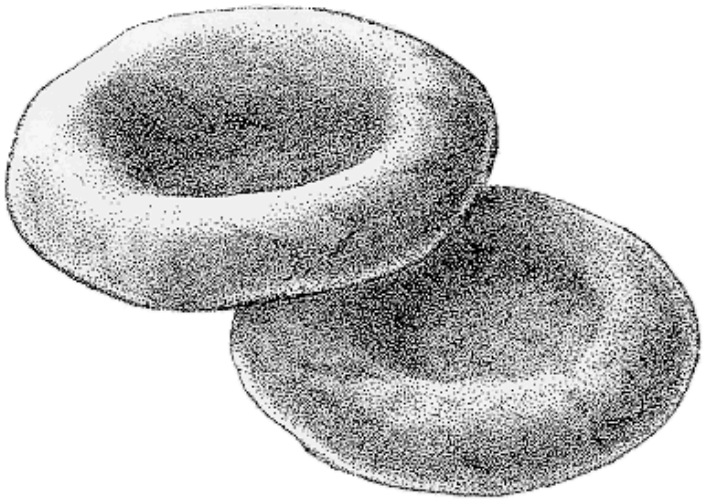


Its not Donat ☺

هذه المذكرة قام بعملها
أخواتكم
Hejaz Gp.

عدل وأضاف عليها :

أحمد العقيل

شكر خاص لـ

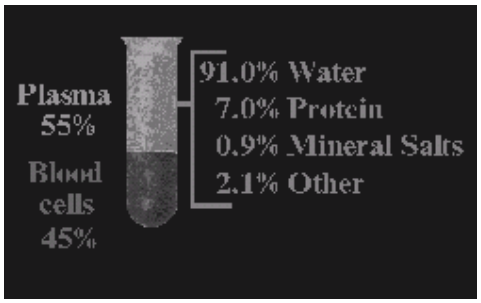
أبو يسرا

Biochemistry

Blood

- Blood is a tissue that circulates in a closed system of blood vessels
- It consist of :
  1. solid element [~ 45%] it includes :
     - red blood cells
     - white blood cells
     - platelets

  2. & is suspended in a liquid medium , the Plasma [~55%]



❖ **Physiological proprieties of blood :**

- PH : 7.4 ± 0.5 (7.35 – 7.45)
- specific gravity : 1.054-1.060 (whole blood) & 1.024-1.028 (plasma)
- blood viscosity : 5-6 ×s water ; it varies **according to** :
  - number of cells
  - temperature
  - degree of hydration
- red cell mass : 30 ml/kg in ♂ & 25 ml/kg in♀
- total blood volume 60-80ml/kg in both (5-6 L) (8% of the body weight)
- plasma volume 45± 5 ml/kg body weight (in children the red cell mass & plasma volume are the same relative to the body weight as in adult)

NOTE : Infants have large blood volume in proportion to body weight than adult .

- osmotic pressure : 7-8 atmospheres at body temperature (freezing point depression -0.537° C)
- Mass : 6-8 % of weight body

❖ **Function of Blood :**

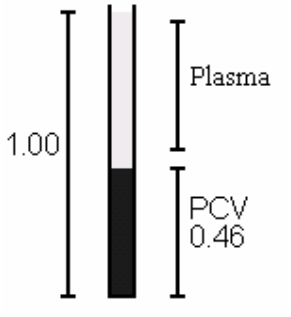
1. Respiration : transport gases (by Hb)
2. nutrition : transport of absorbed food substances
3. excretion : transport of metabolic waste to kidney , lungs & skin for removal
4. transport of hormones to their site of action
5. regulation of body temperature by distribution of heat
6. maintenance of normal Acid-Base balance in body
7. defense aginest infection (WBC & Antibodies)

" Great works are performed not by strength but by perseverance "

❖ **Haematocrit value or Packed cell Volume (PCV) :**

When blood prevented from clotting is centrifuged , cells will stile to the bottom of the tube while the plasma (a straw-colored liquid) will rise to the top

- PCV ~ 0.45 ♂ & ~ 0.41 ♀
↑in Polycythemia , ↓ in Anemia



- Influence of red cell volume & plasma volume on blood cout.

