Erythrocytes Metabolism

By

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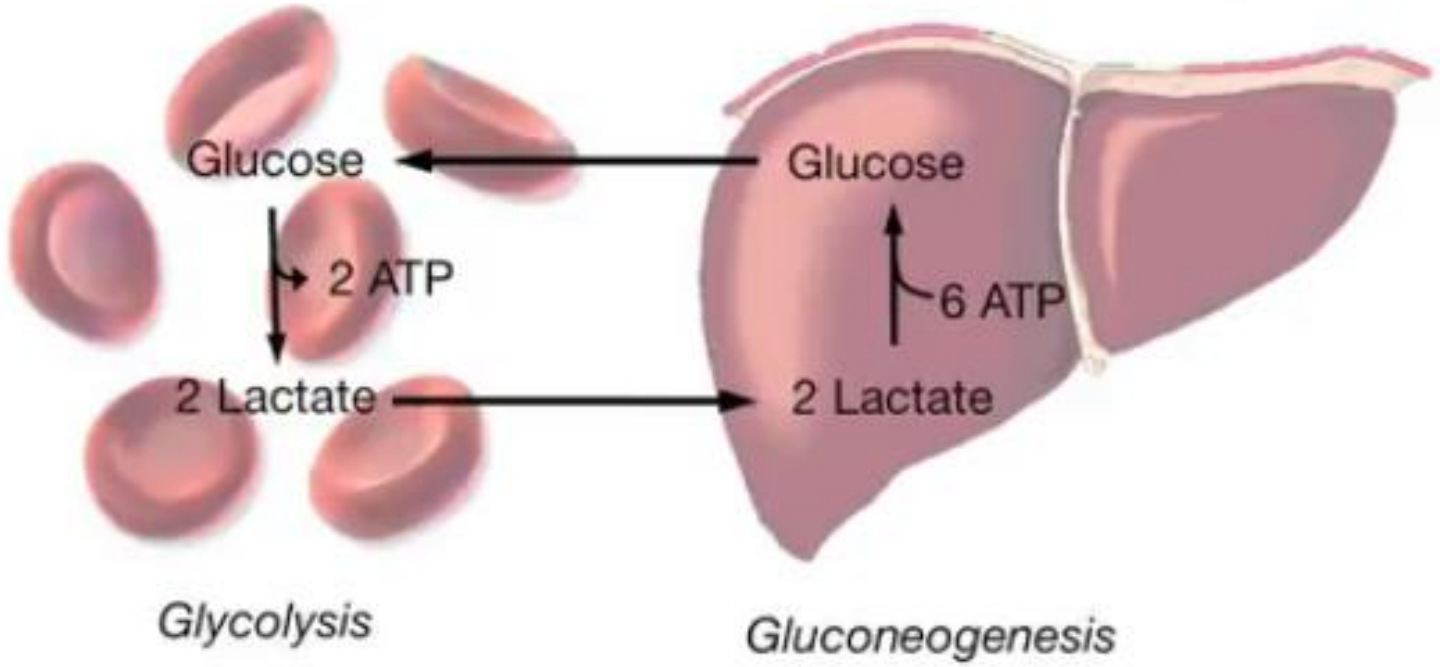
Glycolysis in red blood cells

Pentose phosphate pathway

Cori Cycle


Glucose
2 ATP
2 Lactate
Glucose
6 ATP
2 Lactate
Glycolysis
Gluconeogenesis

Glycolysis
(Embden-meyerhof pathway)

- **Definition:**

  - It is oxidation of glucose or glycogen to pyruvic acid (in presence of $O_{2)}$ or lactic acid (in absence of O_2).

- **Site:**

  - It occurs in the cytosol of every cell. Glucose can only give lactic acid in:

- RBCs (no mitochondria).
- Exercising muscles (O_2 lack).

- **Steps:** The steps of glycolysis can be classified into two phases:

PHASE ONE:

