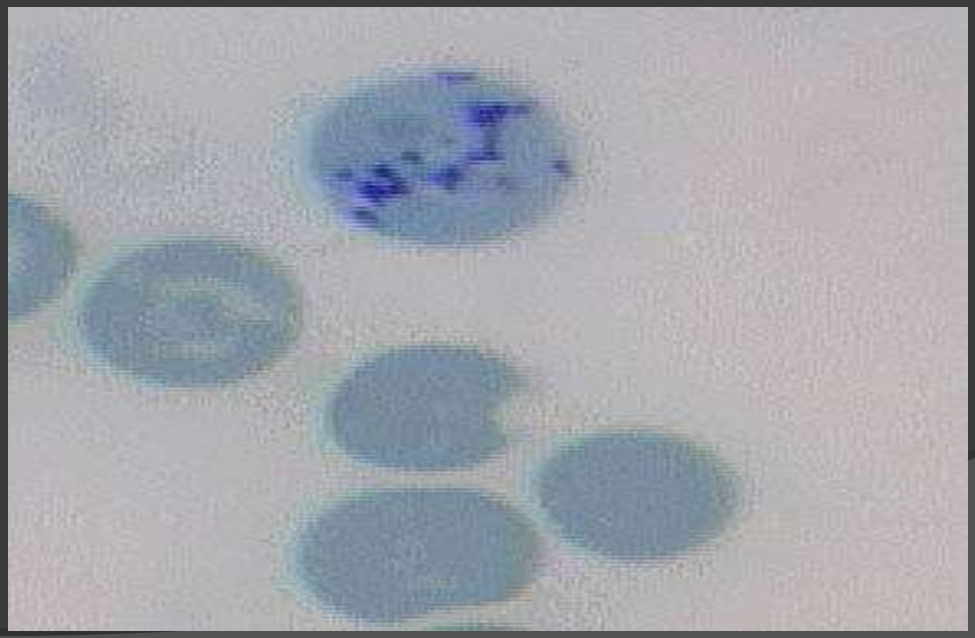
b. Mean corpuscular(cell) haemoglobin(MCH) $= \frac{\text{Hb g/dl x10}}{\text{rbc count}}$ measured in Pico gram (pg).

c. Mean corpuscular haemoglobin concentration (MCHC) $= \frac{\text{Hb g/dl x100}}{\text{PCV\%}}$ it is measured in gram/deciliter(g/dl), decrease in MCHC indicates hypochromic anaemia, increase in MCHC is not detected ,if it is observed it is artifactual.

5- Reticulocyte count

Absolute Reticulocyte Count

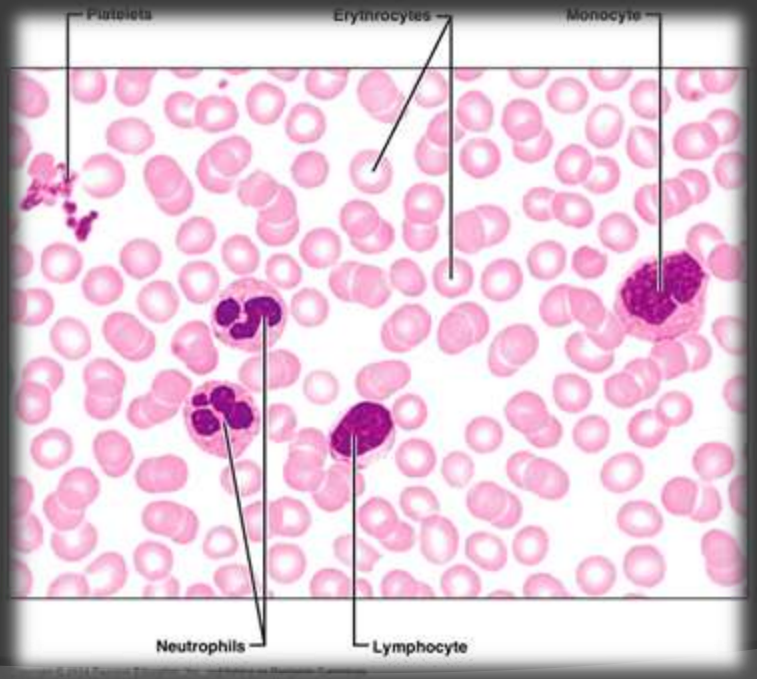
- This is the preferred single index for assessing regenerative response

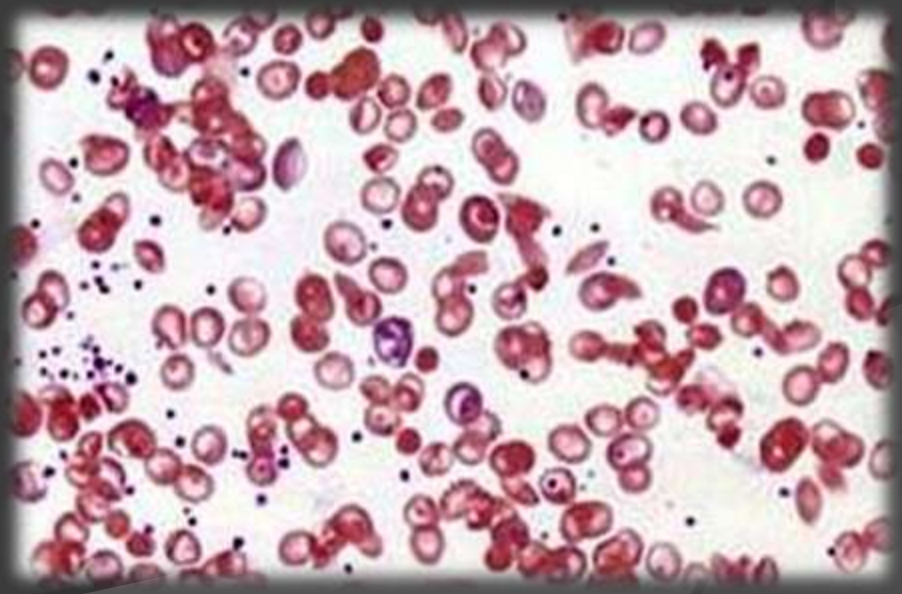


5. Stained peripheral blood film examination:

a. Abnormal rbcs morphology:

1. Abnormalities of shape (Poikilocytosis): It means major deviations from normal shape of erythrocytes for the particular animal sp. (minor deviations are normal).

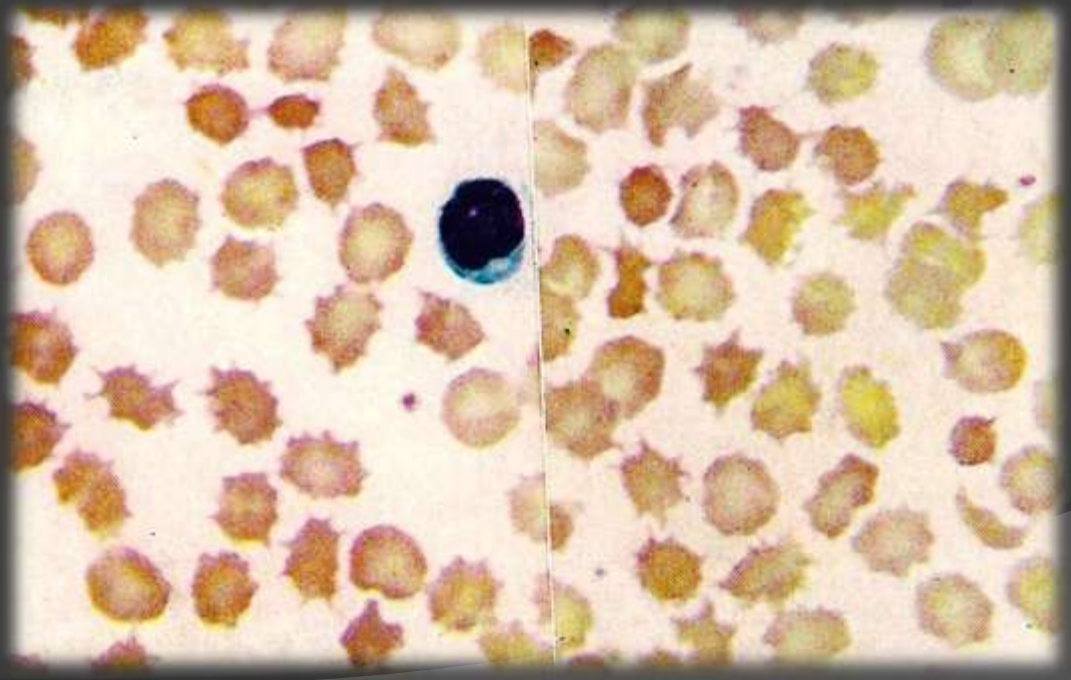




1.a. Leptocyte :Thin, flat, hypochromic rbcs with increased surface area and normal cell volume.They are of two types, target and folded cells, mostly observed in chronic diseases, iron deficiency anaemia, haemolytic anaemia, liver diseases, thalassemia.

Small number is normal in the blood of dogs.

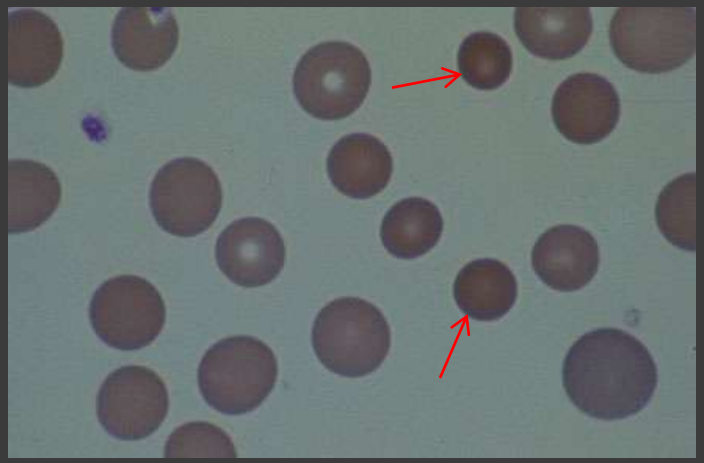
1.b. Acanthocyte: Spiculated rbcs with irregularly spaced and variably-sized spicules, it is formed when cholesterol is present in excess to phospholipid in rbc cell membrane. It is mostly due to increase in blood cholesterol, presence of abnormal plasma lipoprotein and liver diseases.

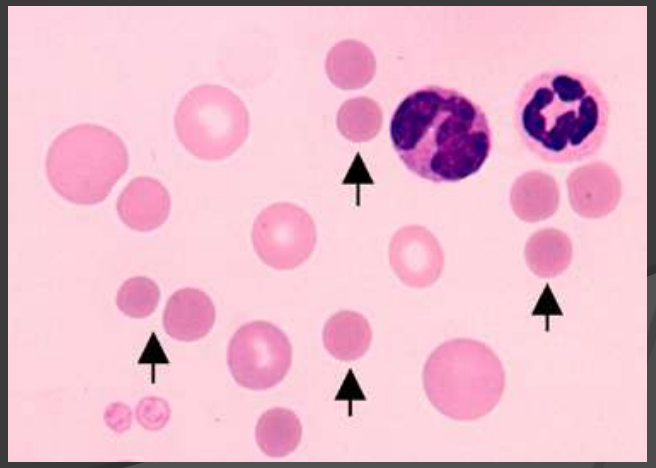


1.c. Crenated RBC: Spiculated, their spicules are relatively evenly spaced and of similar sizes ,it is considered as an artifact resulting from excess EDTA ,prolonged sample storage or delay in the dryness of blood films.