Red cell volume	Plasma volume	Cause	Effect
Normal	High	- Pregnancy	Pseudo anemia
Normal	Low	- Stress - Diuretics - Dehydration - Prolonged bedrest	Pseudo polycythemia
Normal or low	High	- Cirrhosis - Nephritis - Myelomatosis - Marked spleenomegaly	Pseudo anemia or Anemia less sever than idinticated by PCV & RBC
High	Normal or low	- Polycythemia	Acuurate reflexion of polycythemia or polycythemia less sever than apparent

Erythrocyte Sedimentation Rate (ESR)

- ESR is influenced to a gravity by extent to which the red cells form **Rouleaux ,** which sediment more rapidly than single cells
- This is mainly controlled by the concentration of **Fibrinogen** & Other proteins of the acute phase response
  - also **enhanced** by immunoglobulin
  - it is **retarded** by Albumin

- Anemia , by altering the ratio of red cells to plasma , encourage rouleaux formation & sedimentation

- ESR **not specific** screening test to detect the presence of inflammation (MCQ)

- In rheumatoid arthritis or T.B it provides an index of progress of the disease

- By westergren Method at 20± 3 C : 4±3 mm♂ & 6±3 mm♀

N.B ESR influenced by inflammation & **anemia** while Plasma viscosity **only** the protein component

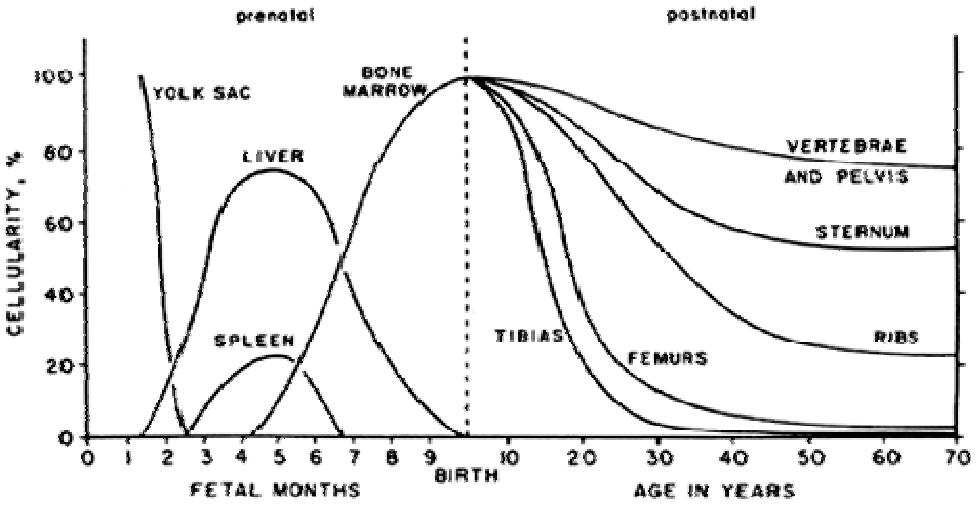
Plasma viscosity :

- ESR has recently been placed by plasma viscosity
- Plasma viscosity is **affected by** concentration of plasma proteins of large molecular weight especially : Fibrinogen & Some Ig
- At room temperature 1.50-1.70mPa/s
- Lower value in Neonates (lower levels of proteins) & **no difference** between male & female
- Independent from effects of **anemia**

Serum :

Serum is deficient from fibrinogen , fibrin , …. Etc , we will loss some protein if we collect the blood in tube without anticoagulant , but if we want to collect all proteins Of the serum we collect the blood in tube with anticoagulant .

❖ **Erythropoietin (Genesis of Blood Cell)**



- 1-2 month ------- yolk sac - 2-6month spleen
- 1-9 month ------- liver
- From 4 month ---------- bone marrow
- At birth --------- bone marrow
- Adult life --------- bone marrow (long bones)