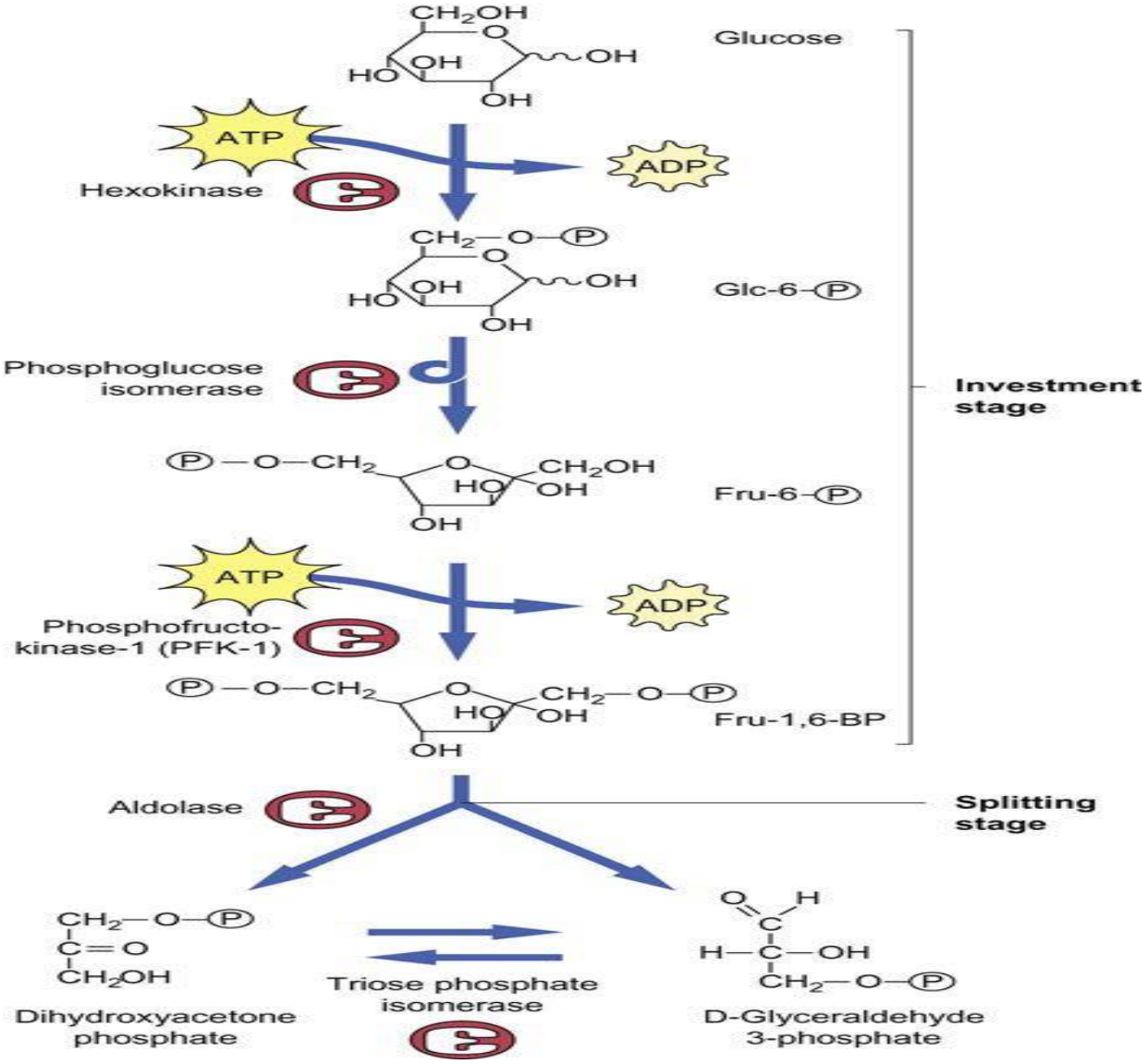
In this phase glucose is converted into two molecules of glyceraldehydes-3-phosphate.

PHASE TWO:

