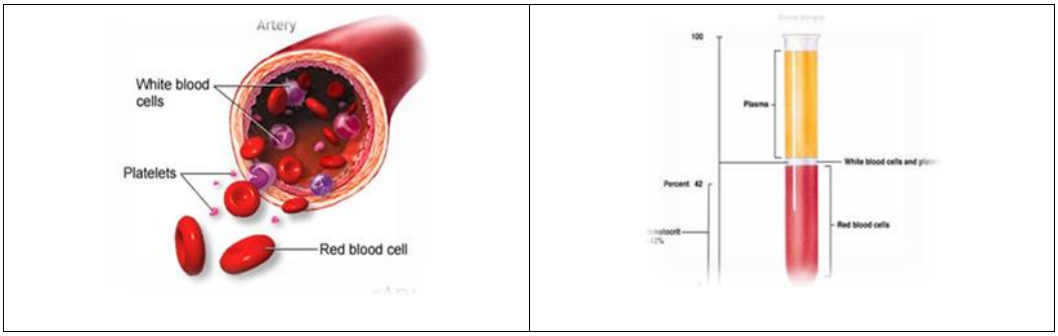
Clinical pathology

- **Clinical pathology**: it means application of different lab. methods on a properly collected sample to evaluate the health or to study the disease in the living or dead subjects; and the subsequent use of the obtained results in making accurate diagnosis and prognosis for the solution of the clinical problem. It makes a link between patient, clinician and lab.
- It includes: Clinical haematology, Clinical biochemistry, Clinical parasitology and Clinical microbiology.

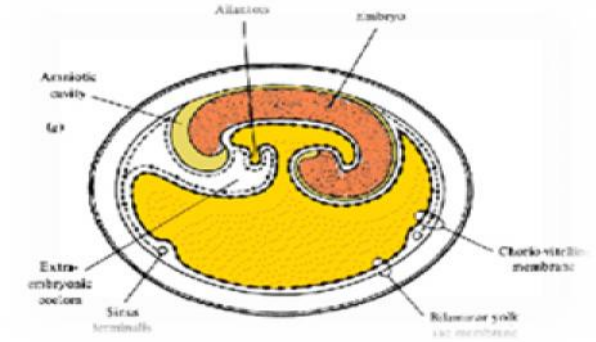
❖ CLINICAL HAEMATOLOGY

- It is the study of blood and blood forming organs. In clinical practice, it 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. Plasma is composed of 90% water, 10% dissolved substances as plasma proteins, electrolytes, hormones, vitamins and other organic compounds.

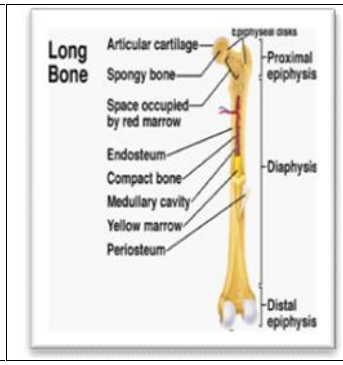


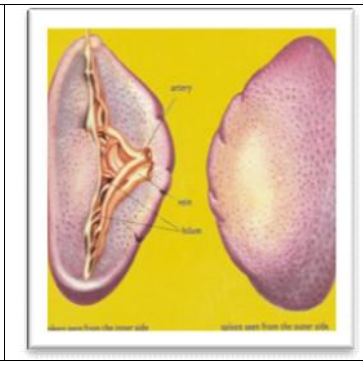
HAEMATOPOIESIS (HAEMOPOIESIS):

- It is the process of blood cell production and platelets which continues throughout life, replacing aged cells (which are removed from the circulation).
- **In prenatal life:**
  - First quarter of gestation: It takes place in the yolk sac, outside the embryo.

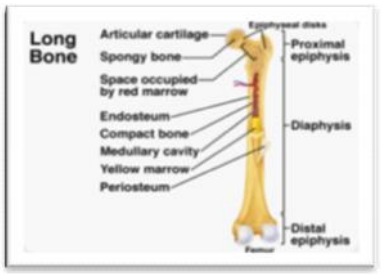


- In the second quarter of gestation: The liver mainly takes part and to a lesser extent the spleen, start of the bone marrow and lymphoid organs to take part in haematopoiesis in mammals.

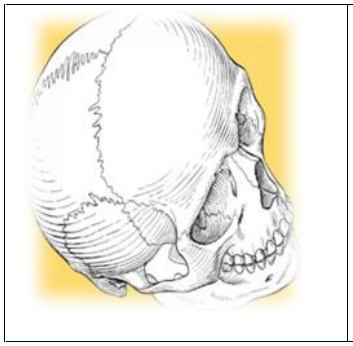
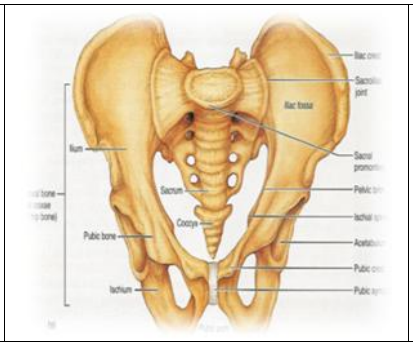
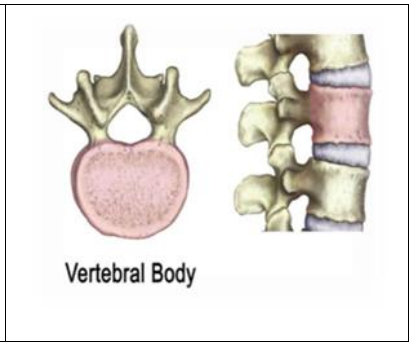


- At the time of birth nearly all blood cells are produced in the bone marrow (MEDULLARY HAEMATOPOIESIS) and haematopoiesis immediately or gradually stops in the liver and spleen.



- In certain disease conditions, when there is great need for blood cells these two organs 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) .The bone marrow has two compartments:

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 which have many pseudopods that may completely encircle the developing blood cells ,in older animals they became loaded with fat producing fatty ,yellow inactive marrow that can turn to active red marrow in need. In addition that there is a group of accessary cells including macrophages, lymphocytes, and natural killer (NK) cells. Also extracellular matrix like collagen.

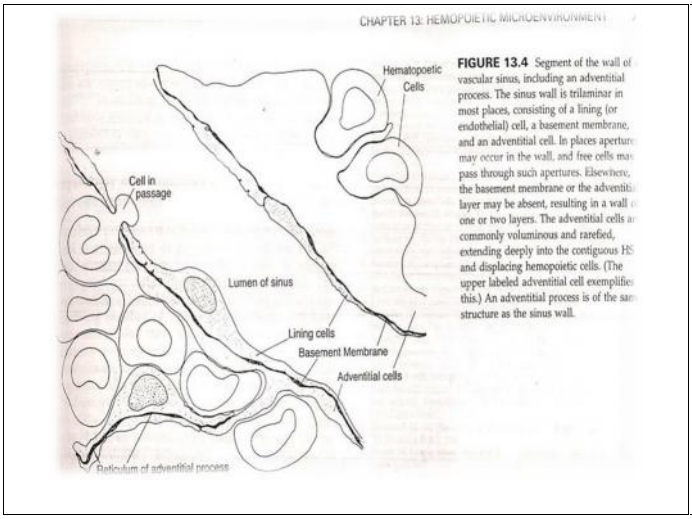
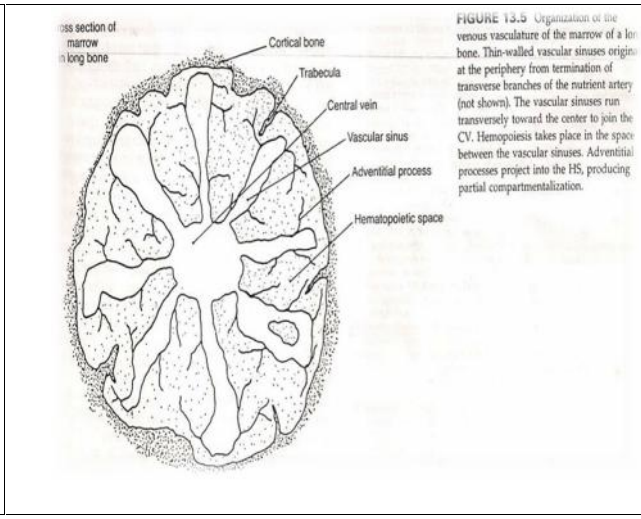


FIGURE 13.4 Segment of the wall of vascular sinus, including an adventitial process. The sinus wall is trilaminar in most places, consisting of a lining (or endothelial) cell, a basement membrane, and an adventitial cell. In places aperture may occur in the wall, and free cells may pass through such 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.



