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.

- K^{+}, Mg^{++} and Zn^{++} concentrations in RBCs are more than in plasma.

- 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, 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)
- No synthesis of DNA and RNA because of lacking nuclei

Free radicals exposure

- Haemoglobin autoxidation ($O_2^{\bullet -}$ release)
- A cell membrane rich in polyunsaturated fatty acids (susceptible to lipid peroxidation)
- Deformation in tiny capillaries; catalytic ions leakage (cause of lipid peroxidation)

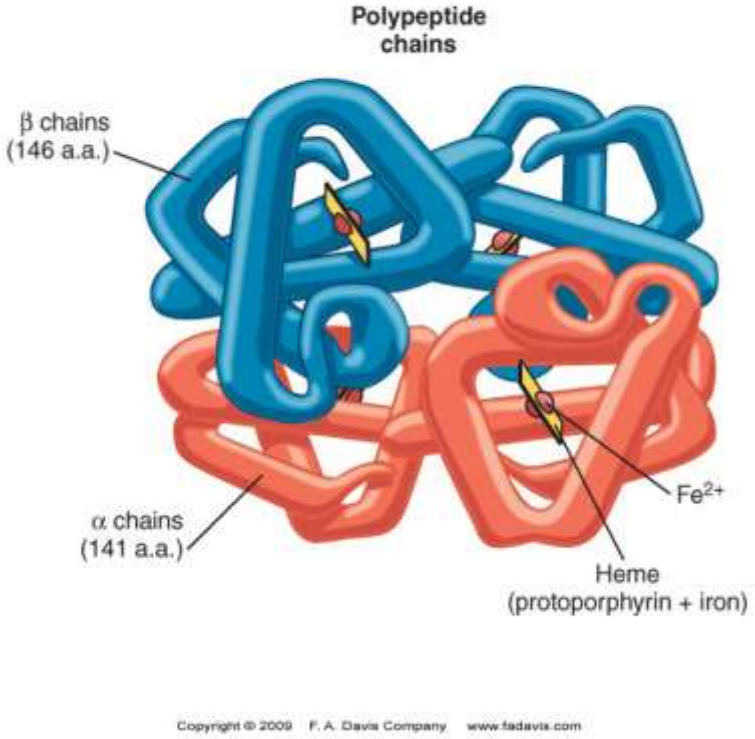
Metabolism of the human RBCs

-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 by diphosphoglycerate mutase generating 2, 3-bisphosphglycerate.

-The pentose phosphate shunt contributes to the redox status of the cell by producing 2 moles of NADPH/ 1 mole of glucose.