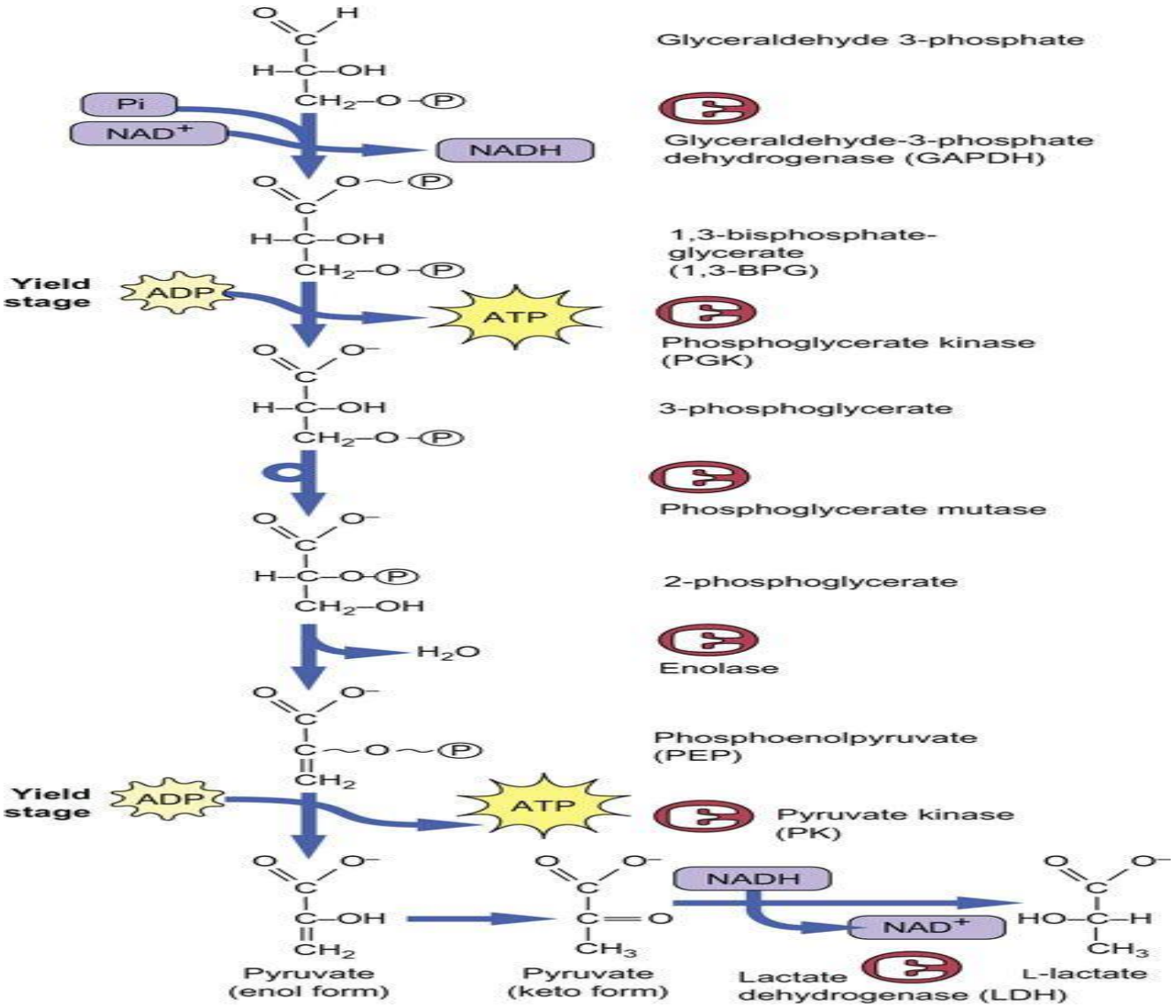
In this phase the 2 molecules of glyceraldehydes-3-phospshate are converted into two molecules of pyruvate (aerobic) or lactate (anaerobic).

