RBCs Metabolism

Biochemical composition of the RBCs

- They contain 35 % solids, hemoglobin, the chief protein.

- Other proteins are present in combination with lipids and oligosaccharide chains, forming the stroma and cell membrane.

What type of energy production happens in Erythrocytes?

Anaerobic (WHY?) Because there is no mitochondria in Erythrocytes so there is no ETC, no TCA, no Ketolysis etc. (since those pathways need oxygen), **BESIDES glycolysis** which can occur aerobic and anaerobic conditions so the enzymes should be very active.

Therefore the energy produced in Erythrocytes in the glycolytic pathway is Substrate Level Phosphorylation.

- K^{+}, Mg^{++} and Zn^{++} concentrations in RBCs (Erythrocytes) are higher than in plasma. **(WHY?)**

- **Zn^{++}** is a co-factor for at least 10 enzymes very active in erythrocytes including the most important 3 enzymes which are:

  Carbonic Anhydrase (CA)

  Copper/Zinc (Cu/Zn) Superoxide Dismutase (SOD)

  Lactate Dehydrogenase (LDH)

- **Mg^{++}** is a co-factor for Kinases in the Glycolytic pathway (Phosphoglycerate Kinase, Pyruvate Kinase). Those 2 enzymes are what produce energy in erythrocytes under Substrate Level Phosphorylation.

  Substrate Level Phosphorylation means a substance that have high energy bond, during the metabolic pathway it will be broken down, this energy instead of being lost, it will be captured enzymatically to join phosphate with ADP for production 1 ATP.

****So we need high amount of Mg^{++} cause the energy produced by glycolysis under anaerobic conditions is very little → 2 ATP**

- K^+ maintains the volume of Erythrocytes so it keeps them in shape. Therefore, high concentration of K^+ intracellularly and Na^+ outside will change the shape of erythrocytes from Biconcave to Spherical form (Spherocytes) which cannot pass through the blood capillaries.

** فـ Na^+/K^+ ATPase pump بتكون Very active في RBC عشان تنظم هاي المعادلة ويكون K^+ عالي في داخل الخلية، مقارنة مع الصوديوم والكالسيوم.

** باختصار يعني لو صار خلل في هاي المعادلة هيتغير شكل RBC.

What is the shape of Erythrocytes?

The biconcave shape allows RBCs to bend and flow smoothly through the body's capillaries and it also facilitates oxygen transport.

- The primary physiological objective of the red blood cell is **Gas exchange and transport** beside several **metabolic function**.

The major metabolic function of RBC is to produce the necessary ATP, NADPH (from Pentose Phosphate pathway), and NADH for **maintaining its osmotic balance**, **electroneutrality** and **fighting oxidative stresses**.

Also, they are necessary for the **biconcave shape of the cell** as well as for the **specific intracellular cation concentrations**.

Erythrocyte exceptions

The RBC is, both structurally and metabolically, the simplest cell in the body, during its maturation, the RBC loses all its subcellular organelles**,** so, No ATP production in oxidative phosphorylation

No ability to replace damaged lipids and proteins (low metabolic activities, with no ability to synthesize new proteins or lipids)

After maturation, the remaining enzymes are used inside erythrocytes and there is no rebuilding of molecules, those enzymes will be used for the rest of erythrocyte lifespan (90-120 days).

No synthesis of DNA and RNA because of lacking nuclei

Free radicals exposure

Erythrocytes are the main cells which are exposed to free radicals. (WHY?)

(Because they carry oxygen, and if this O_2 carried 1 electron it will change to free radical)

1) Haemoglobin autoxidation ($O_2^{\bullet -}$ release) **(explanation)**

2) A cell membrane rich in polyunsaturated fatty acids (susceptible to lipid peroxidation)

3) Deformation in tiny capillaries; catalytic ions leakage (cause of lipid peroxidation)

Oxygen which is carried on hemoglobin inside erythrocytes are susceptible to lipid peroxidation of polyunsaturated fatty acid of cell membrane by saturating the double bond in polyunsaturated fatty acid and convert them into peroxides. Those peroxides will change Erythrocyte cell membrane from flexible to rigid, this can lead to damage to capillaries (no adaptation of capillaries).

Metabolism of the human RBCs

How many types of Haemoglobin in adults?