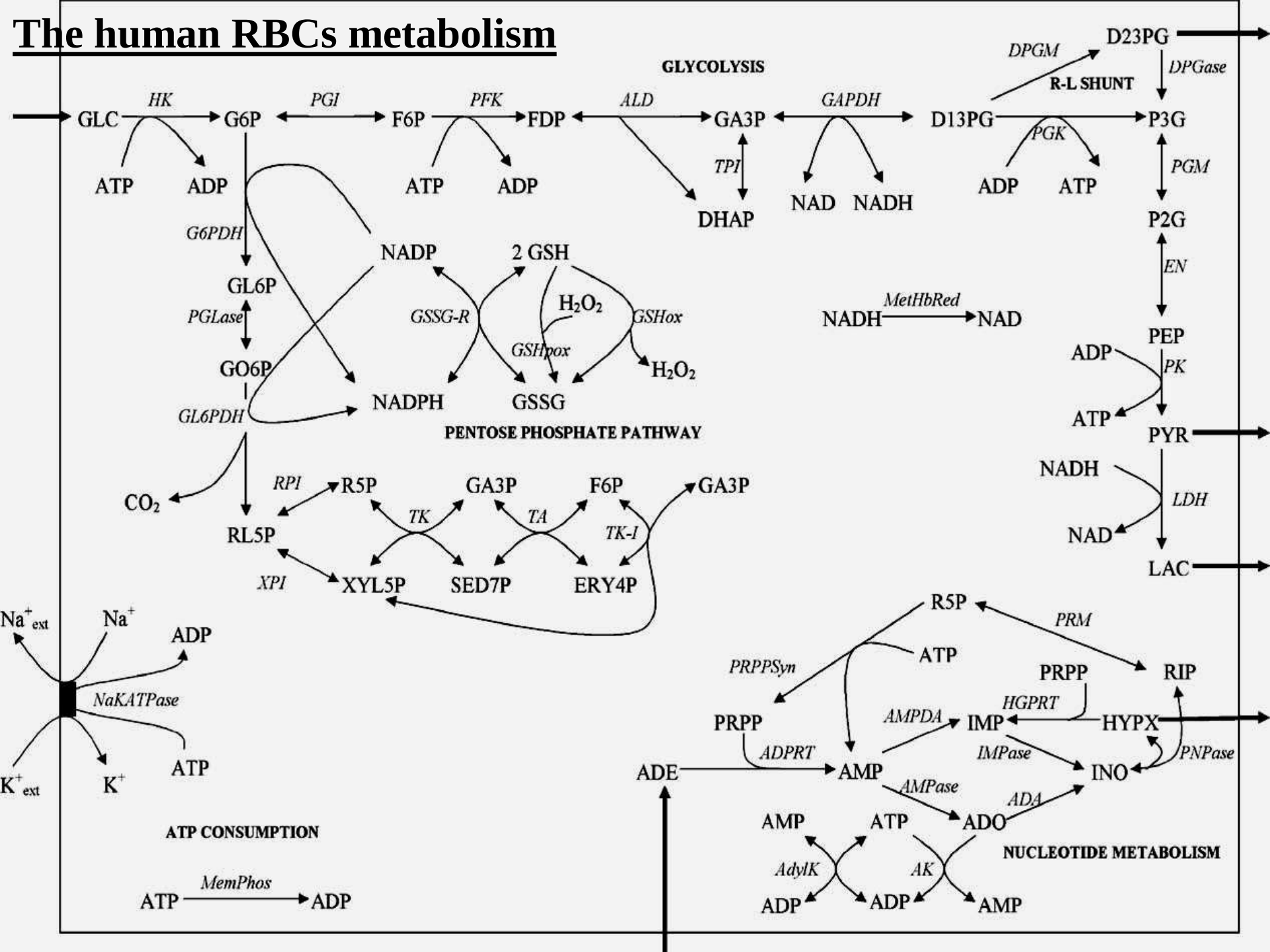
Hemoglobin



The red cell energy requirement is necessary for:

1- Replenishing its adenine nucleotide pool using salvage pathways
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
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.

The human RBCs metabolism



- 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.

- 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.

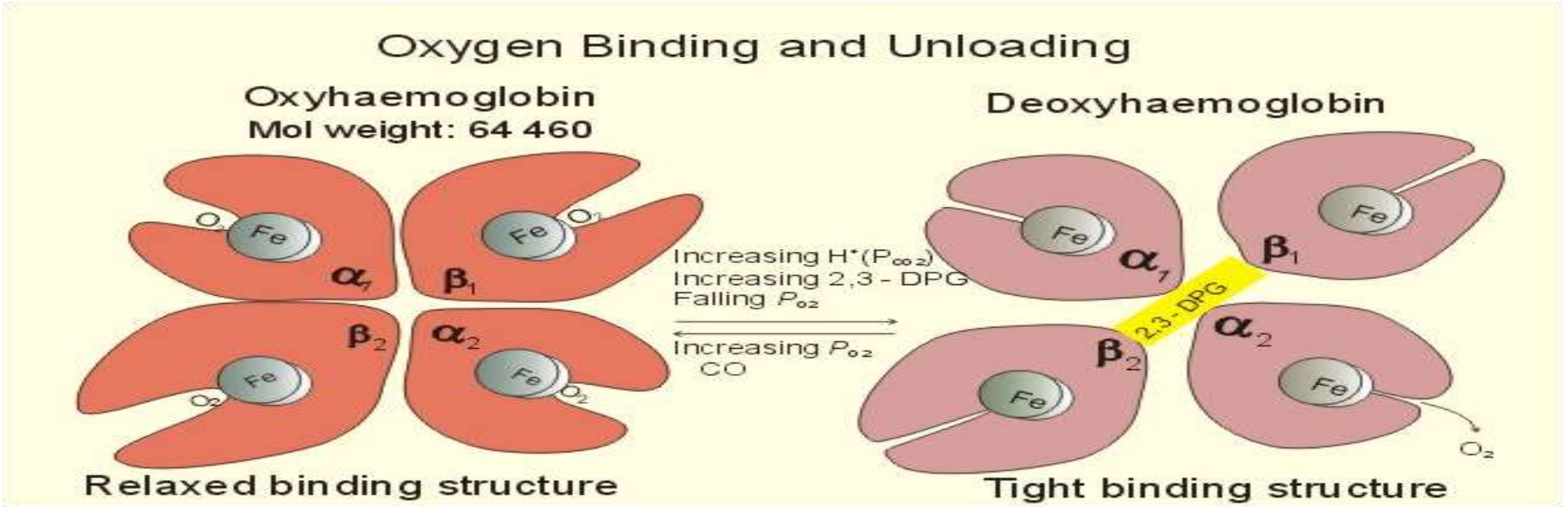
-The pentose phosphate pathway, accounts for about 10% of glucose metabolism in the red cell, this pathway in RBCs has a special role in protection against oxidative stress, while, in nucleated cells, it also serves as a source of NADPH for biosynthetic reactions and pentoses for nucleic acid synthesis.