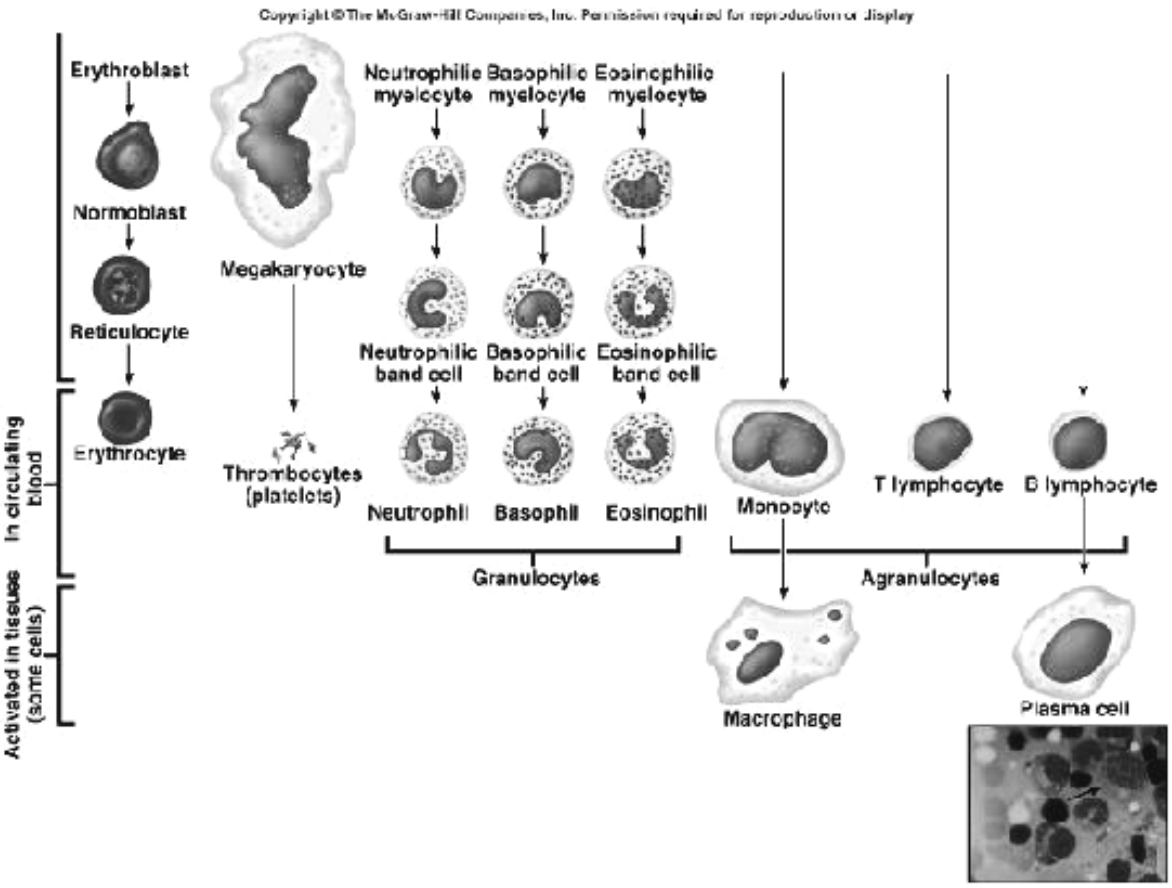
- **Pregnancy** is s ccc by increase erythropoeisis within maternal (& fetal blood)
- **Intrauterine** erythropoeisis occurs **as two waves** :
    1. primitive (extraembryonic sac of human embryo)
    2. definitive in liver & spleen during the 2end trimester , while last trimester in Bone Marrow
- **Infancy all bone** marrow is haemopoietic
- during **childhood** there is progressive fatty replacement of marrow throughout the **Long bones** so that in **adult life** haemopoietic marrow is confined to the **Central skeleton** & **Proximal ends** of the Femurs & Humeri (still in these areas ~50% is fat)

N.B The remaining fatty marrow is capable of reversion to haemopoeisis & in many diseases there is also expansion down the long bones

Liver & spleen can resume their fetal haemopoeitic role (extramedullary haemopoiesis)

Stem cell → pronoblast → basophilic normoblast → polychromatophilic normoblast → orthochromatic normoblast → reticulocyte -→ mature RBC .

Note : Single pronoblast give 16 mature RBC .



- **Erythropoietin**

  - Hormone that is 90% normally produced in **Peritubular interstital cells of the Kidney** & 10% in the **Liver & elsewhere** .