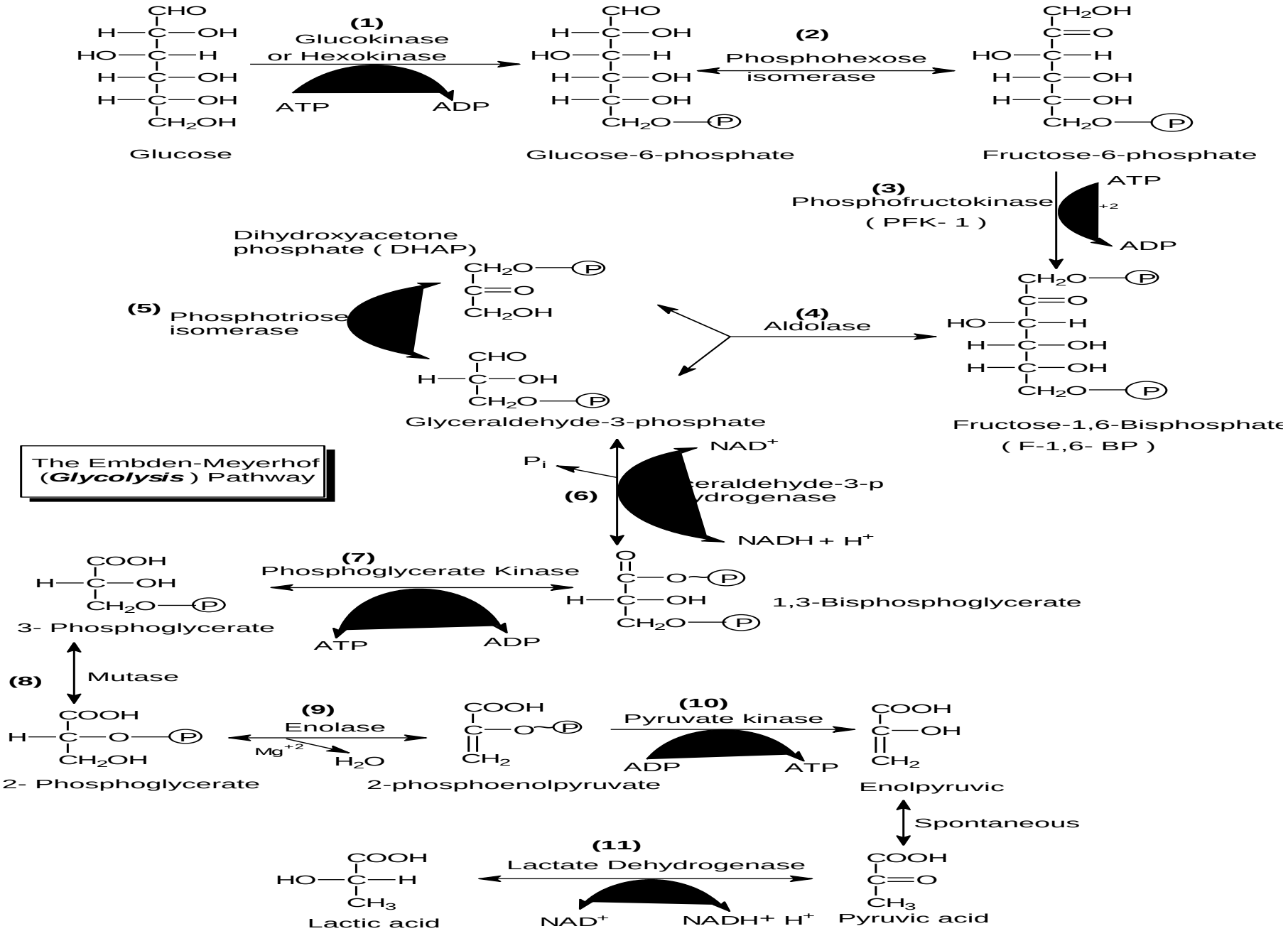
The investment stage of glycolysis

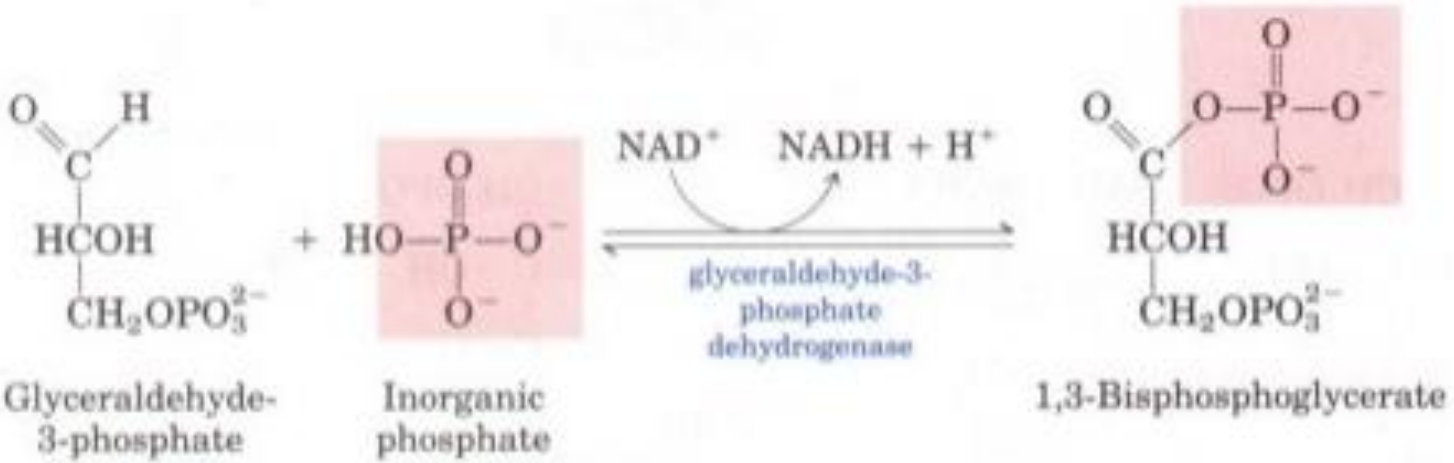
2 ATP are invested to prime the metabolism of glucose by glycolysis