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.



- Glutathione is synthesized from glycine, cysteine, and glutamic acid in a two-step process that requires ATP as a source of energy.
- 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.

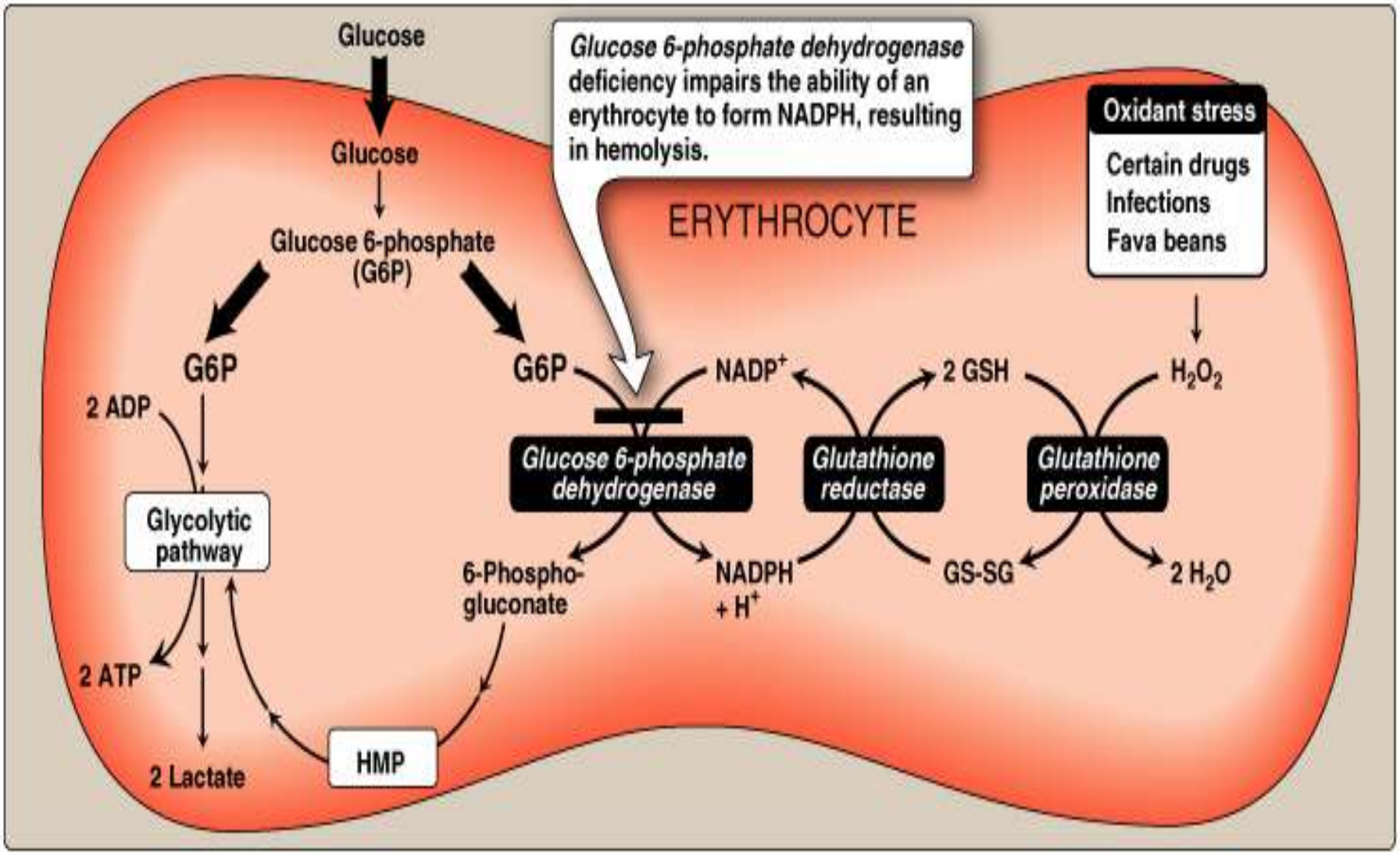