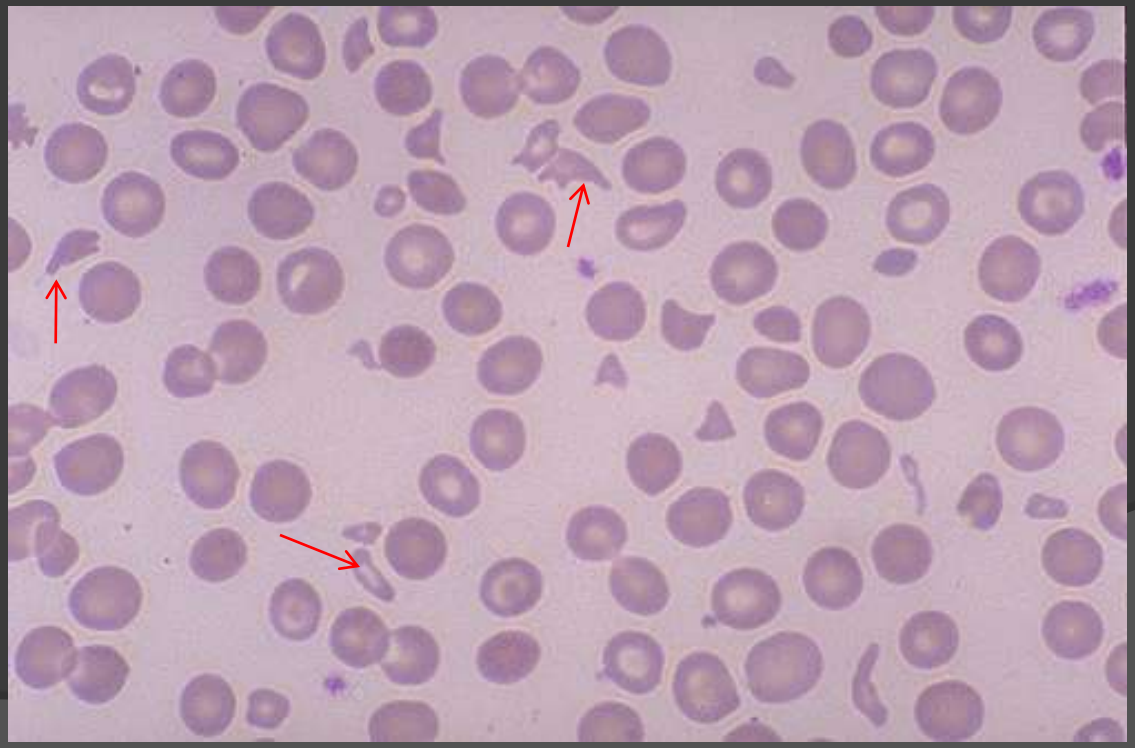
1.d. Stomatocytes: Cup -shaped rbcs that have elongated or slit- like central pallor. It is mostly seen thick stained blood films as an artifact or in hereditary stomatosytosis, liver diseases or chronic anaemias.

1.e. Spherocyte: RBCS lack central pallor with smaller diameter than normal and biconvex surface with spherical shape , it results from cell swelling and/ or loss of part of cell membrane; as in immune mediated haemolytic anaemia e.g. blood parasite infection, snake bite, zink toxicity, it causes anisocytosis, decrease in MCV.

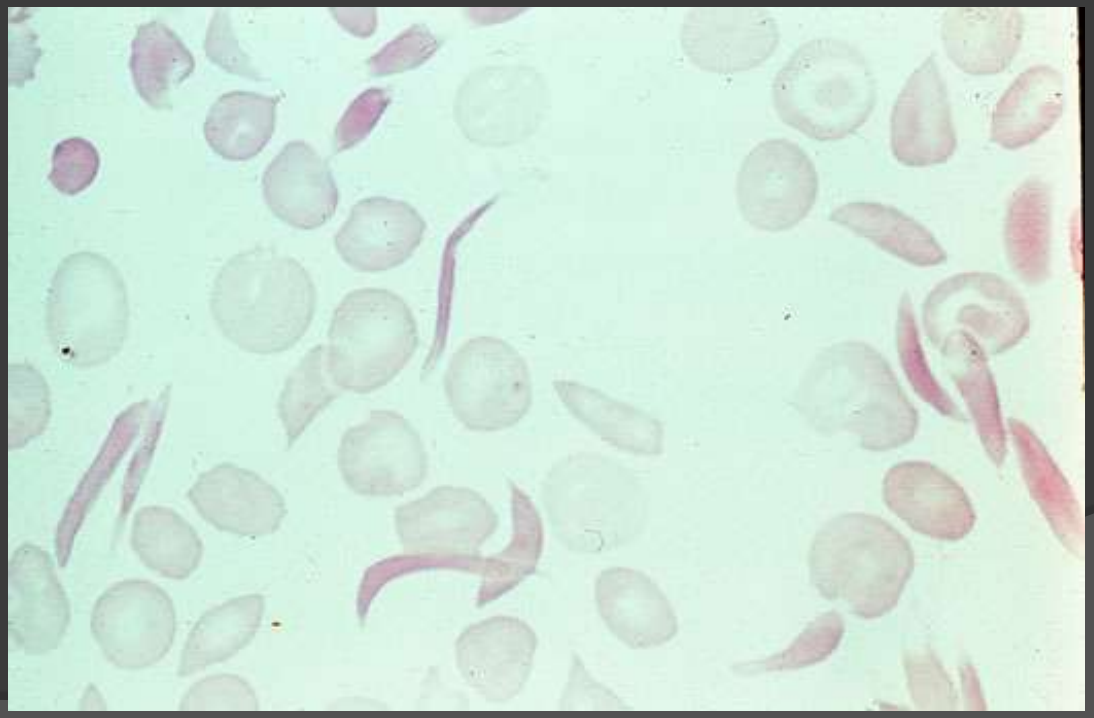




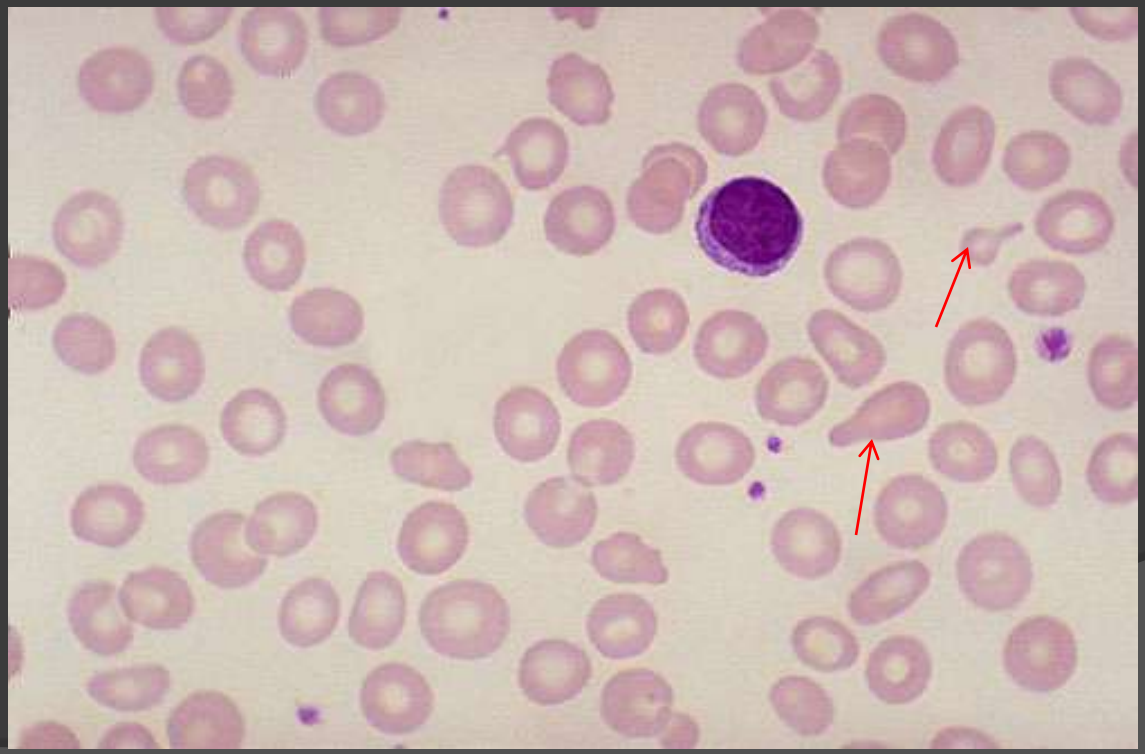
1.f. Schistocyte: Fragment of an rbc with two or three pointed extremities, they are smaller than normal rbcs. Its observation in a stained blood may indicates ;severe iron deficiency anaemia , DIC in dogs fibrin strands may split its rbcs, not the smaller rbcs of cats and horses.



1.g. Sickle cell: Spindle – shaped rbcs ,it is considered normal in deer and young goat(in vitro phenomena due to high o tension + pH between 7.6-7.8.Sickle cell anaemia in man is due to abnormality in amino acid sequences of the β globin chain of haemoglobin (HB-S).



1.h. Dacrocyte: Tear drop-shaped rbcs with single elongated or pointed extremities Seen in blood of dogs and cats with myeloproliferative disorders and dogs with hypersplenism.



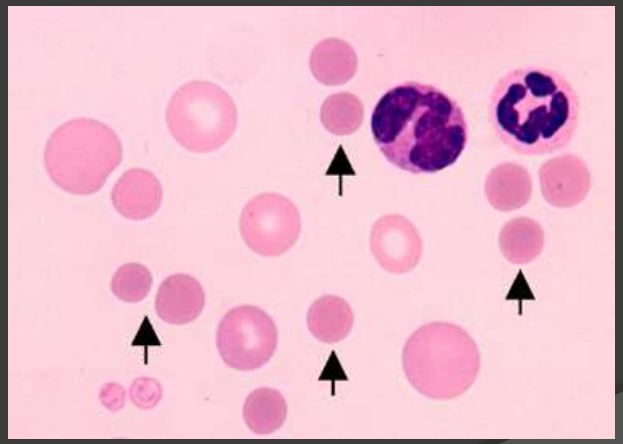
2. Abnormalities and major deviations in size of rbcs (anisocytosis):

Slight anisocytosis is normal in certain animal sp. as cow, less frequently in cat sheep and goat.

The red blood cells here are normal, happy RBC's. They have a zone of central pallor about 1/3 the size of the RBC. The RBC's demonstrate minimal variation in size (anisocytosis) and shape (poikilocytosis). A few small fuzzy blue platelets are seen. In the center of the field are a band neutrophil on the left and a segmented neutrophil on the right.

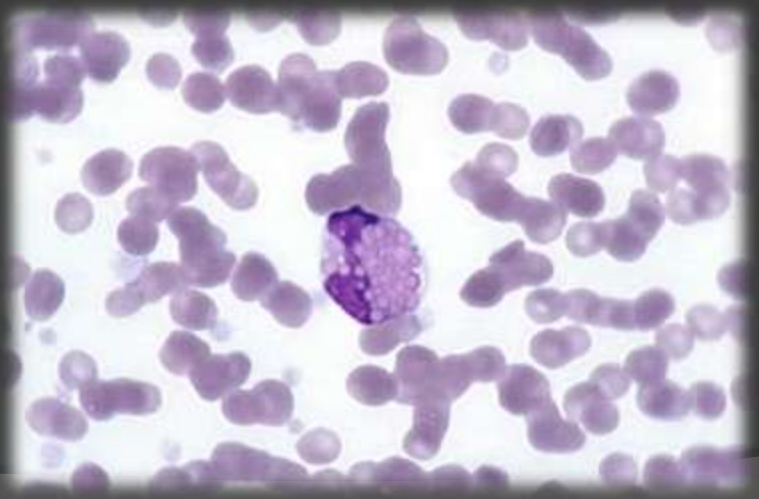
Mostly it is due to the presence of different populations of cells e.g. macrocytes (reticulocytes) in responsive anaemia; or the production of small-sized(microcytes) rbcs as in iron deficiency anaemia.