  - It is heavily glycosylated polypeptide of 165a.a there are no preformed stores
  - If Atmospheric O2 , Cardiac Pulmonary function & blood volume are decrease → decrease Hb conc . or increase O2 affinity → Hypoxia

  - The stimulation to erythropoietin production is the oxygen tension in the tissues of the kidneys
  - Epo prevents programmed cell death upon binding to cell sureface to EpoR which dimerizes & activate specific protein kinase Including (janns family tyrosine protein kinase 2 – phosphoinositol 3-kinase – mitogen activated protein kinase & RAS pathway)

  - It stimulate erythropoesis by incease number of **Pogenitor cells** committed to erythropoeisis where it stimulate late BFU$_E$ & CFU$_E$ which've receptors for erythropoietin (require cooperation of other factor (IL-3) & (insulin like growth factor)
  - There are increased to proliferate differentiate & produce Hb

Notes :

- Commitment of haemopiotic cell to the erythroid include :
TAL -1 , LMO -2 , GATA -2

- genes for alpha & beta chains of Hb are activated & controlled by cis-acting DNA sequence .
- other specific protein : glycoprotein A & EpoR .
- Transferrin & genes of haem are also activated by cis – acting mechanism .
- Other cis – acting promoter region are involved in regulation of genes Coding for enzyme of haem synthesis including :
(porphobilinogen – deaminase – ferrochetalase & samino laveilinic acid synthesis)

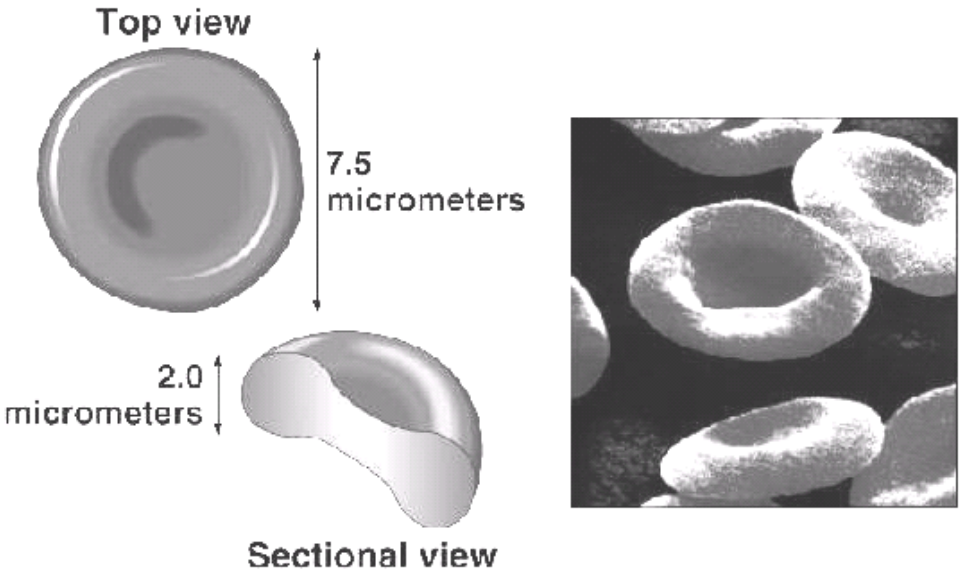
- The RNA for these factor disappear after proerythroblast stage .
- Other trans acting DNA binding protein are the :
(erythroid K factor & the human stem cell leukemia genes)

- The major site of Epo gene expression in the fetus is the Kidney .

"The greatest good is what we do for others"

The Red Blood Cells

- **RBC** are not true cells in the strict sense .
- Contain no Nucleic Acid & cannot reproduce .
- They contain no cell organelles & possess no synthetic activities .