Figure 13.10

Pathways of glucose 6-phosphate metabolism in the erythrocyte.

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.

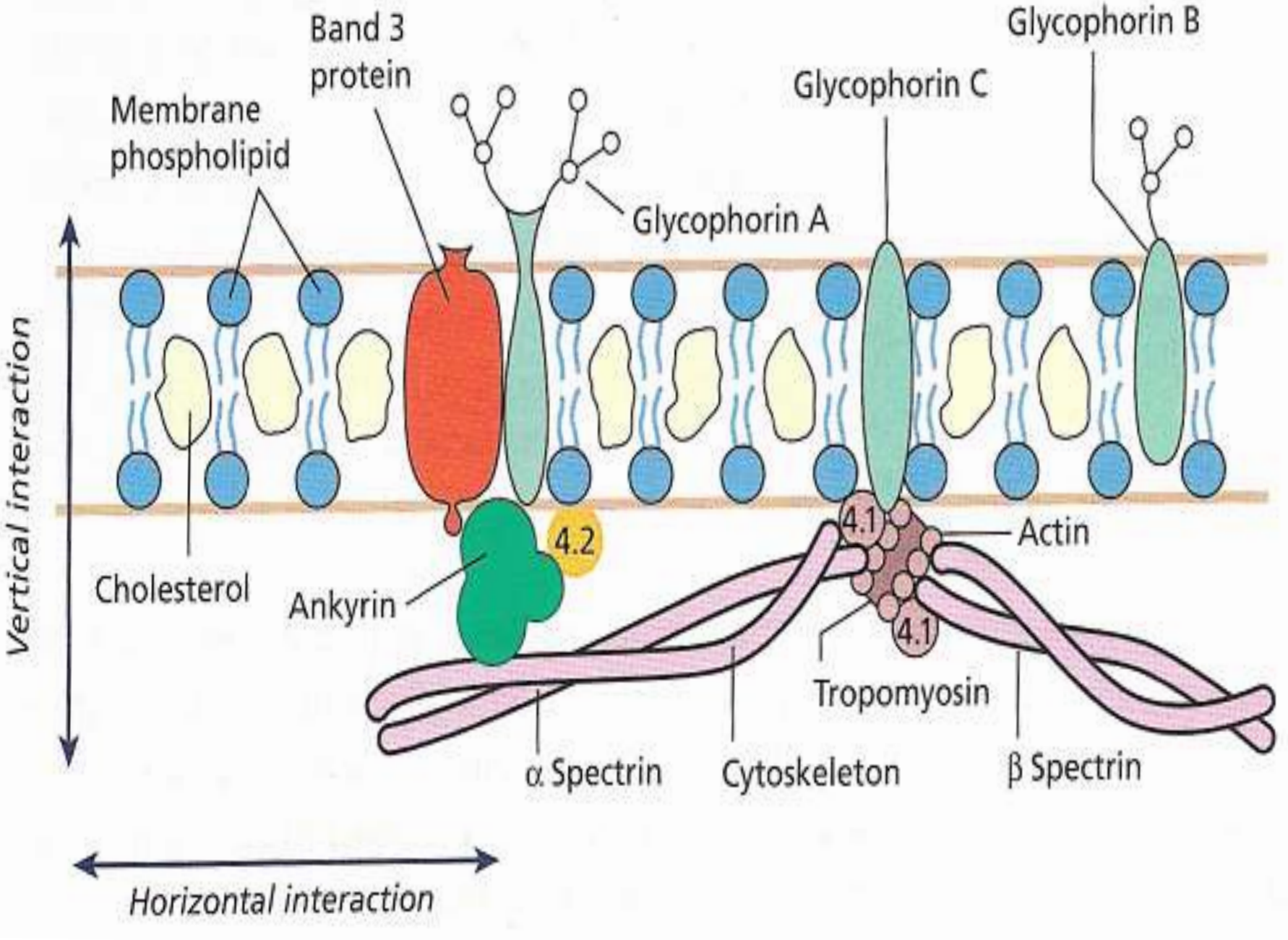
- α & β chains are loosely interconnected forming a dimer, one dimer interact with another, forming a head to head tetramer.