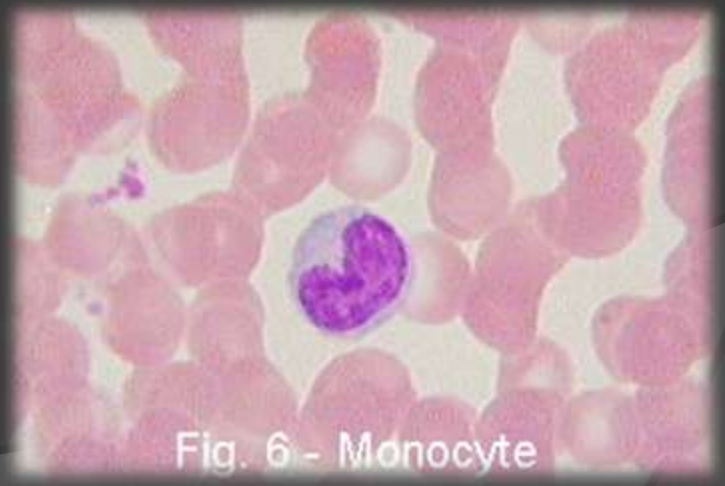
Spherocytes may also leads to anisocytosis.



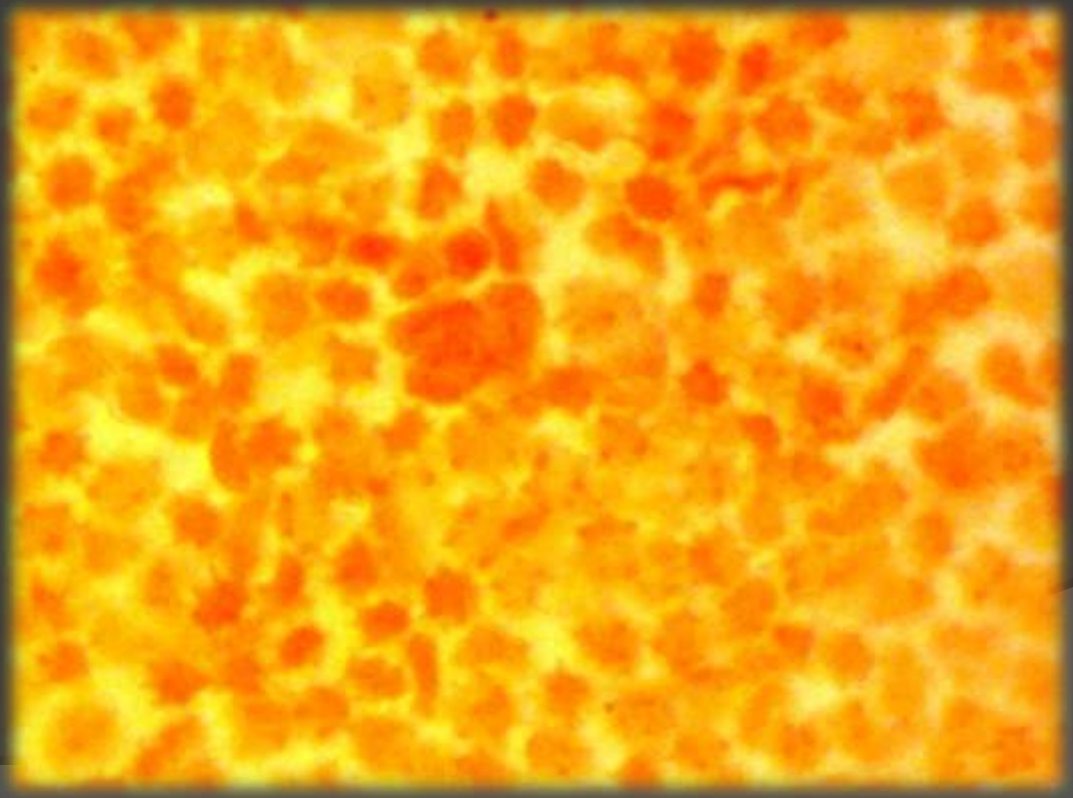
b-Abnormality in arrangement of rbcs: Rouleaux:

It means adhesion of rbcs together like a stalk of coins .This is mostly due to changes in plasma protein con., as increase in fibrinogen and γ – globulin in inflammatory conditions .Extensive rouleaux is normal in equine , moderate to slight rouleaux is normal in cat & dog.





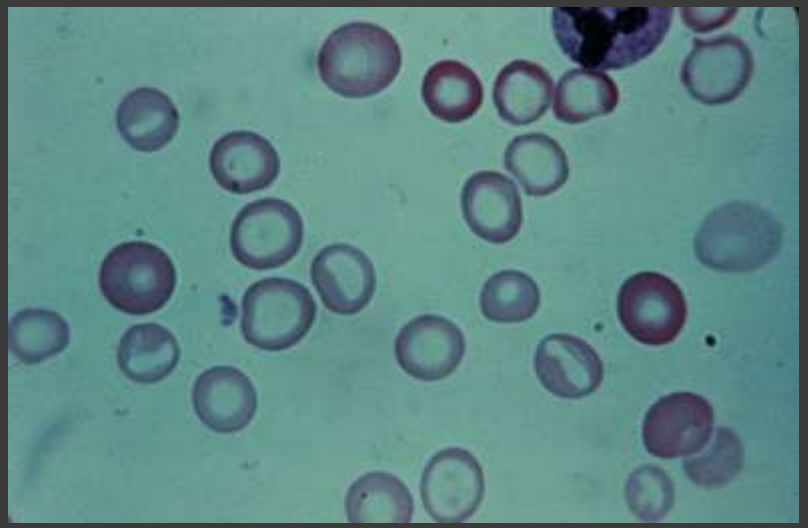
Agglutination: The aggregation or clumping of rbcs in together in clusters not in chains .It is caused by immunoglobulins bound to rbcs surfaces, as in immune – mediated anaemia.

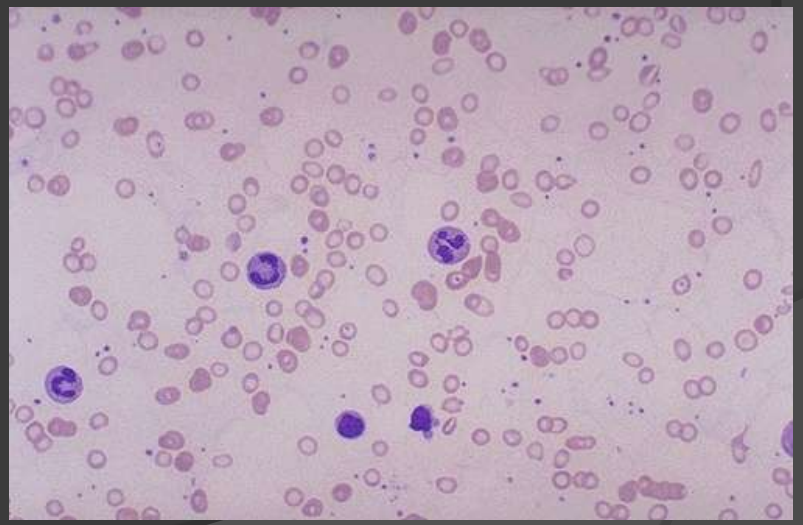


c- Abnormalities in colour of rbcs:

1- Polychromasia: The presence of bluish –red rbcs in stained blood film , due to the presence of a combination of Hb (red) and ribosomes(blue).They are immature rbcs (reticulocytes) present in low number in normal dog(1%-1.5%) ,increased polychromasia indicate reticulocytosis and responsive anaemia.Equine blood does not show polychromasia in disease or in normal conditions

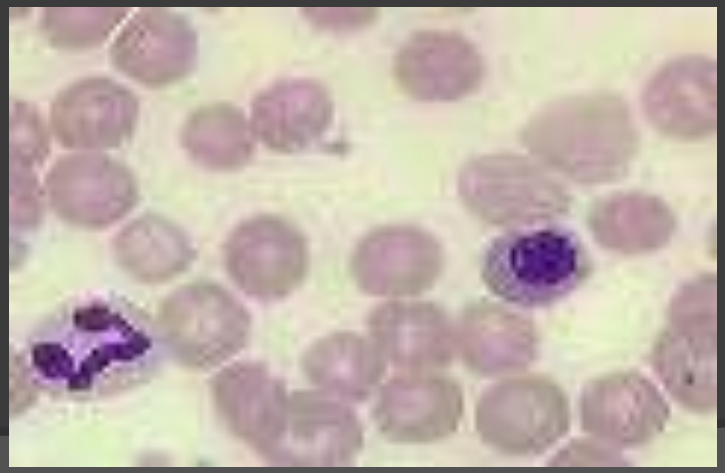
2- Hpochromasia: RBCS with decrease in Hb content and increase in central pallor. It is associated with decrease in MCHC. Increased hypochromasia is associated with iron deficiency anaemia and chronic blood loss.

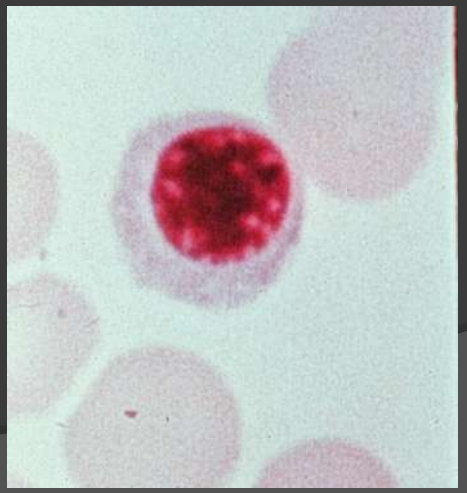


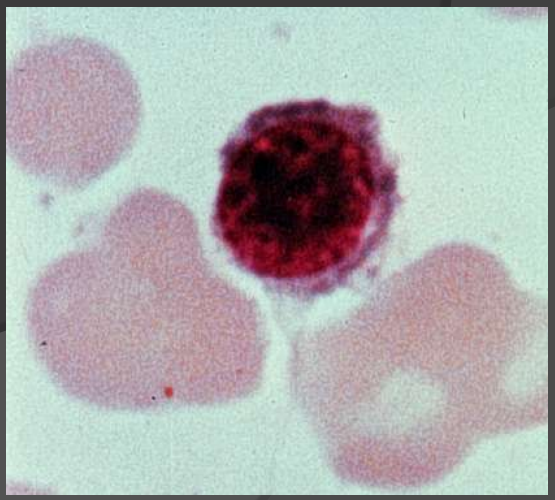


3- RBC inclusions: It means the presence of abnormal bodies inside rbcs.