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 spaces between the vascular sinuses. Adventitial processes project into the HS, producing partial compartmentalization.

Haemopoietic growth factors:

- They are cellular products produced to regulate and control haemopoiesis:

1. **POIETINS:**

   a) **Erythropoietin (EPO);** It is a hormone-like circulatory glycoprotein produced mainly in the kidney; lesser amount is produced in kupffer cells in the liver. EPO stimulate erythropoiesis in response to hypoxia.

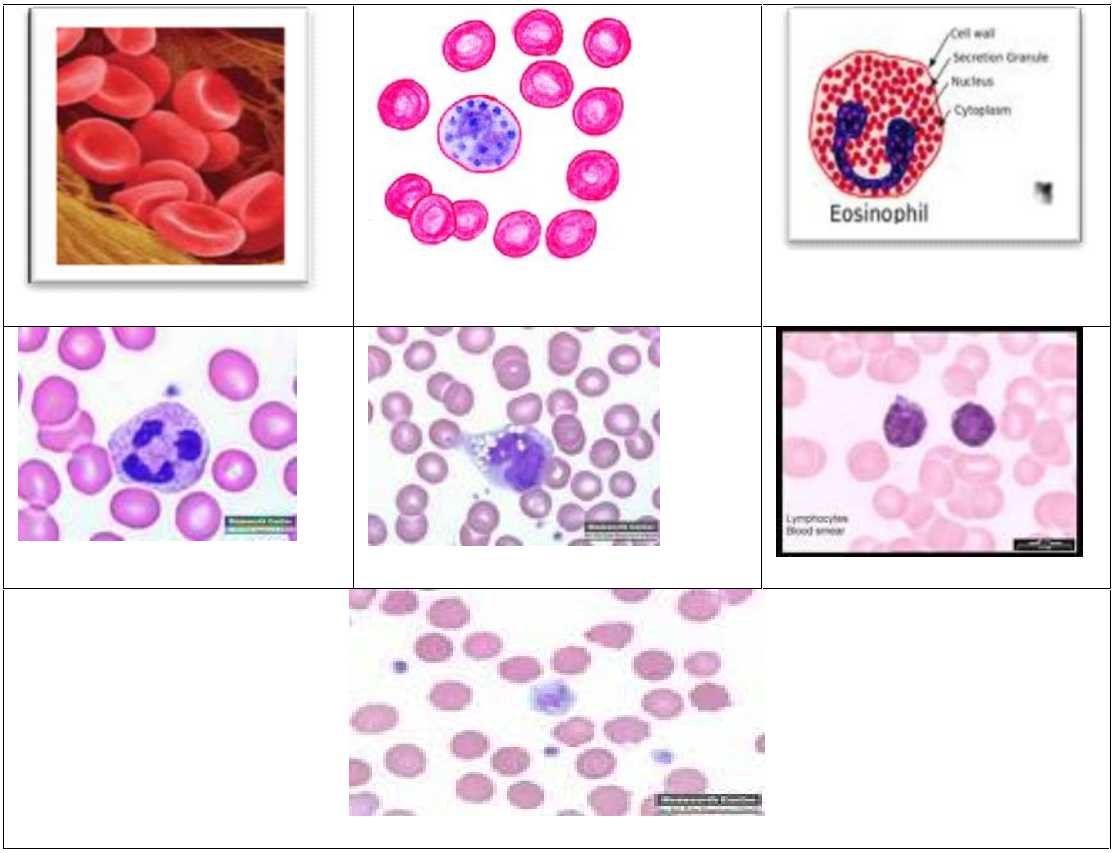
   b) **Thrombopoietin (TPO):** It is synthesized in the kidney and in the liver; it stimulates platelets production on different levels in the bone marrow (BM).

2. **2.Colony stimulating factors(CSFs):**They are glycoproteins act directly on haemopoietic sub –populations in the BM ,produced from adventitial cells, T lymphocytes, macrophages and stromal cells, e.g. stem cell factor(SCF), granulocyte colony stimulating factor(G-CSF), macrophage colony stimulating factor(M-CSF), GM-CSF…..etc.

3. **Interleukins:** It is a family of proteins produced by different cells (cytokines) like fibroblasts, macrophages, activated T lymphocytes, endothelial cells... Etc. they control some aspects of haematopoiesis & immune response.

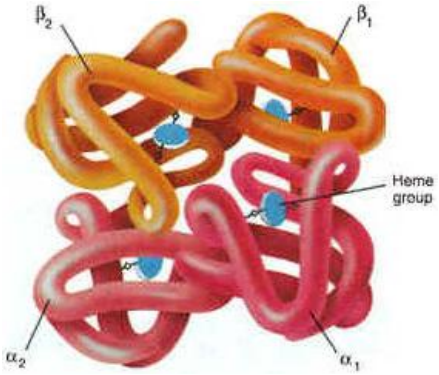
- **Blood cells are:**

1. Red blood cells or erythrocytes (RBCs).
2. White blood cells (WBCs):
   - **a.** Mononuclear WBCs (monocytes & lymphocytes) also knew as agranulocytes.
   - **b.** Polymorphonuclear WBCs (neutrophils, eosinophil, and basophils) also known as granulocytes.
3. Platelets or thrombocytes, they are not true cells but cytoplasmic fragments of the giant BM megakaryocytes.

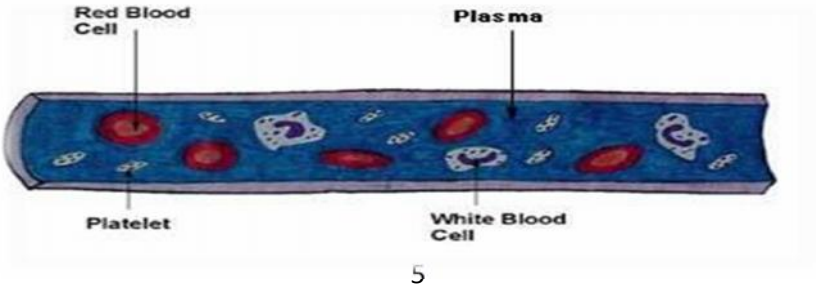


THE ERYTHRON

- It is a term applied to the circulating RBC mass, RBCs precursors in the bone marrow and erythropoiesis stimulating factors. RBCs, are biconcave discs, actually, they are bags that can deformed into almost any shape. They loss their nuclei before leaving the BM.
- **Function:** Transport of oxygen to the tissues &transport of carbon dioxide from tissues to the lungs, this is mediate by haemoglobin that fills these corpuscles. In addition rbcs have a buffering activity since they contain large quantities of the enzyme Carbonic anhydrase.
- **Haemoglobin (HB) consists of:** Haem & Globin, each complete haemoglobin unit is a tetramer or globular unit made up of four subunits; each subunit contains haem conjugated to a polypeptide chain of the globin.
  - **Haem:** is an iron containing porphyrin derivatives (ferrous iron), there are four haem units in each (Hb) molecule.
  - **Globin :** Are polypeptides, two pairs of polypeptides in each haemoglobin molecule, they are of special amino acid sequences .Each two chains are identical 2 α chains and 2 β chains in Adult Hb (Hb -A) .Fetal Hb (Hb- F) contains 2 α and 2 ϒ . Hb-F is soon replaced by Hb-A after birth; Hb type depends on the type of globin chain which is determined by amino acid sequences.

