قسم الكيمياء الحيوية
Biochemistry Department
College of Science - King Saud University

BCH 471

Experiment (7)

HEMOGIOBIN AND ANEMIA, ERYTHROCYTE SEDIMENTATION RATE (ESR) AND HEMATOCRIT (HCT)

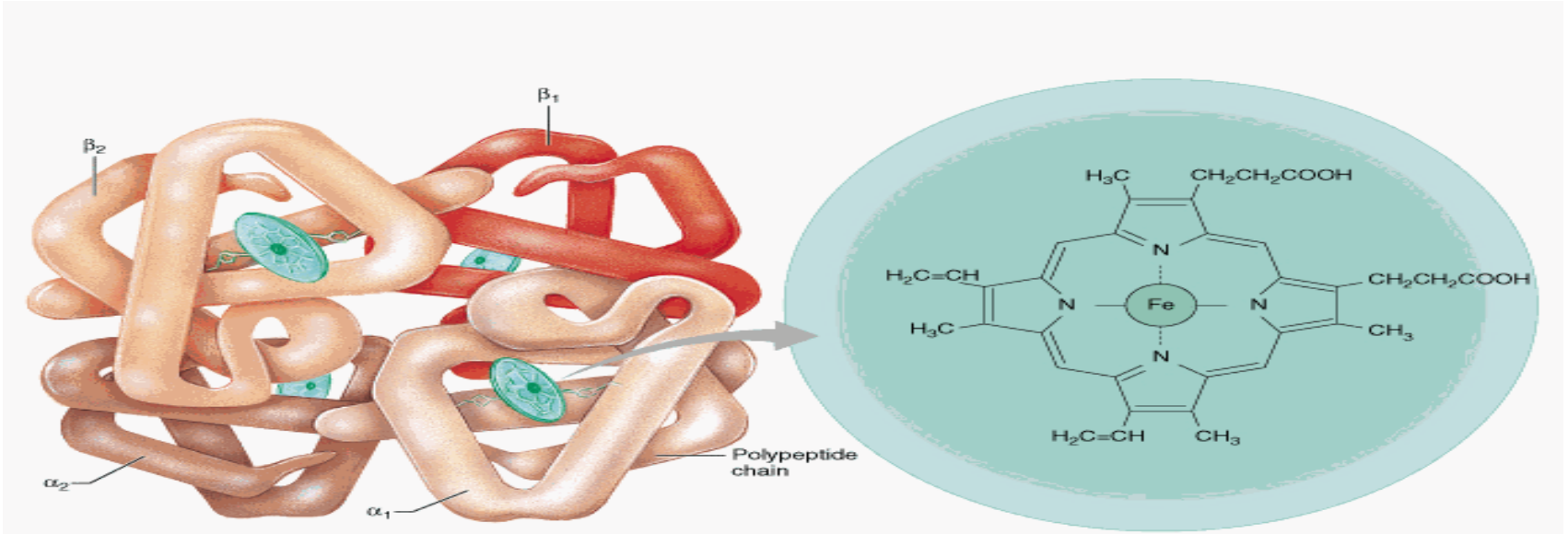
OBJECTIVES

1) Quantitative determination of hemoglobin in a blood sample.
2) Determination of erythrocyte sedimentation rate (ESR).
3) Determination of hematocrit (HCT).
4) To assess the condition of a patient by such tests.

HEMOGLOBIN STRUCTURE

- Hemoglobin (Hb) is a porphyrin–iron (II) protien in RBCs that transport oxygen from the lungs to the rest of the body and carbon dioxide back to the lungs.

- Hb is made up of 4 subunits of globin protein , with a heam (iron containing group).