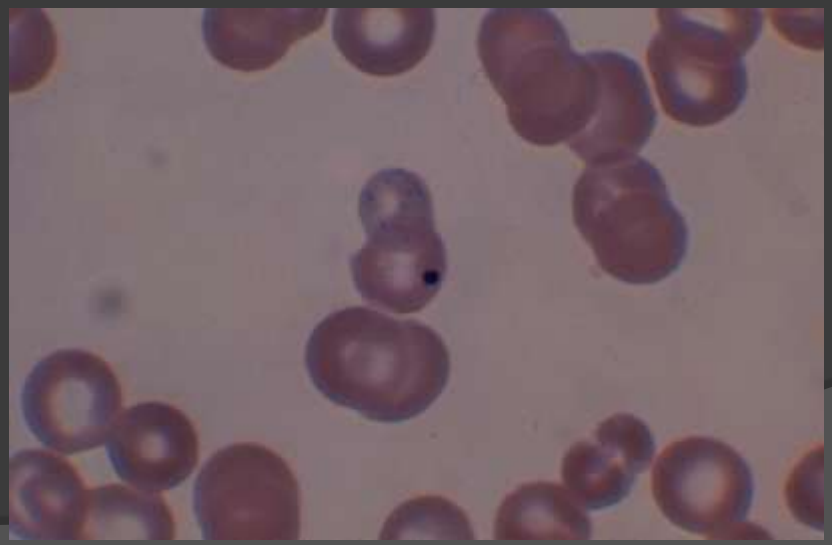
a-Nucleated rbcs: Presence of immature rbcs in peripheral circulation e.g. rubricytes , metarubricytes is seldom in the blood of normal adult mammals, It is seen in regenerative anaemia ,haematopoietic neoplasia and various inflammatory conditions.



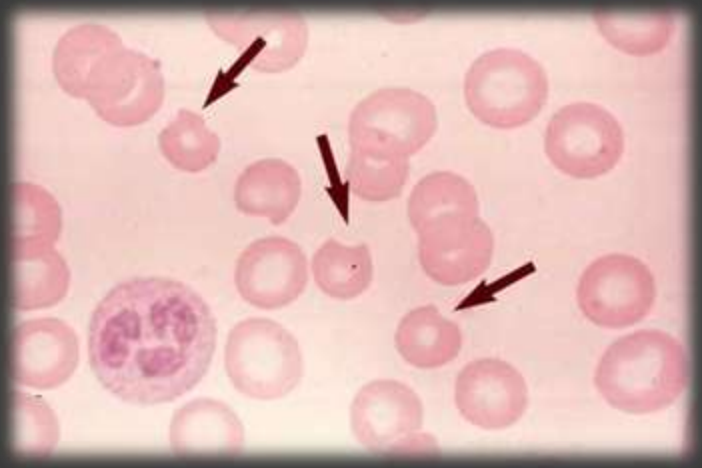


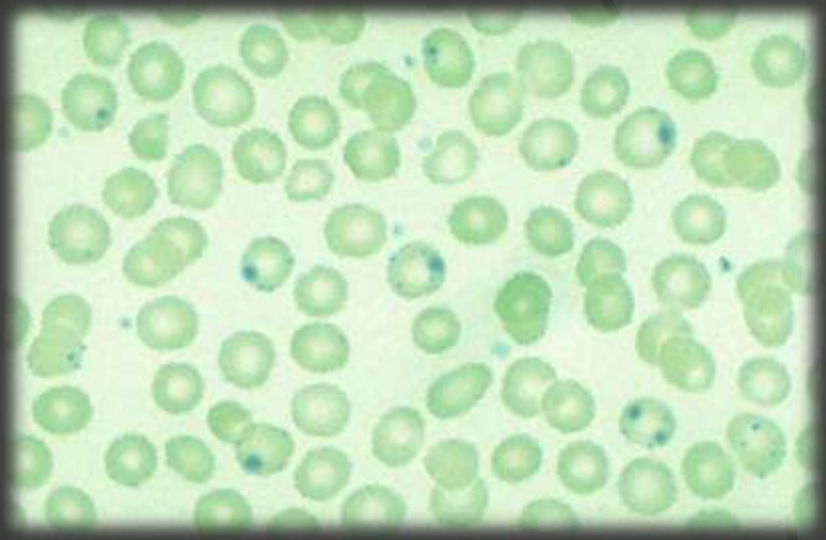


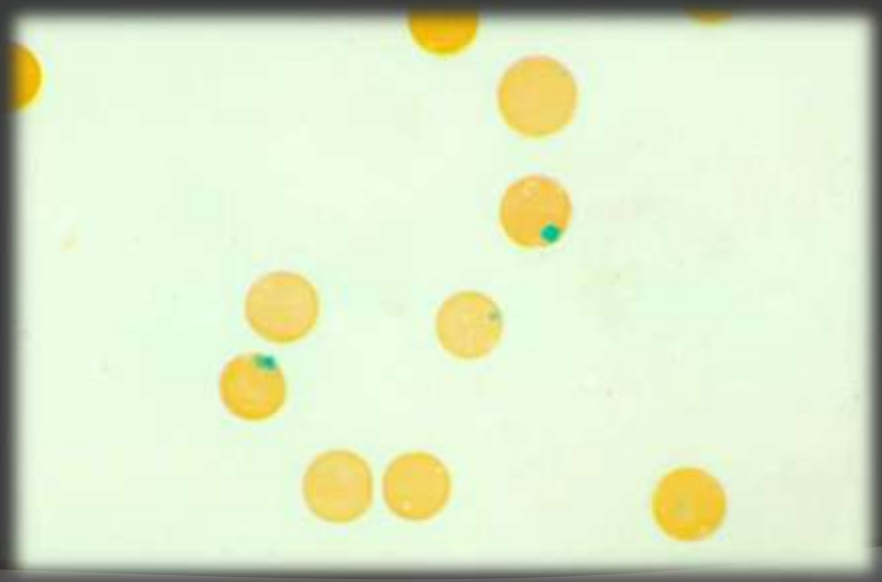
b- Howell-Jolly body: They are small dark blue nuclear remnant (DNA in nature) formed in the bone marrow and it should be removed by pitting activity of the spleen. May be present in low number in rbcs of normal cats and horses, also it is associated with regenerative anaemia and blood films of splenectomized animals.



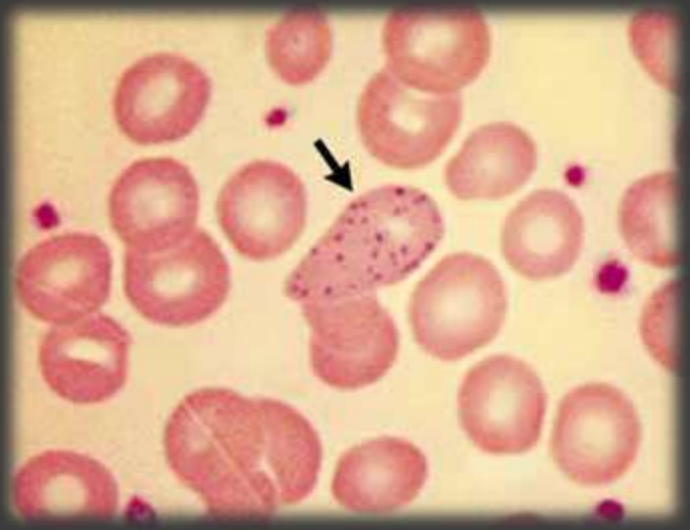
c-Heinz body: Large aggregates of oxidized precipitated Hb attached mostly to the internal surfaces of rbc cell membranes ,they stain pale pink with Romanowsky's stains, dark blue with supra-vital stains. Normal cat blood film may show few Heinz bodies (5-10%).In pathological conditions it is associated with dietary causes as consumption of large quantities of onion by small and large animals ,kale and other Brassica sp. in ruminants,copper toxicity in sheep .It is known as Heinz body haemolytic anaemia.

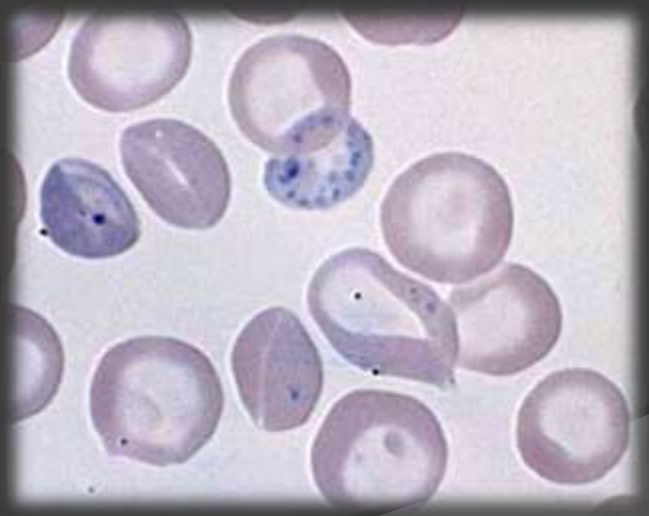




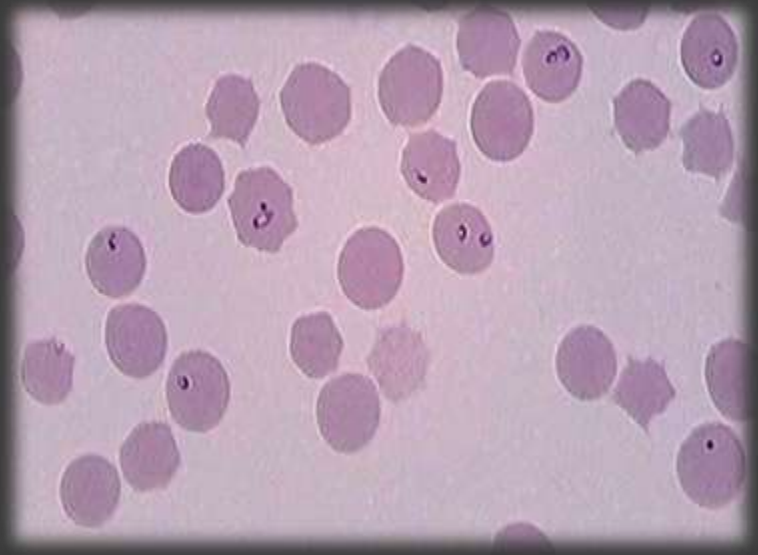


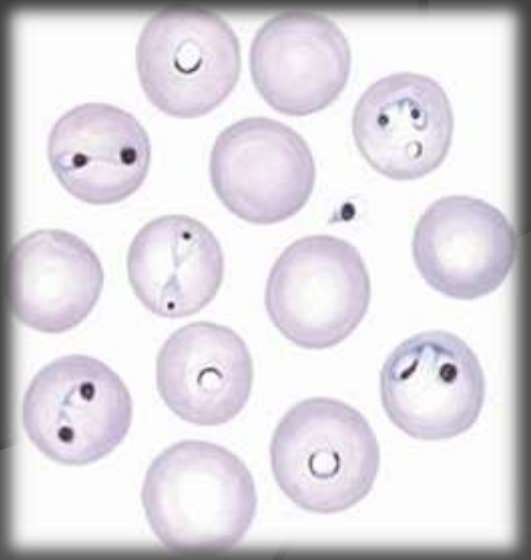
d- Basophilic stippling: Blue staining punctuate inclusions stained with Romanowsky's stains ,it represents RNA of polyribosomes, occur in regenerative anaemia in ruminants and lead toxicity in canine, punctuate may be fine or coarse.



