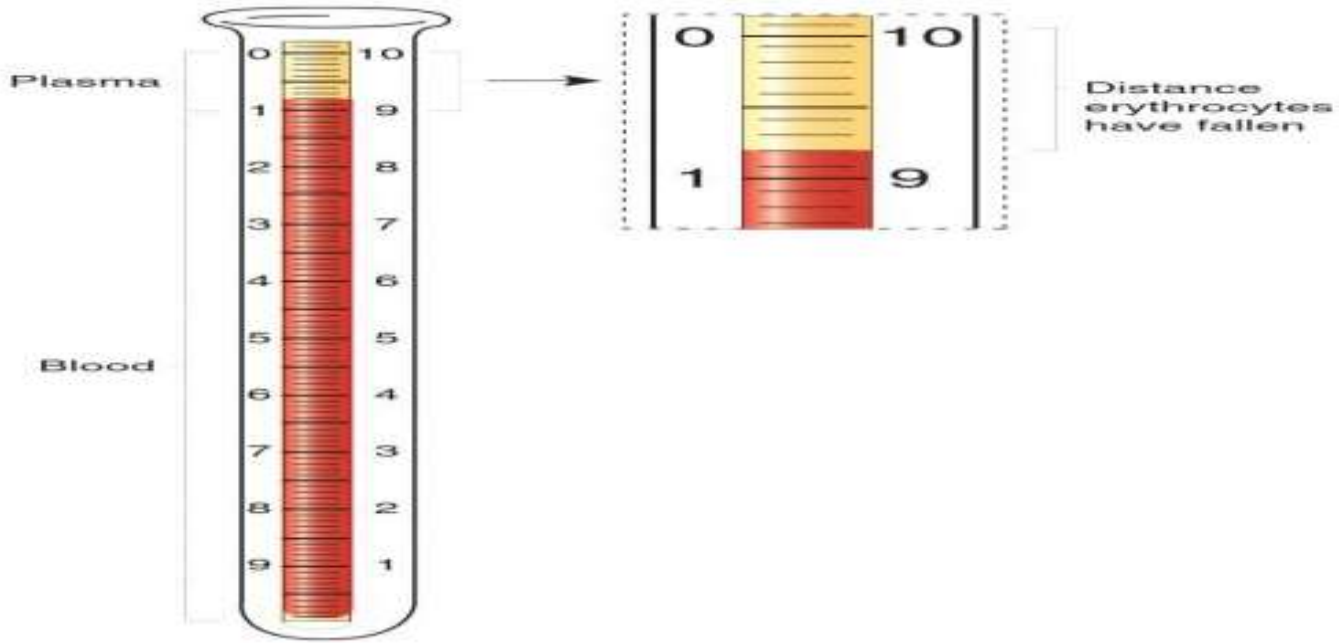
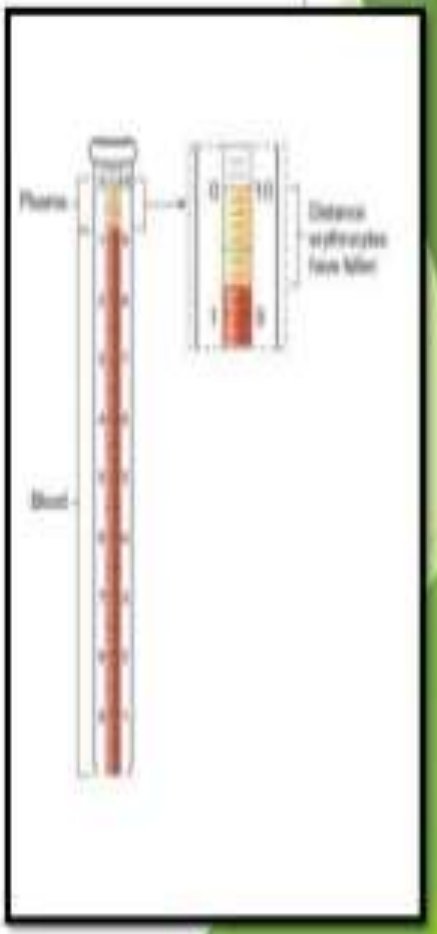
Erythrocyte Sedimentation Rate



Erythrocyte Sedimentation Rate

Measurement of the rate at which the erythrocytes settle from the plasma in anticoagulated blood.



Introduction

- Non specific test indicative of inflammation.
- It is used an initial screening tool and also as a follow up test to monitor therapy and progression or remission of disease.
- Easy to perform.
- Inexpensive.
- Unit - measured in mm/hr.

Mechanism

- ESR is determined by the interaction between factors that promote (fibrinogen) and factors that resist (negative charge of RBC) sedimentation.
- Normally, RBCs settle down slowly as they do not form rouleaux. Instead, they gently repel each other due to the negative charge on their surface.
- Rouleaux are stacks of many RBCs that become heavier and settle down faster.

Mechanism

- Plasma proteins, especially fibrinogen, adhere to the red cell membranes and neutralize the surface negative charges, promoting cell adherence and rouleaux formation.
- ESR is directly proportional to the weight of the cell aggregates.
- It is inversely proportional to the surface area

Stages of Erythrocyte Sedimentation

- Stage of rouleaux formation/aggregation ⟶ 10 minutes
- Stage of sedimentation/settling ⟶ 40 minutes
- Stage of packing ⟶ 10 minutes

Factors That Increase ESR

- Old age
- Female
- Pregnancy
- Anemia
- RBC abnormalities
- Macrocytosis
- Technical factors
- Dilution problems
- Increased temperature of specimen
- Tilted ESR tube
- Elevated fibrinogen levels
- Infection
- Inflammation
- malignancy

Factors That Decrease ESR

- Extreme leucocytosis
- Polycythemia
- RBC abnormalities
- Spherocytosis
- Acanthocytosis
- Microcytosis
- Technical factors
- Dilution problems
- Inadequate mixing
- Clotted blood sample
- Short ESR tube
- Vibrations during testing
- Protein abnormalities
- Hypofibrogenemia
- Hypogammaglobulinemia
- Dysproteinemia

Different Methods

- Westergrens method
- Wintrobes method
- Landau method
- Automated ESR

Westergren Method

- Reference method when undiluted blood is used.

- Requirements:

Westergren pipette

Westergren stand

Anticoagulant diluent solution

Trisodium citrate (4:1)

EDTA

Westergren Method

- Mix anticoagulated blood sample thoroughly. The Westergren tube is filled with blood sample up to the "0" mark. A rubber bulb or a mechanical device should be used for filling. There should be no air bubbles in the blood.
- The tube is placed in a strictly vertical position in the ESR stand and left undisturbed for 1 hr.
- After exactly 1 hr, read the height of the column of plasma above the red cell column in mm.
- ESR is expressed in mm/hr.

Reference Range By Westergren Method

- Male < 50 years: 0-15mm/hr
- Male >50 years: 0-20mm/hr
- Female <50 years: 0-20mm/hr
- Female >50 years: 0-30mm/hr
- Children: 0-10mm/hr

Advantages:

- More reliable and gives accurate result.

Disadvantages:

- More blood is required.
- Difficult to fill blood in the tube.
- PCV cannot be done.
- Mouth pipetting can be hazardous.

Wintrobe's Method

- Used to estimate both ESR and PCV

- Requirement:

Wintrobe's pipette

Wintrobe's stand

Anticoagulated blood

EDTA

Double oxalate

Wintrobe's Method

- Mix the anticoagulated blood thoroughly.
- Fill the wintrobe's tube by using Pasteur pipette upto the "0" mark.
- Place the tube vertically in the stand.
- Note the ESR at the end of 1hr.

Reference Range By Wintrobe's Method.

- Males: 0-9mm/hr
- Female: 0-20mm/hr
- Children: 0-13mm/hr

Sources of Error

- Improper ratio of blood and anticoagulant.
- Haemolysed blood sample.
- Clotted blood.
- Presence of air bubble.
- Error due to sunlight, vibration, small bore size, dirty and wet tube.
- Delay in performing the test.