- 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^-.
- 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.

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


Band 3
protein
Membrane
phospholipid
Glycophorin B
Glycophorin C
Glycophorin A
Vertical interaction
Cholesterol
Ankyrin
4.2
4.1
Actin
4.1
Tropomyosin
α Spectrin
Cytoskeleton
β Spectrin
Horizontal interaction

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

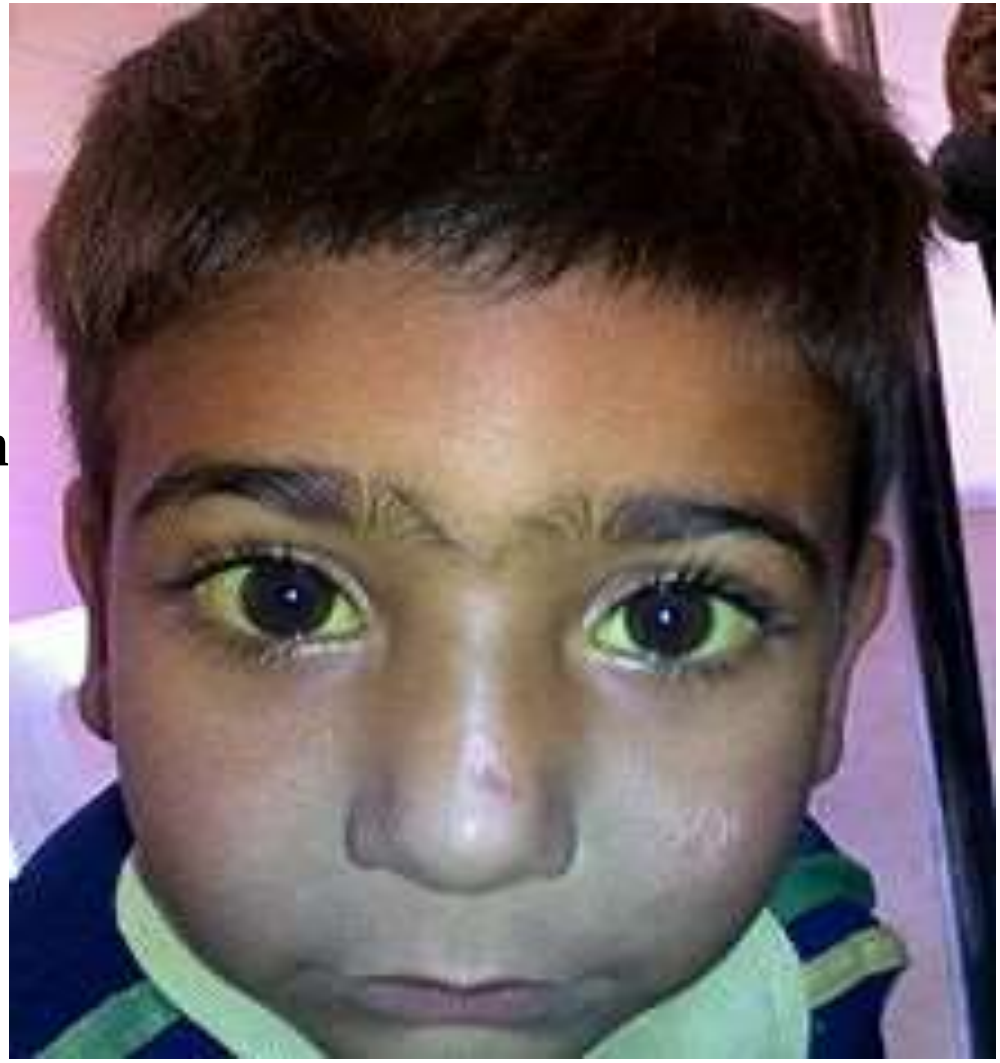
- It is an X-linked recessive hereditary disease, considered the most common human enzyme defect.
- Individuals with the disease may exhibit non- immune hemolytic anemia in response to a number of causes, most commonly infection or exposure to certain medications or chemicals.
- G6PD deficiency is closely 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.
  1- Severe deficiency (<10% activity) with chronic (non- spherocytic) hemolytic anemia
  2- Severe deficiency (<10% activity), with intermittent hemolysis