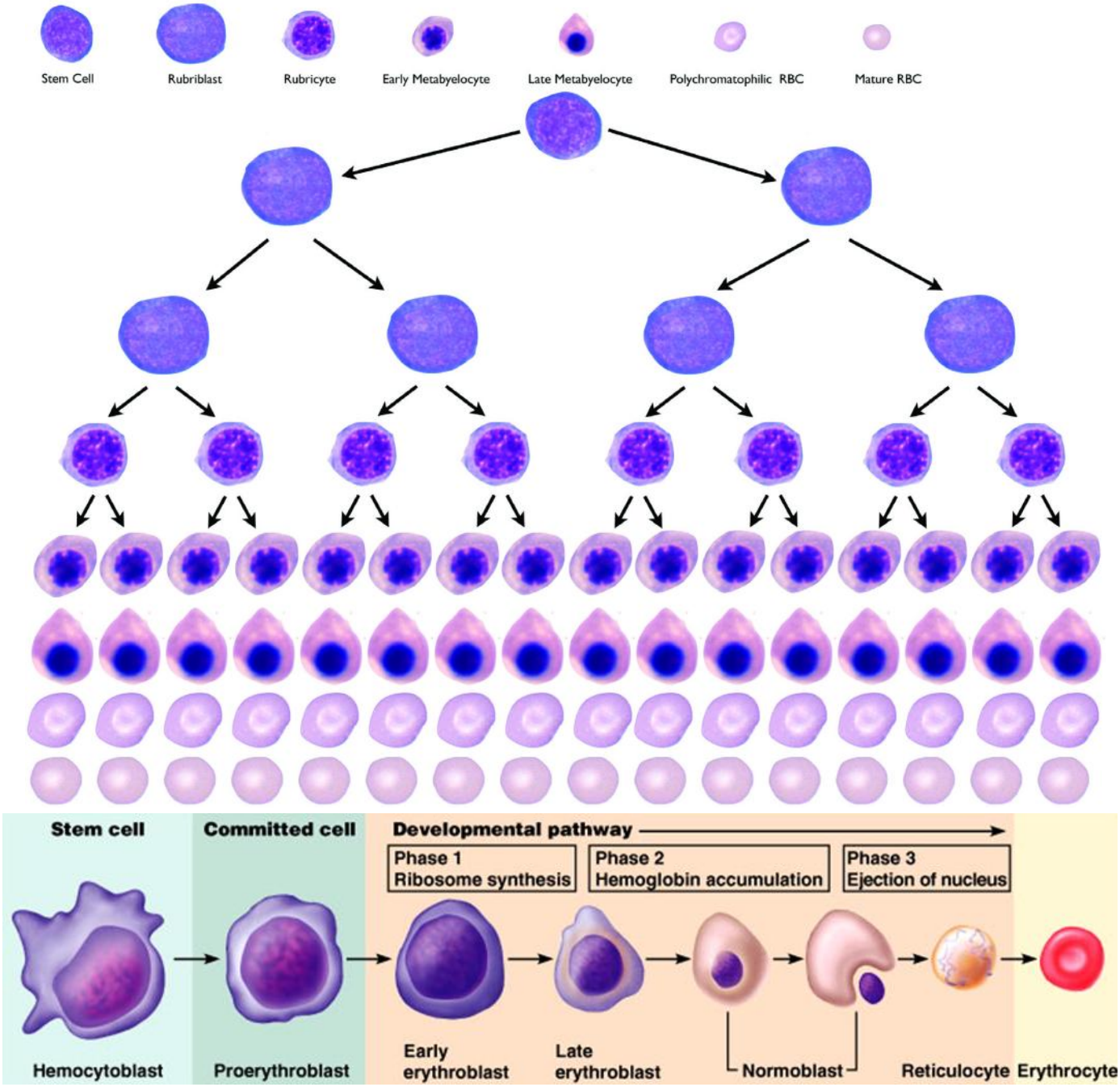


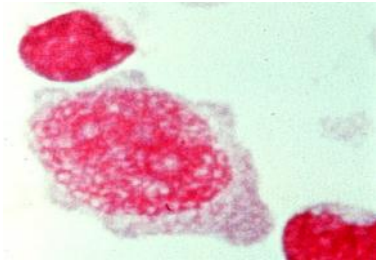
- Oxygen molecule reversibly attached to the ferrous iron in the haem molecule, it is oxygenation and not oxidation, oxidation of ferrous to ferric change haemoglobin to methaemoglobin which is unable to bind oxygen.

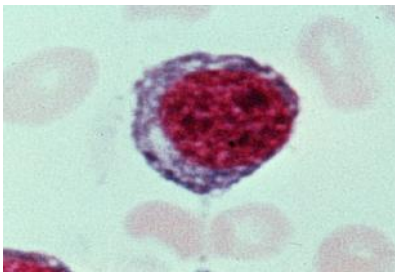


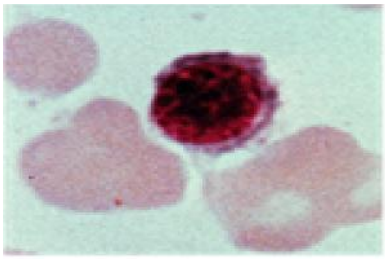
- RBCs may be considered as cell membranes containing haem globin and protective enzymes system that is responsible for ATP production, haemoglobin protection and anaerobic glycolysis. RBCs in mammals lack nuclei, ribosomes, mitochondria, rough endoplasmic reticulum, so they depend on anaerobic glycolysis to obtain energy needed.

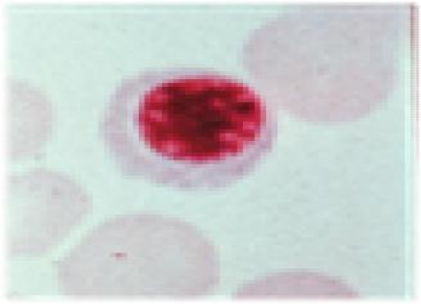
- ***Erythropoiesis:*** pluripotent haemopoietic stem cell (PHSCs) ⟶ Pluripotent myeloid stem cell (PMSCs) ⟶ Burst forming unit erythroid (BFU-E) ⟶ Colony forming unit erythroid (CFU-E) ⟶ Rubriblast ⟶ 2-Prorubricytes 4-Rubricytes (basophilic) 8-Rubricytes (Polychromatophilic) ⟶ 16-Rubricyte (normochromic) ⟶ 32- Metarubricyte ⟶ 32-Reticulocyte ⟶ 32-Erythrocyte.