Pathological conditions associated with an increased ESR

1. Rheumatic fever
2. Rheumatoid arthritis
3. Temporal arthritis
4. Polymyalgia rheumatica
5. Acute myocardial infarction
6. Malignancy
7. Tuberculosis
8. Anemia

Conditions Associated With Very High ESR >100 mm/hr

1. Multiple myeloma
2. Connective tissue disorders - SLE, RA and other autoimmune diseases
3. Tuberculosis
4. Malignancies
5. Severe anemia

Some Conditions With Low ESR

1. Polycythemia
2. Severe Leukocytosis
3. Sickle cell anemia
4. Hereditary spherocytosis
5. Congestive cardiac failure
6. Corticosteroid use
7. Hypofibrinogenemia

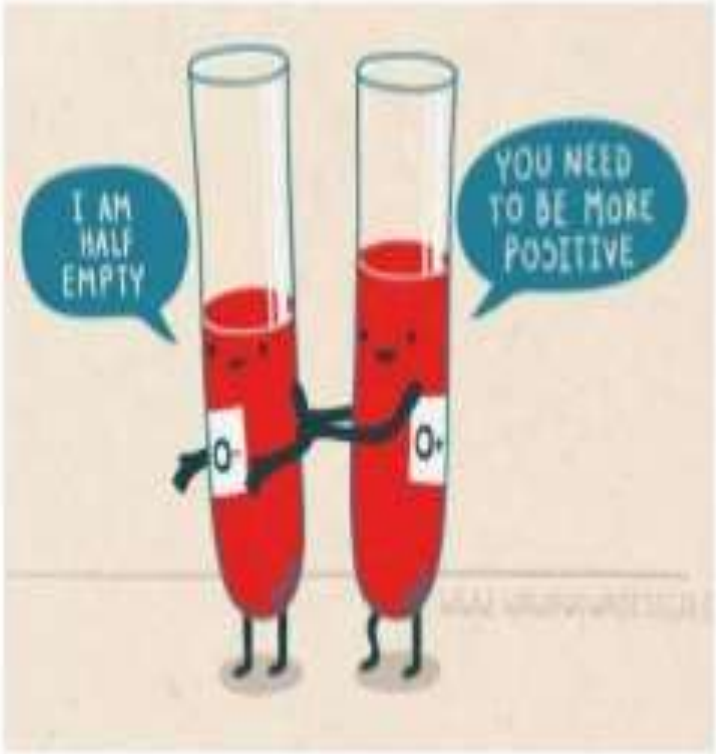
Limitations of ESR

- ESR is a non specific phenomenon and reflects only change in plasma protein pattern and the variation in RBC volume.
- Cannot be used as a diagnostic tool.
- Does not indicate the nature of the disease.

Clinical Significance of ESR

- To follow the course of the disease.
- To establish the prognosis in certain chronic diseases.
- Increase in two or more consecutive tests indicates continuation of the increased activity.
- To distinguish between organic and non-organic disorders.
- To support the diagnosis.

Thank you



RED CELL INDICES

Introduction

- Purpose of determining blood indices is to correlate the fundamental result obtained in CBC
- Also useful to classify anemia

MEAN CORPUSCULAR HEMOGLOBIN

- It is defined as the amount of hemoglobin in average red cell

  Or

  Average amount of Hb in all the red cell
- It is directly proportional to the amount of hemoglobin and the size of the erythrocyte
- Normal range = 27-32pg
- 1pg= 10^{-12} G

Formula

$$\mathrm{MCH} = \frac{Hb(gm/dl)}{RBC\ in\ million} \times 10$$

Interpretation of MCH values

- Low MCH values: are found in microcytic hypochromic anaemias and also when red cells are microcytic and normochromic. In thalassaemia minor the MCH is low even when anaemia is mild (MCHC is often normal)
- *Raised MCH values: found in macrocytic normochromic* anaemia

Mean Corpuscular Volume (MCV)

- It is defined as the average volume of red cell
- It Provides information on red cell size
- It is measured in femtolitres.
- Normal range= 87±5fl
- $1fL = 10^{-15}L$

Formula

$$\mathrm{MCV} = \frac{PCV}{RBC\ in\ million} \times 10$$

Interpretation of MCV values

- Low MCV values: are found in microcytic anaemias particularly iron deficiency, anaemia of chronic disease and thalassaemia
- Raised MCV values: are found in macrocytic anaemias, marked reticulocytosis, and chronic alcoholism

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC)

- The MCHC expresses the average concentration of hemoglobin per unit volume of erythrocytes.
- It is expressed in percentage.
- Normal value= 32-36%

Basic formula

$$MCHC = \frac{Hb(gm/dl)}{PCV} \times 100 \quad \text{or} \quad MCHC = \frac{MCH}{MCV} \times 100$$

Interpretation of MCHC values

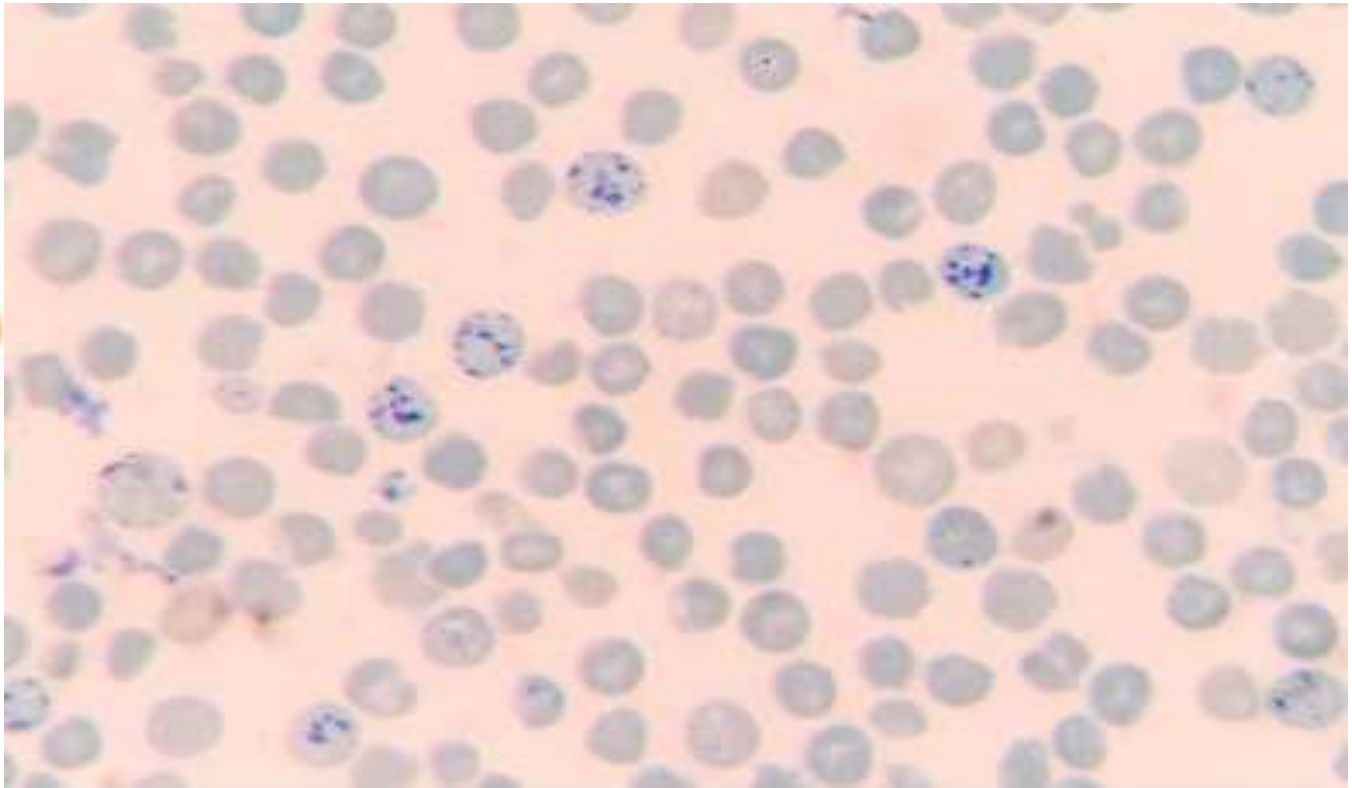
- Low MCHC values are found in iron deficiency anemia and other conditions in which the red cells are microcytic and hypochromic.
- An increased MCHC can occur in marked spherocytosis.