Composition of the RBC :

-Diameter 6.9 μm
-Thickness 1-2 μm

-Range 5.5 +/- 1.0 × 10^{12}L ♂
4. 5 +/- 1.0 × 10^{12}/L ♀

-in Children 4.0 +/- 0.8 × 10^{21} /L
(10-12 yrs) 4.7+/- 0.7 × 10^{21}/L

-Red Cells contain 35% Solids (33% Heamoglobin)
- **Heamoglobin** is the chief protein of Red Cells
-Other protein are present in combination with Lipids & Oligosaccharides chain forming Stroma & Cell membrane

" Spend less time worrying about what you need and more time enjoying what you have"

Erythrocyte composition :

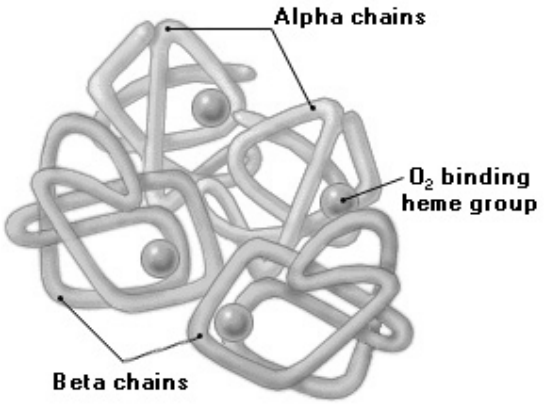
Cation : K^+ (**main**) - Na^+ - Ca^{++} – Mg^{++}
Anion : Cr – HCO^-_3 – Hb – InOrganic Phosphorous – 2,3 DPG

- Potassium – Magnesium – Zinc Conc. In Red Cell are much **Higher** than Plasma.

Gross Composition of Plasma & Red Cells :

Component	Plasma	RBC
Water	91-95%	65%
Solids	8-10%	35%
Protein	6-8%	31-33%
Specific Gravity	~ 1.026	-

NB.
-Heamoglobin is a tetramer : 2α & 2β
in a Diameres $(\alpha - \beta)_1$ & $(\alpha - \beta)_2$



-Bonds **within the same Diameres** are:
1.Hydrophopic Bonds (**mainly**)
2.Hydrogen Bonds
3.Ionic Bonds

-Bonds **between two Diameres** are **weaker** than Hydrophopic Bonds :
(Polar Bonds)
1.Hydrogen bonds
2.Ionic Bonds

-In Heam of the Hb , Iron should be in Ferrus state Fe^{++}
(**in Error**) **Metheamoglobin** :Acquired or Congenital (ferric cannot bind to O2)
Wilson Disease : Serioloplasmin (Copper contained)

- Iron in Heam have **6 bonds** : 4 Pyrol Ring
1 Oxygen
1 Histadine

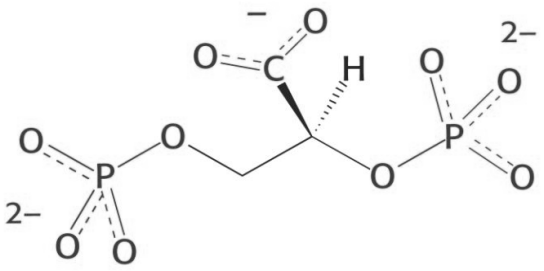
Heamoglobin :

- Hb the main component of RBC (the chief protein)
- Forms about 33% of RBC
- not undergo degradation & resynth. during life of the cell

Responsible for :

1. carrying Oxygen from Lungs → Tissue
2. carrying Carbon Dioxide in the opposite direction
3. Buffering of Carbon Dioxide

2,3 Diphosphoglycerate (Anion) :



2,3-Bisphosphoglycerate
(2,3-BPG)

- one molecule of 2,3BPG binds to each Hb in tetramer
- it's conc. In the erythrocyte is nearly **identical** to that of Hb .

- An **increase** in 2,3BPG : (bind Hb & decrease its affinity to O2)
  1. promotes the release of Oxygen
  2. stabilize the T-state (deoxygenated Form) of Hb by **" cross linking "** the two β-globulin subunit through multiple salt bridge .
  3. occur in response to tissue (**Hypoxia**)
  e.g Anemia – Pulmonary dysfunction – Cigarette smoking – High altitude

NB.
- Hb F keep Oxygen not in favor for tissue **more than Hb A .**
- 2,3BPG cause **shift to the right**

RBC Membrane Structure :

- **RBC** ,must be able to squeeze through some tight spot in Microcirculation, for that RBC must Easily & Reversibly Deformable , it's membrane must be both Flexible & Fluid (**Unlike the Sickle cell Anemia**)

- **About** :
  50% of membrane is Protein
  40% Fat – up to 10% Carbohydrate

- RBC membrane comprise :
  >lipid bilayer (which determine the membrane fluid **)**
  >proteins (responsible for flexibility) , either Peripheral or Integral penetrating the lipid bilayer
  >carbohydrate occur only on the external surface