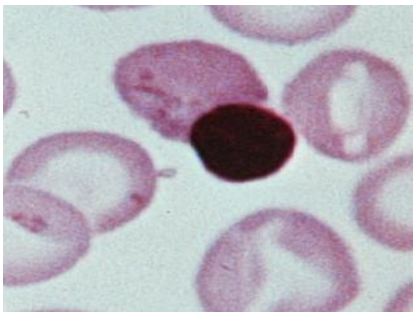


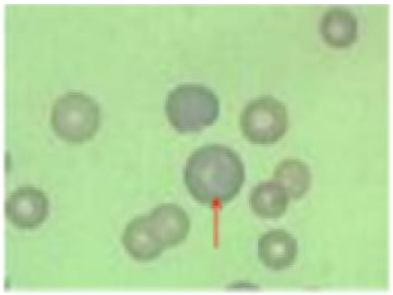

Stem cell


Basophilic rubricyte


Polychromatophilic rubricyte

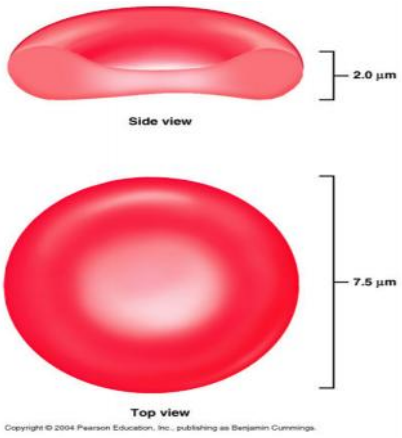

Normochromic Rubricyte


Metarubricyte


reticulocytes

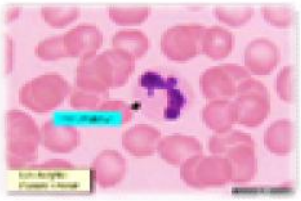
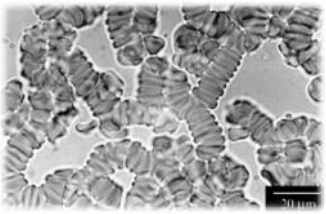
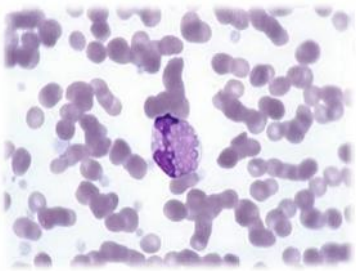
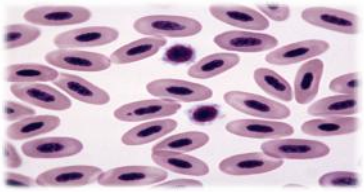
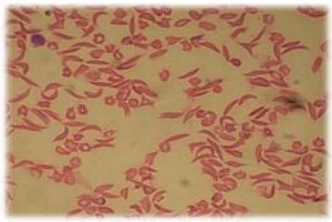
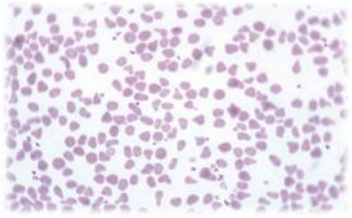
- **Morphology:** Mammalian RBCs are discoid –shaped.
  Benefits: Flexible, Maximum, surface/volume ratio, Size – uniform.
  Diameter of rbcs varies according to animal sp. that of the dog is the largest and that of the sheep and goats are the smallest.

species	RBC count	Morphology	PCV	MCV	MCHC
DOG	5.5-8.5		37-55	60-77	32-36
CAT	5.0-10.0		24-45	39-55	30-36
COW	5.0-10.0		24-46	40-60	30-36
SHEEP	8.0-16.0		24-50	23-48	31-38
GOAT	8.0-18.0		19-38	15-30	35-42
HORSE	6.5-12.5		32-52	34-58	31-37



The shape, size, and number of erythrocytes varies considerably between species.

The MCHC, however, is fairly similar between species

Canine RBCs: are large with central pallor (biconcave discs). Central pallor is slight in feline and bovine RBCs.	
equine rbcs: has shallow concavity lack central pallor with extensive rouleaux formation (grouping of rbcs in the form of chains resembling a stack of coins). 	
Camels and llamas rbcs: have small flat elliptical rbcs (ovalocytes).	
RBCS of birds: are large, oval and nucleated.	
Cervidae: is characterized by sickling phenomena on standing of blood sample in room temp. or in refrigerator.	
Caprine rbcs: are the smallest, round or bluntly triangular lacking central pallor with prominent poikilocytosis (sickle cells)and anisocytosis.	

⦿ ***Evaluation of erythron :***

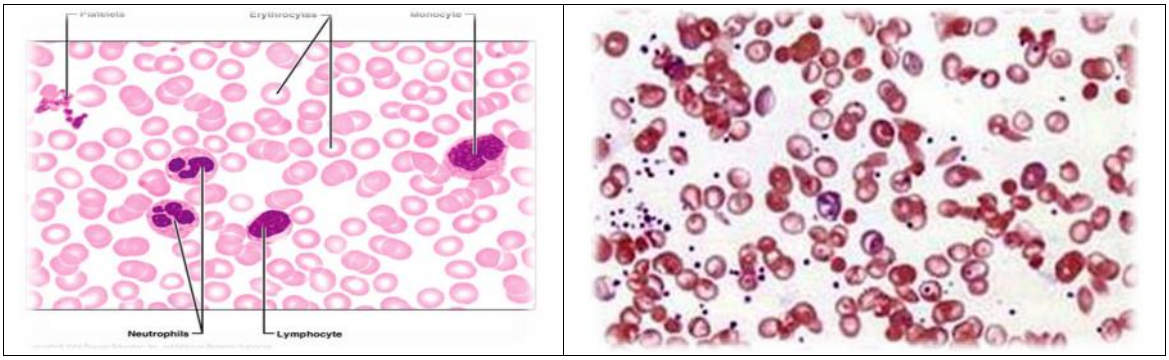
1. RBC count/µl of blood ; decrease in rbcs means anaemia while increase in rbcs means polycythemia.
2. Packed cell volume(PCV%) also known as microhaematocrit value (Hct %) , it is the most efficient method for evaluating the erythron.

3. Meassurment of haemoglobin concentration (Hb g/dl).
4. Erythrocyte indices: 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) = (pcv % × 10)/(rbc 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) = (Hb g/dl x10)/(rbc count) measured in Pico gram (pg).
   c) Mean corpuscular haemoglobin concentration (MCHC) = (Hb g/dl x100)/ (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. Stained peripheral blood film examination:

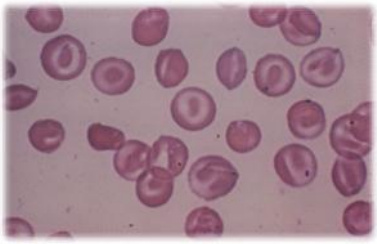
- Stained peripheral blood film examination:

A. Abnormal rbcs morphology:

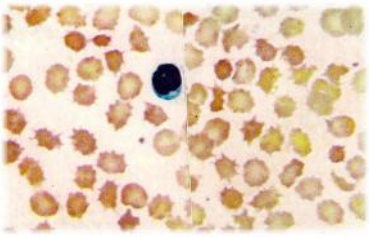
i) Abnormalities of shape (Poikilocytosis): It means major deviations from normal shape of erythrocytes for the particular animal sp. (minor deviations are normal).



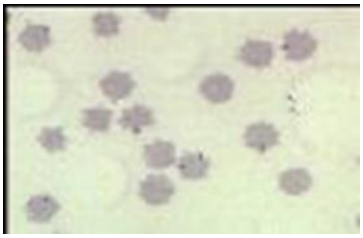
1. **Leptocyte :**Thin, flat, hypochromic rbcs with increased surface area and normal cell volume.They are of two types, target and folded cells, mostly observed in chronic diseases, iron deficiency anaemia, haemolytic anaemia, liver diseases, thalassemia. Small number is normal in the blood of dogs.



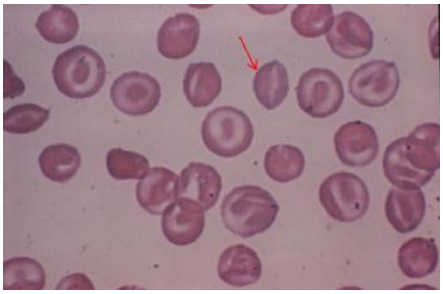
2. **Acanthocyte:** Spiculated rbcs with irregularly spaced and variably-sized spicules, it is formed when cholesterol is present in excess to phospholipid in rbc cell membrane. It is mostly due to increase in blood cholesterol, presence of abnormal plasma lipoprotein and liver diseases.

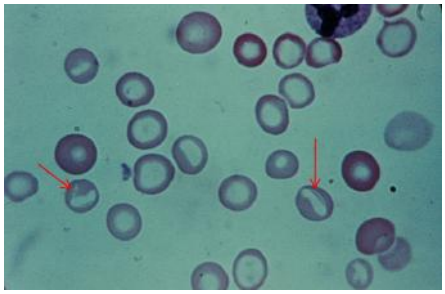


3. **Crenated RBC:** Spiculated, their spicules are relatively evenly spaced and of similar sizes ,it is considered as an artifact resulting from excess EDTA ,prolonged sample storage or delay in the dryness of blood films.