Summary of red cell indices in common anaemias

Anemia	MCV	MCH	MCHC
Normocytic normochromic	N	N	N
Microcytic hypochromic	D	D	D
Macrocytic normochromic	I	I	N

Thank you

RETICULOCYTE COUNT



RETICULOCYTES

- **Reticulocytes** are young , premature, non nucleated red blood cells, contain reticular material (RNA) that stain gray blue.
- Reticulum is present in newly released blood cells for 1-2 days before the cell reach its full mature state.
- RBCs have six stages :

1) Pronormoblast
2) Basophilic normoblast
3) polychromatophilic normoblast
4) orthochromic normoblast
5) Reticulocyte
6) mature red blood cell.

Bone Marrow

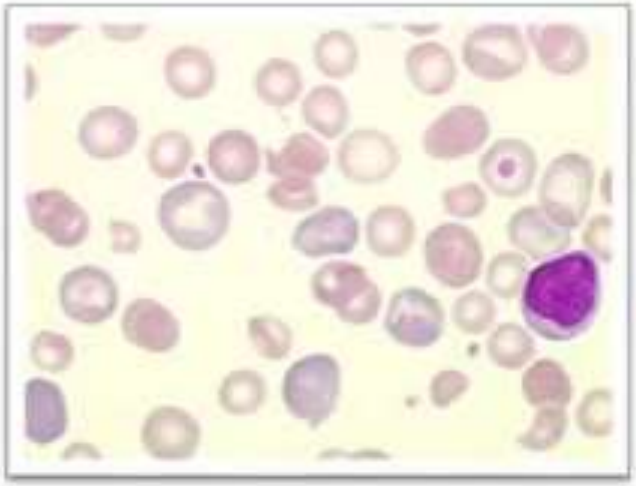
2-3 day B.M. ,
1 day P.B.

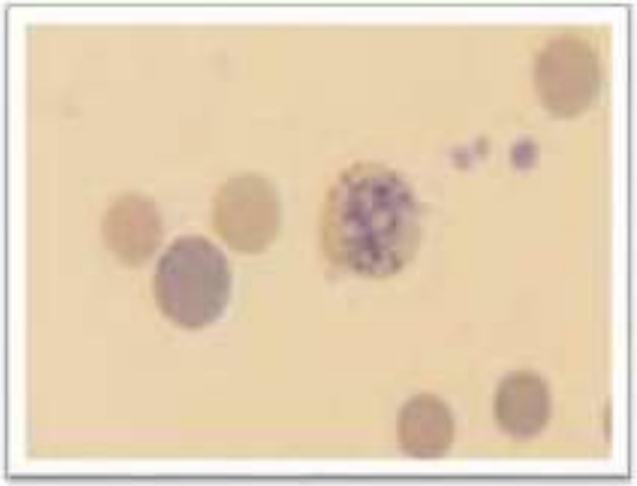
peripheral blood

RETICULOCYTE STAINS

- Reticulocytes are visualized by **supra-vital staining** (such as new methylene blue, Brilliant Cresyl Blue, Pure azure blue) that precipitate the RNA and organelles, forming a filamentous network of reticulum

- On Wright stain. the Reticulocyte appears polychromatophilic or as a Macrocytic blue red cell.





PRINCIPLE

- Whole blood is incubated with **supra-vital staining** (new methylene blue). The vital stain causes the ribosomal and residual RNA to coprecipitate with the few remaining mitochondria and ferritin masses in living young erythrocytes to form dark-blue clusters and filaments (reticulum).
- Smears of this mixture are then prepared and examined. The number of reticulocytes in 1000 red blood cells is determined. This number is divided by 10 to obtain the reticulocyte count in percent.

SPECIMEN

- Whole blood that is anticoagulated with either EDTA or heparin is suitable.
- Capillary blood drawn into heparinized tubes or immediately mixed with stain may also be used.
- Red blood cells must still be living when the test is performed therefore it is best to perform it promptly after blood collection.
- Blood may be used up to 8 hours after collection.
- Stained smears retain their color for a prolonged period of time.

REQUIREMENTS

1. Commercially prepared liquid new methylene blue solution. It should be stored in a brown bottle. If precipitate is a problem on the smear, the stain should be filtered prior to use.
2. Microscope slides
3. Microscope
4. 10 x 75 mm test tubes
5. Pasteur pipets (with bulb if pipets are glass)
6. Capillary tubes
7. Miller ocular (if available)

PROCEDURE

Preparation of smears

1. Add 3-4 drops of new methylene blue solution to 3-4 drops of thoroughly mixed EDTA anticoagulated blood to a glass 10 x 75 mm test tube.
2. Mix the contents by gently shaking and allow to incubate at room temperature for a minimum of 10 minutes.
3. At the end of 10 minutes, gently mix the blood/stain solution.
4. Using a capillary tube, place a drop of the mixture on each of three slides near the frosted edge as you would when making a peripheral smear.
5. Using the wedge smear technique, make acceptable smears not too thick or thin.
6. Label the slides with patient name, ID# and date.
7. Allow to air dry. (Do not blow to hasten to drying.)

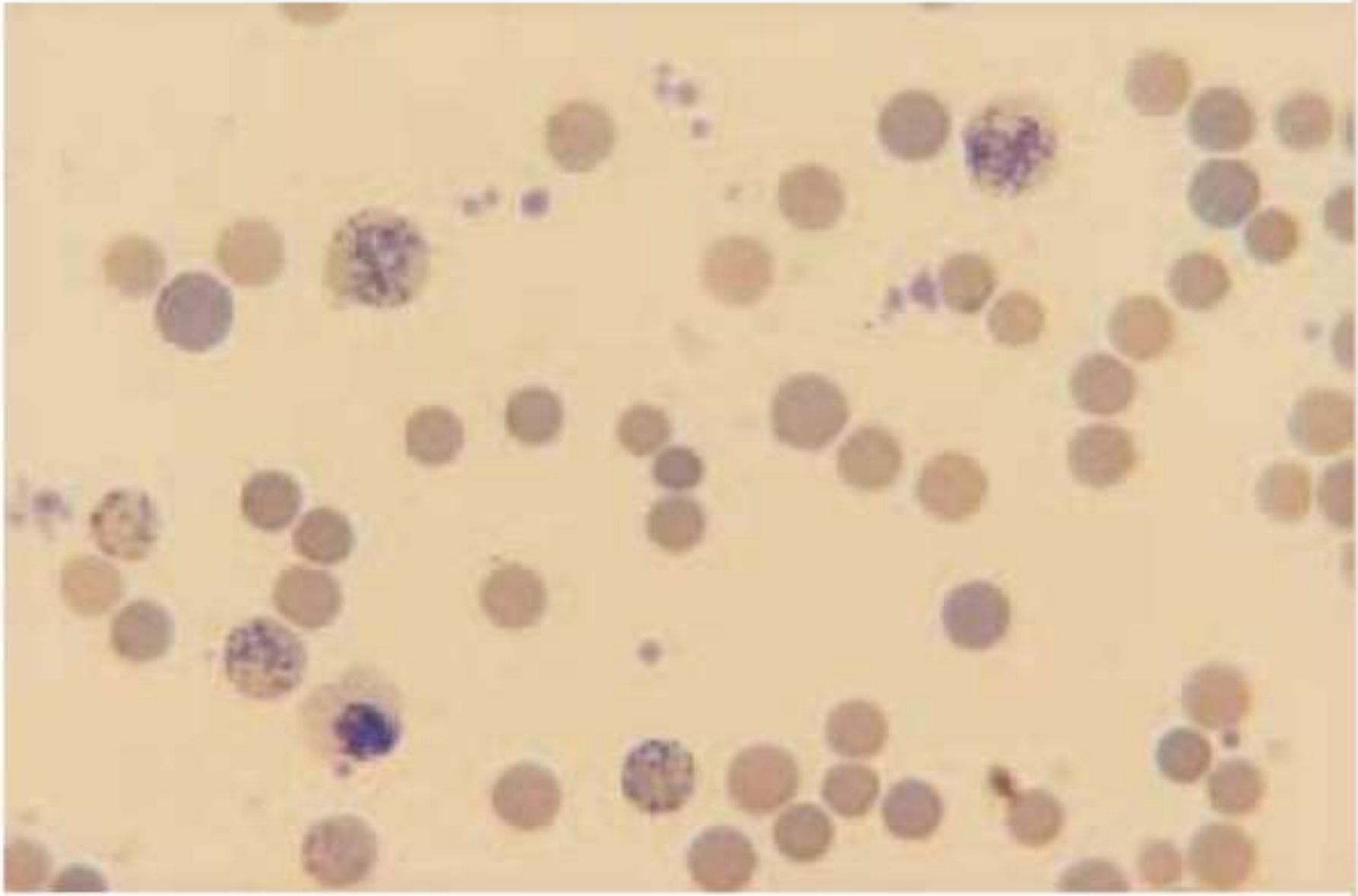
Counting procedure

- Place the first slide on the microscope stage and, using the low power objective (10x), find an area in the *thin portion of the smear in which the red cells are evenly distributed and are not* touching each other.
- Carefully change to the oil immersion objective (100x) and further located an area in which there are approximately 100 red cells per oil immersion field.
- Do this by finding a field where the cells are evenly distributed and mentally divide the field into 4 quadrants. Count the cells in 1 quadrant. If there are about 25, you are in a good area. There will be a lot of open space between the red cells.