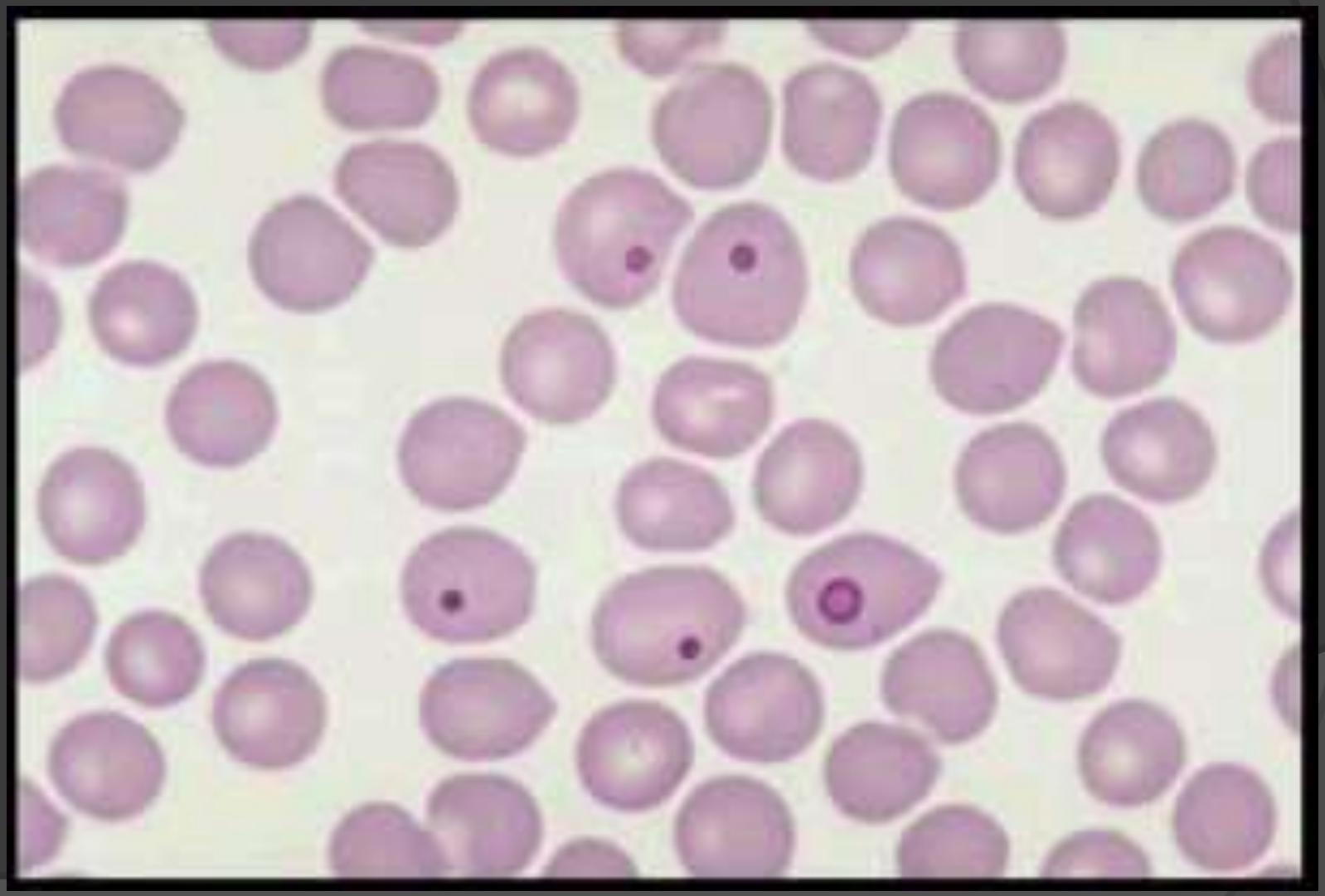


e- Infectious agents: As protozoa e. g . Babesia, Theileria, Malaria. Bacteria as the rickettsial microorganism, anaplasma. Viral inclusions e.g. Canine distemper viral inclusions appear as red or orange mostly rounded bodies (it should be differentiated from Howell-Jolly bodies.





Canine distemper inclusion:



Haematology

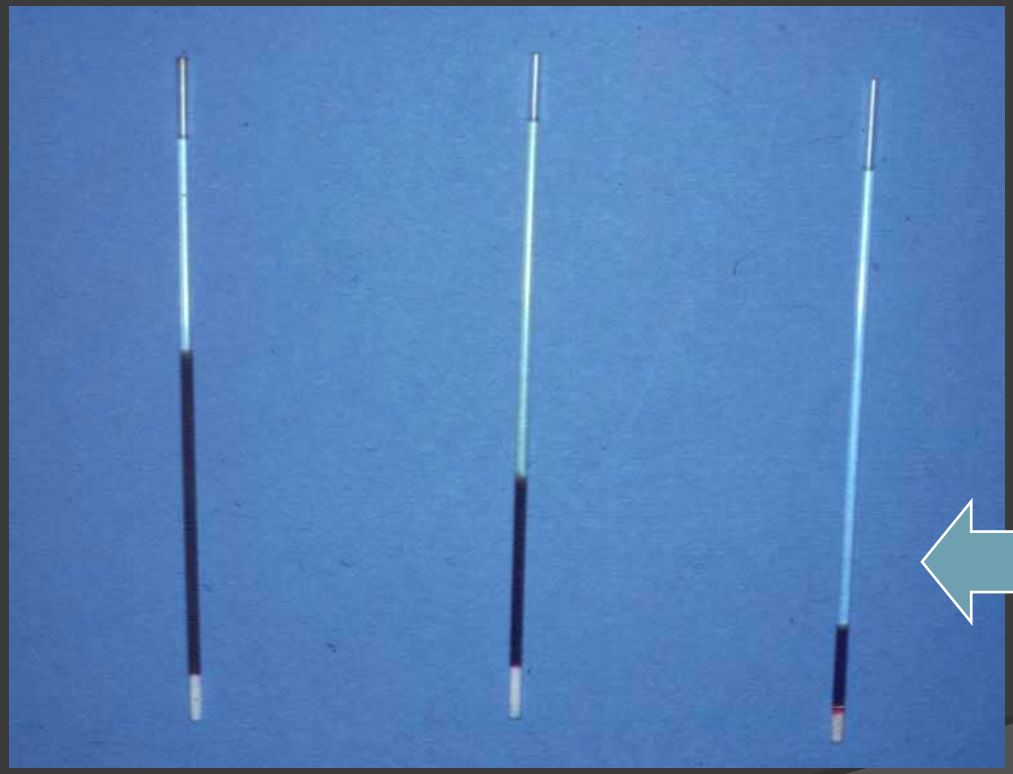
ERYTHROCYTE (RBC) DISORDERS: POLYCYTHAEMIA AND ANAEMIA

Diseases of RBCs

Anaemia: It is a sign not a disease; it is a problem not a diagnosis.

ANAEMIA

A decrease in **PCV**, **Hb** concentration and/ or **RBC co**



Clinical signs of anaemia:

1.Pale mucous membranes (Icteric if haemolytic).

2.Weakness and exercise intolerance.

3.Tachycardia and polypnea particularly after exercise .

4. Increased sensitivity to cold.

5. Syncope and depression.

6.Heart murmur caused by decrease in viscosity and increase in turbulence of blood.

7. Weak or fluttering pulse.

8.Shock, if one third of blood is lost rapidly.

ANAEMIA: CLINICAL IMPLICATIONS

-Signes which may be associated with cause of anaemia

- Icterus
- Bleeding (petechiae,ecchymoses, melena, haematuria, haematomas)
- Fever
- Splenomegaly

Anaemia is classified in various ways to assist in determining its specific cause so that effective treatment can be provided in addition to:

*History,

*Clinical signs,

*Other lab. findings and other test procedures (e.g. ,diagnosing images).

Classification of anaemia according to the cause:

a-Blood loss anaemia (Haemorrhagic).

b-Anaemia due to increase in RBCs destruction(Haemolytic anaemia).

c- Anaemia caused by decrease in RBCS production.

a-Blood loss anaemia(Haemorrhagic):,

1.parasitic infestation as fleas, blood sucking lice, ancylestoma (hookworm), Hemonchus .Trauma, surgery and coagulative disorders- e.g. vit.K deficiency, sweet clover toxicity (dicoumarol) in cattle, inherited coagulation factors deficiency(hemophilia A& B),platelets disorders, neoplasia, gastrointestinal ulcers etc.

b-Anaemia due to increase in RBCs destruction(Haemolytic anaemia):

Destruction of rbcs or their lyses may be caused by different agents and different mechanisms.

1-Site of RBC destruction-

Intravascular haemolysis or extracellular haemolysis Within the circulation it means outside macrophages of the spleen.

Extravascular haemolysis or intracellular haemolysis; means destruction of rbcs inside splenic macrophages outside the vascular system:

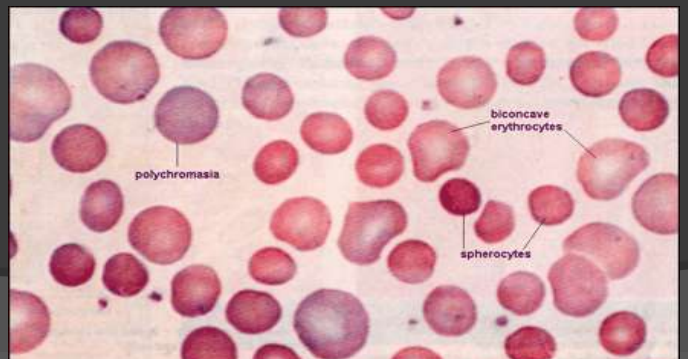
2-Cause of destruction-

a- Extracorpuscular ;(abnormal elements in vascular bed that “attack” RBCs, like Immune mediated: Autoimmune (drug, virus, lymphoid malignance) vs Alloimmune (transfusion reaction), Infection, chemical agents (spider venom), etc.

b- Intracorpuscular (erythrocyte defects- membrane abnormalities, metabolic disturbances, disorders of hemoglobin).

- **Membranopathies: hereditary spherocytosis**
- **Enzymopathies: G6PD**
- **Hemoglobinopathies: Sickle cell disease**

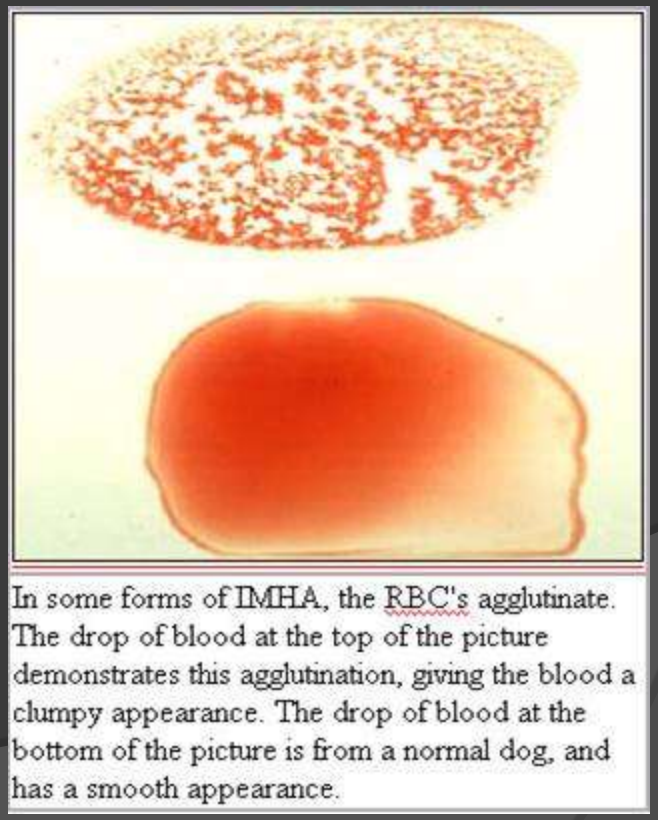
Comparison of normal biconcave erythrocytes and a spherocyte lacking central pallor.