The yield stage of glycolysis:




Glucose
(1)
Glucokinase
or Hexokinase
ATP
ADP
Glucose-6-phosphate
(2)
Phosphohexose
isomerase
Fructose-6-phosphate
(3)
Phosphofructokinase
(PFK- 1)
ATP
+2
ADP
Fructose-1,6-Bisphosphate
(F-1,6- BP)
(4)
Aldolase
Dihydroxyacetone
phosphate (DHAP)
(5)
Phosphotriose
isomerase
Glyceraldehyde-3-phosphate
The Embden-Meyerhof
(Glycolysis) Pathway
Pi
NAD+
(6)
NADH + H+
(7)
Phosphoglycerate Kinase
ATP
ADP
3- Phosphoglycerate
1,3-Bisphosphoglycerate
(8)
Mutase
2- Phosphoglycerate
(9)
Enolase
Mg+2
H2O
2-phosphoenolpyruvate
(10)
Pyruvate kinase
ADP
ATP
Enolpyruvic
Spontaneous
(11)
Lactate Dehydrogenase
NAD+
NADH+ H+
Lactic acid
Pyruvic acid


NAD+
NADH + H+
glyceraldehyde-3-phosphate dehydrogenase
Glyceraldehyde-3-phosphate
Inorganic phosphate
1,3-Bisphosphoglycerate

Energy gain of glycolysis:

- **Energy consumed:**

Step (1) by glucokinase: One ATP is lost (spared if we start with glycogen).

Step (3) by phosphofructokinase: One ATP is lost. So, the total lost 2 ATPs

- **Energy gained:**

Step (6) by glyceraldehyde -3 P dehydrogenase: 2 $NADH+H^+$ (6 ATPs) gained only in the presence of O_2.

Step (7) by phosphoglycerokinase: 2 ATPs gained.

Step (10) by pyruvate kinase: 2 ATPs gained. So, the total gains 10 ATPs.

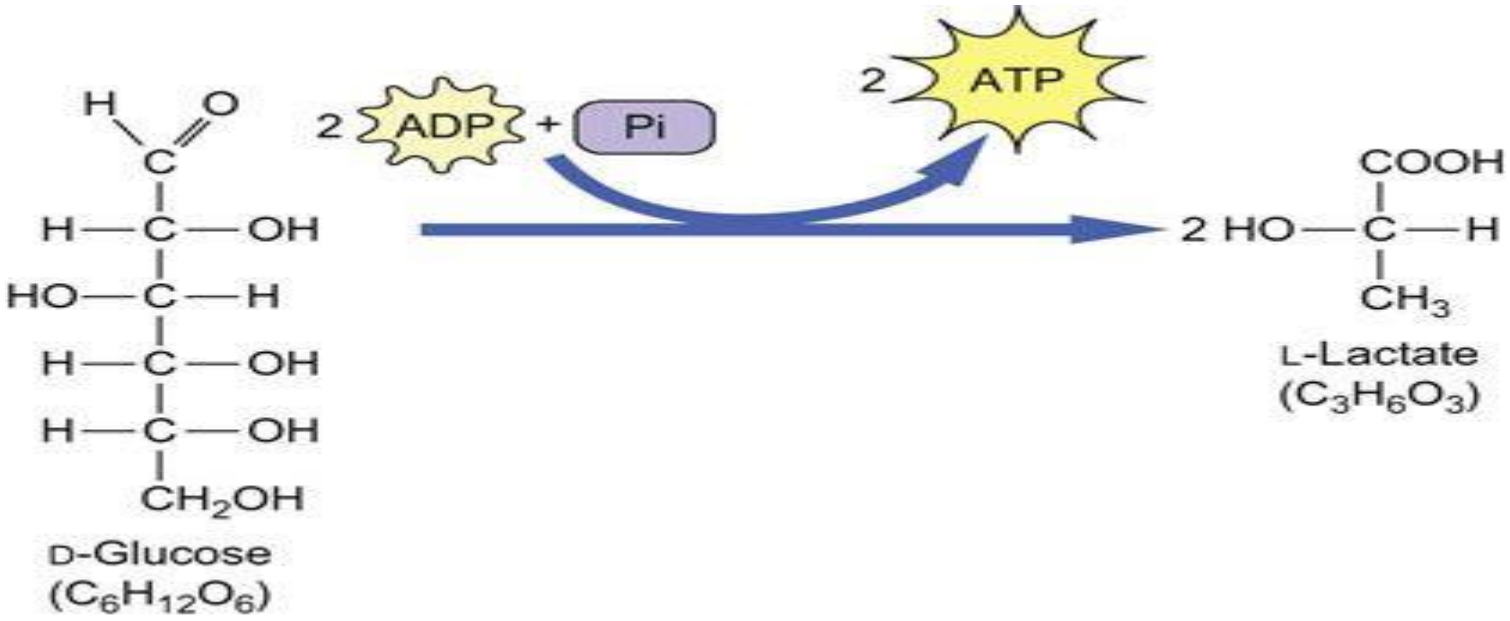
So, Energy gained under anaerobic condition (i.e.) Glucose to 2 molecules of lactic acid is 2 ATPs
and 3 ATPs if we start with glycogen.