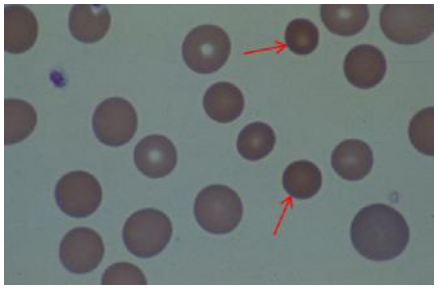


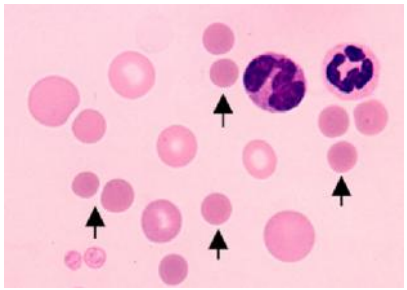
4. **Stomatocytes:** Cup -shaped rbcs that have elongated or slit- like central pallor. It is mostly seen thick stained blood films as an artifact or in hereditary stomatosytosis, liver diseases or chronic anaemias.



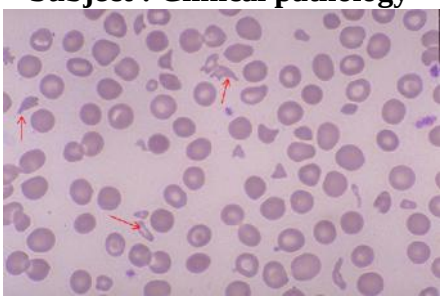


5. **Spherocyte:** RBCS lack central pallor with smaller diameter than normal and biconvex surface with spherical shape , it results from cell swelling and/ or loss of part of cell membrane; as in immune mediated haemolytic anaemia e.g. blood parasite infection, snake bite, zinc toxicity, it causes anisocytosis,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

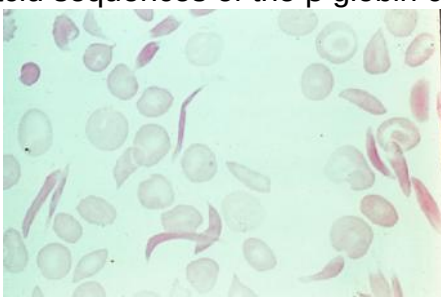




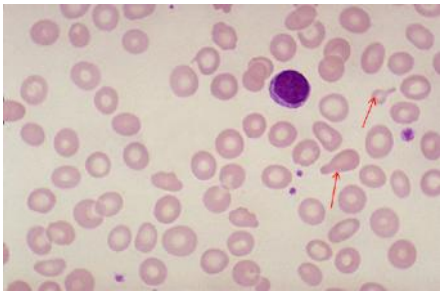
6. **Schistocyte:** Fragment of an rbc with two or three pointed extremities, they are smaller than normal rbcs. Its observation in a stained blood may indicates ;severe iron deficiency anaemia , DIC in dogs fibrin strands may split its rbcs, not the smaller rbcs of cats and horses.



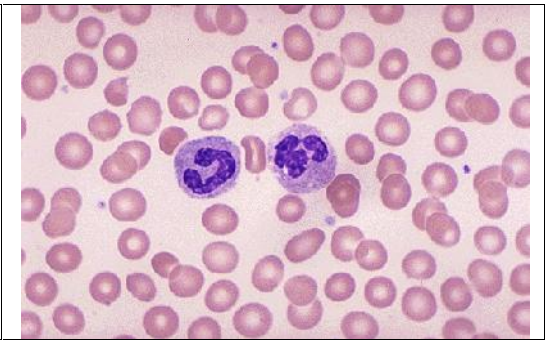
7. **Sickle cell:** Spindle – shaped rbcs ,it is considered normal in deer and young goat(in vitro phenomena due to high o tension + pH between 7.6-7.8. Sickle cell anaemia in man is due to abnormality in amino acid sequences of the β globin chain of haemoglobin (HB-S).



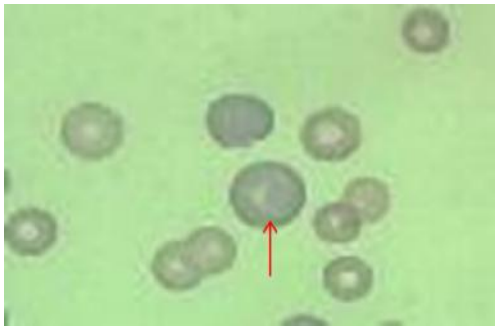
8. **Dacrocyte:** Tear drop-shaped rbcs with single elongated or pointed extremities Seen in blood of dogs and cats with myeloproliferative disorders and dogs with hypersplenism.

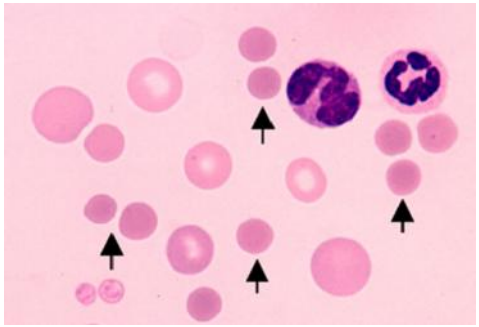


ii) Abnormalities and major deviations in size of rbcs (anisocytosis): Slight anisocytosis is normal in certain animal sp. as cow, less frequently in cat sheep and goat.

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.	

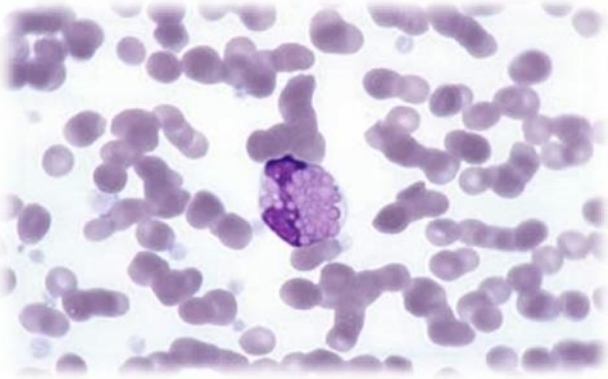
- Mostly it is due to the presence of different populations of cells e.g. macrocytes (reticulocytes) in responsive anaemia; or the production of small- sized (microcytes) rbcs as in iron deficiency anaemia. Spherocytes may also leads to anisocytosis.

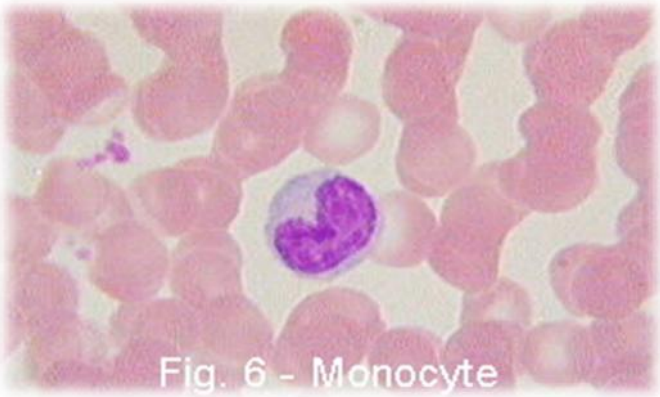




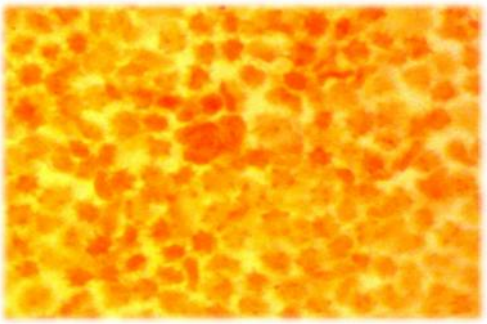
B. Abnormality in arrangement of rbcs:

1. **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.