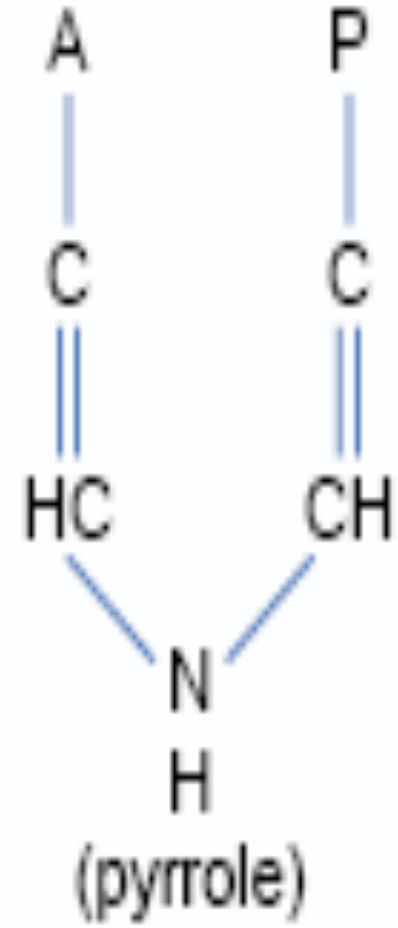


Hemoglobin Synthesis

- The circulation blood of normal adult contain about 750 g of Hb and of this about 7 – 8 g are degraded daily.

- **This amount has to be newly synthesized each day because:**
  - The globin part of Hb can be reutilized only after catabolism into its constituent amino acid.
  - The free heam is broken down into bile pigment which is excreted.
  - Iron alone is reutilized in the synthesis of Hb.

- **The rate of Hb synthesis (Rate of RBC formation) depends on**
  - The amount of oxygen reaching the blood
  - Capacity of the blood to carry oxygen ,which in turn depend on the amount of circulating hemoglobin

I. 2 succinyl-CoA + 2 glycine → 

(pyrrole)