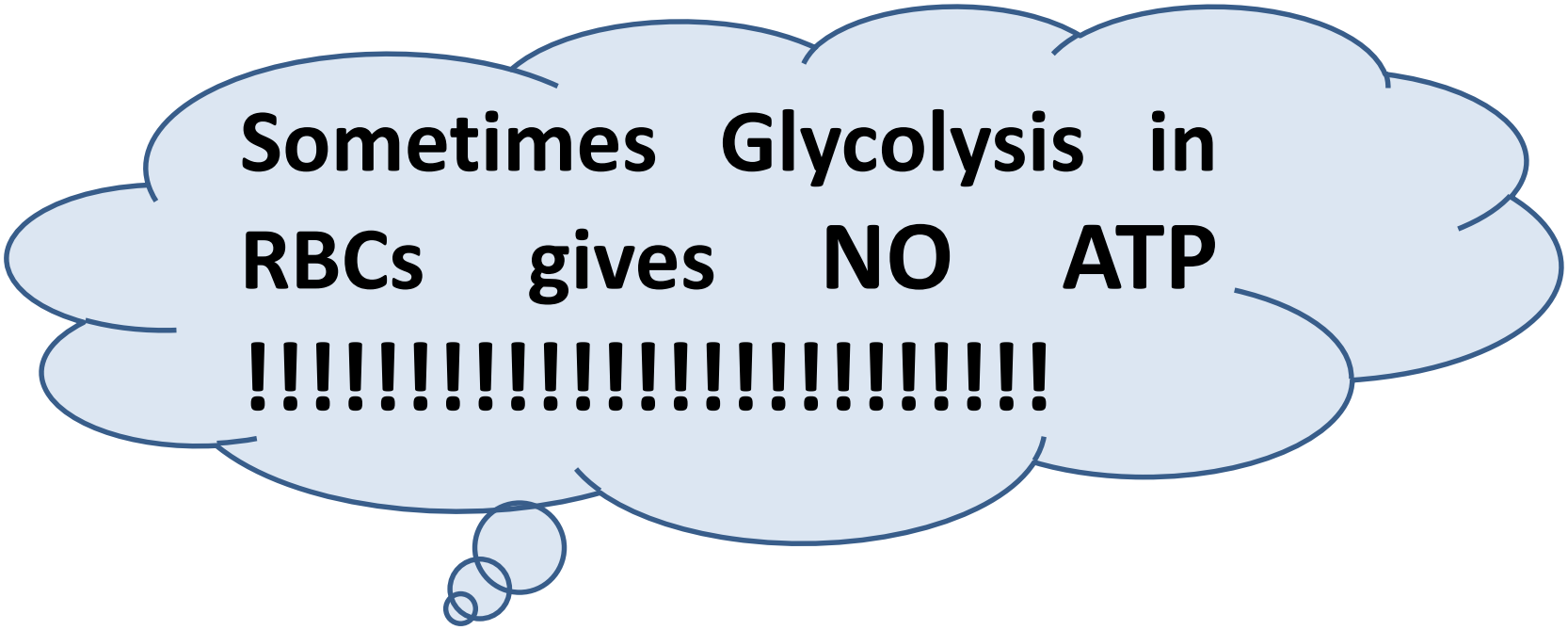
Energy gained under aerobic condition (i.e.) Glucose to 2 molecules of pyruvic acid and 2 NADH +H+

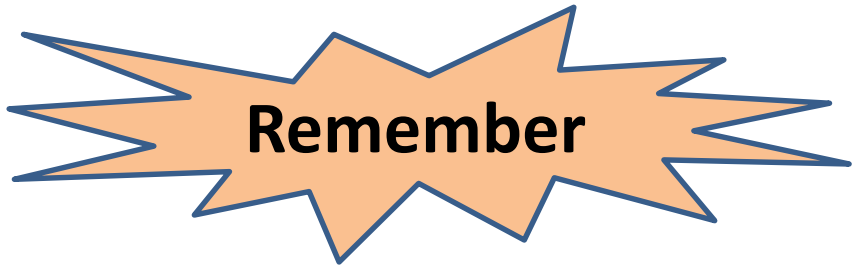
= 2 ATPs + 6 ATPs (from 2 $NADH+H^+$) = **8 ATPs** and 9 ATPs if we start with glycogen.