1) Haemoglobin a (Adult Haemoglobin).. 4 polypeptide chains 2 alpha 2beta, at its center Heme molecule and 1 Ferrous at the center of heme molecule (to be enable to carry oxygen). (ferric cant carry oxygen)

2) Haemoglobin a2 (1-1.5% in our body) and it has alpha2 delta2

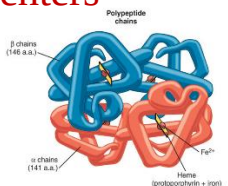
3) Haemoglobin a1c (aka Glycosylated Haemoglobin or Glycated Haemoglobin) it doesn't respond fast to the changes of blood glucose level, so they use it for diabetes tests. HbA1C is non-enzymatic reaction between glucose and haemoglobin so its found normally (5% in our body), its level changes according to glucose concentration in plasma.

- The main red cell energy source is glucose because of lacking mitochondria, it is metabolized through the glycolytic pathway (anaerobically) for producing **2 moles of ATP** and **2 lactate molecules** as end products/glucose mol.
No ability to oxidize fats.

The glycolytic pathway is modified by the **Rapoport-Luebering shunt (R-L-shunt)** by **diphosphoglycerate mutase** generating **2, 3-bisphosphglycerate (2,3 BPG).**

2,3 BPG decrease affinity of haemglobin to oxygen allowing the release of oxygen to the cells (cellular oxygenation), so if **diphosphoglycerate mutase** didn't exist there will be no oxygen release to cells.

The pentose phosphate shunt contributes to the redox status of the cell by producing 2 moles of NADPH/ 1 mole of glucose. It makes ribose-5-phosphate which enters the structure of RNA and deoxy ribose-5-phosphate which enters the structure of DNA and NADPH+H



The red cell energy requirement is necessary for:

1) Replenishing its adenine nucleotide pool using **salvage pathways**.
Nucleotide is made by 2 ways:
1) de novo synthesis
2) **salvage pathway**: reutilization of catabolites (recycling of wastes)

2- Protecting the cell against oxidative stress

3- Controlling its volume by membrane Na^+/K^+ ATPase (cation pump), which maintains high K^+, low Na^+ and Ca^{++} in the cell (high K^+ inside, high Na^+ and Ca^{++} outside)

4- Maintaining the plasticity of its membrane and the biconcave shape (If energy is decreased, RBCs will be logged with Ca^{++} and Na^+, while K^+ is depleted causing a change of biconcave into spherical form).

5- Preventing the accumulation of methemoglobin

6- Modulating oxyhemoglobin

7- Keeping the sulfhydryl groups of RBCs enzymes, Hb and membranes in the active reduced form. (this S-H group comes from cysteine amino acid, the more its found in reduced form → more active and functioning)

The human RBCs metabolism

Glucose enter erythrocyte by GLUT1 (insulin independent) into the glycolytic pathway by 2 reactions (Phosphoglycerate Kinase, Pyruvate Kinase) so each one makes 2 ATP (total 4 ATP), 2 moles will be removed by activation reactions catalyzed by Hexokinase and PhosphoFructoKinase 1 (at the end, total 2 ATP) and it result at the end to make Lactic Acid and Pyruvate. (Zn^{++} plays a role in this pathway since it's a cofactor for Lactate Dehydrogenase) then lactate passing outside to the liver by Cori cycle (liver convert lactate coming from organs like erythrocytes and skeletal muscles which have anaerobic glycolysis to glucose) then the cycle continues.
Pyruvate is converted to lactate which needs NADH (NADH comes from glyceraldehyde-3-phosphate dehydrogenase 6 تفاعل رقم), but pyruvate have much more molecules than NADH **(WHY?)** cause some NADH will react with methemoglobin reductase (when hemoglobin is converted to methemoglobin, it will be reduced to hemoglobin using NADH)