II. 4 pyrrole → protoporphyrin IX

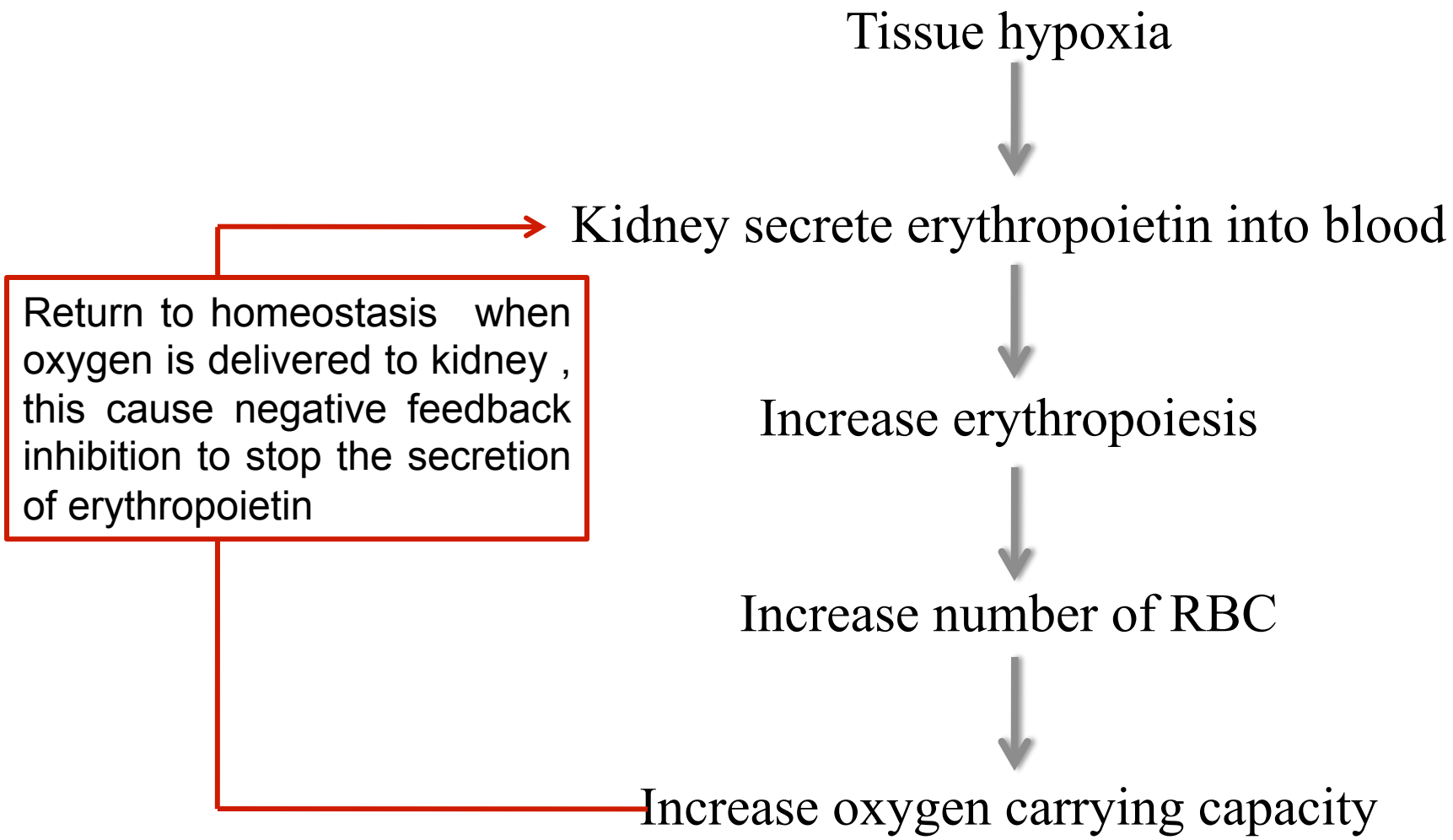
III. protoporphyrin IX + Fe^{++} → heme

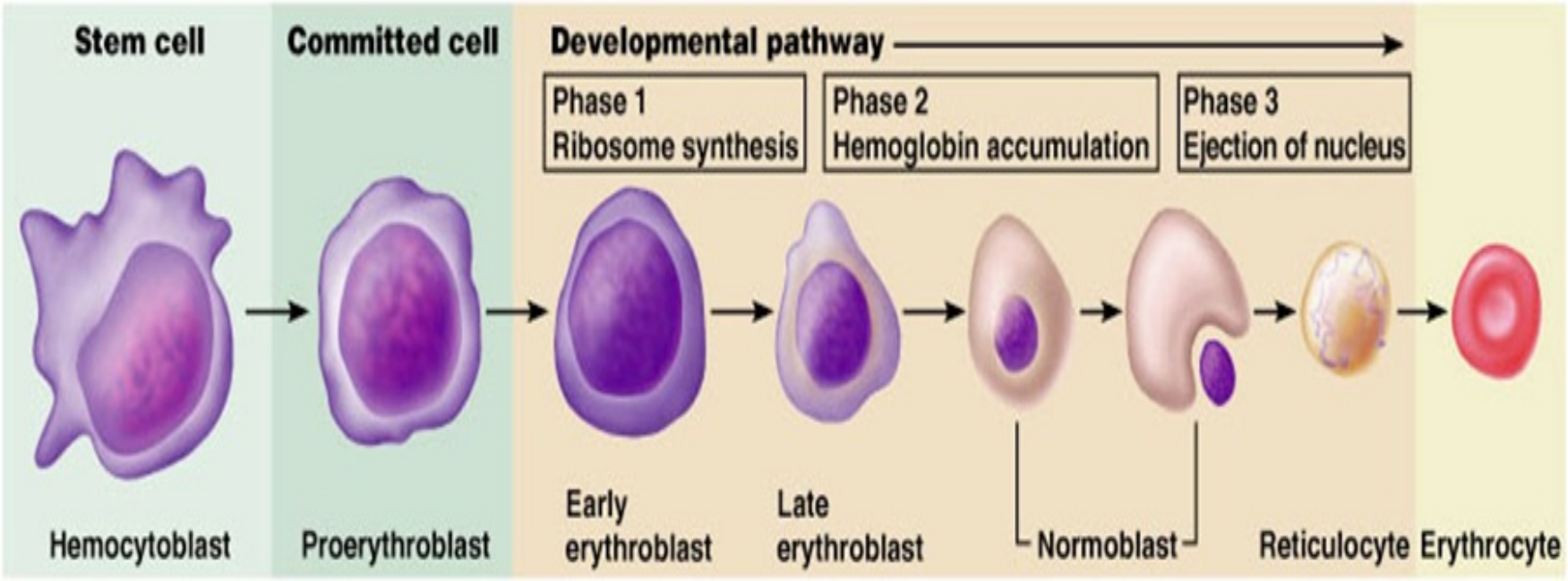
IV. heme + polypeptide → hemoglobin chain (α or β)

