ERYTHROCYTES

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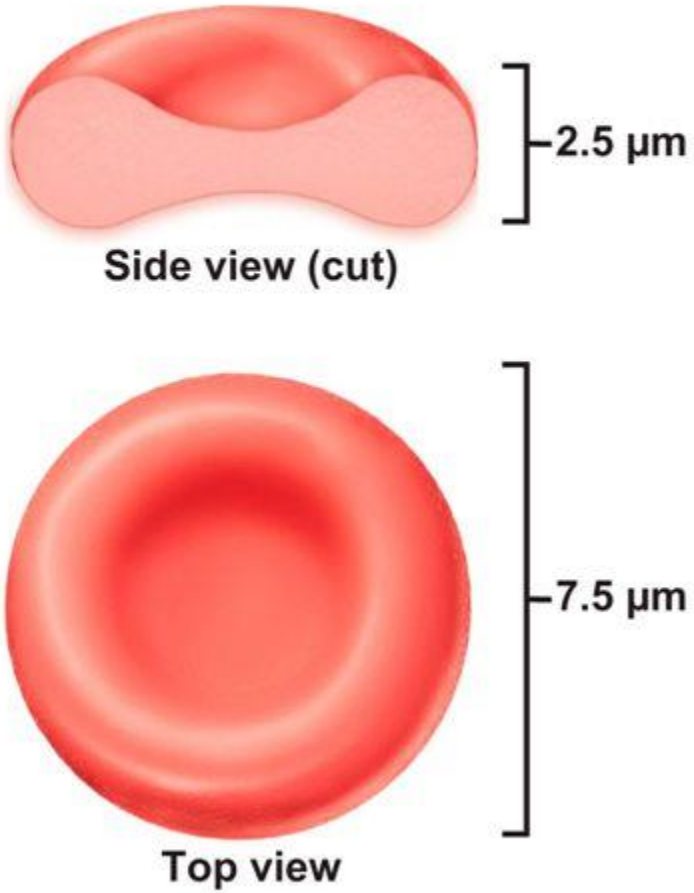
CBU SCHOOL OF MEDICINE

Erythrocytes (Red Blood Cells)

- Main function is to carry oxygen
- Biconcave disks
- Essentially bags of hemoglobin; few organelles
- Anucleate (no nucleus)
- Outnumber white blood cells 1000:1
- Contain the plasma membrane protein spectrin and other proteins
- Major factor contributing to blood viscosity



- The R.B.C. (red cells or *erythrocytes)* are non-nucleated circular biconcave discs that have *an average life span of about 120 days.*

- Their average diameter is 7.5 microns, thickness 2 microns and volume 87 cubic microns. Their normal count averages 5 *million/mm^3 (5.4 million in adult males and 4.8 million in adult females)*

POLYCYTHEMIA (= Erythrosis or Erythremia)

(1) Primary polycythemia (= polycythemia vera): This is a condition in which there is excessive production of R.B.C.s due to *an unknown cause.*

- Their count may reach 7-8 million/mm^3 leading to increased hematocrit, blood volume and viscosity. The increased blood volume tends to increase the venous return while the increased viscosity tends to decrease it (so the cardiac output usually remains normal).

- On the other hand, the increased peripheral resistance (due to the increased blood viscosity) tends to elevate the arterial blood pressure. The condition also leads to stagnant hypoxia (due to the sluggish blood flow) which is associated with cyanosis

(2) Secondary (= physiological) polycythemia : This normally occurs whenever the body suffers *O2 lack* (which stimulates red cell production in the bone marrow).

- The condition occurs in individuals living at *high altitudes* (in whom the red cell count may reach up to *6-8 million/mm3)* and also in *newly born infants* (due to the relative O2 lack during intrauterine life) in whom the hematocrit is about 60 %.

- The excess red cells are gradually hemolyzed so that the hemoglobin concentration falls from about 20 to about 12 gm% within the first *3 months after birth.*

- The iron released from the degraded hemoglobin (plus that stored in the body) produces a sufficient reserve for normal erythropoiesis up to *the first 4-6 months of life* (after which exogenous iron must be supplied, otherwise the infant would suffer iron deficiency anemia)

STRUCTURE OF THE RED BLOOD CELLS

- The red cell is formed of a viscous solution (cytoplasm) enclosed by a cell membrane. The cytoplasm is formed mainly of hemoglobin. Each red cell contains about 30 pg Hb

- It also contains electrolytes (specially K+ and HCO,) and several enzymes e.g. carbonic anhydrase and glucose-6-phosphate dehydrogenase (G6PD)

- The red cell membrane is formed of 3 layers

(a) An outer layer formed of glycoprotein
(b) A middle layer formed of phospholipids
(c) An inner layer formed of mucopolysaccharide.

- The biconcave shape of the red cells is produced by 2 proteins in their membranes called ankyrin and spectrin (their defects have been reported as causes of spherocytosis)

- It also contains electrolytes (specially K+ and HCO,) and several enzymes e.g. carbonic anhydrase and glucose-6-phosphate dehydrogenase (G6PD)

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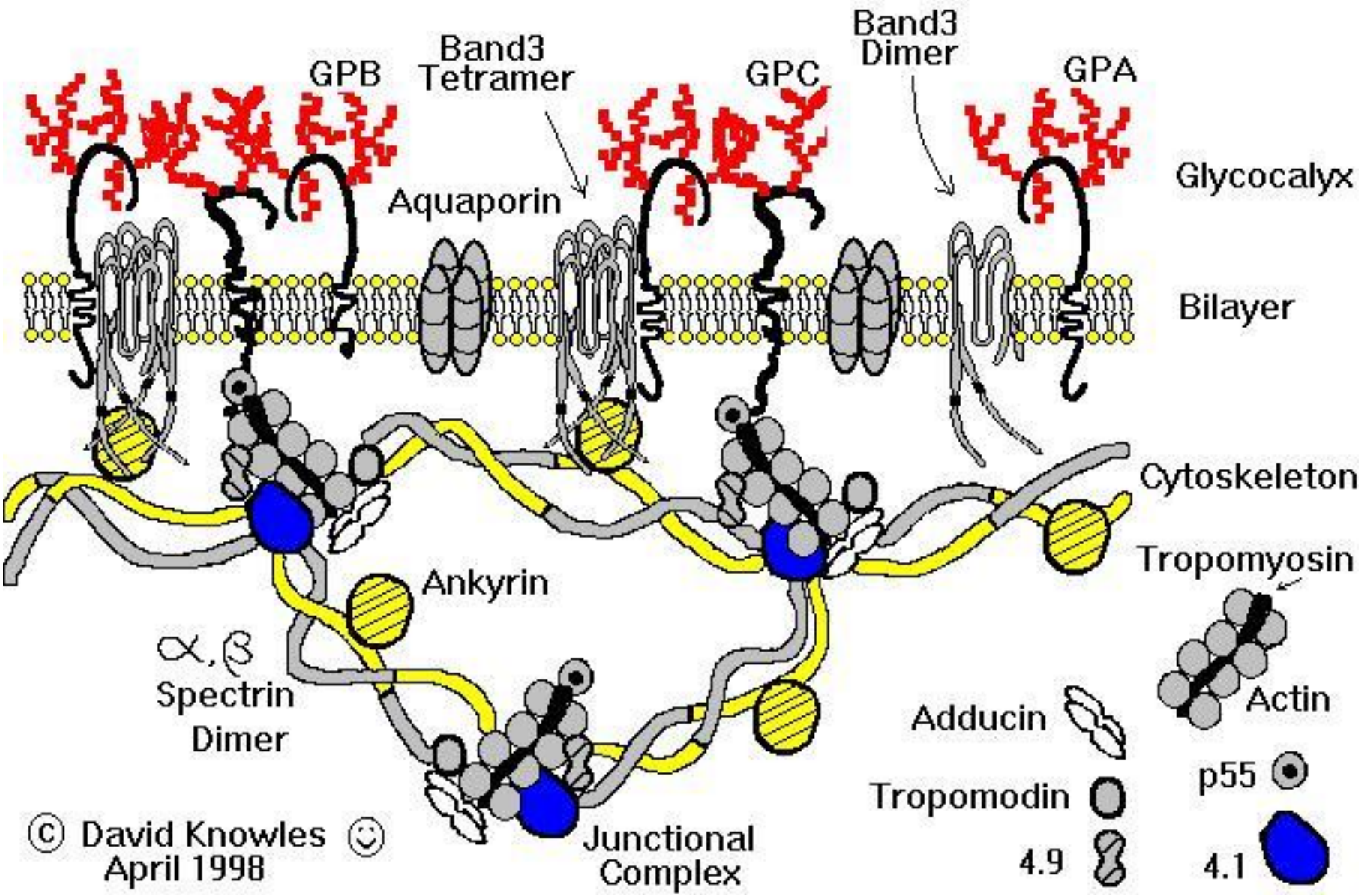
Erythrocyte Membrane Cartoon



- The major constituent of this cytoskeleton is spectrin, a dimer consisting of α and β sub-units

- The membrane skeleton also contains filaments of actin. The integral proteins e.g. protein (Band) 3 and glycophorin A are tightly bound to the membrane and act as anchors for the underlying membrane skeleton

- Defects of this protein cytoskeleton are thought to explain some inherited abnormalities of erythrocyte shape e.g. hereditary spherocytosis and elliptocytosis

- Integral membrane protein subserve other functions. One has binding sites for several glycolytic enzymes and provide transport for HCO- and Cl- leaving or entering the red cell.