2. Be sure to count all cells that contain a blue-staining filament or at least 2 or more discrete blue aggregates of reticulum in the erythrocyte.
3. Count 1000 red cells in consecutive oil immersion fields. Record the number reticulocytes seen.
4. You may count 500 cells on two slides each. They should agree within **± 15%** of each other. If they do not, repeat the reticulocyte count on the third smear.
5. Calculate the result as follow:

    %Retix= Reticulocyte counted/10

RETICULOCYTE



INTERPRETATION

- **The Reticulocyte count is an important diagnostic tool:** The number of Reticulocytes is a good indicator of bone marrow activity, because it represents recent production. It is used to differentiate anemia's caused by bone marrow failure from those caused by hemorrhage or hemolysis.
- It used also to check the effectiveness of treatment in prenicious anemia and folate and iron deficiency.
- To assess the recovery of bone marrow function in aplastic anemia and to determine the effects of radioactive substance on exposed workers.
- A low reticulocyte count may mean a need for a bone marrow biopsy. This can tell if is a problem with how new reticulocytes are made by the bone marrow.

- Reticulocytosis (Increased RBC Production)
  - Reticulocyte Index >3%, Reticulocyte Count >1.5%
  1. Acute blood loss or hemorrhage
  2. Post-Splenectomy
  3. Acute Hemolytic Anemia (Microangiopathic Anemia)
  4. Hemoglobinopathy
     - Sickle Cell Anemia
     - Thalassemia major
  5. Post-Anemia Treatment
     - Folate Supplementation
     - Iron Supplementation
     - Vitamin B12 Supplementation

- Reticulocytopenia (Decreased RBC Production)
  - Reticulocyte Index <1%, Reticulocyte Count <0.5%

1. Aplastic Anemia
2. Bone Marrow infiltrate
3. Bone Marrow suppression or failure
   1. Sepsis
   2. Chemotherapy or radiotherapy
4. Disordered RBC maturation
   1. Iron Deficiency Anemia
   2. Vitamin B12 Deficiency
   3. Folate Deficiency
   4. Sideroblastic Anemia
   5. Anemia of Chronic Disease
   6. Hypothyroidism
5. Blood transfusion
6. Liver disease

FACTORS AFFECTING THE TEST

Reasons you may not be able to have the test or why the results may not be helpful include:

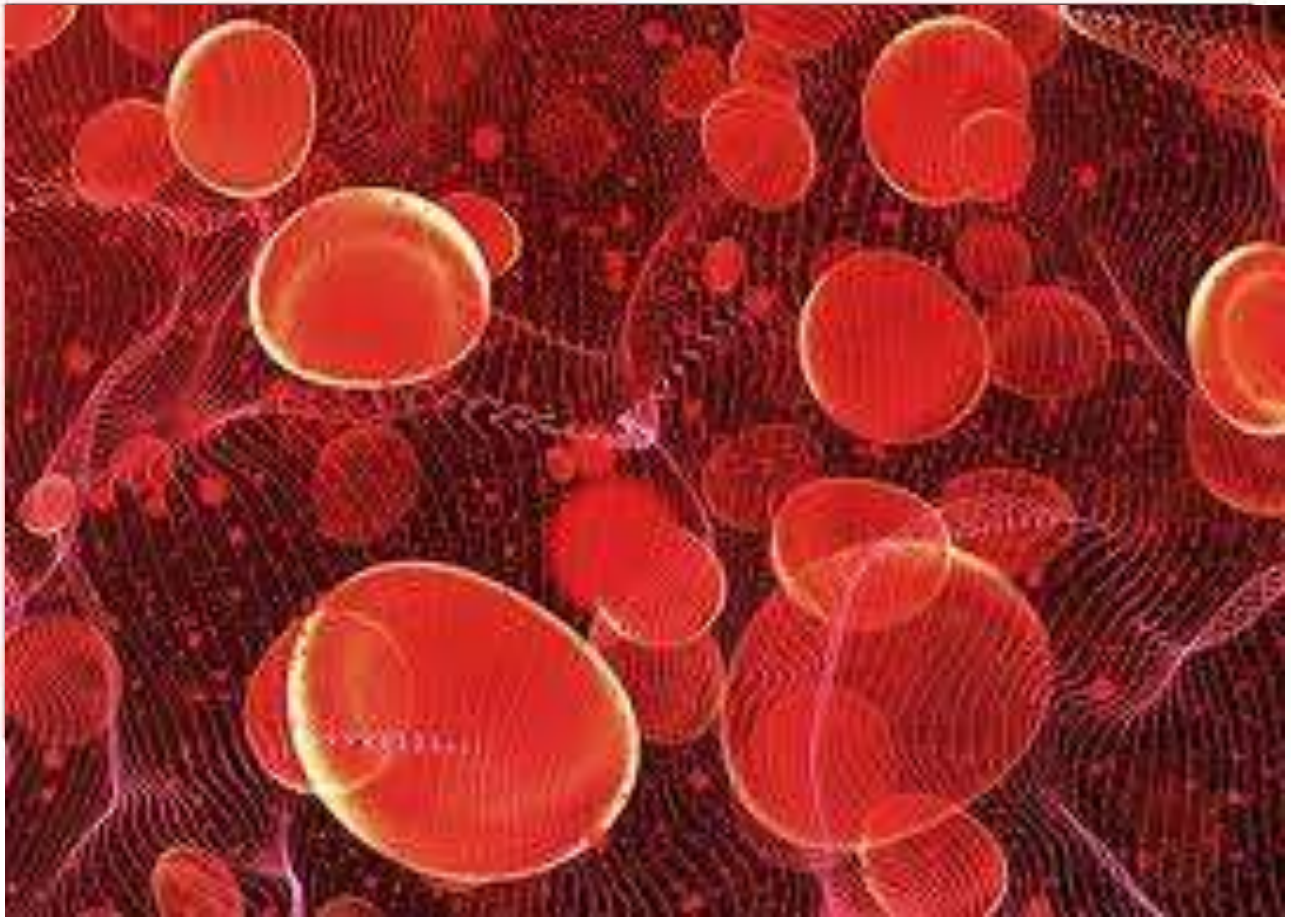
- Taking medicines, such as levodopa, corticotropin, azathioprine (Imuran), chloramphenicol (Chloromycetin), dactinomycin (Cosmegen), medicines to reduce a fever, medicines to treat malaria, and methotrexate and other cancer chemotherapy medicines.
- Getting radiation therapy
- Taking sulfonamide antibiotics (such as Bactrim or Septra)
- Being pregnant
- Having a recent blood transfusion

Error sources

1. A refractile appearance of erythrocytes should not be confused with reticulocytes.
2. Filtration of the stain is necessary when precipitated material is present which can resemble a reticulocyte.
3. Erythrocyte inclusions should not be mistaken for Reticulocytes.
   - Howell-Jolly bodies appear as one or sometime two, deep-purple dense structures.
   - Heinz bodies stain a light blue-green and are usually present at the edge of the erythrocyte.
   - Pappenheimer bodies are **more often confused** with reticulocytes and are the most difficult to distinguish. These purple-staining iron deposits generally appear as several granules in a small cluster. If Pappenheimer bodies are suspected, stain with Wright-Giemsa to verify their presence. Hemoglobin H inclusions will appear as multiple small dots in every cell.

4. Falsely decreased reticulocyte counts can result from under staining the blood with new methylene blue. Be sure the stain/blood mixture incubates the full 10 minutes.
5. High glucose levels can cause reticulocytes to stain poorly.
6. There is high degree of inaccuracy in the manual reticulocyte count owing to error (± 2%) in low counts and ± 7% in high counts) and a lack of reproducibility because of the inaccuracy of the blood film. This inaccuracy has been overcome by the use of **automated** instruments **using flow cytometry**.
7. If no reticulocytes are observed after scanning at least two slides, report "**none seen**".