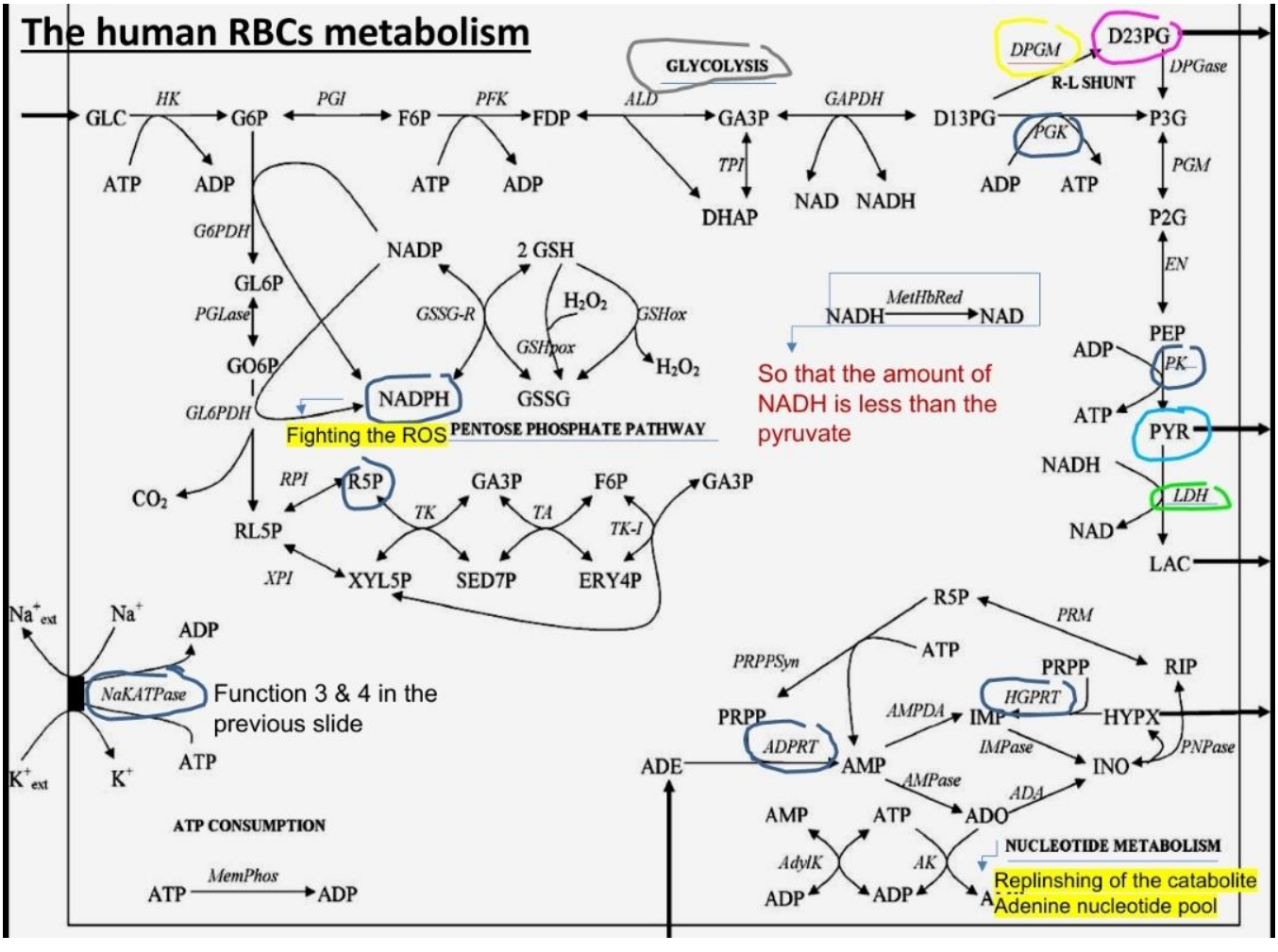
** باختصار هاي العملية ما بتحوّل كل الـ pyruvate الى lactate والباقي بروح على liver عشان يتحول الى جلوكوز.

- In RBCs, which lack mitochondria and pyruvate dehydrogenase multienzyme complex, pyruvate is reduced to lactic acid (anaerobic glycolysis), consistent with the primary role of the RBC in oxygen transport and delivery, rather than its utilization.

- Each mole of glucose yields 2 moles of lactate to maintain electrochemical and ion gradients across its plasma membrane, then lactate is excreted into blood.

- By R-L-Shunt in RBCs, 10-20% of the glycolytic intermediate, 1,3- DPG, is converted into 2,3- BPG, an allosteric regulator of the O_2 affinity of Hb. (decreasing the affinity of Hb to O_2) then 2,3-BPG is catalyzed by phospotase enzyme and remove the phosphate group in position 2 to become 3-BPG and continues the glycolytic pathway.