- Other proteins include Na+,K+ ATPase of the sodium pump and Ca2+,Mg2+ ATPase which mediate active efflux of Ca2+ from the cell.

FUNCTIONS OF THE RED BLOOD CELLS

(1) The red cells play an important role in producing *blood viscosity* *w*hich is essential for maintenance of the diastolic arterial B.P.

(2) Hemoglobin is essential for O2 carriage. It is also essential for CO2 transport, and the carbonic anhydrase enzyme is important for this function. In addition, its an important buffer

(3) The main function of the red cell membrane is to keep Hb inside the red cells to prevent its escape into the plasma.

- Its glycoprotein layer contains the specific agglutinogens that determine the blood group

- In addition, it allows free diffusion of O2 and CO2 in both directions, and the biconcave shape of the red cells provides the largest possible surface area for this purpose

HEMOGLOBIN (Hb)

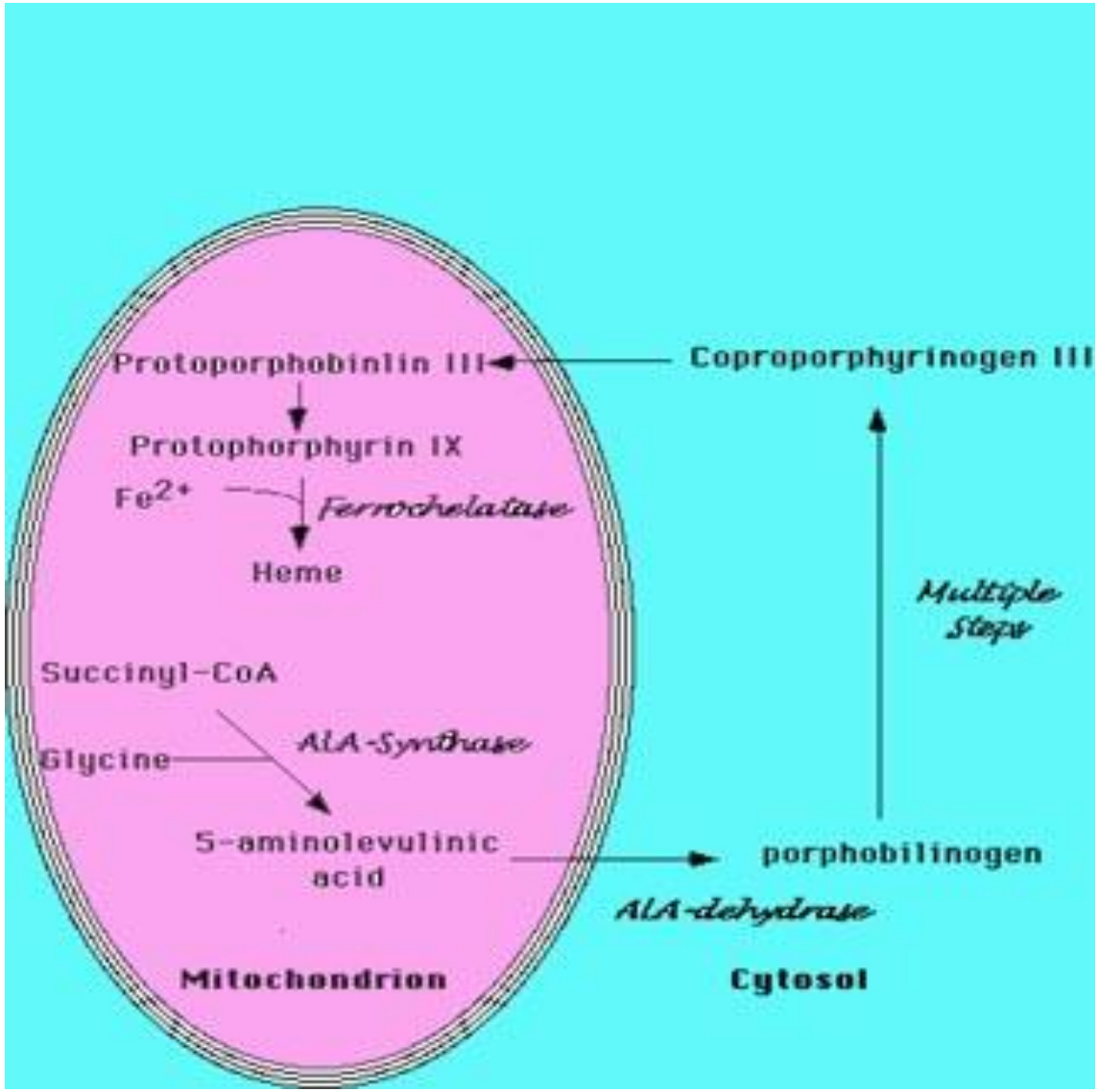
- This is a chromoprotein with a molecular weight 64450. Its molecule is globular in shape, and is made up *of 4 subunits* each of which is formed of a *heme molecule conjugated to a polypeptide chain*

- The heme is formed of *a porphyrin derivative containing one ferrous (Fe2+) atom.* Thus, each Hb molecule contains *4 Fe2+ atoms and 2 pairs of polypeptide chains,* which are collectively called *globin.*

- The Hb in normal adult human beings is called HbA, and its normal amount averages 15 gm% (about 16 gm % in males and 14 gm % in females).

- It contains 2 alpha polypeptide chains (each containing 141 amino acids) and 2 beta polypeptide chains (each containing 146 amino acids), so it is referred to as alpha2, beta2, Hb

HEMOGLOBIN (Hb)



Red-cell metabolism

- Without the benefits of mitochondria and ribosomes for synthesizing protein, the erythrocyte survives for more than 4 months in the face of repeated oxidant stress from high O2 conc and repeated mechanical stress from passages through capillaries of diameter smaller than that of the cell.

- This cellular longevity depends on simplified metabolic organization with three main functions:

a) To provide energy for maintaining cellular volume
b) To provide reducing power to protect the cell against oxidation\
c) To help control the affinity of Hb for oxygen

- About 95% of the glucose consumes by the red cells is metabolized by anaerobic glycolysis, 5% is used by the pentose-phosphate-pathway (PPP)

- The glycolytic pathway provides energy for the Na+,K+ ATPase as well as maintain a supply of reduced NADH

- Reduced NADH is a co-enzyme for a reductase enzyme which helps to maintain the iron in the Hb in the ferrous state.

- If the iron is oxidized to the ferric state, the methaemoglobin so formed cannot combine reversibly with O2.

- Methaemoglobin may also be reduced with the aid of the coenzyme NADPH which is produced by the PPP

- The reducing power of NADPH is also made available to the cell through its linkage with the tripeptide, glutathione.

- It provides protection against the oxidation of sulphydryl groups of enzymes, globin and constituents of the membrane.

- It also counteracts auto-oxidation of membrane lipids and helps dispose of any hydrogen peroxide that forms

- A side reaction of the glycolytic pathway in erythrocytes leads to the synthesis of 2,3 bisphosphoglycerate from 1,3-bisphosphoglycerate.

- 2, 3 BPG combines with HB to reduce its affinity for O2, thus promoting delivery of O2 to the tissues.

- Conditions causing hypoxia, lead to increased synthesis of 2,3-BPG and therefore increased release of O2 from Hb.

ABNORMALITIES OF HEMOGLOBIN PRODUCTION

(1) The hemoglobinopathies: These are conditions in which abnormal Hbs (i.e. having diiferent polypeptide chains from those of Hb A) are produced.

- Such abnormalities are due to mutations in the globin genes, and over 1000 of abnormal Hbs have been described in humans. Some of these Hbs are harmful e.g. HbS while most of the others are harmless e.g. *HbA1 , HbC, HbE, HbI and HbJ*

- About 2.5 % *of Hb in normal adult persons is HbA* which contains *2 alpha and 2 delta polypeptide chains.*

- Also normally, there are small amounts *of glycated Hb A* derivatives. One of them (Hb A_{1c}) has a glucose molecule attached to each beta chain.