ANEMIA

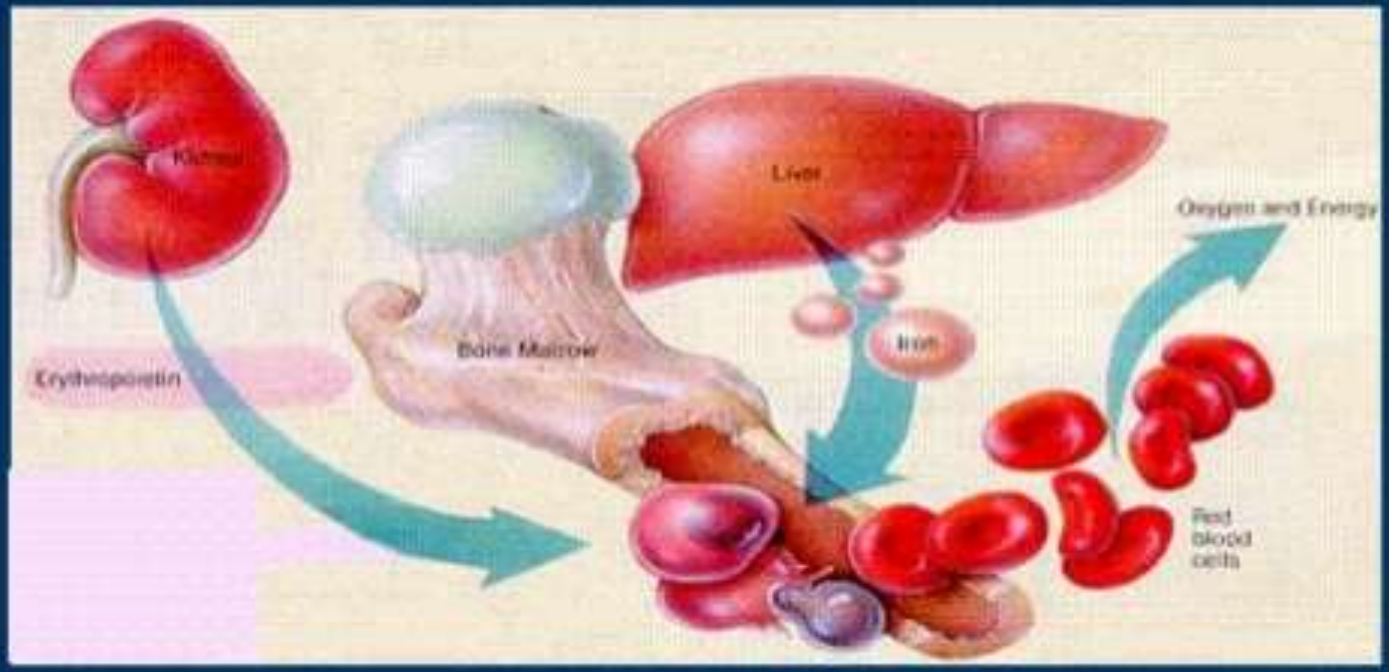


DEFINITION

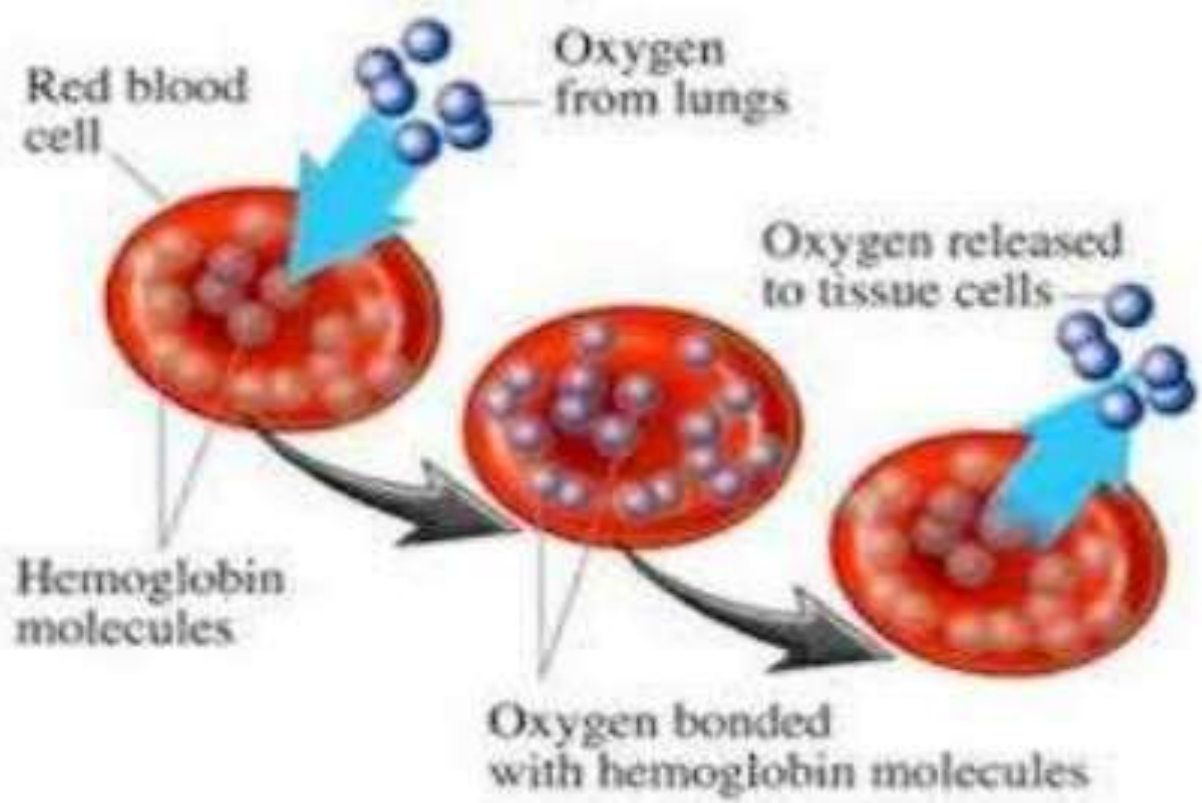
- Anemia (An-without,emia-blood)is a decrease in the RBC count, hemoglobin and/or Hematocrit values resulting in a lower ability for the blood to carry oxygen to body tissues .