- The pentose phosphate pathway (Hexose Monophosphate Shunt), accounts for about 10% of glucose metabolism in the red cell, this pathway in RBCs has a special role in **protection against oxidative stress**, while, in nucleated cells, it also serves as a **source of NADPH for biosynthetic reactions and pentoses for nucleic acid synthesis.** (pentose phosphate pathway can produce energy by utilizing intermediates such as Glyceraldehide-3-phosphate & Fructose-6-Phosphate which are components of Glycolysis and can produce little amount of energy)



Glucose utilization in the red cell

- Glucose is transported through RBC membrane by a facilitated diffusion by glucose transporters [(GLUT-1) insulin-independent i.e. insulin does not promote glucose transport to RBCs)].

- In a 70-kg person, there are about 5 L of blood and a little over 2 kg (2 L) of RBCs.

- These cells constitute consume about 20 g of glucose/day, representing about 10% of total body glucose metabolism.

- The RBC has the highest specific rate of glucose utilization of any cell in the body, approximately 10 g of glucose/kg of tissue/day, compared with ~2.5 g of glucose/kg of tissue/day for the whole body.

- In the RBC, about 90% of glucose (~18 g/day) is metabolized via glycolysis, yielding ~0.2 mole of lactate.

- Despite its high rate of glucose consumption, the RBC has one of the lowest rates of ATP synthesis of any cell in the body, ~0.2 mole of ATP/day.

Synthesis of 2, 3-bisphosphoglycerate

- 2,3-BPG is an important product of glycolysis in the RBC, sometimes reaching 5 mmol /L concentration, comparable with the molar concentration of Hb in the RBC.

- It is the major phosphorylated intermediate in the RBCs, present at even higher concentrations than ATP (1-2 mmol/L) or inorganic phosphate (1 mmol/L).

- 2,3-BPG is a negative allosteric effector of O_2 affinity to Hb.

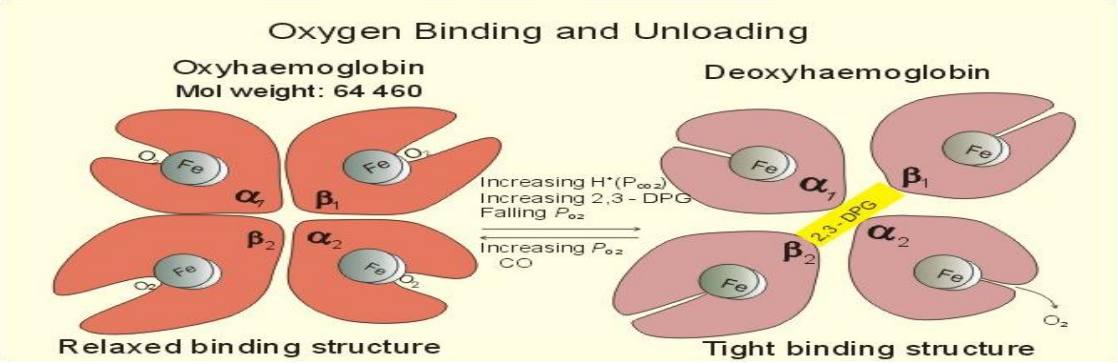
- It decreases the O_2 affinity of deoxy Hb, promoting the release of O_2 in peripheral tissue.

- The presence of 2,3-BPG in the RBC explains the observation that the O_2 affinity of purified HbA is greater than that of whole RBCs.

- 2,3-BPG concentration ↑in the RBC during adaptation to high altitude and in anemia, promoting the release of $O_2$to tissues when the PO_2 and saturation of Hb is ↓ in the lung.

- HbF is less sensitive than HbA to the effects of 2,3-BPG, promoting efficient transfer of O_2 across the placenta from HbA to HbF.

↑ 2,3 DPG formation	↓ 2,3 DPG formation
Anemia ↓PaO2 (high altitude) ↑H+ ↑PCO2	Polycythemia ↑PaO2 ↓H+ ↓PCO2



Glutathione (tri-peptide) is synthesized from Glycine, Cysteine, and Glutamic acid in a two-step process that requires ATP as a source of energy. (each molecule of Glutathione utilizes 2ATP) Glutathione has Sulfhydryl group so its found in both oxidized and reduced form, so it can act as hydrogen carrier

Catalase and glutathione peroxidase serve to protect the red cell from oxidative damage.

The maturation of reticulocytes into erythrocytes is associated with a rapid decrease in the activity of several enzymes and it occurs much more slowly or not at all with ageing.