3- Mild deficiency (10-60% activity), hemolysis with stressors only
4- Non-deficient variant, no clinical sequelae
5- Increased enzyme activity, no clinical sequelae

This is a 4-year old boy diagnosed with glucose-6-phosphate dehydrogenase deficiency showing jaundice in the sclera



- Most individuals with G6PD deficiency are asymptomatic.
- Symptomatic patients are almost exclusively male, due to the X-linked pattern of inheritance, but female carriars 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.
- 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

2- Hemolytic crises in response to:

a- Illness (especially infections) b- Diabetic ketoacidosis

c- Sulpha drugs, antimalarial drugs and aspirin.

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

3- Very severe crises can cause acute renal failure.

H_2O_2 accumulated will cause:

1- Convert F.A.s on cell membrane to peroxide which will cause hemolysis of RBCs.

2- Convert Hb to methemoglobin that leads to increase cell membrane fragility.

Diagnosis

- 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 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);

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.

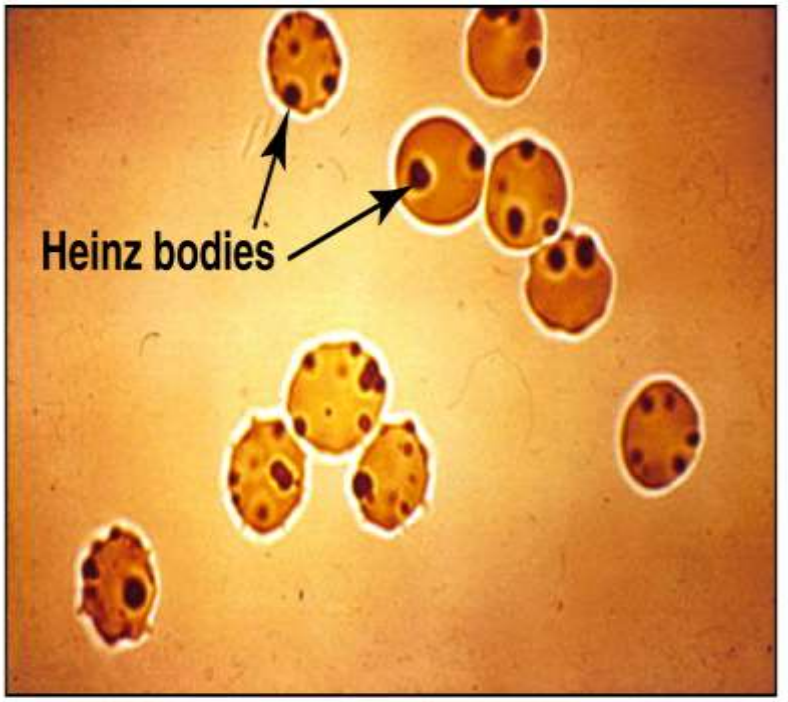


Figure 13.11

Heinz bodies in erythrocytes of patient with *G6PD* deficiency.

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).
-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.