Sickle cell anaemia

1-Haemolytic anaemia associated with immune response(immune mediated): It is caused by the binding of immunoglobulins to the surface of rbcs or their precursors. The two major diseases in animals are :

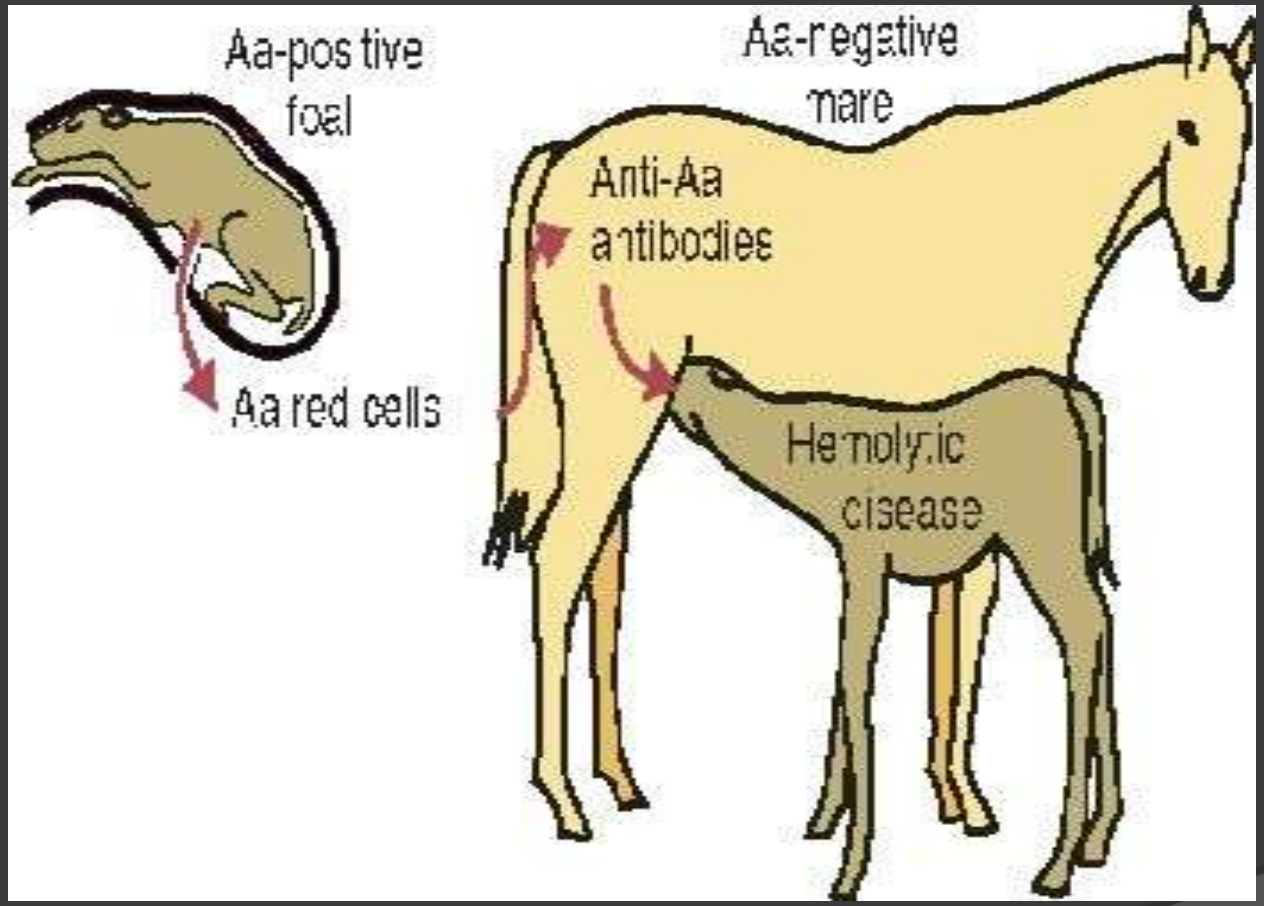
Springer Spaniel presented with "yellow skin". Notice the jaundiced sclera and skin on interior ear.



In some forms of IMHA, the RBC's agglutinate. The drop of blood at the top of the picture demonstrates this agglutination, giving the blood a clumpy appearance. The drop of blood at the bottom of the picture is from a normal dog, and has a smooth appearance.

- a-Neonetal isoerythrolysis (NI) or isoimmune haemolytic anaemia: It is a haemolytic disease of horse & mule foals ,rarely calves & kittens. A dam is bred more than once to the same stallion with different blood group------ antigens of the male rbcs are acquired by the fetus-----

Hemolytic anemia of the newborn. By permission from Tizard IR, Veterinary Immunology An Introduction, Saunders, 2001



Preventing the foal from sucking colostrum

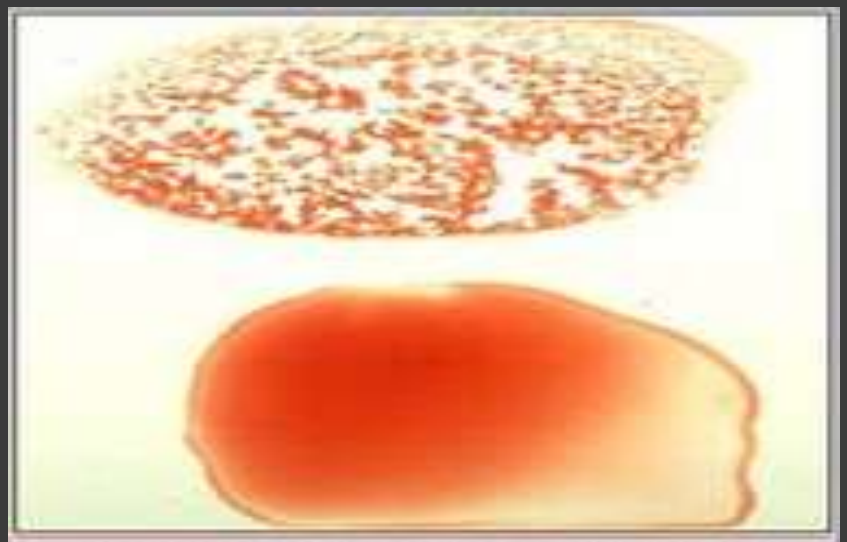
Neonatal isoerythrolysis; paleness and icterus in mucosa.

-----the dam develop antibodies against the fetal red cells------- passes to colostrums ingested by foal --------absorbed intact to the circulation------ bind to neonatal rbcs----- causing haemagglutination and intravascular haemolysis.

Characterized by haemoglobinuria, jaundice pale mucous membranes weakness &collapse, if not treated it is fatal.

b-Autoimmune haemolytic anaemia (AIHA): May be classified as:

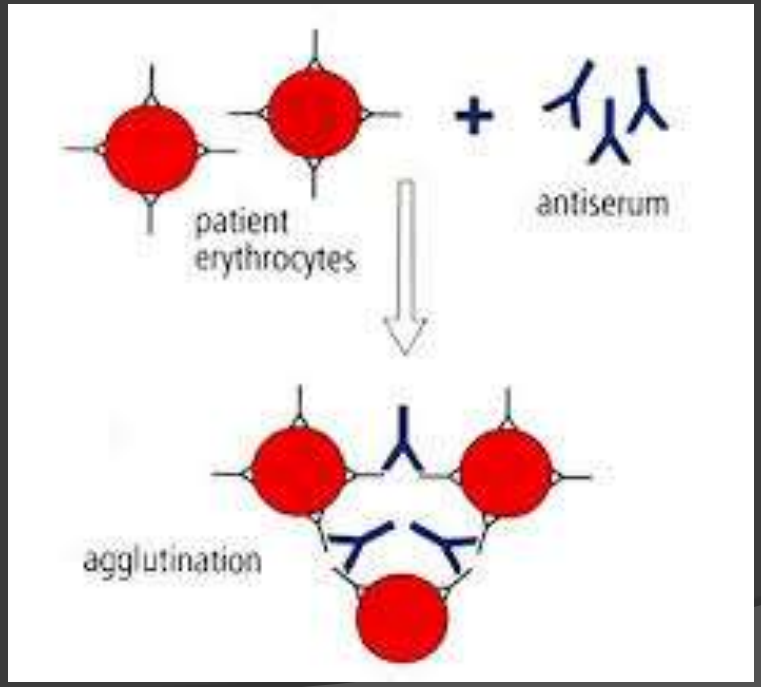
1- Primary or idiopathic: In the absence of any other clinical condition or disease, it means that it is of unknown cause. Abs is directed against self antigens on rbcs.



In some forms of IMHA, the RBC's agglutinate. The drop of blood at the top of the picture demonstrates this agglutination, giving the blood a clumpy appearance. The drop of blood at the bottom of the picture is from a normal dog, and has a smooth appearance.

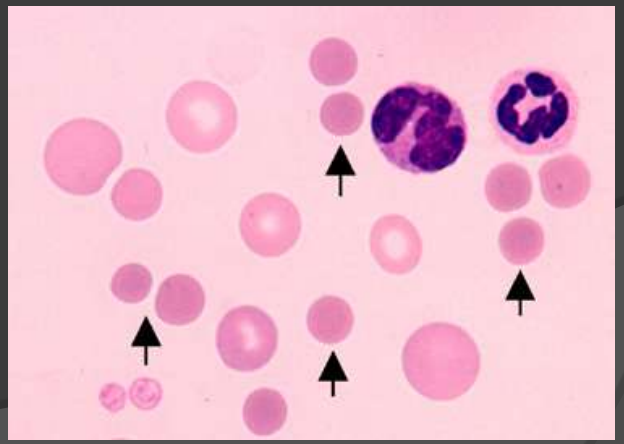
2-Secondary AIHA: When it occurs as a result of concurrent disease e.g. viral, richettsial, bacterial or protozoal infections, neoplasia especially lymphoma, SLE & different toxin or drug exposure

- **Parts of the drug, toxin or infectious agent will associate with the rbcs, so they are going to be recognized as foreign by the immune system and Abs are formed against them.**



Laboratory findings in AIHA:

- 1. Low rbcs, Hb & PCV values.
- 2.Presence of spherocytes in stained blood films. Spherocytosis is caused by partial phagocytosis of sensitized rbcs and removal of part of the cell membrane, spherocytes have short half life because of their rigidity.