- لما يطلع NADPH ويستهلك داخل erythrocytes عن طريق Glutathione reductase enzyme هيوخد الـ 2 hydrogens الموجودات في NADPH

- ويعطيهم الى GSSG عشان يتحول الى GSH

- بعدها تستهلك GSH عن طريق Glutathione peroxidase ويوخد الـ 2hydrogens مع **Hydrogen peroxide** ويعمل منهم 2 H2O**(not free radical)**

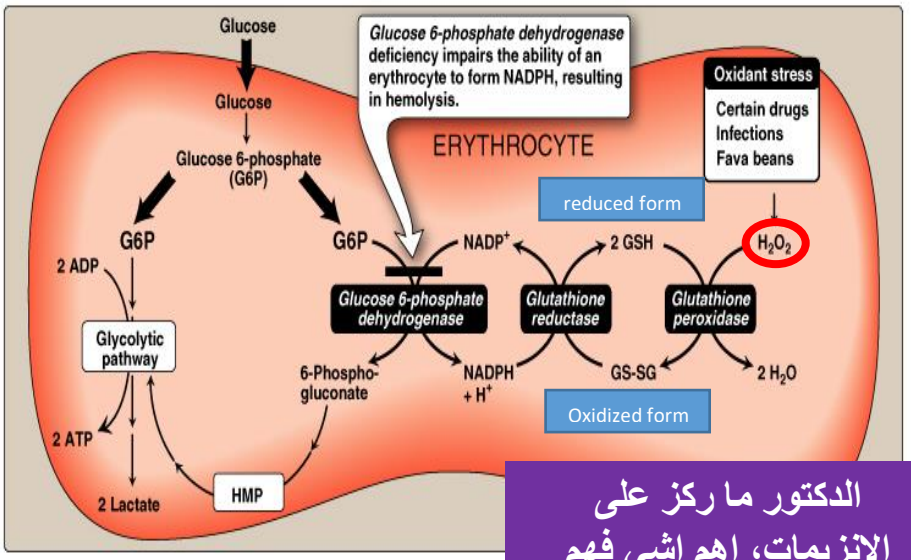


Figure 13.10
Pathways of glucose 6-phosphate metabolism in the erythrocyte.

الدكتور ما ركز على الانزيمات، اهم اشي فهم الـPathway بشكل عام

What is the co-factor of Glutathione Peroxidase? Selenium (anti-oxidant enzyme)

RBCs membrane structure

RBCs must be able to squeeze through capillaries, so, RBCs must be easily & reversibly deformable.

Its membrane must be both **fluid & flexible**.

About **50% of membrane is protein**, **40% is fat** & up to **10% is carbohydrate**.

RBCs membrane comprises a **lipid bilayer** (which determine the **membrane fluidity**), **proteins** (which is responsible for **flexibility**) that are either peripheral or integral penetrating the lipid bilayer & carbohydrates that occur only on the external surface.

The membrane skeleton is four structural proteins that include α & β spectrin, ankyrin, protein 4.1 and actin.

Spectrin is major protein of the cytoskeleton & its two chains (α & β) are aligned in an antiparallel manner *(dimeric form from **head to tail**)*

** N terminal end of α chain meet C terminal end of β chain and so on in dimeric form but the association is weak (no covalent bond between the 2 molecules)

α & β chains are loosely interconnected forming a dimer, one dimer interact with another, forming a head to head tetramer.

Ankyrin binds **spectrin** & in turn **binds tightly to band 3** securing attachment of spectrin to membrane.