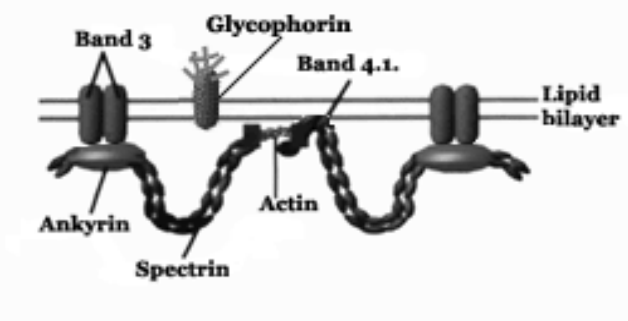
- Major lipid classes are : Phospholipids & Cholesterol
  Glycoshingolipids as Gasngliosides & Complex series including ABO blood group substances constitute 5-10% of total lipids

- Glycophorines A,B,C are **Transmembrane Glycoproteins**

- **Glycophorin A** (**more –ve**) contain binding site for Influenza Virus & Plasmodium Falciparum

- Defect of protein may explain some of the abnormalities of shape of RBC membrane as **Hereditary Spherocytosis** & **Elleptocytosis**

- Alteration in lipid composition because of Congenital or Acquired abnormalities in plasma cholesterol or phospholipids (PL) , may be associated with other membrane abnormalities as **Target Cell** .



- The membrane Skeleton is **4 structural** proteins that include :
  1. A& B Spectrin
  2. Ankyrin
  3. Protein 4.1
  4. Actin

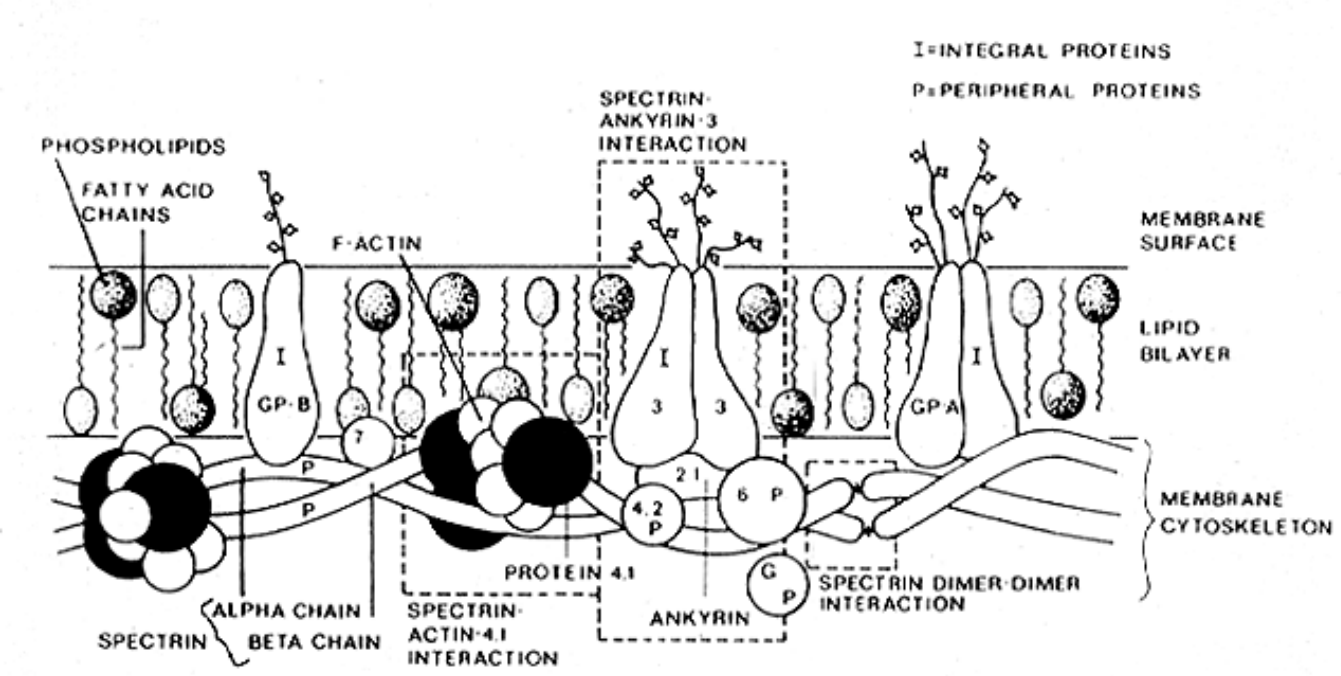
- 4 links :
  1. Head-Head tetramer called specrtin head association
  2. Ankyrin : pear-shape that attached to Band 3
  3. Protein 4.1 & 4.Actin both at the same time attached to bilayer through Glycophorin