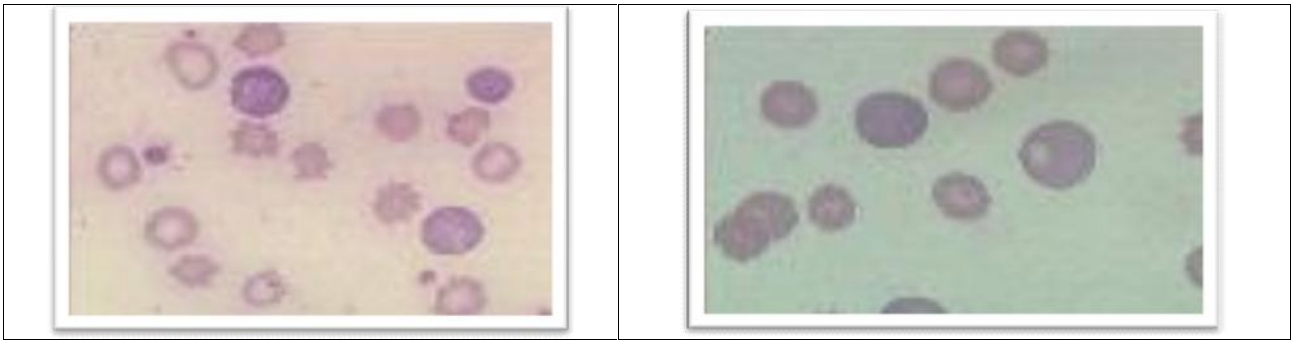


2. **Agglutination:** The aggregation or clumping of rbcs in together in clusters not in chains .It is caused by immunoglobulins bound to rbcs surfaces, as in immune – mediated anaemia.

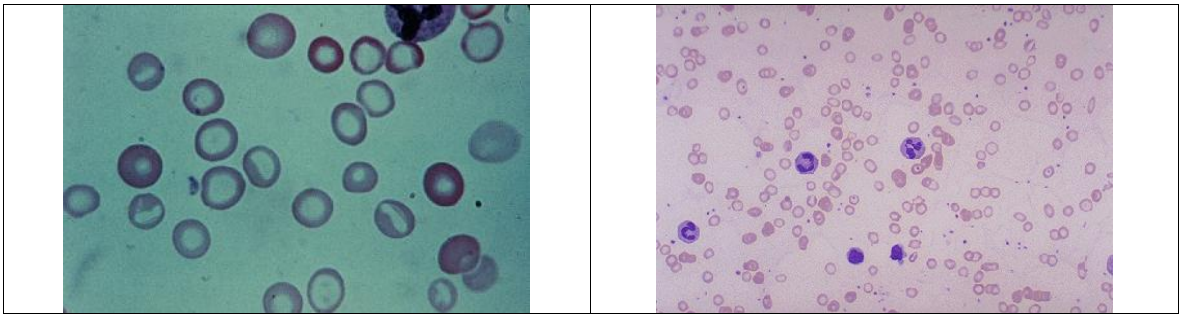


C. Abnormalities in colour of rbcs:

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.

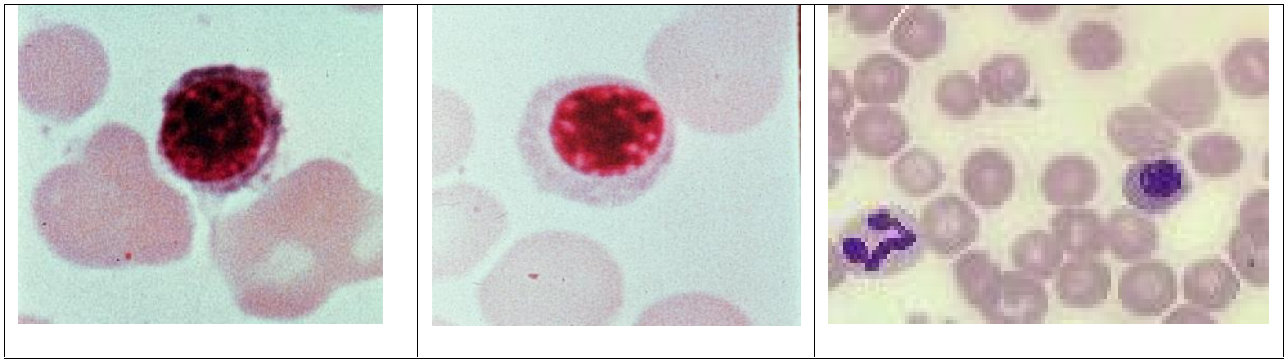


2. **Hpochromasia:** RBCS with decrease in Hb content and increase in central pallor. It is associated with decrease in MCHC. Increased hypochromasia is associated with iron deficiency anaemia and chronic blood loss.

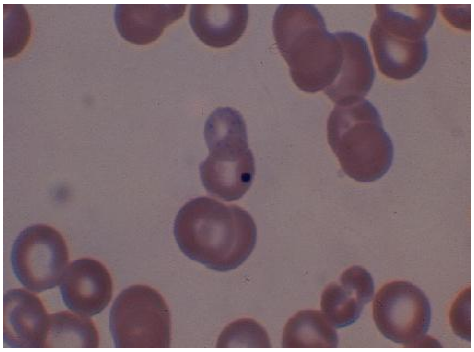


3. **RBC inclusions:** It means the presence of abnormal bodies inside rbcs.

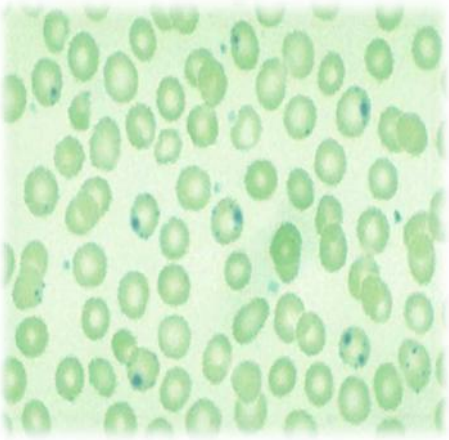
   a) **Nucleated rbcs:** Presence of immature rbcs in peripheral circulation e.g. rubricytes, metarubricytes is seldom in the blood of normal adult mammals, It is seen in regenerative anaemia, haematopoietic neoplasia and various inflammatory conditions.

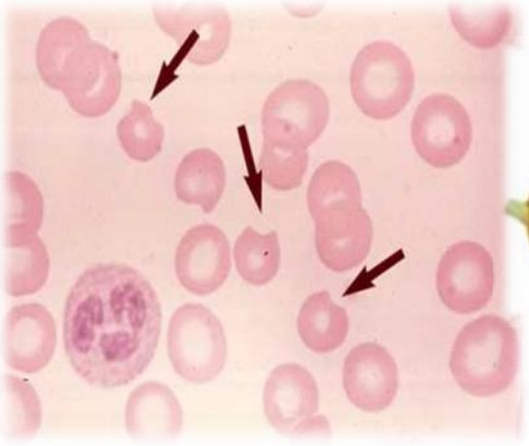


   b) **Howell-Jolly body:** They are small dark blue nuclear remnant (DNA in nature) formed in the bone marrow and it should be removed by pitting activity of the spleen. May be present in low number in rbcs of normal cats and horses, also it is associated with regenerative anaemia and blood films of splenectomized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

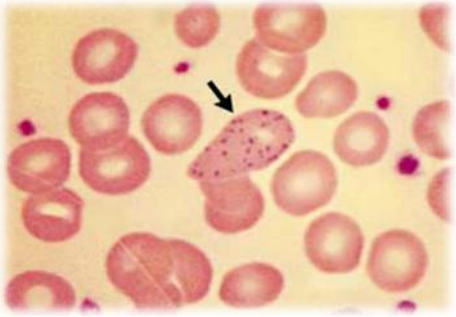


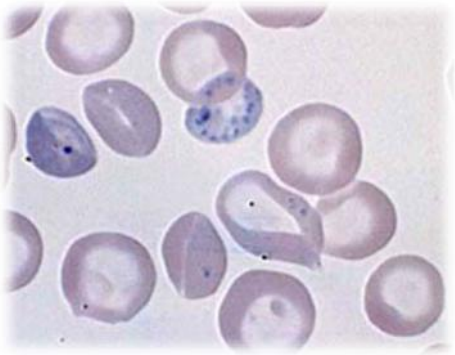
c) **Heinz body:** Large aggregates of oxidized precipitated Hb attached mostly to the internal surfaces of rbc cell membranes, they stain pale pink with Romanowsky's stains, dark blue with supra-vital stains. Normal cat blood film may show few Heinz bodies (5-10%).In pathological conditions it is associated 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.