PATHOPHYSIOLOGY

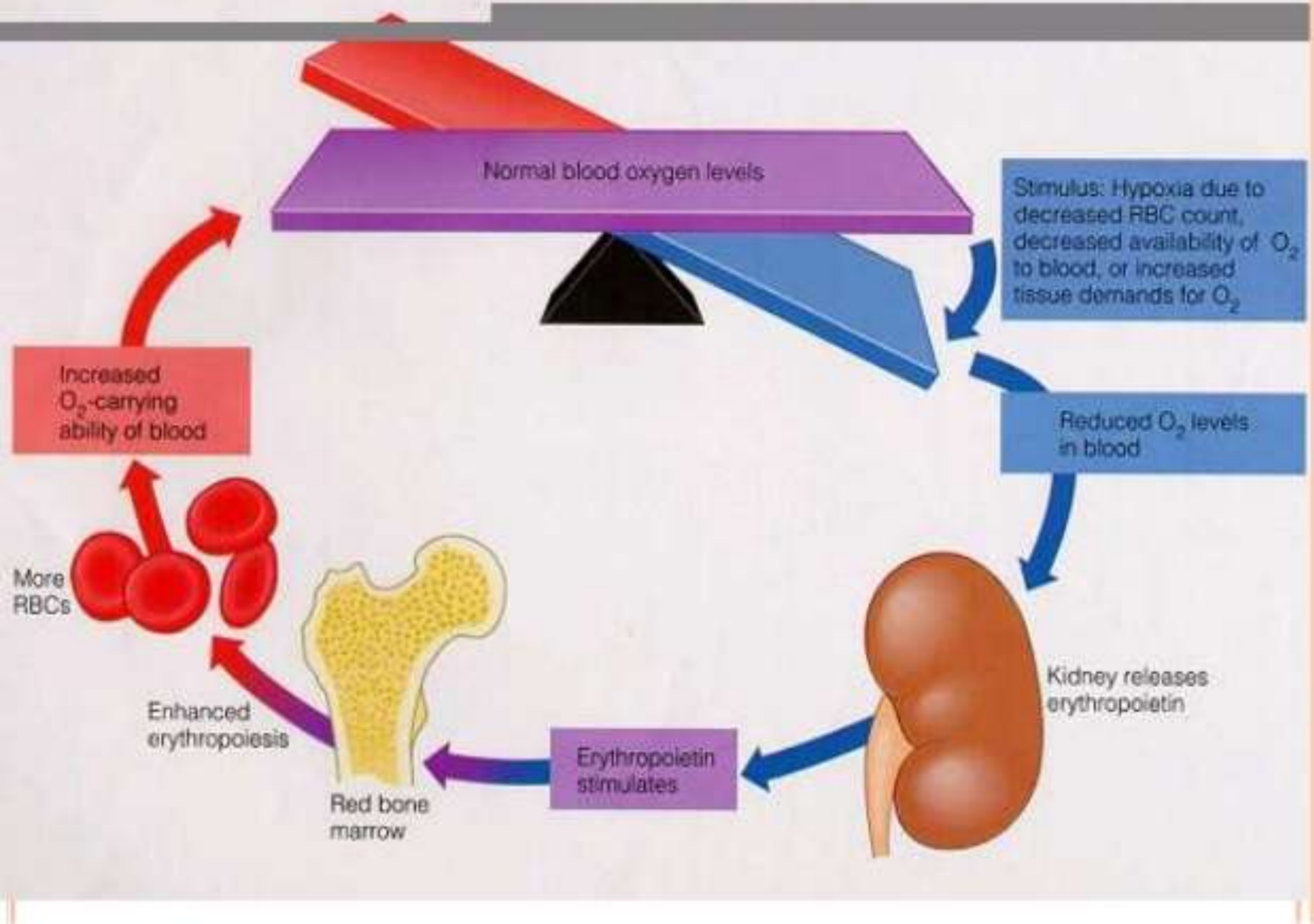
Normal Erythropoiesis



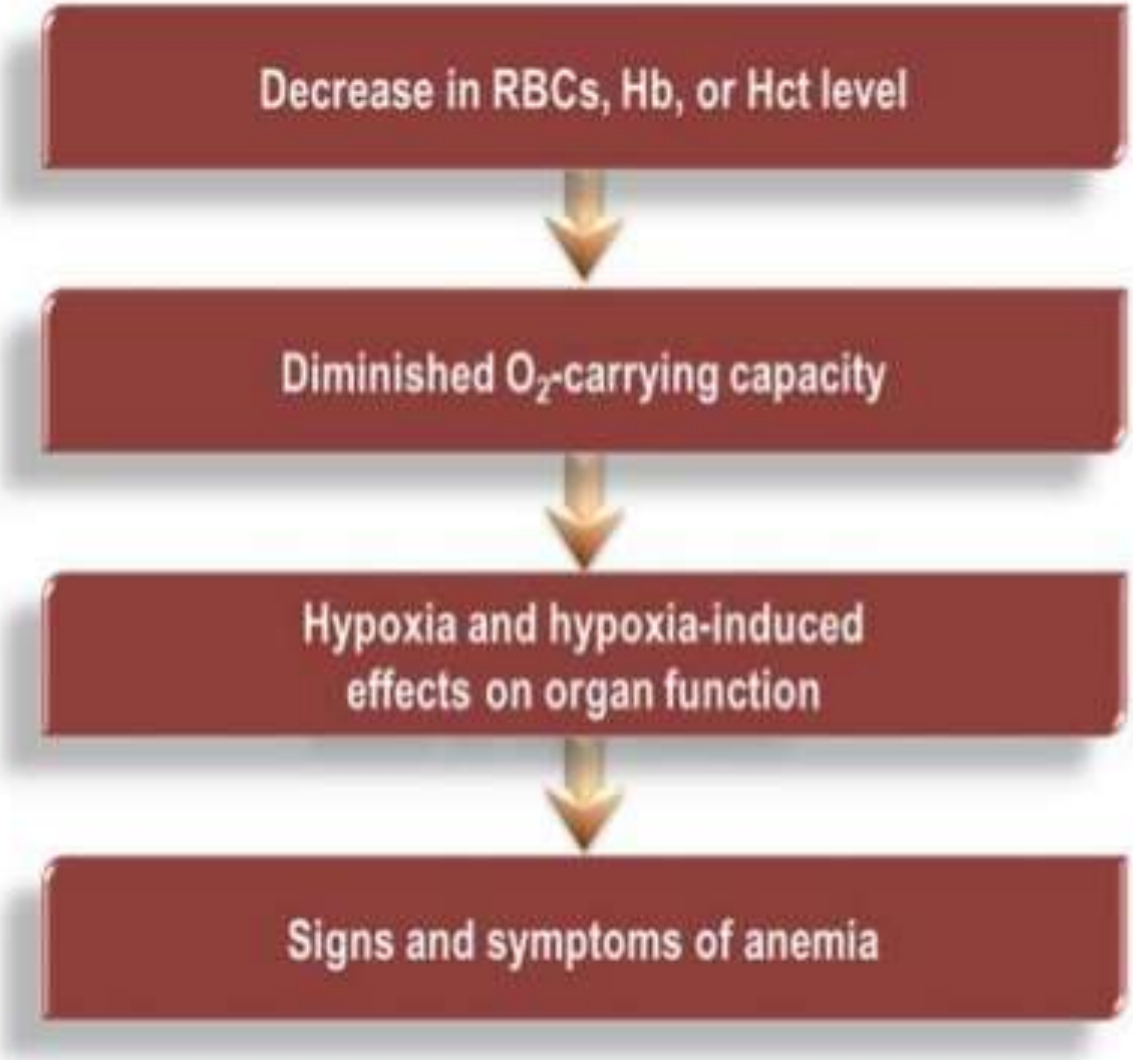
Adapted from Schott et al. US Pharmacist. 1997;22:HS5-HS12


Red blood cell
Oxygen from lungs
Oxygen released to tissue cells
Hemoglobin molecules
Oxygen bonded with hemoglobin molecules

Erythropoietin mechanism for regulating the rate of erythropoiesis (Figure 18.6)