Glycolysis in red blood cells

- RBCs have no mitochondria so, glucose oxidation by Glycolysis gives **2 lactic acids** and only **2 ATPs.**




Sometimes Glycolysis in RBCs gives NO ATP !!!!!!!!!!!!!!!!!!!!!!!!!!



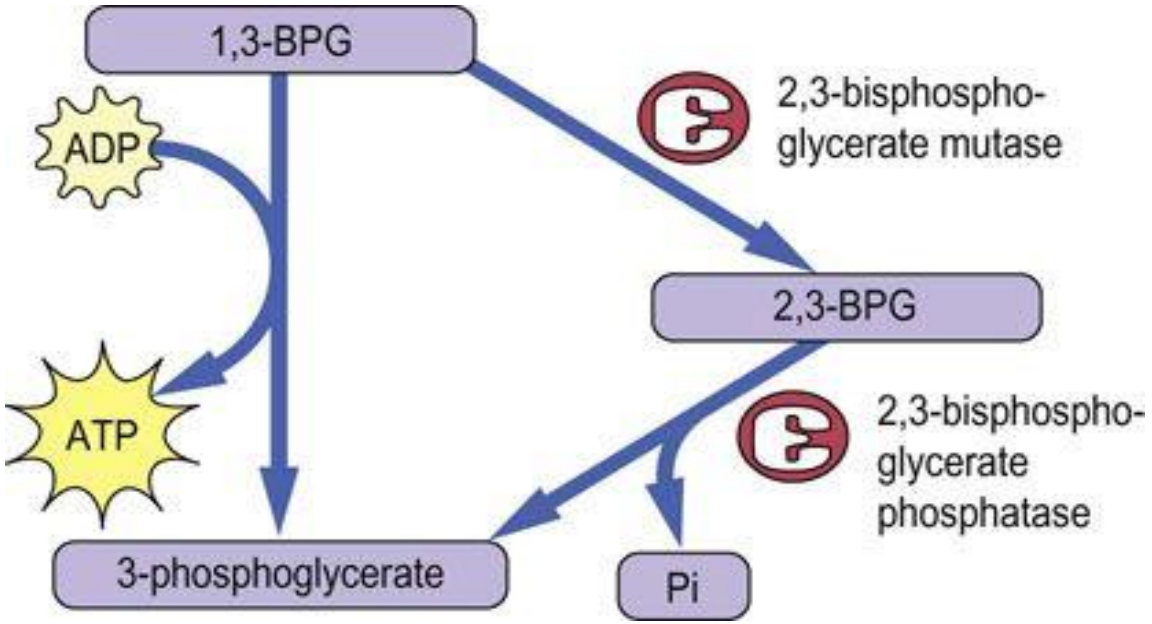
- The 2,3-bisphosphoglycerate (BPG) molecule carries 5 negative charges and is derived from oxidation of glucose (glycolysis) in red cells.

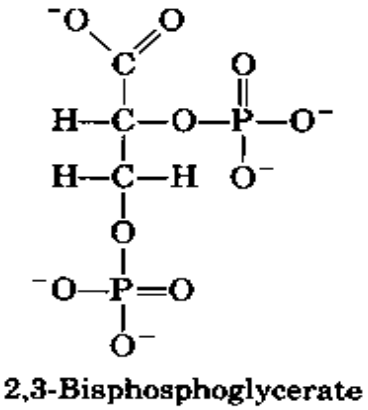
- It binds to a positively charged pocket in Hb between the 2 β chains (small cavity in the center of the four Hb subunits)

- Binding favors the T- form of Hb, reducing affinity for oxygen and helping delivery of oxygen to tissues.

- BPG increases in red blood cells in cases of chronic anemia and in hypoxia. This helps delivery of oxygen to tissues.

Pathway for biosynthesis and degradation of 2,3-bisphosphoglycerate.