- **Spectrin** is major protein of the cytoskeleton & it's two chains (α & β) are ligned in antiparallel manner .

- (α & β)chains are loosly interconnected forming a Dimer , One Dimer interact with another forming a Head-to-Head tetramer
- Ankyrin binds spectrin & in turn binds highly to Band 3 secure attatch of spectrin to membrane **(Spectrin Ankyrin-3 Interaction)**
- Band 3 : is Anoin exchange protein, permits exchange of Cl^- for HCO^-_3

"You have to have confidence in your ability, and then be tough enough to follow through"

- Actin binds to the tail of spectrin & to protein 4.1 while in turn binds to integral protein Glycophorins A&C **(Spectrin Actin4.1 Interaction)**



NB.

Hereditary Spherocytosis :

-Autosomal Dominant , present as Hemolytic Anemia

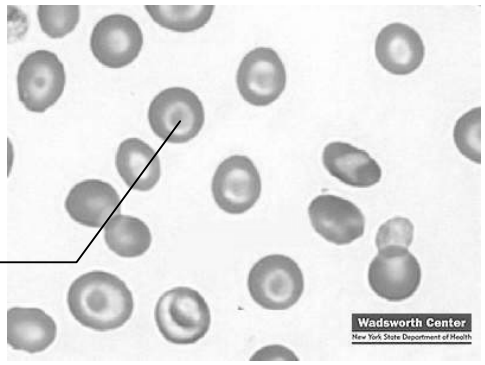
-Decrease surface area (osmotic fragility test)

-Pigments stone , Spleenomegaly

Target cell :

- Congenital or Acquired abnormalities in cholesterol & phospholipids
- An abnormal red blood cell with a ringed appearance; associated with anemia

- seen in Liver Disease
- Target cells (codocytes) are erythrocytes with a central color spot in the area of pallor, resembling a target. They are seen in many hemolytic anemias, especially sickle cell, HbC disease, and thalassemia.



"The secret of success is constancy of purpose"

In Laboratory :

- when you take your blood sample keep it in an **Isotonic Solution** .
- Chloroform – Salt – Hypotonic solution
  → Hemolysis (Ghost Appearance for RBC)

Red Cell Aging :

Example of changes occur in Aging Red Cell :

☺	↑ **in Old Cells**	↓ **in Old Cells**
Hb	Glycosylated Hb * use to follow up DM treatment	Bisphoglycerate
Membrane	Osmotic fragility Na^+ Binding to IgG	Siatic Acid K^+ Lipids Proteins
Enzymes	-	G6PD Pyruvate Kinase Hexokinase others
General	Cell density Spheriaty	Deformability Disc-like shape

General points about Hb :

1) allosteric protein (bind to 4 O2)&(show sigmoid curve)
2) cooperative effect
3) Affinity for O2 depend on :
 - PO2 , PCO2 & H2 & 2,3 DPG level .
4) Carboxy Hb (has high affinity to CO)

5) HB → oxyhemoglobin
 Need : neutral PH , cool (lung) , high O2 , low CO2

6) oxyhemoglobin → Hb
 Need : Acid PH , warm (tissue) , high CO2 , low O2

7) The Bohr effect :
In lung : High pO2 , low H&CO2 → high affinity for O2
(O2 dissociation curve shift to left)
In tissue : low pO2 , high H&CO2&2,3BPG → low affinity of Hb for O2 .
(O2 dissociation curve shifts to right)

8) Hb F cannot bind 2,3 DPG & has higher affinity for O2 .