❑The quantity of Hb A_{1c} increases in poorly controlled diabetes mellitus and it decreases with insulin treatment, so its measurement is used as an index in evaluating the control of that disease

(2) The thalassemias : These are conditions in which the Hb polypeptide chains are normal in structure but they are produced in decreased amounts (or some of them may be absent) because of defects in the regulatory portion of the globin genes

❑There are 2 types of this abnormality, *alpha and beta* (according to the deficient polypeptide chain). The red cells are *hypochromic and also rapidly hemolyzed in the circulation* (due to an intrinsic defect) leading to a severe type of anemia called *mediterranean or Cooley's anemia*

HEMOGLOBIN F

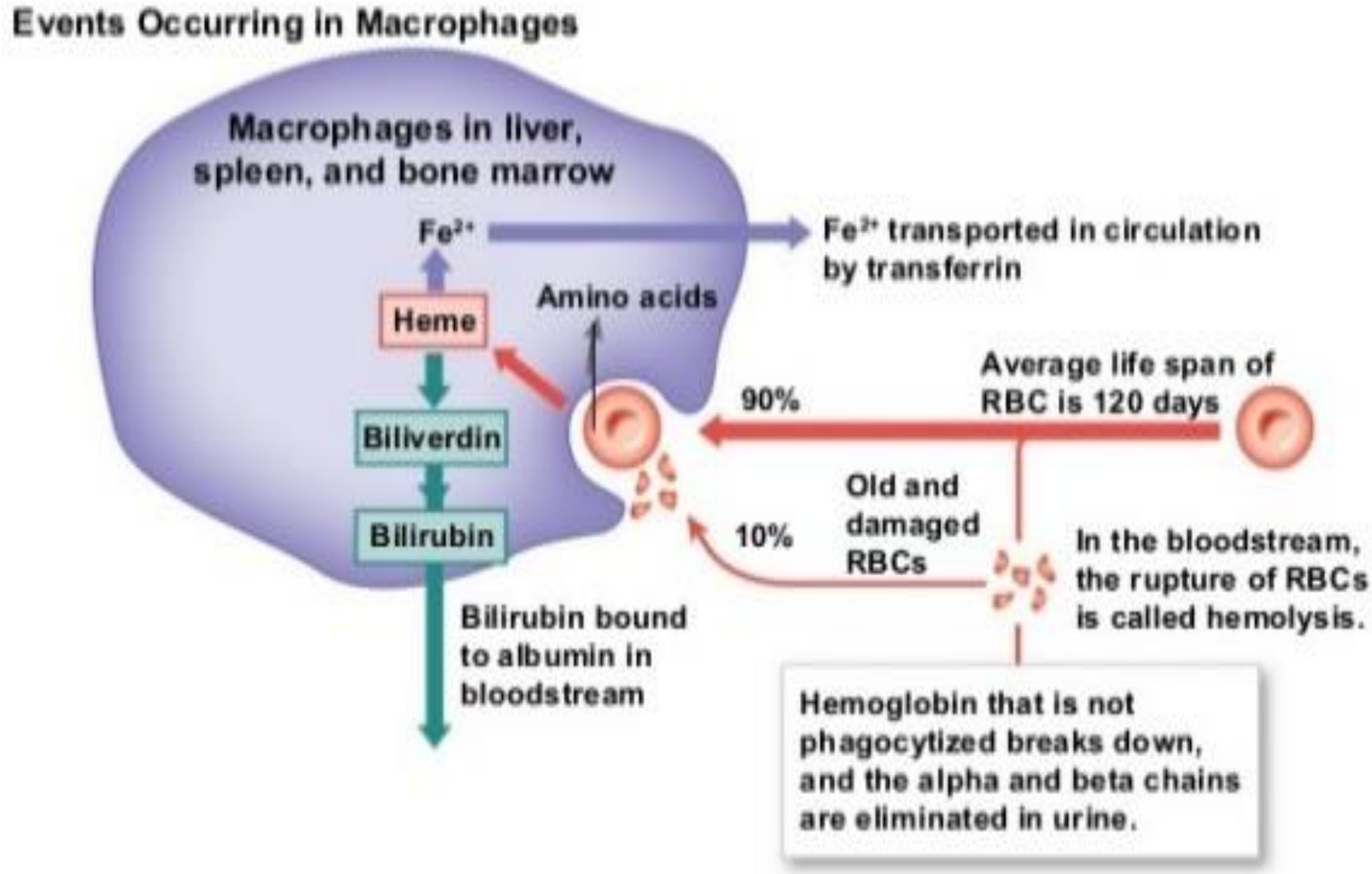
- This is the Hb present in the red cells of *the fetus a*nd it is normally replaced by HbA soon after birth. Its O2 carrying capacity is greater than that of HbA and it contains *2 alpha and 2 gamma polypeptide chains* (= *alpha 2 gamma 2 Hb)*

HEMOGLOBIN S

- This is a type of Hb in which the alpha polypeptide chains are normal but in the *beta chains* one glutamic acid residue is replaced by a valine residue. At low O2 tensions, Hb S polymerizes and this causes the red cells to become sickle shaped, hemolyze and form aggregates that block blood vessels

- The result is a severe hemolytic anemia called sickle cell anemia. This anemia is more common in black races. and although it is dangerous (and may be fatal), it has a benefit in Africa because the sickle cell gene also provides resistance to one type of malaria.

FATE (METABOLISM) OF Hb



Outline of Degradation of hemoglobin

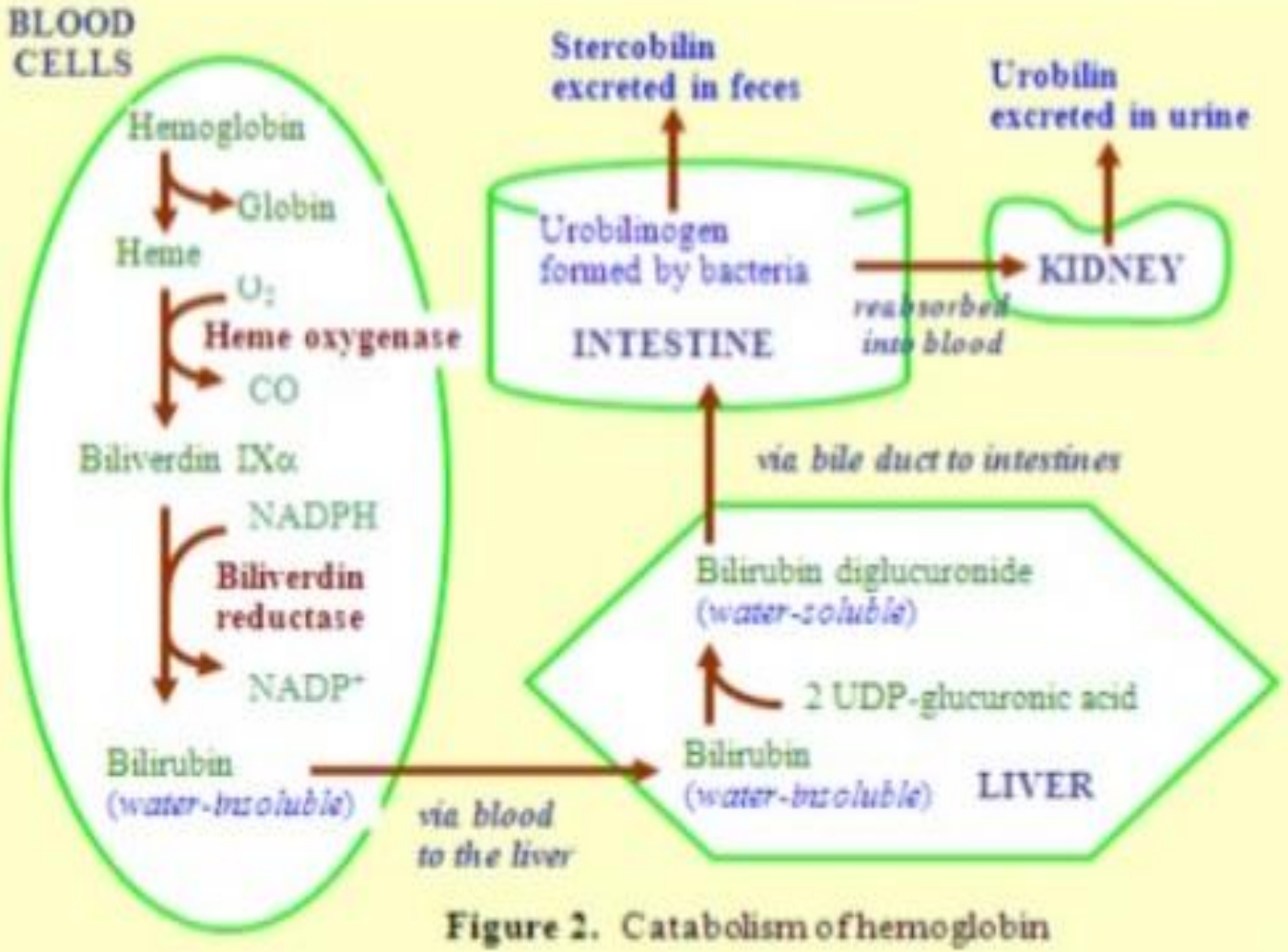


Figure 2. Catabolism of hemoglobin

“Run from anything that stimulates youthful lusts. Instead, pursue righteous living, faithfulness, love, and peace. Enjoy the companionship of those who call on the Lord with pure hearts”

SITE OF FORMATION (ORIGIN) OF THE RED CELLS

- During early fetal life, red cells are produced in the *area vasculosa of the yolk sac* then, till the end of the 6th month, they are formed in the *liver and spleen* (= *extramedullar origin).*

- During the last 3 months of pregnancy and after birth, they are formed in the bone marrow (=medullary origin). In children, all bones contain active (red) marrow.

- However the red marrow gradually decreases with age, so that at the age of about 20 years the active marrow becomes restricted to the upper ends of long bones (specially the femur and humerus*)* and *the flat bones* (the ribs, sternum and vertebrae)

PHYSIOLOGICAL FACTORS THAT AFFECT RED CELL COUNT

(1) Age : It is *high in* new born *infants* due to polycythemia and low in old individuals.

(2) Sex : It is higher in males mainly because erythropoiesis is stimulated by *the male hormone (*testosterone).

(3) Exercise, hot weather and emotions (in which the red cell count is increased due to *spleen contraction* induced by adrenaline secretion).