V. 2 α chains + 2 β chains → hemoglobin A

Regulation of Hb Synthesis:

- **Hb synthesis is stimulated by** anoxia or hypoxia, whether due to oxygen deficiency or due to anaemia.
    - *Anoxia:* means a total depletion in the level of oxygen, an extreme form of hypoxia or "low oxygen"
- There is a strong evidence that the marrow response to the stimulus of hypoxia is dependent upon **erythropoietin.**
- **Erythropoietin is a** glycoprotein hormone formed in kidney in response to decrease oxygen carrying capacity (hypoxia or anoxia), in order to stimulate the erythropoiesis

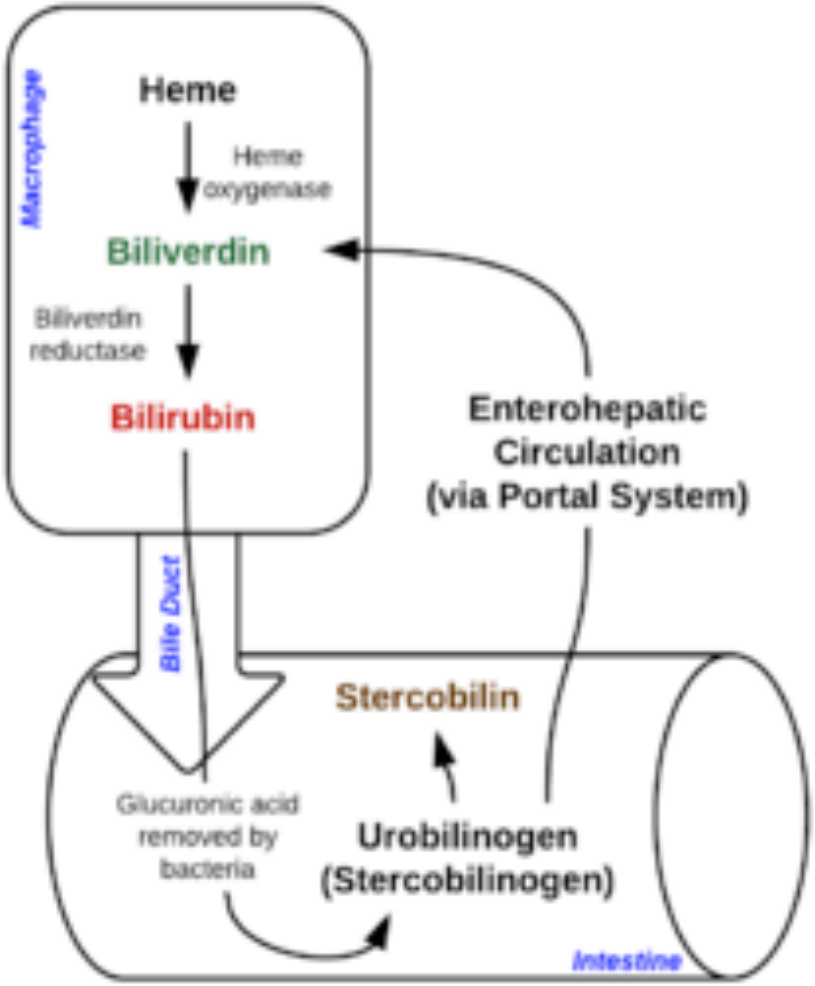

Tissue hypoxia
Kidney secrete erythropoietin into blood
Increase erythropoiesis
Increase number of RBC
Increase oxygen carrying capacity
Return to homeostasis when oxygen is delivered to kidney , this cause negative feedback inhibition to stop the secretion of erythropoietin