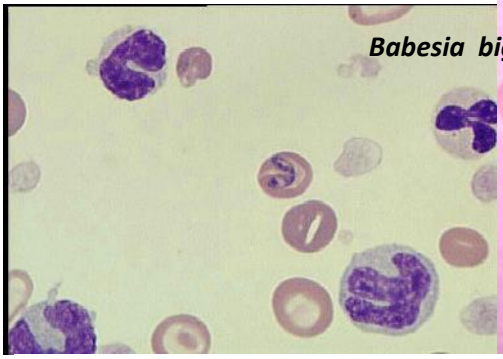


d) **Basophilic stippling:** Blue staining punctuate inclusions stained with Romanowsky's stains ,it represents RNA of polyribosomes, occur in regenerative anaemia in ruminants and lead toxicity in canine, punctuate may be fine or coarse.

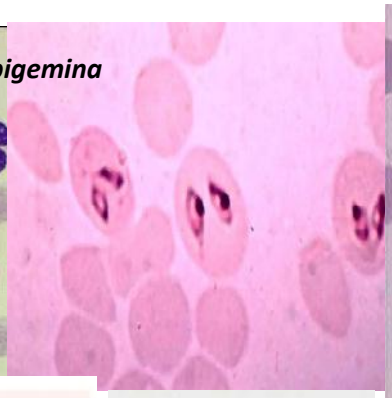


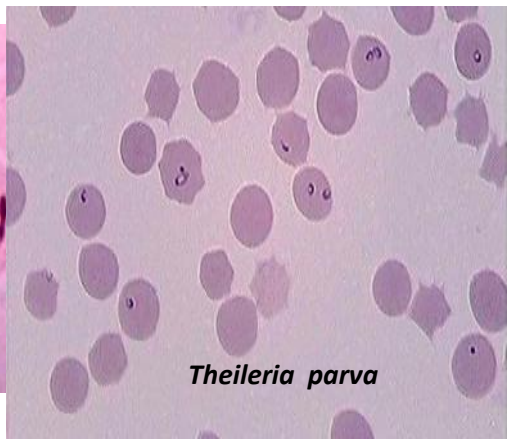


e) **Infectious agents:** As protozoa e. g. Babesia, Theileria, Malaria. Bacteria as the rickettsial microorganism, anaplasma. Viral inclusions e.g. Canine distemper viral inclusions appear as red or orange mostly rounded bodies (it should be differentiated from Howell-Jolly bodies.

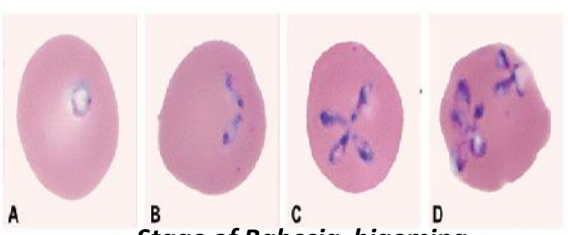


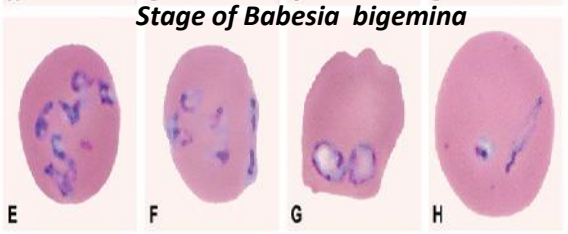
Babesia bigemina



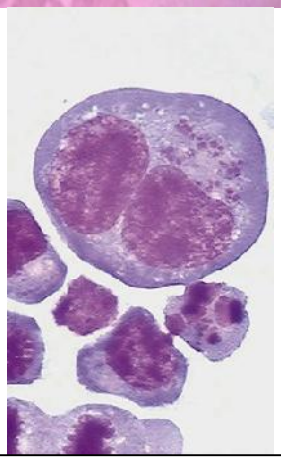


Theileria parva

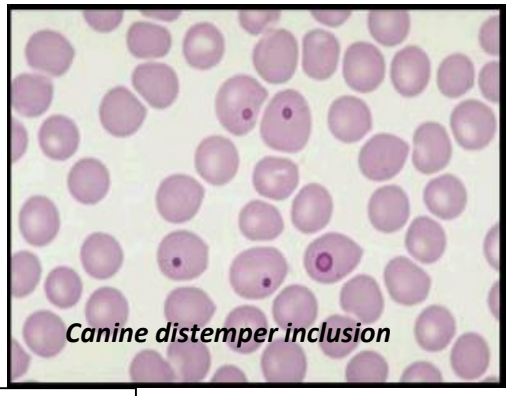




Stage of Babesia bigemina



Lymph smear showing schizonts of Thieleria



Canine distemper inclusion

Diseases of RBCs

I. Anaemia:

- True or absolute anaemia, may be defined as a decrease in erythrocyte mass in the body. HTC, HB and RBCs mass are usually below their normal range. Anaemia is a sign not a disease; it is a problem not a diagnosis.
- 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, and 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):

May be caused by ,different kind of 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; this may take place within the circulation (Intravascular haemolysis or extracellular haemolysis) it means outside macrophages of the spleen.
- Extravascular haemolysis or intracellular haemolysis; means destruction of rbcs inside splenic macrophages outside the vascular system:

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 animal s are:

1) Neonetal isoerythrolysis (NI) or isoimmune haemolytic anaemia:

It is a haemolytic disease of horse & mule foals ,rarely calves & kittens, problem arises when a dam is bred more than once to the same stallion which has different blood group; antigens of the male rbcs are transmitted to the fetus; then the dam develop antibodies against the fetal red cells. The colostrums then will contain antibodies against rbcs of the new born who ingested them they are then absorbed intact to the circulation, bind to neonatal rbc causing haemagglutination and intravascular haemolysis characterized by haemoglobinuria jaundice pale mucous membranes weakness &collapse, if not treated it is fatal.

2) Autoimmune haemolytic anaemia (AIHA): May be classified as:

a) 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.

b) Secondary AIHA: When it occurs as a result of concurrent disease e.g. viral, rickettsial, 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:
  - ❖ Low rbc, Hb & pcv values.
  - ❖ 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.
  - ❖ Anaemia produced is regenerative in most cases & characterized by reticulocytosis, anisocytosis &increase in MCV.
  - ❖ 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.
  - ❖ 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 coombs test positive.
  - ❖ Hyper- bilirubinaemia & bilirubinuria, haemoglobinuria is observed when intravascular rbcs destruction takes place.

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:**

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

ii. **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.

iii. **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):**

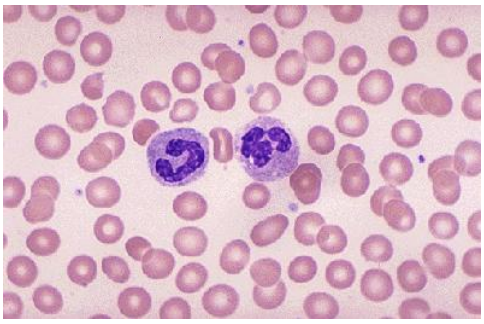
- Normochromic = normal MCHC.
- Hypochromic=decreased MCHC.
- Anaemia is not classified as. hyperchromic because high MCHC is an artifact.