(4) High altitudes (the red cells increase due to polycythemia).

(5) Diurnal variation : About 5 % change in the red cell count occurs during 24 hours, being highest in the evening and lowest during sleep.

ERYTHROCYTE SEDIMENTATION RATE

- This is the rate at which the red cells sediment (sink) when blood is placed vertically in a narrow tube.

- Red cells sediment because
(a) Their density is greater than that of the plasma
(b) They tend to aggregate to form rouleaux shapes

- ESR is determined by the *Westergren method* as follows : A blood sample from the subject is anticoagulated by addition of 3.8% Na citrate solution at a ratio of *1 part citrate to 4 parts blood* (e.g. 0.5 ml citrate to 2 ml blood).

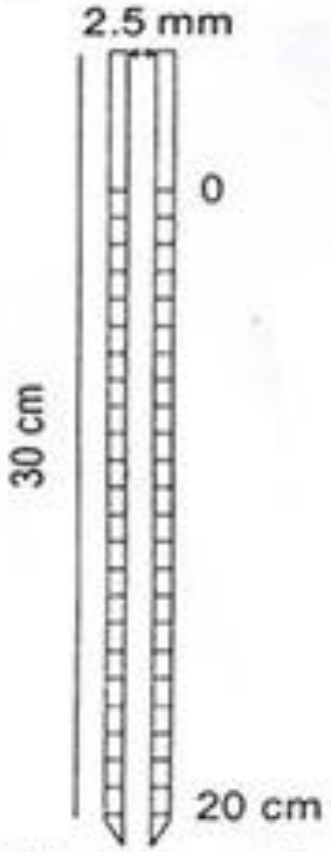
- Blood is then placed in a Westergren tube (length 30 cm and diameter 2.5 mm) which is fixed in a vertical position.

- The cells sediment (at first slowly then rapidly as they form rouleaux shapes) and after one hour, the height of the column of clear plasma (in mm) on top of the sinking red cell is measured and this represents the ESR in mm/hour.

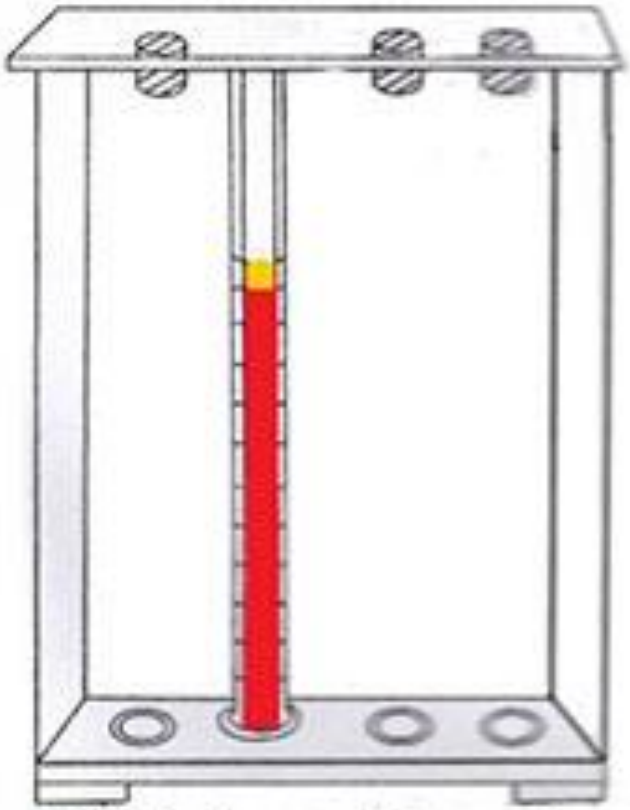
- A second measurement can be obtained after 2 hours and in this case, the ESR is calculated as follows:
ESR =(first hour reading + 1/2 second hour reading) *I* 2

NORMAL VALUES OF THE ESR

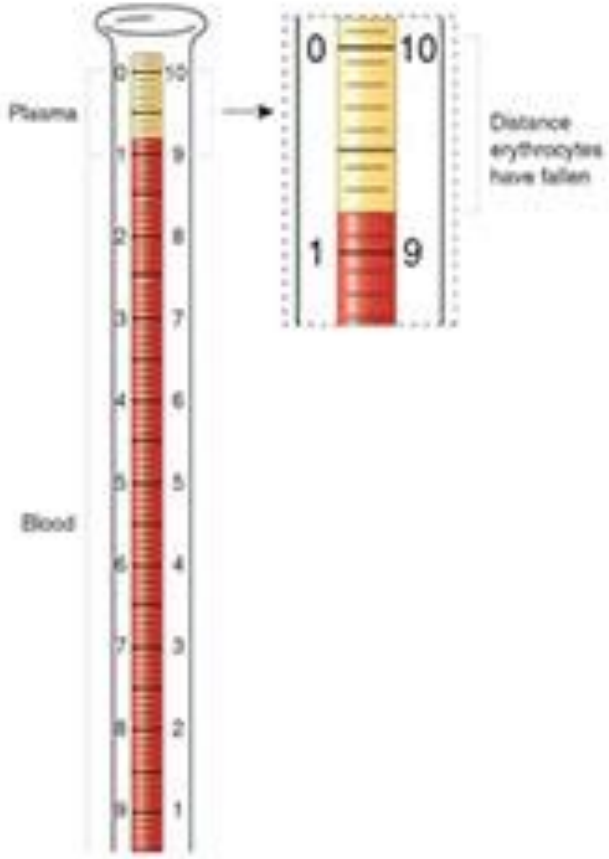
In males, it is *4-6 mm/hour* while it is higher in females *(6- 10 mm/hour)* because the red cell count in females is lower. It *may be zero if the plasma is viscous.*



a) Westergren tube



b) Westergren tube on the rack



Wintrobe tube

FACTORS THAT AFFECT THE ESR

(1) Plasma proteins : The ESR increases if the *albumin level is decreased* and when the level of fibrinogen *or globulins increases*. The latter effect is probably due to decrease of the electronegative charge of the red cells (which reduces their repulsion and helps rouleaux formation).

(2) Red cell count : The ESR is decreased in polycythemia and increases in anemia *except in iron deficiency* (probably due to reduction of the intrinsic ability of the red cells to sediment)

COMMON CAUSES THAT INCREASE THE ESR

(A) Physiological causes : The ESR is normally high in females during *menstruation* (about 15mm/hour) and *pregnancy* (20 mm hour or more) and in cases of *increased temperature* and after *muscular exercise.*

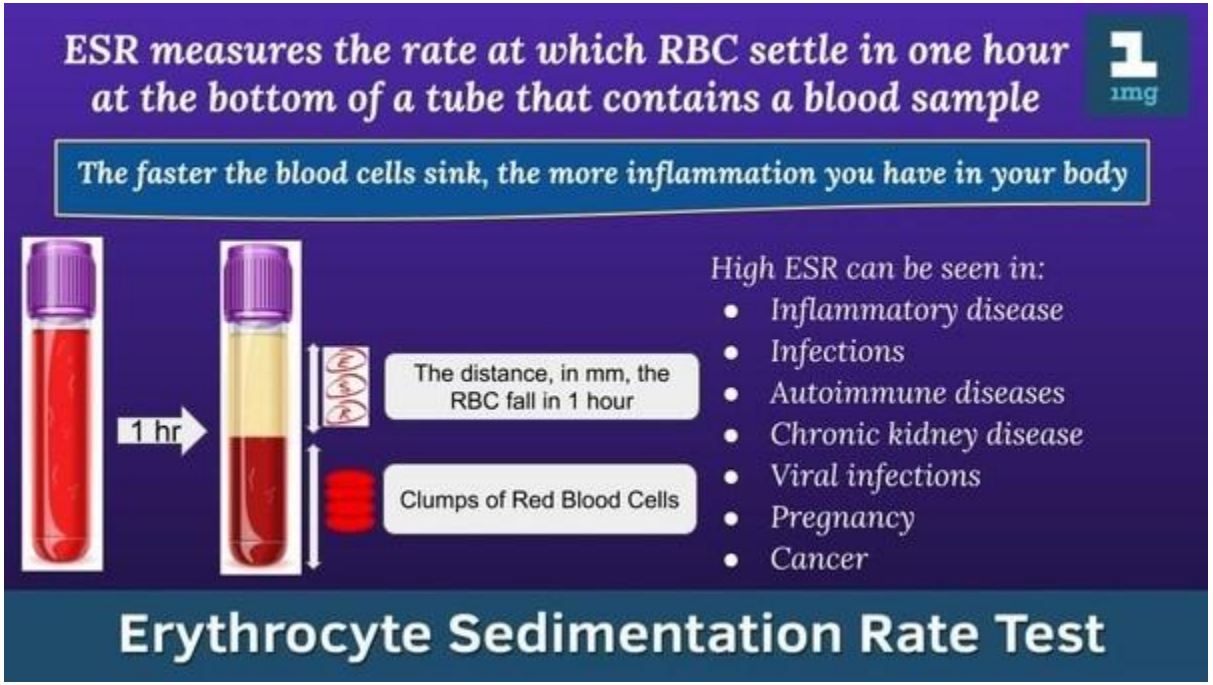
(B) Pathological causes: The ESR increases in cases of *inflammation and tissue destruction* due to increased fibrinogen or globulins Thus it increases in