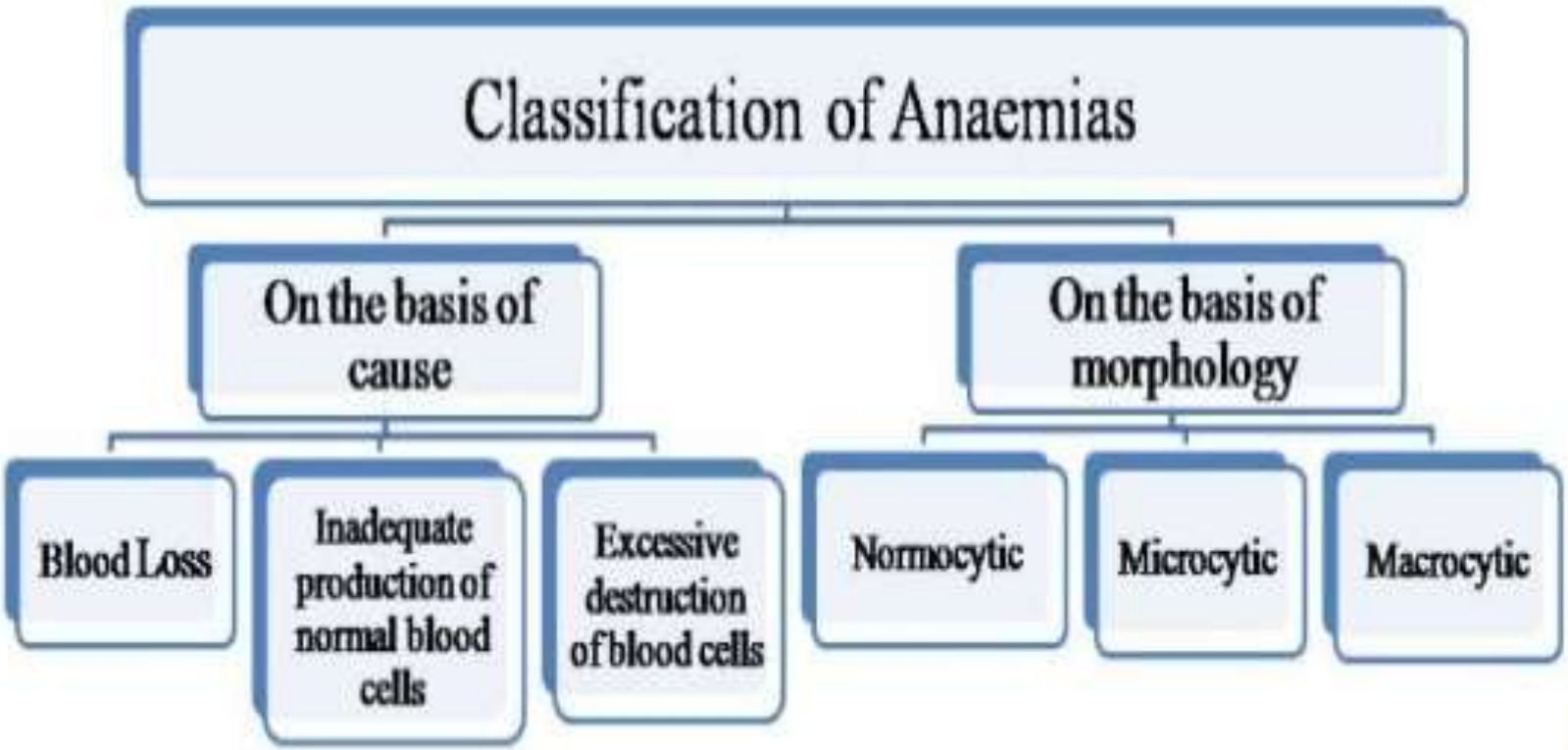
PATHOPHYSIOLOGY



NORMAL VALUES

Category Values	Reference
Men	>13 g/dl
Women	>12 g/dl
Pregnant women	>11g/dl
Infants from 2 to 6 months	>9.5 g/dl
Children from 6 months to 24 months	>10.5 g/dl
2yrs to 11 yrs	>11.5 g/dl
Children from 12 years	>12 g/dl

CLASSIFICATION



TYPES OF ANEMIA

Based on clinical picture-

- Iron deficiency anemia.
- Megaloblastic anemia.
- Pernicious anemia.
- Hemorrhagic anemia.
- Hemolytic anemia.
  - -Thalassemia anemia
  - -Sickle cell anemia
- Aplastic anemia

TYPES OF ANEMIA

- Iron deficiency anemia

♣ excessive loss of iron .

♣ Women are at risk. ---- For menstrual blood and growing fetus.

- Megaloblastic anemia

♣ Less intake of vitamin B 12 and folic acid.

♣ Red bone marrow produces abnormal RBC.

e.g cancer drugs

- Pernicious anemia

♣ Inability of stomach to absorb vitamin B 12 in small intestine.

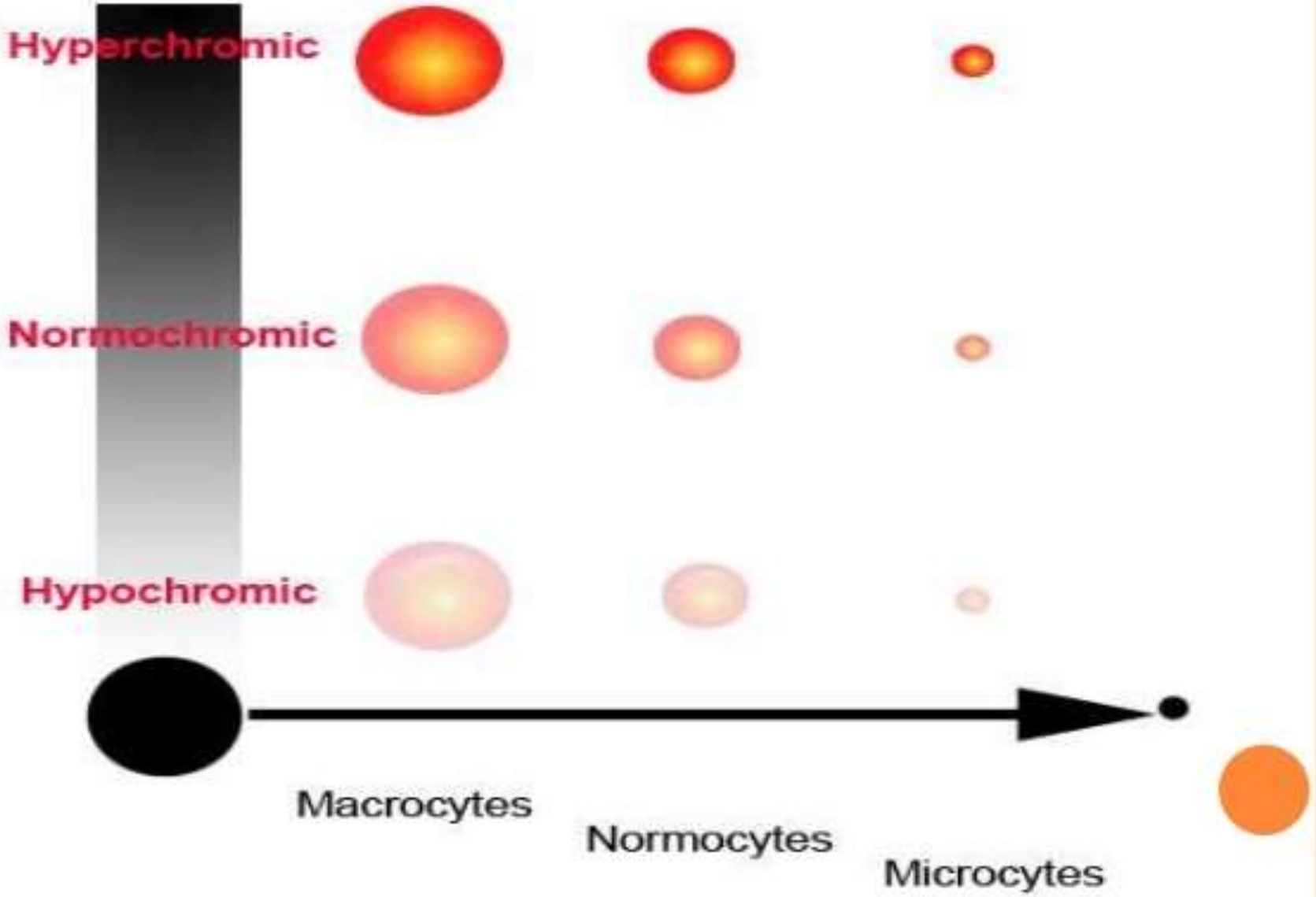
TYPES OF ANEMIA

- Hemorrhagic anemia
- ♣ Excessive loss of RBC through bleeding,stomach ulcers,menstruation
- Hemolytic anemia
- ♣ RBC plasma membrane ruptures.
- ♣ may be due to parasites,toxins,antibodies.
- Thalassemmia
- ♣ Less synthesis of hemoglobin .Found in population of Mediterranean sea.
- Sickle cell anemia
- ♣ Hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape.
- Aplastic anemia
- ♣ destruction of red bone marrow .
- ♣ caused by toxins,gamma radiation.

TYPES OF ANEMIA

- **Normochromic, normocytic anemia (normal MCHC, normal MCV).** These include:
  - anemias of chronic disease
  - hemolytic anemias (those characterized by accelerated destruction of rbc's)
  - anemia of acute hemorrhage
  - aplastic anemias (those characterized by disappearance of rbc precursors from the marrow)
- **Hypochromic, microcytic anemia (low MCHC, low MCV).** These include:
  - iron deficiency anemia
  - thalassemias
  - anemia of chronic diseases
- **Normochromic, macrocytic anemia (normal MCHC, high MCV).** These include:
  - vitamin B_{12} deficiency
  - folate deficiency

TYPES OF ANEMIA



RISK FACTORS

- Poor socio economic class
- Multiparity
- Teenage pregnancy
- Menstural problem

CAUSES

Increased Requirements	• Menstruating females • Pregnancy • Lactation • Growing infants and children • Erythropoietin treatment
Increased Loss	• GI bleeding • Menorrhagia • Persistent hematuria • Intravascular hemolytic anemias • Regular blood donors • Parasitic infections
Decreased Intake	• Vegetarian diet • Socioeconomic factors
Decreased Absorption	• Upper GI pathology (eg: Celiac and Crohn's disease) • Gastrectomy • Medications (antacids, Zantac)