- The erythrocytes are derived from primitive nucleated cells in the bone marrow by successive processes of mitosis and maturation.
- A primitive stem cell divides to form two cells, one of which retains its behavior as a stem cell while the other successively divides to form the mature non-nucleated fully haemoglobionized erythrocyte

Haemoglobin Catabolism:

- In the reticuloendothelial system, erythrocytes are destroyed and haemoglobin is released.
- Globin is separated from haem and haematin is formed (the ferrous iron Fe^{+2} oxidized to ferric iron Fe^{+3})
- The porphyrin ring is then opened and the iron is removed with formation of straight chain compund **biliverdin** which is converted to **bilirubine** by reduction
- The iron and amino acid of the globin are retained but pyrrole ring are excreted as bilirubin.



The role of some factor affecting on the native of haemoglobin:

1) *Vitamins and cofactor*: Biotin (B7), pantothenic acid (B5), folic acid (B9), coenzyme A and pyrodixal phosphate are essential for haem synthesis .

2) *Trace metals* : Only copper and cobalt are known to play a role .

- (Copper is playing a role in the absorption of iron while Cobalt is essential constituent of vitamine B12 (Cobalamin))

3) *Glucose -6-phsphatase dehydrogenase (G6PD)*: it is an enzyme responsible for the conversion the glucose in the pentose phosphate pathway (PPP) to form 6-phosphogluconate , this pathway provide ***NADPH*** which is used to produce reduced glutathion, which is necessary for cell integrity by neutralizing free radicals that cause oxidative damage.

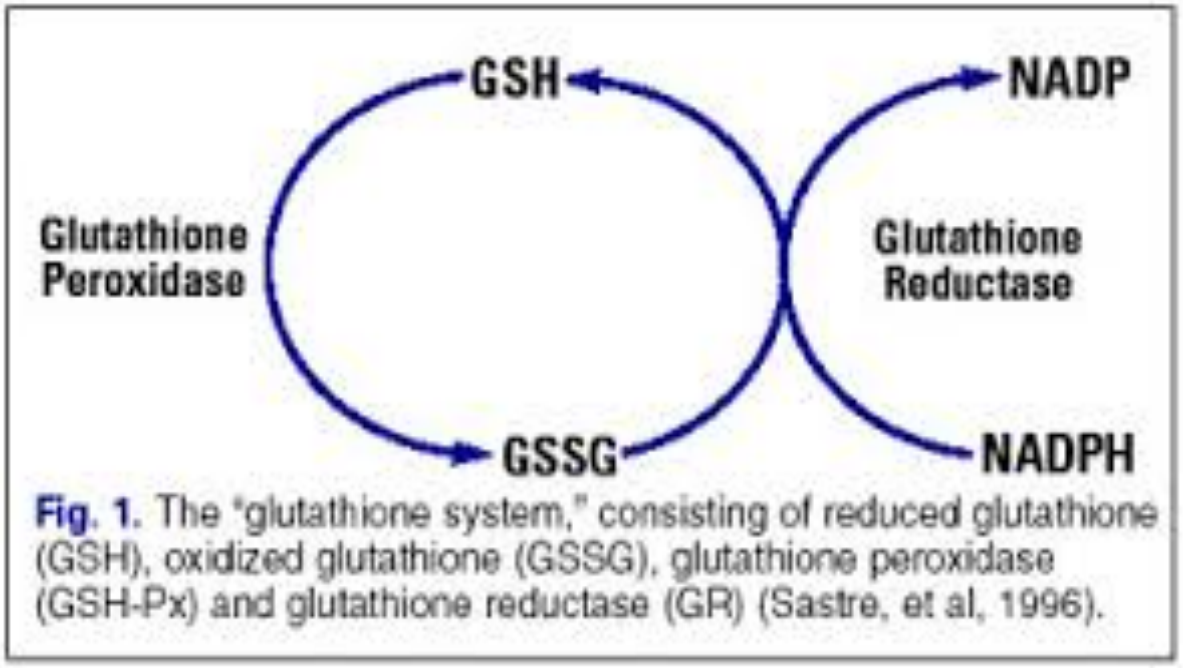


Fig. 1. The "glutathione system," consisting of reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) (Sastre, et al, 1996).