(1) Acute and chronic infections e.g tonsillitis, appendicitis and tuberculosis
(2) Severe trauma e.g. in fractures
(3) Myocardial infarction
(4) Degenerative and neoplastic diseases
(5) Rheumatic arthritis

CLINICAL IMPORTANCE OF ESR ESTIMATION

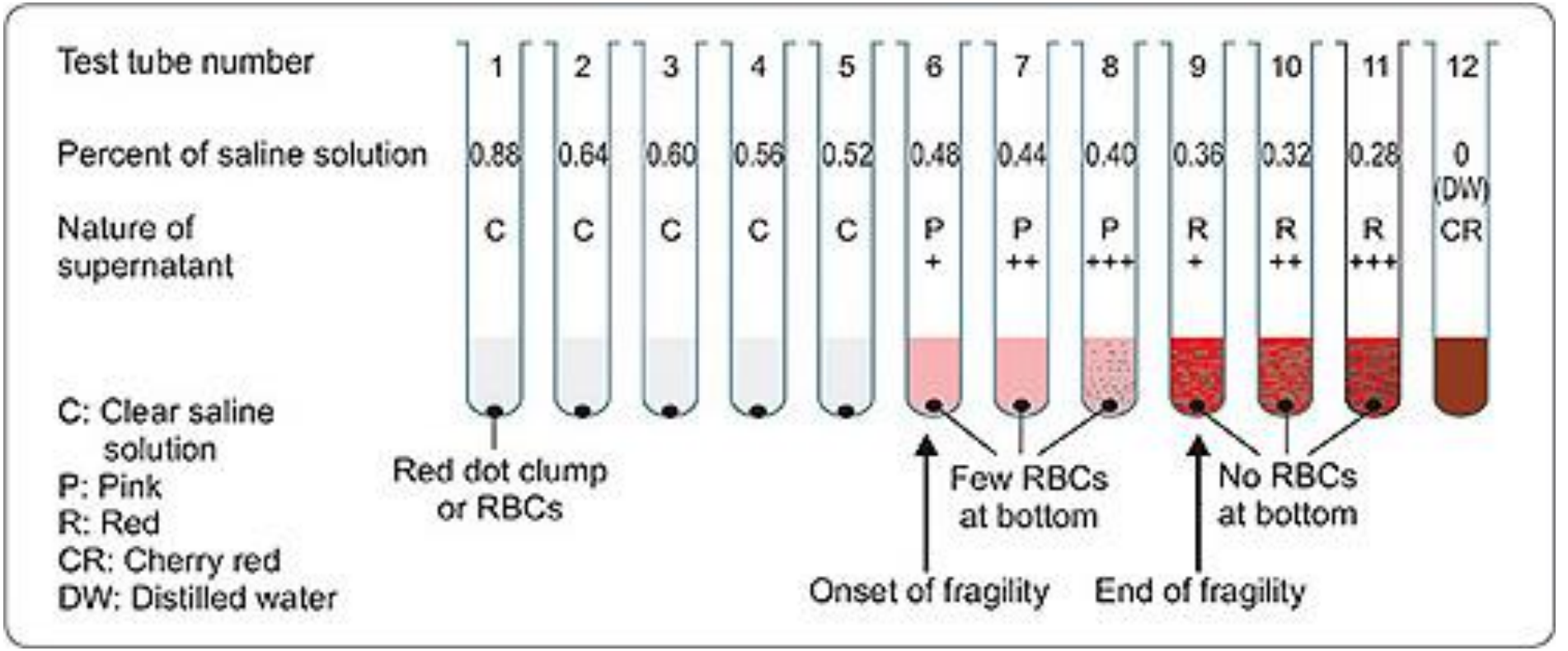
- Because the ESR is changed in a great variety of conditions, its alteration is *not specific (i.e. not diagnostic) for any particular disease* and gives no indication about the nature of the underlying disorder.

- However, *repeated ESR estimations help in prognosis* and follow up of the activity of the disease and its response to treatment



OSMOTIC FRAGILITY OF THE RED BLOOD CELLS

- It is a test that measures the resistance to hemolysis of red blood cells exposed to hypotonic solution. The biconcave shape of the red cells allows 45-65 % increase in their volumes before they rupture.



- This range indicates that the *R.B.C.s vary in their fragility* (probably because the *older cells are more fragile and rupture before the young ones)* so that *hemolysis normally starts in about 0.5 % NaCI solution and is complete in about 0.35 % NaC/ solution*

FACTORS THAT AFFECT RED CELL FRAGILITY

(1) Congenital and inherited defects of hemopoiesis

(a) *Sickle cell anemia and thalassemia*: These conditions are associated with increased fragility of the red blood cells which renders these cells to be readily hemolyzable

(b) *Hereditary spherocytosis* : The red cells are spherical due to abnormalities of the protein network that maintains the shape and flexibility of their membranes, particularly *spectrin and ankyrin*

- The spherocytes are smaller than normal cells and are more fragile, so they *start to hemolyze at about 0. 7 % saline solution* and are completely hemolyzed at about *0.45 % saline solution*

(2) Acquired anemias: In iron deficiency anemia, the red cells are flattened but they are not fragile, so they resist hemolysis more than normal. On the other hand, in macrocytic anemias, the abnormal red cells are fragile, so they are more readily hemolyzable than normal.

(3) Ionic changes in the blood: Normally, the red cells in the venous blood become slightly spherocytic (and accordingly more fragile) due to the chloride shift phenomenon

(4) Red cell age : In old red cells the *power of the Na+ pump is decreased.* Accordingly, Na accumulates inside these cells and the resulting rise of the osmotic pressure causes water diffusion into them, so they become fragile and hemolyze more easily than normal.

(5) The G6PD enzyme : This enzyme catalyzes glucose oxidation in the red cells. Such oxidation generates *NADPH* (Nicotinamide-Adenine Dinucleotide Phosphate) which is *needed for maintenance of normal red cell fragility.* Accordingly, the red cells become fragile and hemolyze easily if the G6PD enzyme is congenitally deficient.

CAUSES OF HEMOLYSIS

(1) Intravenous injection of excess amounts of *hypotonic fluids.*
(2) *Incompatible (mismatched) blood transfusion*.
(3) Deficiency of the *G6PD* enzyme.
(4) *Snake venom,* some chemicals (e.g. arsenic) and bacterial toxins.
(5) *Hereditary spherocytosis, abnormal Hbs (e.g. Hb S) and thalasemia.*
(6) *Blood freezing* :The formed ice crystals cause disruption of the red cells and hemolysis during thawing of blood (i.e. liquefaction by heat).
(7) *Fat solvents* e.g. ether (destroy the lipoprotein in cell membranes).
(8) Immune reactions that cause formation of *red cell antibodies.*

EFFECTS AND DANGERS OF HEMOLYSIS

(1) *Jaundice* (due to excessive formation of bilirubin).
(2) Increase of the plasma colloid osmotic pressure (from 25 mmHg to about 70 mmHg), which prevents tissue fluid formation.
(3) *Hemoglobinuria (*= Hb excretion in the urine). Hb is also precipitated in the renal tubules leading to *oliguria* and may be *renal failure.*
(4) *Hb dilution* by the plasma. This *shifts the O2 dissociation curve to the left* leading to *less O2 delivery to the tissues*
(5) *Tissue hypoxia* due to both Hb loss and Hb dilution.
(6) *Acidosis* due to (a) Renal insufficiency (b) Decreased blood buffering power after loss of Hb (c) The decreased O2 delivery to the tissues.
(7) *Shock due to V.D.*, which occurs secondary to release of certain *V.D. substances* from the hemolyzed red cells specially *histamine.*
(8) *Hyperkalemia* due to release of K+ from the hemolyzed red cells.