SYMPTOMS

Common symptoms of anemia

- Easy fatigue and loss of energy
- Unusually rapid heart beat, particularly with exercise
- Shortness of breath and headache, particularly with exercise
- Difficulty concentrating
- Dizziness
- Pale skin
- Leg cramps
- Insomnia

Anemia Caused by Iron Deficiency

People with an iron deficiency may experience these symptoms:

- A hunger for strange substances such as paper, ice, or dirt (a condition called pica)
- Upward curvature of the nails, referred to as koilonychias
- Soreness of the mouth with cracks at the corners

Anemia Caused by Vitamin B12 Deficiency

People whose anemia is caused by a deficiency of Vitamin B12 may have these symptoms:

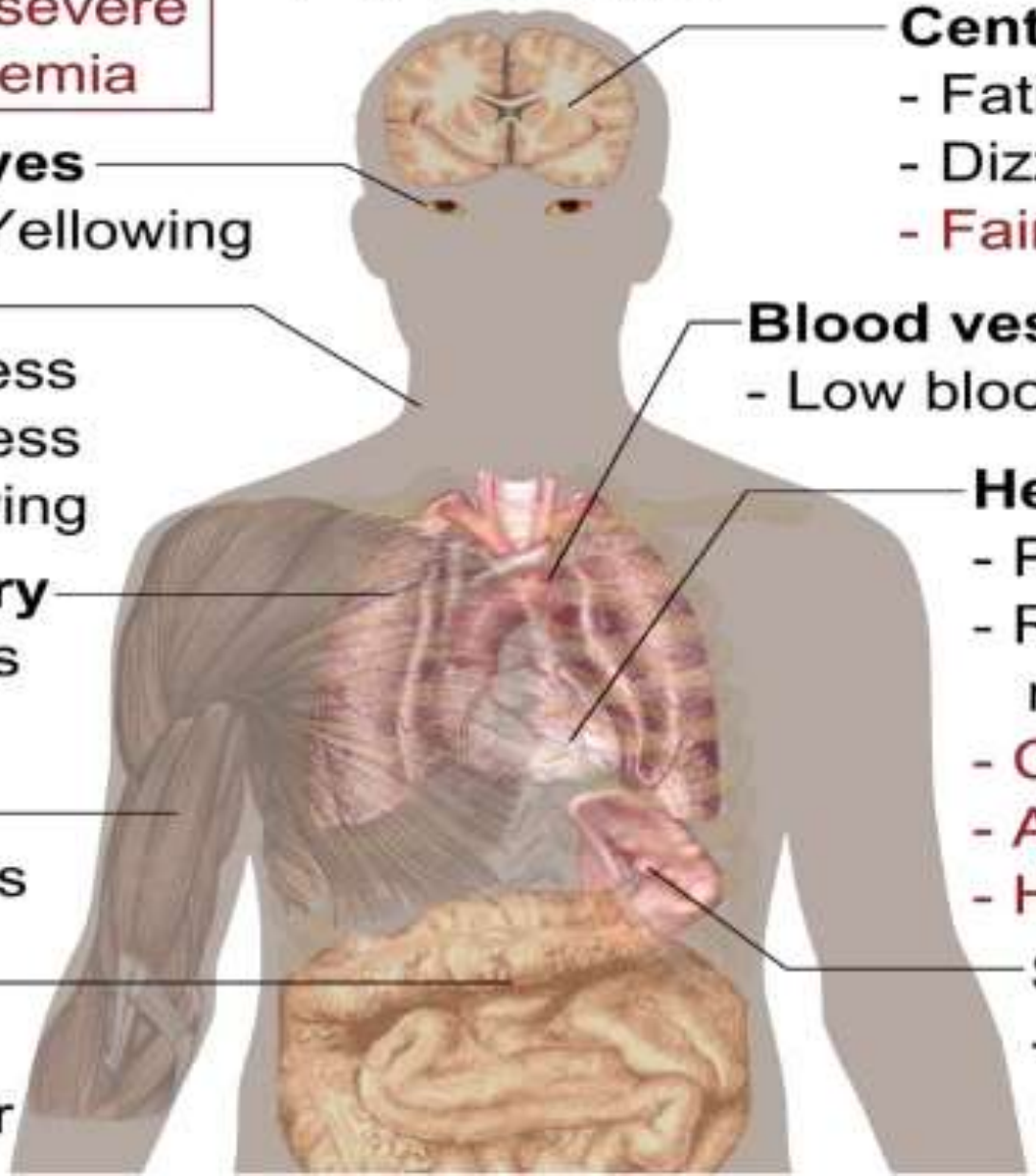
- A tingling, "pins and needles" sensation in the hands or feet
- Lost sense of touch
- A wobbly gait and difficulty walking
- Clumsiness and stiffness of the arms and legs
- Dementia
- Hallucinations, paranoia, and schizophrenia

SIGNS OF ANAEMIA

- Brittle nails
- Koilonychia (spoon shaped nails)
- Atrophy of the papillae of the tongue
- Angular stomatitis
- Brittle hair
- Dysphagia and Glossitis
- Plummer vinson/kelly patterson

Symptoms of Anemia

Red = In severe anemia



Central
- Fatigue
- Dizziness
- Fainting

Eyes
- Yellowing

Skin
- Paleness
- Coldness
- Yellowing

Blood vessels
- Low blood pressure

Heart
- Palpitations
- Rapid heart rate
- Chest pain
- Angina
- Heart attack

Respiratory
- Shortness of breath

Muscular
- Weakness

Intestinal
- Changed stool color

Spleen
- Enlarge-ment

INVESTIGATIONS

The red cell population is defined by

1.Quantitative parameters:

- Volume of packed cells i.e. the hematocrit
- Hemoglobin concentration
- Red cell concentration per unit volume.

2.Qualitative parameters:

- Mean corpuscular volume
- Mean corpuscular hemoglobin
- Mean corpuscular hemoglobin concentration.

INVESTIGATIONS

- Hematocrit (Packed cell volume): It is the proportion of the volume of blood sample that is occupied by RBCs.
  - Men -42-52%
  - Women -36-48%

- Cell Volume Hemoglobin Concentration: It is the amount of hemoglobin per unit volume of blood.(Gms/Dl)
  - Women - 12-16gms/dl
  - Men - 14-17 gms/dl

- Red Cell Count: Total number of Red Cells per unit volume of blood sample. [No.of RBC/ cu.mm]
  - Men - $4.2\text{-}5.4*10^{6}/mm^{3}$
  - Women- $3.6\text{-}5.0* 10^{6}/mm^{3}$

INVESTIGATIONS

- Mean Corpuscular Volume: It is the average volume a RBC. [fL]
- Normal 82-98mm^3or 82-98fL

- Mean Corpuscular Hemoglobin: It is the average hemoglobin content per RBC.
- Normal value is 27 to 31 pL

- Mean Corpuscular Hemoglobin Concentration: It is the average concentration of hemoglobin in a given Red Cell Volume. [Gms/ dL]
- Normal 32-36 g/Dl

THANK YOU

OSMOTIC FRAGILITY TEST

Introduction:

Osmosis = water conc.

Hypotonic = high water+ low salt.

Hypertonic=low water+ high salt.

Cells in the body path in extra cellular fluid which is isotonic in nature(i.e the salt and water inside the cells = to salt and water outside the cells).

The plasma membrane of body cells are semipreamable (i.e preamble to water only).

Objective:

The test is used to diagnosis different type of anemia in which the physical properties of the RBC are altered.

The main factors affecting the osmotic fragility test is the shape of RBC which in turn is depended on the volume, surface area and functional state of RBC membrane.

Material and instrument:

1.test tubes
2.heprinized blood.
3.hypotonic solution (distilled water).
4.hypertonic solution.
5.isotonic solution (physiological normal saline).
6.centrifuge .

Composition of physiological normal saline 0.9%:

1. Nacl 9gm.
2. 1000 ml of distilled water.

Procedure:

1.prepare three test tubes: contain distilled water , normal saline, hyper tonic.

2.put few drops of heprinized blood in each tube.

3.mixed for few mints. Manually or by stirrer.

4.put the 3 samples in the centrifuge .

Results:

1.the fluid portion of hypotonic (D.W) will colored because the RBC swelling and finally rupture and hemoglobin release .

2.the fluid portion of normal saline remain clear because the RBC remain in normal condition

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3.the fluid portion of hypertonic will colored because the RBC shirk and finally the plasma membrane destruct and hemoglobin release .