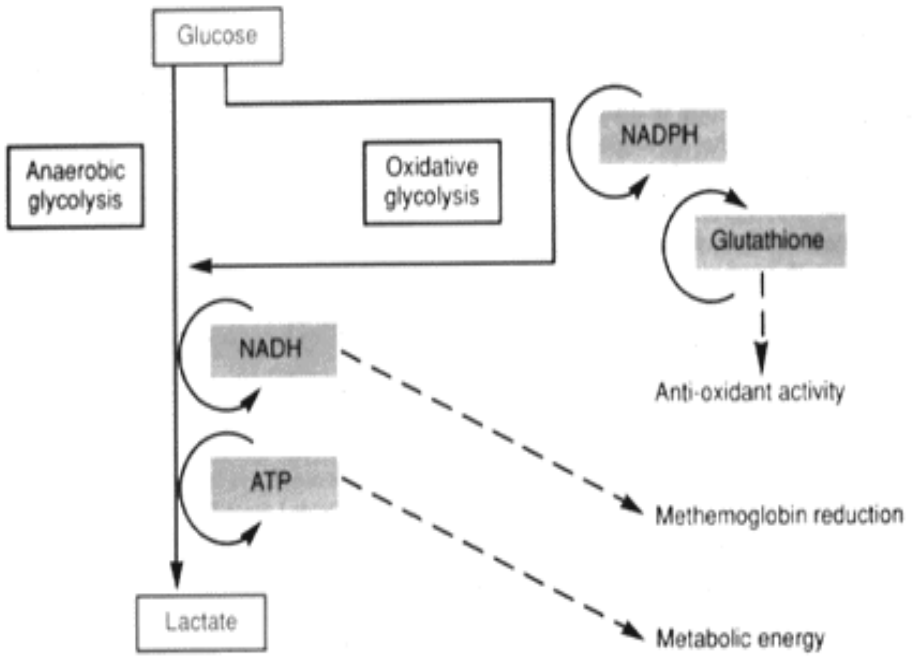
RBC Metabolism

- **Erythrocyte contain :**
  1. no mitochondria , so there is no respiratory chain .
  2. no citric acid cycle .
  3. no oxidation of fatty acid or ketone body .

- **Energy** is optained from the Glycolytic Breakdown of glucose with production of lactate (anerobic) .

N.B: RBC needs ATP via Glycolysis and there are 3 ways :

1. Embden-Meyerhof Pathway
   end with: Lactate + 2ATP
2. Rapapord- Luebering Pathway
3. HMP Pathway
   mainly In producing : reducing equivalents



❖ **ATP** producing being used for :
- Na^+ pump
- RBC membrane structure & flexibility

❖ **Rapoport & Luebering** described a special enzyme in glycolysis bisphosphoglycerate mutase

- Bisphosphoglycerate Mutase **converts** :

1,3 bisphosphoglycerate (1,3BPG) → 2,3 bisphosphoglycerate (2,3BPG)

- this reaction waste the high energy bond in 1,3BPG without generate of ATP
- this explain the fact that the RBC utilize more glucose than is required to maintain their vitality

- **RBC** contain an active Pentose Phosphate Pathway that supplies NADPH

- NADPH is important in keeping glutathione in Reduced Glutathione
- Reduced Glutathione plays a very important role in survival of RBC (membrane structure – Hb sulfhydral oxidation ; Hinz body)

❖ **Defecting in G-6-P dehydrogenase** leads to reduced RBC survival.

- The **erythrocyte** contain Carbonic Anhydrase enzyme

- CO_2 combine with H_2O only after it enters the RBC
- Hb is the most important **buffer** for the resulting Carbonic Acid is present

N.B : Carbon dioxide reacts with water to give bicarbonate, carbonic acid freed protons via the reaction, which is catalyzed by carbonic anhydrase:

$$CO_2 + H_2O <-> HCO_3^- + H^+$$

❖ **RBC** also contain " Rhodanese Enzyme " responsible for the detoxification of Cyanide

* An average 70Kg adult male produce 2.3 × 10 6 red cell / sec .

أول ما في الحياة ... شروق
وأجمل ما فيها إبتسامة ... طفل
وأوسع ما فيها ... الخيال
وأضيق ما فيها ... فكرٌ ضيق
وآخر ما فيها .....
غروب