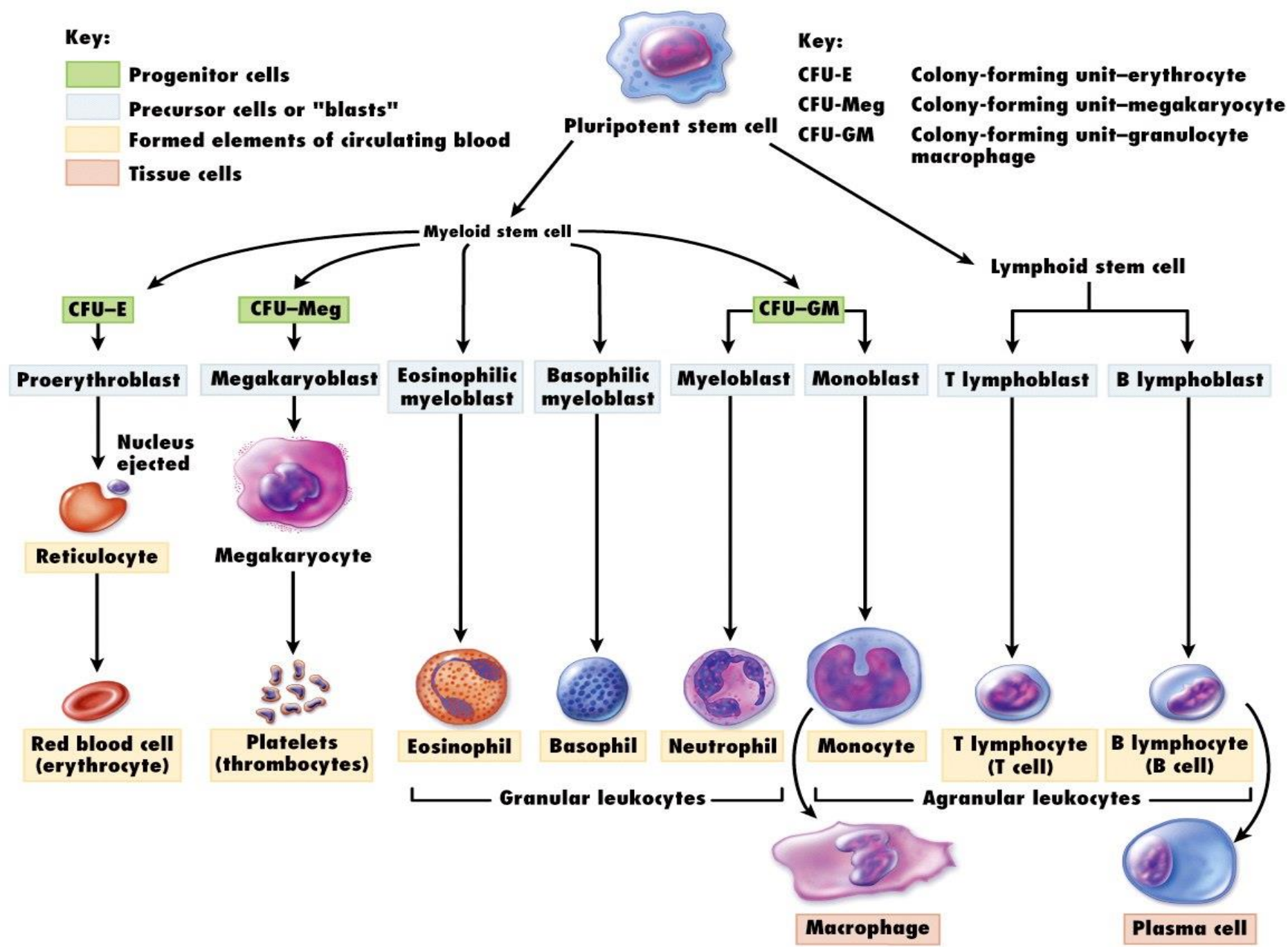


Figure 19-3 Principles of Anatomy and Physiology, 11/e

THE RIGHTEOUS SHOULD **CHOOSE** HIS **FRIENDS CAREFULLY**, FOR THE WAY OF THE WICKED LEADS THEM ASTRAY

ERYTHROPOIESIS

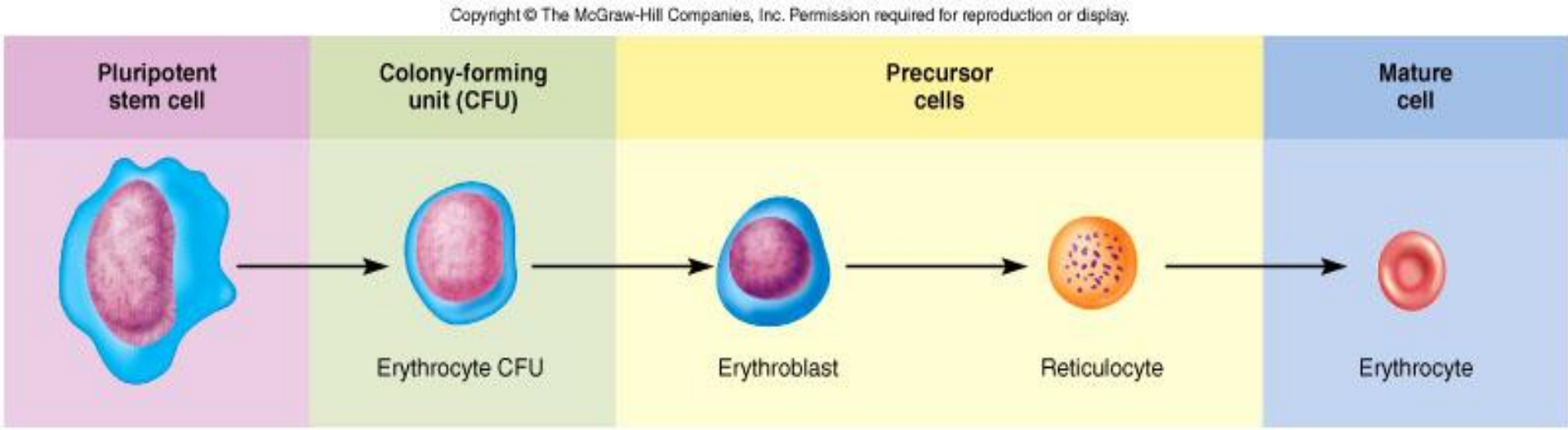
- The process of red-cell production is called erythropoiesis.

- In a Romanowsky-stained film of marrow, the earliest recognizable red-cell precursor is a large nucleated cell, the proerythroblast (pronormoblast) – basophilic cytoplasm

- This cell develops into an early (basophilic) erythroblast (normoblast), and the cells subsequently become smaller (intermediate and late erythroblasts) with increasingly acidophilic cytoplasm as hemoglobin is synthesized and the capacity for mitosis is lost as nuclear pyknosis.

- Extrusion of the nucleus then leaves cells, reticulocytes, which contain remnants of RNA, and ribosomes and continue making hemoglobin.

- Reticulocytes mature for 1-2 days in the marrow and are then released into the blood where, after a further 1-2 days they lose their remaining ribosomes to become mature RBCs



- Reticulocytes can be identified in blood films stained with a dye such as new methylene blue which reacts with the ribosomes to form precipitates visible as dark blue granules

- The reticulocyte count of blood is a useful index of the erythopoietic activity of the bone marrow

FACTORS THAT AFFECT ERYTHROPOIESIS (R.B.C.s FORMATION)

(1) BLOOD O2 TENSION

- Erythropoiesis is stimulated when the blood O2 tension is decreased (e.g. in high altitudes and after hemorrhage) and vice versa. *O2 lack (hypoxia) is the most powerful stimulus for erythropoiesis* through inducing secretion of the hormone erythropoietin from the kidneys

(2) THE KIDNEYS

The endothelial cells of the peritubular capillaries in the kidneys secrete a hormone called *erythropoietin* (a glycoprotein having a m.w. 34000) which stimulates red cell formation in the bone marrow through *increasing the number of the erythroid committed stem cells*

- The release of erythropoietin is stimulated by *O2 lack*, *the male sex hormone (androgen) and cobalt salts*, and is facilitated by catecholamines and in conditions of *alkalosis* (e.g. at high altitudes). About 15% of erythropoietin is secreted by the hepatocytes and Kupffer cells of *the liver.*

(3) HORMONES

Erythropoiesis is stimulated by several hormones specially *androgens, corticosteriods, growth hormone* and *thyroxine.*

(4) THE LIVER

The liver forms the globin part of Hb, stores vitamin B12 and several minerals required for red cell formation specially iron, and also secretes some erythropoietin

(5) THE BONE MARROW

A healthy bone marrow is essential for normal hemopoiesis (including erythropoiesis), so its damage (e.g. by radiation or disease) results in a severe type of anemia known as *aplastic anemia*

(6) DIET

For adequate erythropoiesis, the diet should contain the following

(a) *Proteins of* high *biological value,* for synthesis of globin portion of Hb).

(b) *Iron,* for synthesis of the heme part of Hb.

(c) Other minerals specially *copper and cobalt,* which act as cofactors in the process of Hb synthesis, and they are required in *trace amounts.*

(d) *Vitamins:* Almost all vitamins are required particularly *vitamin B and folic acid (iron and vitamin B12 are the most important nutritional factors required for erythropoiesis).*

IRON METABOLISM

❑Iron is an *essential element* since it is a component of Hb, myoglogin and many enzymes (e.g. cytochrome oxidase). The body contains about *4 (2-5) gm of iron, 60*-70 % of which is in Hb, 4-5 % in myoglobin and the remainder in some enzymes, ferritin

IRON REQUIREMENTS

The daily requirement of iron must equal the amount lost and is about 0.6 mg in males and 1.2 mg in females (due to blood loss in menstruation). However, its usual daily intake is about 20 mg of which the required amount only is absorbed (i.e. about 3- 6 % of the ingested amount)

❑Serum iron is lower in women than in men and decrease in pregnancy, iron deficiency and severe infections.

IRON ABSORPTION

- Iron is absorbed mostly in the *duodenum* and only in the *ferrous (Fe 2+) form* by an *active process*. Iron in heme and in other compounds are both absorbed.

- Its absorption increases when the iron stores in the body are depleted and when erythropoiesis is increased (by an unknown mechanism) and is affected by the following factors :

(1) Gastric HCl : The iron in diet is mostly in the ferric (Fe 3+) state. HCl dissolves iron and permits it to form soluble complexes with substances that aid its reduction to the Fe 2+ form (specially vitamin C).

(2) Nature of the iron-containing compound : Iron in inorganic compounds is more readily absorbed than that in organic compounds.

(3) Iron salt solubility :Iron is not absorbed from insoluble salts e.g. iron oxalate and phosphate, and its salts with phytic acid (present in cereals) .