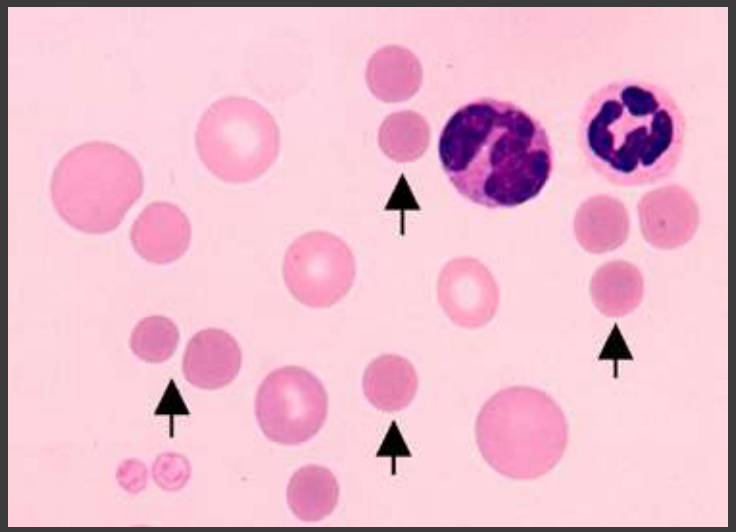
Hemolysis

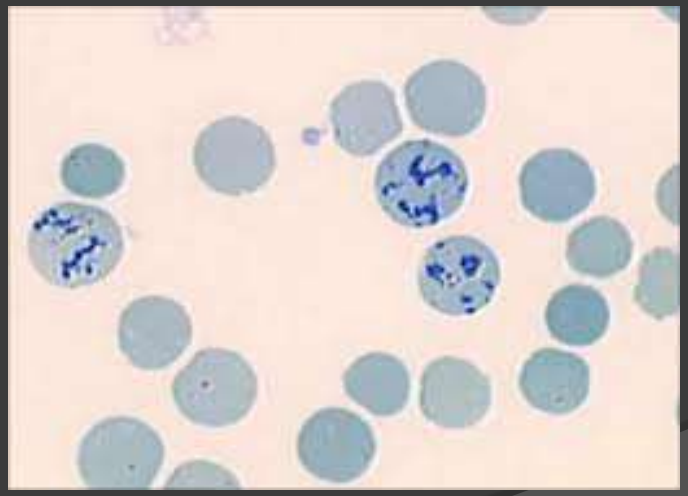
Signs of Hemolysis

Autoagglutination

Indicates immune mediated hemolytic anemia (IMHA)

- 3.Anaemia produced is regenerative in most cases & characterized by reticulocytosis, anisocytosis &increase in MCV.





- Note: If the cause of AIHA leads to injury or inhibition to bone marrow non-regenerative anaemia will result(normocytic normochromic) as in EIA in horses (Equine infectious anaemia).

- 4. Coomb,s test or direct antiglobulin test(DAT): It is a test used to detect anti-rbcs Abs when visible agglutination is absent, all cases of AIHA are Coomb,s test positive.

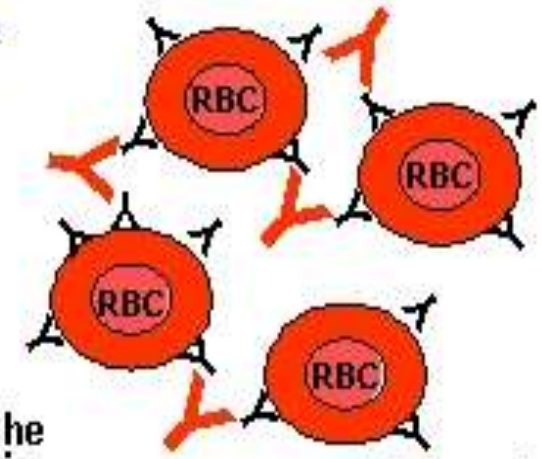
DIRECT COOMB'S TEST

+

anti- Hu IgG
Coomb's
Reagent

=

Patient
Sample



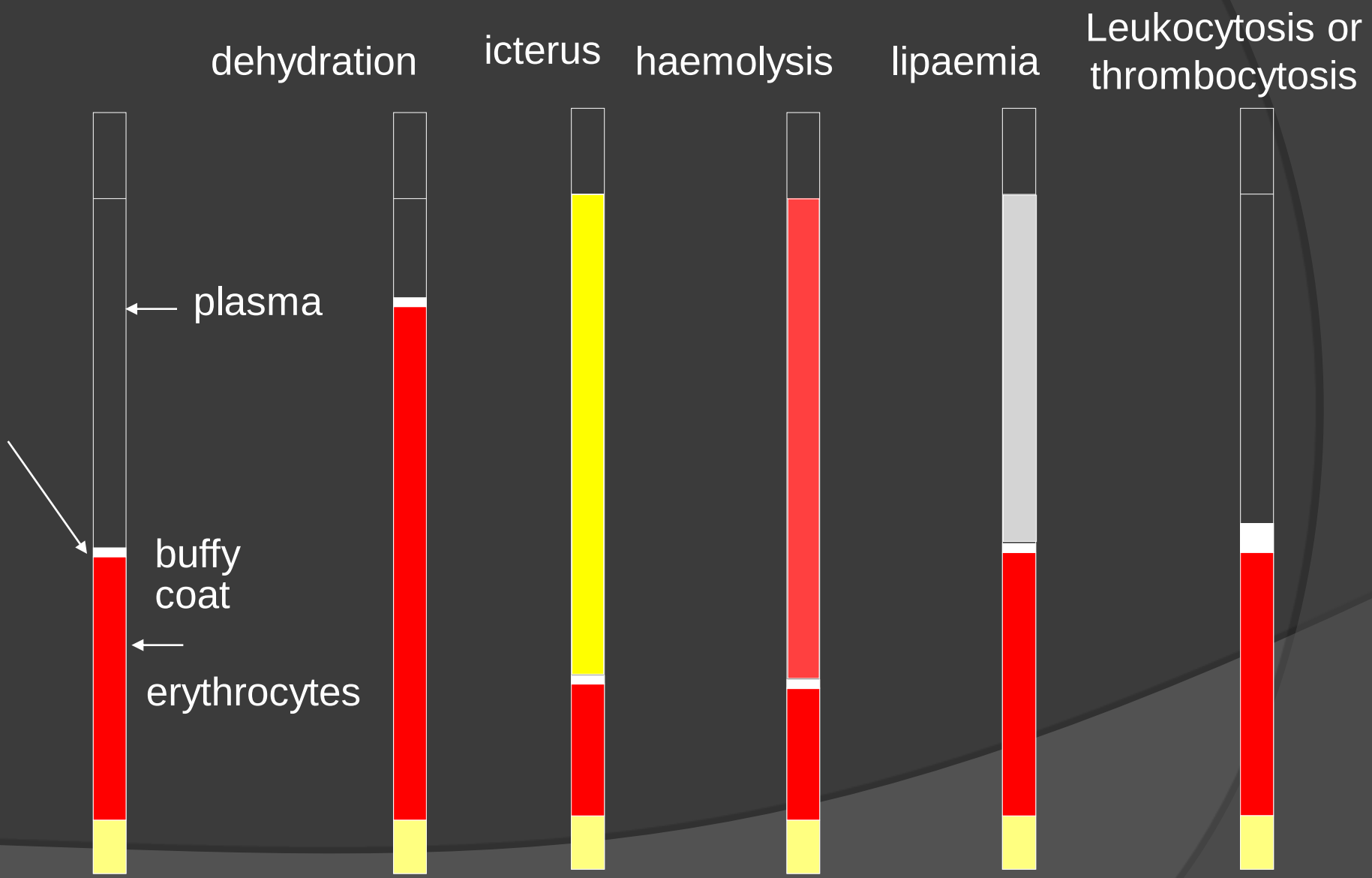
Example: The baby's sample is positive for the presence of the mother's Ab on the surface of RBCs in erythroblastosis fetalis

Agglutination

- 5. Hyper- bilirubinaemia & bilirubinurea, haemoglobinuria is observed when intravascular rbcs destruction takes place.

Color of plasma in pilot tubes varies from normal, lipaemic, icteric and hemolyzed (from right to left)

CAPILLARY TUBE SECTIONS AND PLASMA COLOURS



C- Anaemia caused by decrease in RBCS production: Such anaemia lacks evidence of bone marrow response or non-regenerative(non-responsive).It results from reduced or defective erythropoiesis:

1-Reduced erythropoiesis:

- chronic renal diseases(decrease in EPO).
- chronic diseases as inflammation and neoplasia.
- endocrine diseases as hypothyroidism hypoadrenocorticism.

- cytotoxic damage to bone marrow.
- infectious agents like Ehrlichiosis.
- feline leukemia virus infection (FeLV),
- myelophthisis as in leukemia, lymphoma, multiple myeloma metastatic tumors etc.

2-Defective erythropoiesis: It means abnormal or incomplete erythropoiesis, it includes:

a-Disorders of haem synthesis:

Mostly due to iron, copper or B6 deficiency all are needed for complete and normal synthesis of haem.

b-Disorders of nucleic acid synthesis:

Due to B12 and folic acid deficiency, both are coenzymes needed and are essential for nucleic acid synthesis to accomplish mitosis of the developing rbc, both are rare in domestic animals, in man they cause megaloblastic anaemia characterized by macrocytic normochromic anaemia.

c-Abnormal maturation of rbcs: Caused by erythroleukemia, or myelodysplastic syndrome.

Classification of anaemia according to erythrocyte indices:

Anaemia may also be classified using MCV&MCHC values to assist in determining the cause of anaemia.

- Terms used to express size:
- Macrocytic = increase in MCV.
- Normocytic = normal MCV.
- Microcytic =decrease in MCV.

- Terms used for MCHC (haemoglobin concentration):
- Normochromic = normal MCHC.
- Hypochromic=decreased MCHC.
- Anaemia is not classified as. hyperchromic because high MCHC is an artifact.

Comparison of classification of anaemia by rbc indices and etiology:

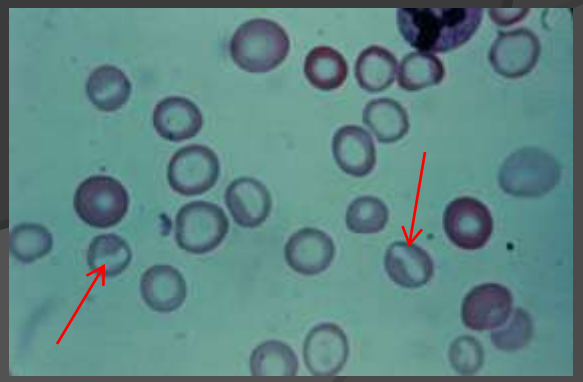
Normocytic normochromic: With poor or no bone marrow response associated with:

- Acute haemorrage(after < 3days).
- Acute haemolytic disease (before sufficient time has relapsed for sufficient reticulocyte production).
- Chronic inflammations and neoplasia.
- Chronic renal failure.

- Endocrine insufficiency .
- Selective erythroid aplasia
- Aplastic and hypoplastic bone marrow.

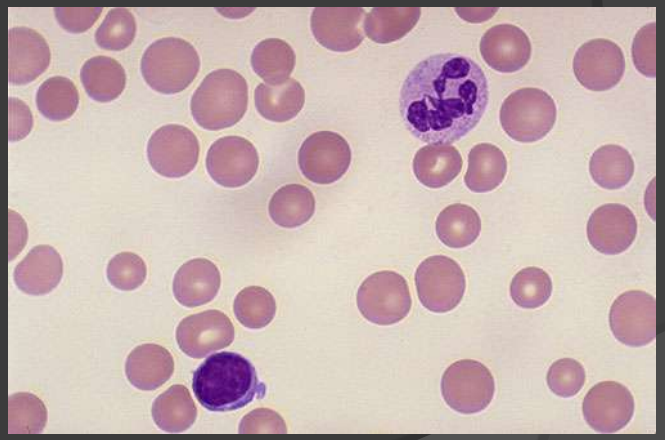
Macrocytic hypochromic:

- Regenerative anaemia with marked reticulocytosis.
- Hereditary stomatocytosis in dog.