- **Deficiency of G6PD** lead to Decrease level of NADPH.
- Under oxidative stress, Hb is oxidized to met-Hb, which aggregates together causing hemolytic anemia.

Anemia :

- It is in general decrease in the amount of RBC or the normal amount of Hb in blood. It can also be defined as a lowered ability of the blood to carry oxygen.

Causes:

I. Genetics → RBC membrane Defect

II. Acquired

- Megaloblastic Anemia
- Iron-Deficiency Anemia

Iron-deficiency anemia:

Deficiency of iron is essentially due to blood loss with failure to replace the iron stores because of :

- Dietary deficiency or
- Increase requirement or
- Defective absorption.

Megaloblastic Anemia:

This may be due to deficiency of folic acid or cobaltamin (Vit. B12)

RBC membrane defects:

- In this condition there is a defect of the erythrocyte membrane and an abnormality in the soduim pumps.
- The best-known disorders are hereditary spherocytosis and hereditary elliptocytosis.

Estimation of blood haemoglobin:

Principle:

- The ferrouse (Iron II) in each haem in RBC is oxidized by ferricyanide to Fe(III)-methaemoglobin .
- A cynide group (CN^-) is then attached to the iron atom (because it is positively charge) by reaction with KCN to give the brown cyanomethamoglobin (stable) which can be estimated quantitatively

Normal Hb conc.: for men: 14 - 18 g/dl, for women : 12 - 16 g\dl

↑ Level of Hb is associated with polycythemia and dehydration

↓ Level of Hb is associated with aneamia

METHOD

Pipette into clean dry test tubes

	Test	Blank
Hemoglobin reagent	2 ml	2 ml
Blood sample	0.01 ml (10µl)	_____
Mix, allow to stand at room temperature for 3 min and read the absorbance at 540 nm against hemoglobin reagent		

- Hb conc (g/dl) = 29.4 x Abs of test

ERYTHROCYTE SEDIMENTATION RATE (ESR)

- ESR is the rate at which erythrocytes settle out of anticoagulated blood in 1 hour.
- It is used clinically as a non-specific screening test to:
  - detect the presence of infection in the body in general.
  - monitor the status of chronic inflammatory disease such as rheumatoid arthritis.
- ESR is not diagnostic of any particular disease, but rather is an indication that a disease process is ongoing and must be investigated.
- This test is based on the fact that inflammatory and necrotic processes cause an alteration in blood proteins, resulting in an aggregation of red cells, which make them heavier and more likely to fall rapidly when placed in a special vertical tube.

PRINCIPLE:

- In this technique, anticoagulated whole blood are allowed to sediment under the effect of gravity, using a narrow vertical tube called *Westergren's tube*.
- The length of the column of clear plasma at the top is noted at the end of 1 hour.

Normal Range

	After 1 hour	After 2 hours
Men	0 – 5 mm	7 – 15 mm
Women	0 – 10 mm	10 – 20 mm

HEMATOCRIT (HCT)

- *HCT or packed cell volume (PCV)* is the volume percentage (%) of RBCs in blood
- It is used as a simple screening test for anemia.
- Blood is collected in heparinised *capillary tube*, which is then sealed, centrifuged and the red cell volume expressed as a percentage of the whole blood.