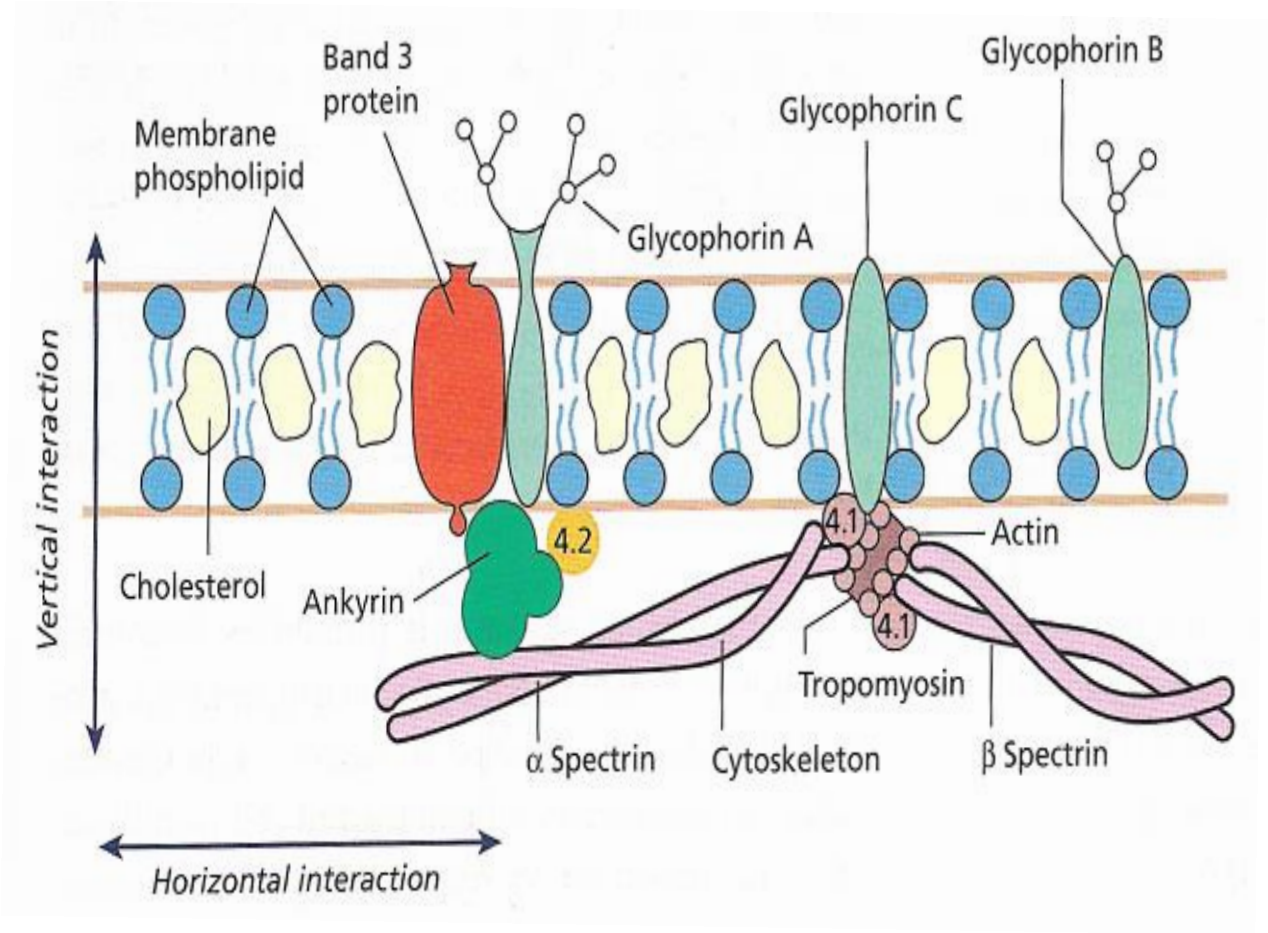
Band 3 is **anion** exchange protein permits exchanges of Cl^- for HCO_3^-. (responsible for Chloride Shift)

Actin binds to **spectrin** & to **protein 4.1** which in turn **binds to integral proteins, glycophorins A, B & C**.

(Glycophorins A,B,C are transmembrane glycoproteins).

****The association between those 8 proteins (Spectrin, Ankyrin, Band 3, Actin, Protein 4.1, Glycophorins A,B,C) are important for maintenance of elasticity and biconcave shape. This association is maintained by energy so any defect in energy inside erythrocyte will defect in the association between the protiens and damage the structure of erythrocytes and hemolysis.**

Defects of proteins may explain some of the abnormalities of shape of RBCs membrane as (hereditary spherocytosis & elliptocytosis).



Glycosylated haemoglobin (HbA_{1c})

-Formed by hemoglobin's exposure to high plasma levels of glucose
- Non-enzymatic glycolysation (glycation)- sugar bonding to a protein
- Normal level HbA_1- 5%; a buildup of HbA_1- increased glucose concentration
- The HbA_1 level is proportional to average blood glucose concentration over previous weeks; in individuals with poorly controlled diabetes, increases in the quantities of these glycated hemoglobins are noted (patients monitoring)

Sugar CHO + NH_2 CH_2 Protein

↓

Sugar CH N CH_2 Protein

Schiff base

↓ **Amadori reaction**

Sugar CH_2 NH CH_2 Protein

Glycosylated protein

Glucose-6-phosphate dehydrogenase deficiency (Folism)

- It is an **X-linked recessive hereditary disease**, considered the most common human enzyme defect.
- Individuals with the disease may exhibit non- immune hemolytic anemia in response to a number of causes, most commonly infection or exposure to certain medications or chemicals.
- G6PD deficiency is closely linked to favism, a disorder characterized by a hemolytic reaction to consumption of bean (*fava*).
- The name favism is sometimes used to refer to the enzyme deficiency as a whole, although this is misleading as not all people with G6PD deficiency will manifest a physically observable reaction to consumption of broad beans.