(4) Role of the intestinal mucosal cells (the enterocytes) :
- Most of iron in the diet is in the ferric (Fe 3+) form. Fe 3+ is first converted to Fe 2+ by the *ferric reductase enzyme* at the brush borders of the enterocytes and Fe 2+ is then transported into these cells by an apical *membrane iron transporter (DMT 1)*

- Heme is also transported into the enterocytes by a special *heme transporter (HT)* then the Fe 2+ in heme is released by the *heme oxygenase enzyme (HO)* and is added to the intracellular Fe 2+ pool.

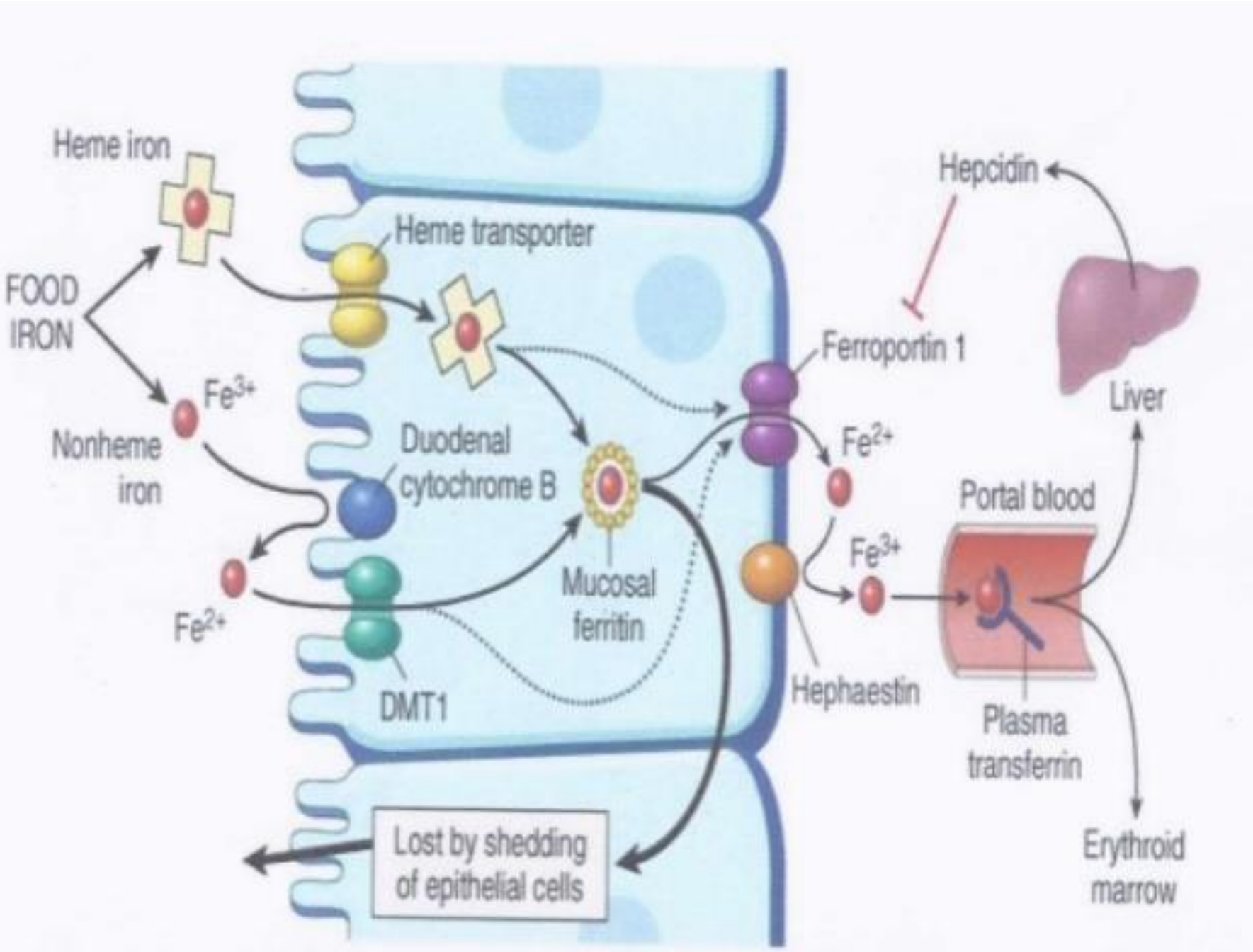
- Some of the intracellular Fe 2+ is converted to Fe 3+ and combine to a protein called apoferritin to form ferritin

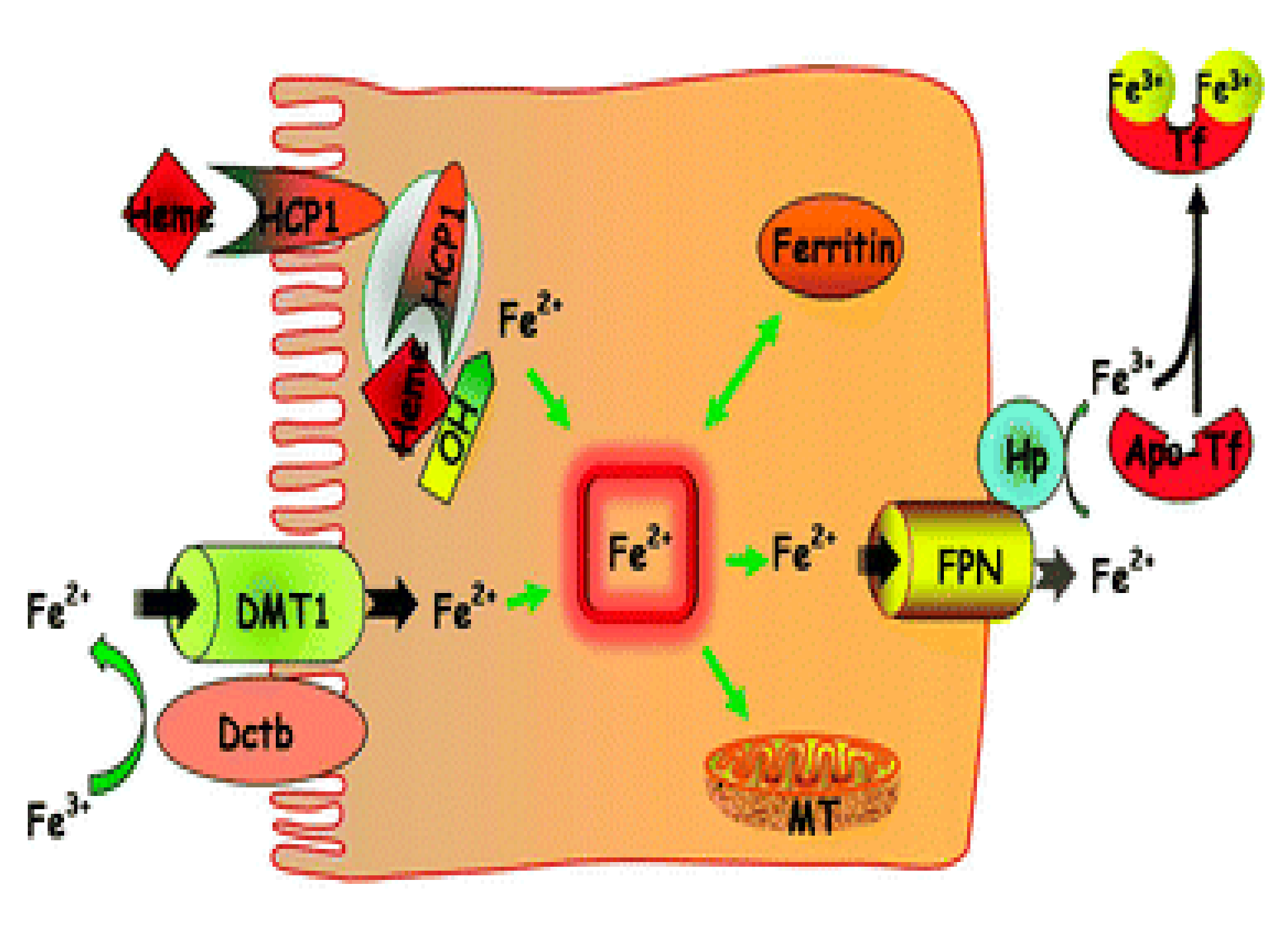
- The remaining Fe 2+ is transported to the interstitial fluid at the basolateral borders of the enterocytes by the transporter *called ferroportin I (FP)* and such transport is aided by a protein called *hephaestin (Hp)*

- Fe 2+ then diffuses into the blood and in the plasma, Fe 2+ is converted to Fe 3+ which becomes bound to the iron transport protein called *transferrin (TF).* Normally, the plasma iron level is about *130 and 110 microgram %* in males and females respectively.

- In many tissues (specially the liver), Fe 3+ also combines to apoferritin to form ferritin. Ferritin molecules in the lysosomal membranes may aggregate in deposits called *hemosiderin* (that contains as much as 50 % iron).

- In cases of iron overload, hemosiderin accumulates in the tissues(= *hemosiderosis)* and may lead to *hemochromatosis* (a syndrome that may be hereditary or acquired and is characterized by skin pigmentation, pancreatic damage with diabetes called *bronze diabetes,* liver cirrhosis with a high incidence of hepatic carcinoma, and gonadal atrophy).