Calculation :

$$HCT = \frac{\text{Length of column of RBC}}{\text{Total length of blood component}} \times 100$$

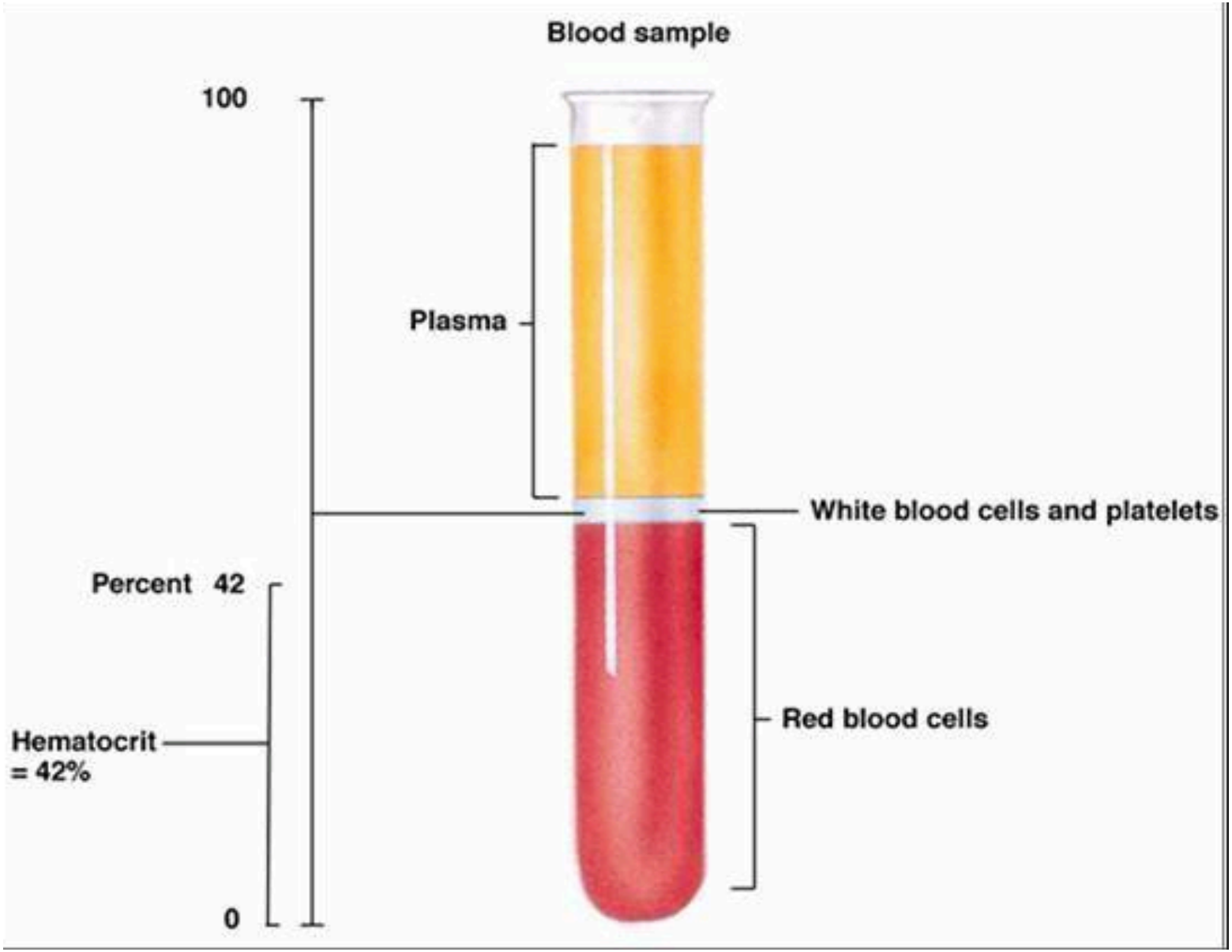
- A normal hematocrit percentage depends on age and gender.

Normal ranges

Male: 40.7 - 50.3% Female: 36.1 - 44.3%

Interpretation :

Low HCT cause of anemia High HCT cause of Polycythemia


Blood sample
100
Plasma
White blood cells and platelets
Percent 42
Hematocrit
= 42%
Red blood cells
0