Classification

The WHO classifies G6PD genetic variants into five classes, the first three of which are deficiency states. (based on activity) – (this activity is based on site of mutation in the gene encoding for this enzyme)

** يعني ممكن يكون تركيزه 100% بس الـ activity تكون 10%

1- Severe deficiency (<10% activity) with chronic (non- spherocytic) hemolytic anemia (very dangerous) (which is non immune mediated)

2- Severe deficiency (<10% activity), with intermittent hemolysis (very dangerous)

3- Mild deficiency (10-60% activity), hemolysis with stressors (predisposal factors) only

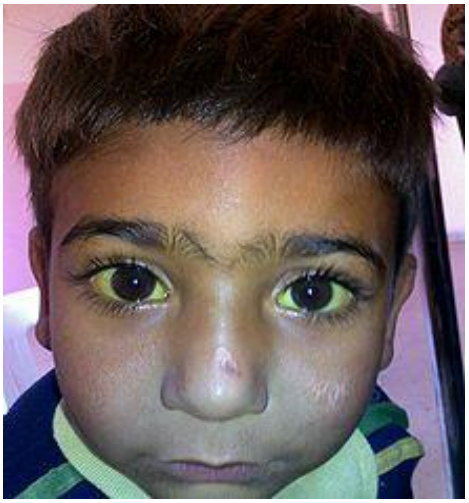
4- Non-deficient variant, no clinical sequelae

5- Increased enzyme activity, no clinical sequelae

ما بيعملوا مشاكل

This is a 4-year old boy diagnosed with G6PD deficiency showing jaundice in the sclera

Hemolysis of the erythrocytes + yellow discoloration of sclera as a result of bilirubin accumulation



- Most individuals with G6PD deficiency are **asymptomatic**.

Symptomatic patients are almost exclusively **male**, due to the X-linked pattern of inheritance, but female carriers can be clinically affected due to **unfavorable lyonization**, where random inactivation of an X-chromosome in certain cells creates a population of G6PD-deficient red blood cells coexisting with normal red cells.

**__Unfavorable lyonization__: random inactivation of some X-chromosomes in some immature erythrocytes. It can release some erythrocytes which have G6PD deficiency in females which results same manifestation in males.

A typical female with one affected X chromosome will show the deficiency in approximately half of her red blood cells.

However, in rare cases, including double X deficiency, the ratio can be much more than half, making the female almost as sensitive as a male.

Abnormal red blood cell show hemolysis in G6PD deficiency can manifest in a number of ways, including the following:

1- Prolonged neonatal jaundice, possibly leading to kernicterus (mental retardation due to increased amount of unconjugated (indirect) bilirubin, hence it can pass BBB)

2- Hemolytic crises in response to:

a- Illness (especially infections) b- Diabetic ketoacidosis

c- Sulpha drugs, **antimalarial drugs** and **aspirin (acetylsalicylic acid).**

d- Certain foods, most notably broad beans e- Certain chemicals

3- Very severe crises can cause **acute renal failure**. **(most dangerous)**

H_2O_2 accumulated will cause:

1- Convert F.A.s on cell membrane to peroxide which will cause hemolysis of RBCs. **(cell membrane become Rigid)**

2- Convert Hb to methemoglobin that leads to increase cell membrane fragility. **(cell membrane become Fragile)**

Diagnosis **(definite diagnosis=DNA sequencing to the gene responsible of producing G6PD)**

It is suspected when patients from certain ethnic groups develop anemia, jaundice and symptoms of hemolysis after challenges from any of the causes, especially when **there is a (+) family history**.

- **Generally, tests will include**:

1- Complete blood count (CBC) and reticulocytes count; in active G6PD deficiency, Heinz bodies can be seen in RBCs on a blood film.