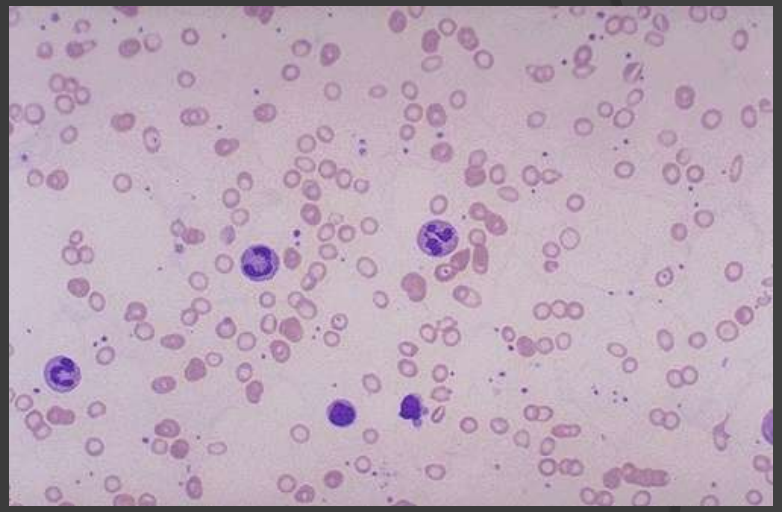
Macrocytic normochromic:

- Regenerative anaemia (decrease in MCHC is not always present).
- Infection with Feline leukemia virus(FeLV) due to dyserythropoiesis and maturation arrest.
- Normal regenerative response in equine.



Microcytic normochromic:

- Chronic iron deficiency.
- Anaemia of chronic diseases usually normocytic).
- Portosystemic shunt.
- copper &pyridoxine (B6) deficiency.



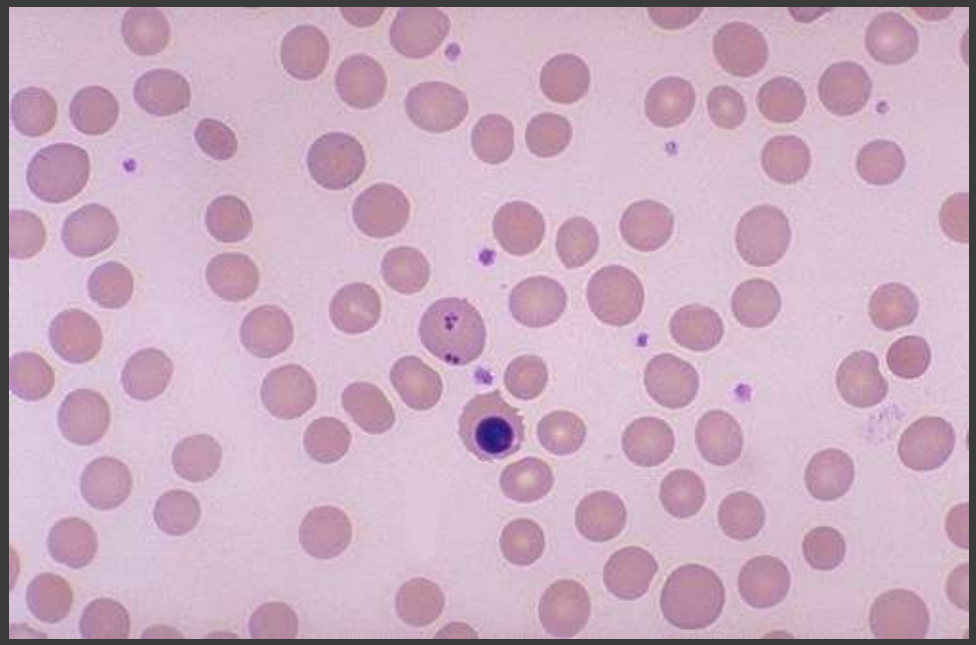
Classification of anaemia according to bone marrow response:

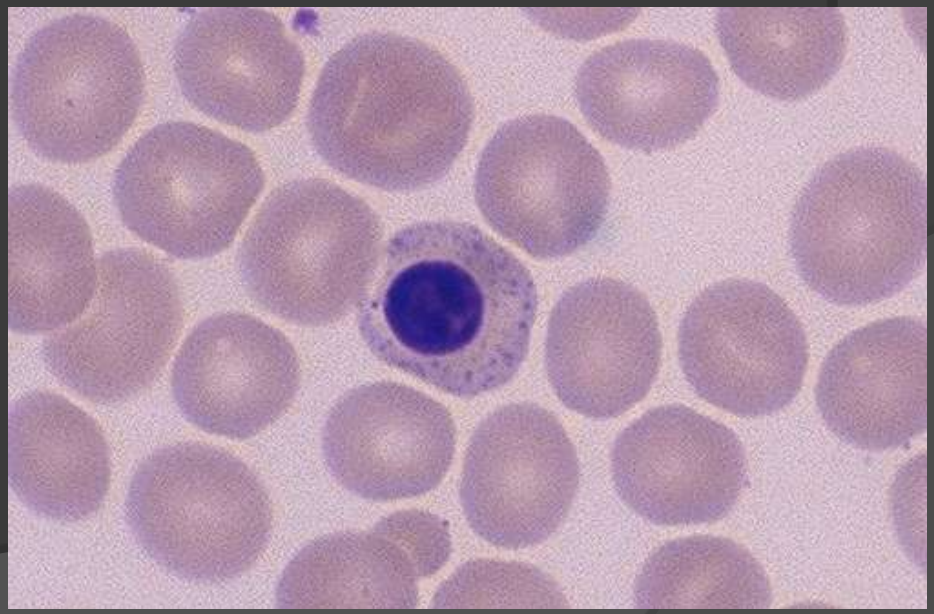
a-Regenerative or responsive anaemia:

It is characterized by good bone marrow response which is associated with the following lab. Results:

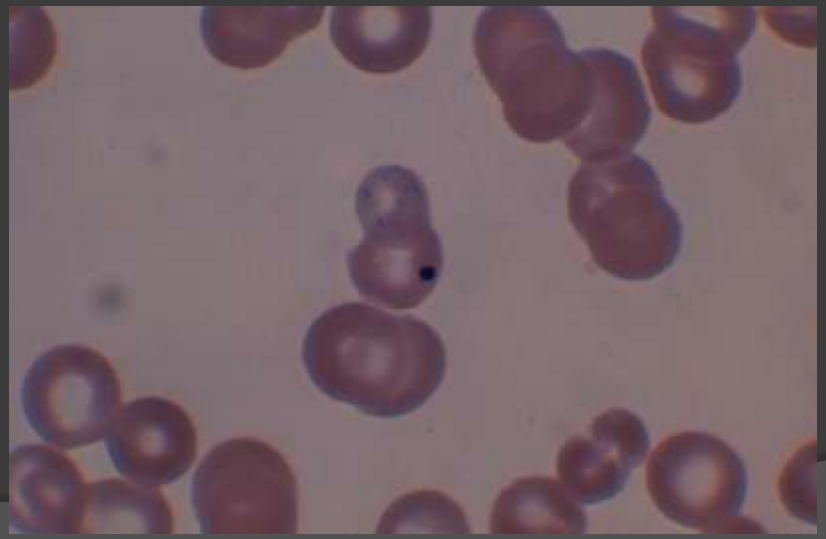
- Reticulocytosis: except in equine they do not release reticulocyte to the peripheral circulation but macrocytes with increase in MCV.
- Examination of stained blood film:

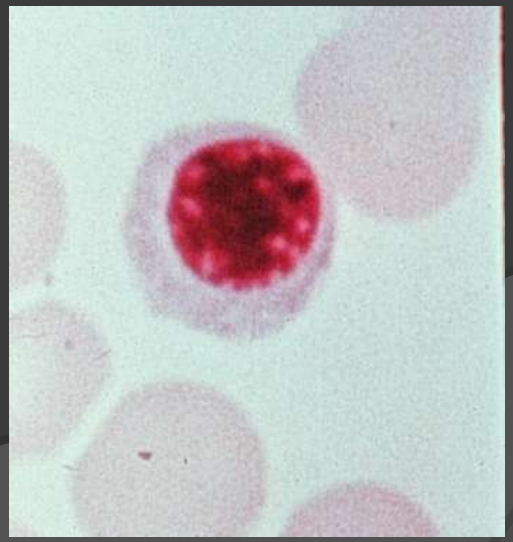
1. Increase in polychromasia which indicate reticulocytosis.





2. Anicocytosis due to the presence of large immature rbcs.
3. Presence of nucleated rbcs (metarubricytes, rubricytes).
4. Howell- Jolly bodies.
5. Basophilic stippling, in ruminants rarely other animals(in lead toxicity in all other species).





Expected causes of responsive anaemia:

- Acute blood loss of more than three days duration, and some cases of short term chronic blood loss before iron deficiency develop.
- Haemolytic diseases e.g. deficiency of certain important rbc enzymes like pyruvate kinase. Immune-mediated haemolytic anaemia. Infection associated haemolysis as babesiosis, bacillary haemoglobinuria, anaplasmosis etc.
- Chemical or toxin - induced haemolytic anaemia.

b-Non-responsive or non-regenerative anaemia:

Bone marrow response is not evident in spite of the presence of anaemia, there is no reticulocytosis or anisocytosisa anaemia is normocytic normochromic .

Causes:

- Blood loss of less than three days.
- Diseases associated with suppression of erythropoiesis e.g. Chronic inflammatory diseases ; renal failure,(decrease in EPO), drugs and toxins affecting erythropoiesis selectively in the bone marrow.
- Infections, e.g. Parvo virus infection in dogs.
- Bone marrow diseases e.g. Myelofibrosis, pure red cell aplasia (autoimmune in nature), marrow necrosis, neoplasia .
- Radiation.

ERYTHROSYTOSIS (POLYCYTHEMIA)

It refers to increase in HCT, Hb and RBC count above the normal reference range. Normal reference range can vary between species and breeds. Polycythemia is either absolute or relative.

Relative polycythemia: The PCV is high but total RBC mass is normal. It is caused by:

a- splenic contraction (transitory or physiologial polycythemia): As occur in excitement, fear, pain, or exercise after the release of adrenaline from the adrenal medulla.

b- Dehydration: Causes polycythemia as from water loss after diarrhea vomiting excessive diuresis, sweating or water deprivation. Plasma protein will increase also, clinical signs of dehydration may be detected by examination.

Absolute polycythemia: Real polycythemia characterized by real increase in circulating RBC mass, it may be

primary secondary

Primary absolute polycythemia: Also known as *Polycythemia Vera*, it is an erythrocytosis that result from myeloproliferative disorder of unknown cause, there is normal or decreased EPO level in the blood. It is EPO- independent Autonomous proliferation of rbcs precursors, mostly observed in man, dogs & cats. Familial erythrocytosis has been described in highly inbred Jersey calves .There is persistence of a moderate or marked increase in pcv. Arterial blood gas is normal.

Secondary absolute polycythemia: Mostly characterized by increase in EPO level in the blood & increase in EPO production.

It is caused by :

a- ***Chronic hypoxia*** as seen in chronic lung diseases, heart defects with right to left shunting of blood, living in high altitudes, methaemoglobinaemia.

b- ***Renal disorders:*** Causing local hypoxia in renal tissue like tumors , renal cysts hydronephrosis .

C- ***EPO – secreting tumors:*** As nephroma, hepatoma.

This kind of polycythemia is characterized by :

In chronic hypoxia there is increase in Pco2 in and decrease in Po2 when arterial blood gases are measured.

There is increase in EPO production . Clinical examination & diagnostic images to differentiate between lung and heart diseases.