Heme iron
Heme transporter
FOOD IRON
Fe3+
Nonheme iron
Duodenal cytochrome B
Fe2+
Mucosal ferritin
DMT1
Lost by shedding of epithelial cells
Hepcidin
Ferroportin 1
Fe2+
Fe3+
Hephaestin
Liver
Portal blood
Plasma transferrin
Erythroid marrow


Heme
HCP1
HCP1
Fe2+
Ferritin
Fe3+
Fe3+
Tf
Fe3+
Hp
Apo-Tf
Fe2+
Fe2+
DMT1
Fe2+
Fe2+
FPN
Fe2+
Fe2+
Dctb
Fe3+
MT

CAUSES OF NEGATIVE IRON BALANCE

- Increased physiological demands
- Inadequate absorption
- Loss of blood

THERE IS A WAY THAT SEEMS RIGHT TO A MAN,
BUT ITS END IS THE WAY OF
DEATH/DESTRUCTION

VITAMIN B12 (CYANOCOBOLAMIN)

- This is a *cobalt-containing vitamin* that is also called *hematinic principle.* It is required together *with folic acid* for red cell maturation (so both are called *erythrocyte maturation factors)* because both are essential for *synthesis of DNA* (therefore, when they are lacking there will be failure of nuclear maturation and division).

- The daily requirement of vitamin B12 is 1-3 micrograms, and besides its role in erythrocyte maturation, it also promotes growth, stimulates other hemopoietic processes in the bone marrow and is essential for formation of myelin in the nervous tissues

ABSORPTION OF VITAMIN B12

- This occurs *in the distal part of the ileum* and it requires a special glycoprotein factor secreted by the *parietal cells of the stomach called* the *intrinsic factor.*

- Vitamin B12 binds to the intrinsic factor and the formed complex is taken up by a lipoprotein present in special receptors in the distal ileum called *cubilin.*

- This triggers absorption of the complex by endocytosis. In the ileal enterocytes, vitamin B12 is released then it is transferred to a special protein called transcobolamin ll which is absorbed and transports the vitamin in the plasma.

- A large amount of vitamin B12 is stored in the liver from which it is released to the bone marrow

CAUSES OF VITAMIN B12 DEFICIENCY

(1) *Lack of the gastric intrinsic factor* (the commonest cause) often due to autoimmune destruction of the parietal cells or after total gastrectomy.

(2) *Certain diseases of the distal ileum* or congenital absence of the specific ileal receptors at which the vitamin is absorbed.

(3) *Intestinal diseases characterized by decreased absorbing power* (the malabsorption syndromes) e.g. sprue.

*** *Lack of vitamin B12 in diet is very rare,* since it is present in most foods or animal origin and its daily requirement is very little

VITAMIN B 12 DEFICIENCY (PERNICIOUS ANEMIA)

- Deficiency of vitamin B12 results in a type of *malnutrition failure anemia* called *pernicious anemia*. In most cases deficiency of this vitamin occurs secondary to *absence of the intrinsic factor* as a result of *autoimmune destruction of parietal cells*. This anemia is characterized by :

(1) Appearance of large red cells called *megaloblasts* in the bloodstream which are *nucleated and larger than mature red cells* However they are fragile and their *life span is shorter than normal*, so they hemolyze easily and the resulting anemia is called *megaloblastic or macrocytic anemia*. The Hb content in the megaloblasts is greater than normal but the mean corpuscular Hb concentration is normal

(2) *Leukopenia and thrombocytopenia* due to less synthesis of DNA.
(3) *Neurological symptoms :* These occur due to degeneration of the dorsal and lateral columns of the spinal cord as well as the peripheral nerves a condition known as *subacute combined degeneration.*

Treatment of pernicious anemia : *Administration of vitamin B 12 by injection and not by mouth* (because most cases occur secondary to lack of the intrinsic factor).

THE ANEMIAS

- *A*nemia is a *pathological condition* characterized by lowering of the circulating red cell mass below the normal level or reduction of the Hb concentration (or both together), leading to *a decrease in the O2 carrying capacity of the blood*

- Generally, it is due to either *a decrease in the production or an increase in the destruction of the red blood cells* and it may be classified in one of the following 2 ways :

(1) Hemorrhagic anemia : This is anemia caused by acute or chronic *blood loss* (e.g. due to bleeding peptic ulcers or piles or *bilharziasis)*

(2) Hemolytic anemia : This is anemia caused by excessive hemolysis (breakdown) of the R.B.C.s. Hemolysis may occur due to either :

(a) *Intracapsular (congenital) causes* e.g. abnormalities in the red cell membranes (as in congenital spherocytosis) or in Hb (as in sickle cell anemia and thalassemia) or deficiency of the G6PD enzyme.

(b) *Extracapsular (acquired) causes* e.g. certain drugs, chemicals and toxins (as some snake venoms), certain infections, malaria. hypersplenism, incompatible blood transfusion and autoimmunity (i.e. formation of abnormal antibodies that attack the red blood cells).

(3) Aplastic anemia : This is anemia caused by *damage of the bone marrow* e.g. due to

(a) Exposure to excessive X ray or gamma ray radiation
(b) Certain drugs (e.g. chloramphenicol) and chemicals (e.g. arsenic)
(c) Leukemia (in which the cxcessively-proliferating white cells in the bone marrow decrease red cell formation)
(d) Certain chronic infections

(4) Nutritional (deficiency) anemias : This is anemia caused by deficiency of the nutritional factors that are required for erythropoiesis e.g. :

(a) Anemia due to deficient supply of iron (iron deficiency anemia).
(b) Pernicious anemia (due to vitamin B12 deficiency).
(c) Macroctyic anemia (due to folic acid deficiency).

(II) According to red cell size and Hb concentration

(1) Normochromic normocytic anemia : In this anemia, the size of the red blood cells and their Hb content are *normal* but their count is less than normal. It occurs in

(a) Hemorrhagic and hemolytic anemias
(b) Some cases of aplastic anemia
(c) Hepatorenal disease
(d) Chronic infections.

(2) Hypochromic microcytic anemia : In this anemia, the red cell size and their Hb content are decreased but their count is almost normal. It occurs in

(a) Iron deficiency anemia
(b) Thalassemia
(c) Defective synthesis of porphyrin and heme e.g. in lead intoxication
(d) Hypothyroidism

(3) Macrocytic anemia : In this anemia, both the red cell volume and its Hb content are increased but the mean Hb concentration is almost normal. There are 2 types of this anemia:

(a) Megaloblastic (maturation failure) anemia : This occurs in *pernicious anemia and in cases of fo*lic *acid deficiency.*

(b) Non-megaloblastic anemia : This occurs after recent hemorrhage or hemolysis and in some cases of aplastic anemia as well as in some cases of anemia resulting from hepatic disease.

EFFECTS OF ANEMIA

(1) Reduction of the blood viscosity and O2 lack. The former decreases the peripheral resistance while the latter causes V.D. Both effects increase the venous return and consequently the cardiac output, which increases the work load on the heart.

(2) Increase of the blood velocity (due to reduction of viscosity) and this increases the blood turbulence which accentuates the heart sounds and may produce functional murmurs

(3) Decrease of the O2 content in the arterial blood which leads to tissue hypoxia and rapid fatigue

THE BLOOD INDICES

(I) Colour Index (CI) : This is a rough (approximate) indication of *the amount of Hb in the red cells.* It is calculated as follows :

$$CI = \frac{\text{Hb content (\% of normal)}}{\text{Red cell count (\% of normal)}} \text{ . Its normal value is } \frac{100\ \%}{100\ \%} = 1$$

(***normal range 0.85 - 1.1***). Lower values suggest decreased Hb content, most commonly as a result of iron deficiency.

(2) Mean Corpuscular Hb (MCH) : This is the average amount of Hb in one red blood cell in picograms (pg). It is calculated as follows :

$$\mathbf{MCH = \frac{\text{Hb content in one litre blood}}{\text{Red cell count in millions/mm}^3} \text{ . Its normal value is } \frac{150}{5} = 30\ pg}$$

(***normal range is 26 to 34 pg***). Higher values (up to 38 pg) are found in macrocytic anemias while lower values are obtained in microcytosis and when Hb synthesis is decreased or defective e.g. in hypochromic anemias.

(3) Mean Corpuscular Hb Concentration (MCHC) : This is the average amount ofHb per 100 ml of red cells. It is calculated as follows:

MCHC = $\frac{\text{Hb (gm) in 100 ml blood}}{\text{Packed cell volume}}$ **x 100 . Its normal value is** $\frac{15}{45}$ **x 100**
= *33-35 gm /100 ml red cells* (= *normochromic cells*). Below 31 gm, the cells are ***hypochromic. In macrocytic anemia there is normochromia*** .

(4) Mean corpuscular volume (MCV): This is the average volume of one red blood cell. It is calculated as follows :

MCV = $\frac{\text{Packed cell volume / one litre blood}}{\text{Red cell count in millions/mm}^3}$ **. Its normal value is** $\frac{450}{5}$ **=**
90 microns^3 ranging from 78 to 94 microns^3 (= ***normocytes***). Red blood cells with more volumes are ***macrocytes*** (e.g. in pernicious anemia) while those with less volumes are ***microcytes*** (e.g. in iron deficiency anemia).

And wisdom and knowledge shall be the stability of thy times, and strength of salvation: the fear of the LORD is his treasure.

MERCI