2- Liver enzymes (to exclude other causes of jaundice).

3- Lactate dehydrogenase (**elevated in hemolysis and a marker of hemolytic severity)**

4- Haptoglobin (**decreased in hemolysis**); when erythrocytes breakdown it will release haemoglobin, since it has low molecular weight it can pass and precipitate inside the renal tubules and cause renal failure (this haptoglobin bind with haemoglobin to increase its molecular weight so it cant pass into the pores of glomural filtration in kidney. So if there is G6PD deficiency haptoglobin will be reduced)

5- A "direct antiglobulin test" (coombs' test) - this should be **negative**, as hemolysis in G6PD is **not immune-mediated**.

Treatment

- Avoid the drugs and foods that cause hemolysis (inevitable).

- Vaccination against some common pathogens (e.g. Hepatitis A and Hepatitis B) may prevent infection-induced attacks.

- In the acute phase of hemolysis, **blood transfusions** might be necessary, or even **dialysis** in acute renal failure.

- Blood transfusion is an important symptomatic measure, as the transfused red cells are generally not G6PD deficient and will live a normal lifespan in the recipient's circulation.

- Some patients may benefit from **splenectomy** as this is an important site of red cell destruction.

- **Folic acid** should be used in any disorder featuring a high red cell turnover.

- Antioxidants as **vitamin E** and **selenium**.

Pyruvate kinase deficiency:

- It is one of the most common enzymopathy associated with chronic hemolytic anemia, which usually occurs in compound heterozygotes for two different mutant alleles and in homozygotes.

- The increased 2, 3-BPG levels eases the anemia by lowering the oxygen-affinity of hemoglobin.

- Phenotypically, the clinical picture varies from severe hemolysis causing neonatal death, to a well-compensated hemolytic anemia and only very rare cases can present with hydrops fetalis.

- More than 180 gene mutations in had been reported to be associated with PK deficiency.

- Most of these mutations (70%) are the missense mutants c.1456C→T(Arg486Trp), c.1529G→A (Arg510Gln), c.994G→A (Gly332Ser), and the nonsense mutant c.721G→T (Glu241stop).

Nonsense mutation: substitution of single base pair that leads to appearance of stop codon. (its more dangerous than the missense mutation)

- They affect conserved residues in structurally and functionally important domains of PK.

Triose phosphate isomerase deficiency

- TPI catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate and plays an important role in several crucial metabolic pathways.

- The metabolic pattern of TPI deficient erythrocytes is characterized by high levels of dihydroxyacetone phosphate and a relatively minute decrease of ATP.

- Dihydroxyacetone phosphate accumulation has been reported to be toxic for cellular functions and responsible for the severity of TPI enzymopathies but its mechanism of toxicity is not well understood.

- The defect leads to hemolytic anemia coupled with neurological dysfunction.

Phosphoglucose isomerase deficiency

- Phosphoglucose isomerase catalyzes the reversible isomerization from G6P to F6P, an equilibrium reaction of glycolysis.

- Glucose turnover reacts, therefore, only on deficiency below a very low critical residual activity of PGI but then with a decline of lactate formation, i.e., decrease in glycolytic flux.

- The consequence of a limitation by the PGI reaction is an increase of the G6P level which causes a feedback inhibition of hexokinase resulting both in a lower rate of glycolysis and increased PPP activity associated, in turn, with the recombination of F6P formed in PPP with glycolytic pathway.

- With the effect of hexokinase inhibition, ATP, D23PG and GSH regeneration decreases.

- This is the third most common enzymopathy in the world.

Diphosphoglycerate mutase deficiency

- Disphosphoglyceromutase is a multifunctional enzyme which catalyzes both the synthesis and dephosphorylation of D23PG in human red blood cells.

- With lowering of disphosphoglyceromutase, the turnover via D23PG declines in favor of substrate phosphorylation catalyzed by phosphoglycerate kinase and pyruvate kinase leading to changes of the metabolic pattern. ATP, FDP, triose phosphates, P3G, P2G, PEP are enhanced, ADP, D23PG, F6P, G6P are diminished.

Phosphoglycerate kinase deficiency

- PGK is a key enzyme for ATP generation in the glycolytic pathway, catalyzing the conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate bypassing the Rapoport-Luebering shunt.

- A significant accumulation of D23PG, and a decreased concentration of ATP were observed in patients with PGK deficiency.

- Also, diminished glucose consumption was reported.