◘ ***Comparison of classification of anaemia by rbc indices and etiology:***

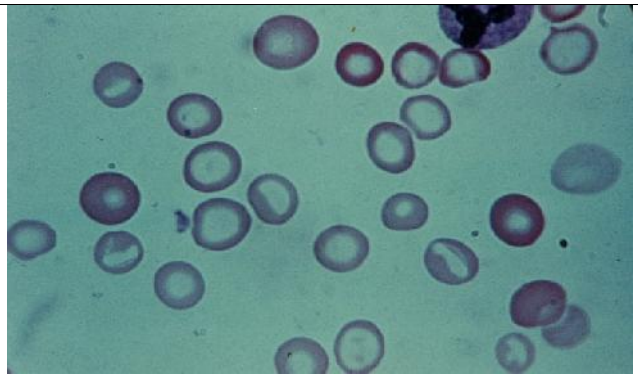
◆ **Normocytic normochromic**: With poor or no bone marrow response associated with:

- 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.

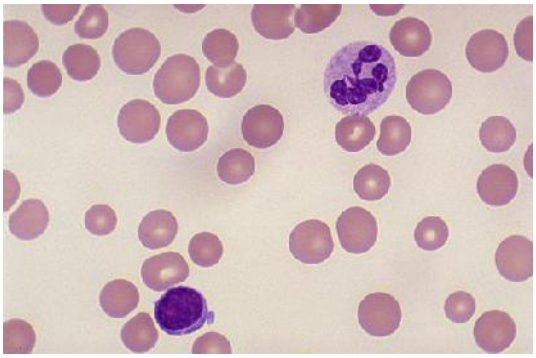


- **Macrocytic hypochromic:**
  - Regenerative anaemia with marked reticulocytosis.
  - Hereditary stomatocytosis in dog.



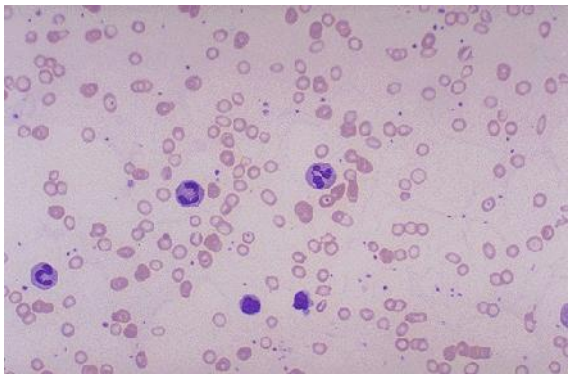
- **Macrocytic normochromic**:
  - Regenerative anaemia (decrease in MCHC is not always present).
  - Infection with Feline leukemia virus(FeLV) due to dyserythropoiesis and maturation arrest.

- Normal regenerative response in equine.



◆ **Microcytic normochromic**:

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

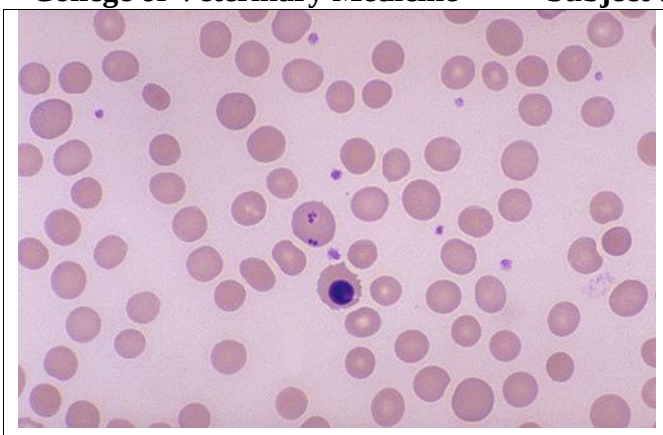


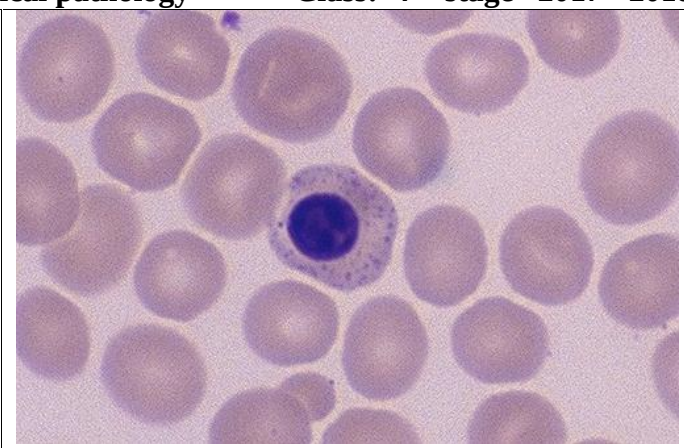
⦿ **Classification of anaemia according to bone marrow response:**

a) Regenerative or responsive anaemia: It is characterized by good bone marrow response which is associated with the following lab. Results:

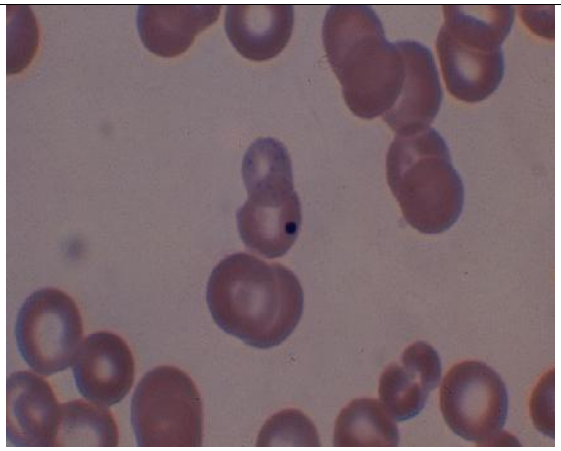
- Reticulocytosis: except in equine they do not release reticulocyte to the peripheral circulation but macrocytes with increase in MCV.
- Examination of stained blood film:

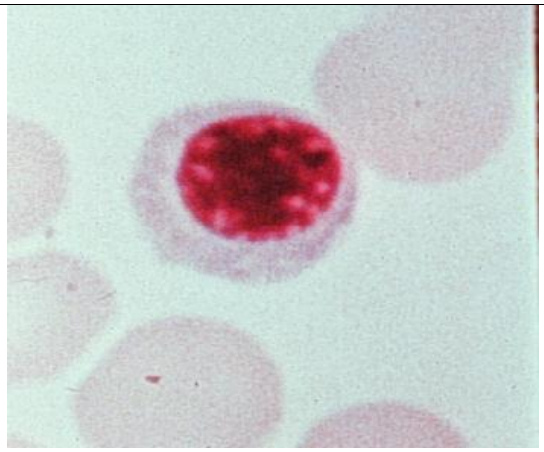
1. Increase in polychromasia which indicate reticulocytosis.





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 non – 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 < 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.
- ***Clinical signs of anaemia*:**
  - Pale mucous membranes (Icteric if haemolytic).
  - Weakness and exercise intolerance.
  - Tachycardia and polypnea particularly after exercise.
  - Increased sensitivity to cold.
  - Syncope and depression.
  - Heart murmur caused by decrease in viscosity and increase in turbulence of blood.
  - Weak or fluttering pulse.
  - Shock, if one third of blood is lost rapidly.

II. 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.

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

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

   ii. **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.

2) **Absolute polycythemia:** Real polycythemia characterized by real increase in circulating RBC mass, it may be: **primary** or **secondary.**

   a. **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.

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

   - **It is caused by :**

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

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

     3. ***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.