- **This pathway discovered by Rapoport-Lubring and called Rapoport-Lubring cycle.**
- **About 15 to 25% of the glucose utilized in red cells is utilized through BPG shunt.**

- So RBCs need 2, 3 bisphosphoglycerate as its increase will decrease the oxygen affinity for hemoglobin to oxygen and helping oxygen delivery to tissues.
- RBCs 1, 3 diphosphoglycerate is changed by mutase to 2, 3 diphosphoglycerate which by phosphatase is changed to 3-phosphoglycerate to continue glycolysis till pyruvic acid.

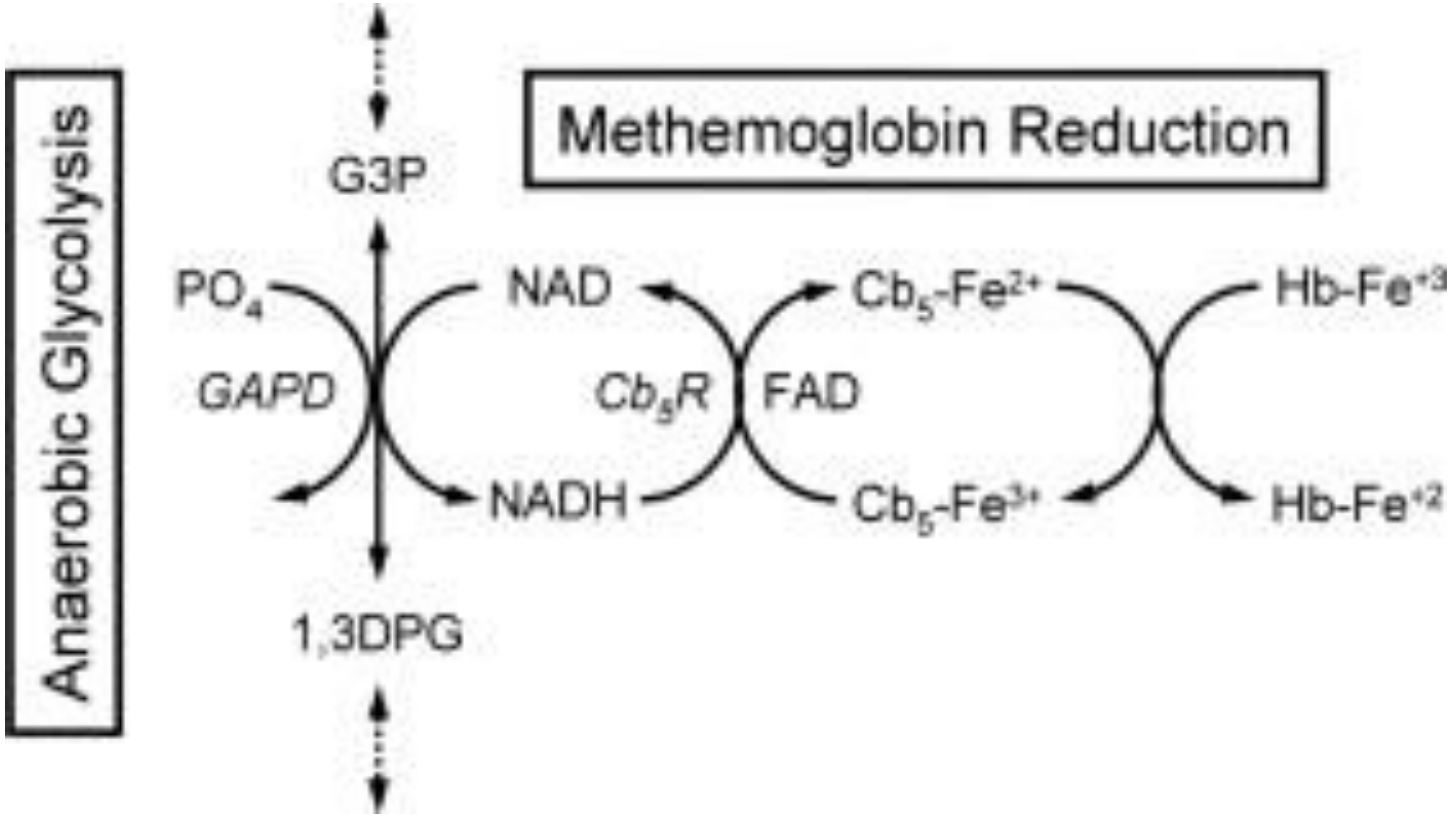
•Importance of glycolysis in Red cells:

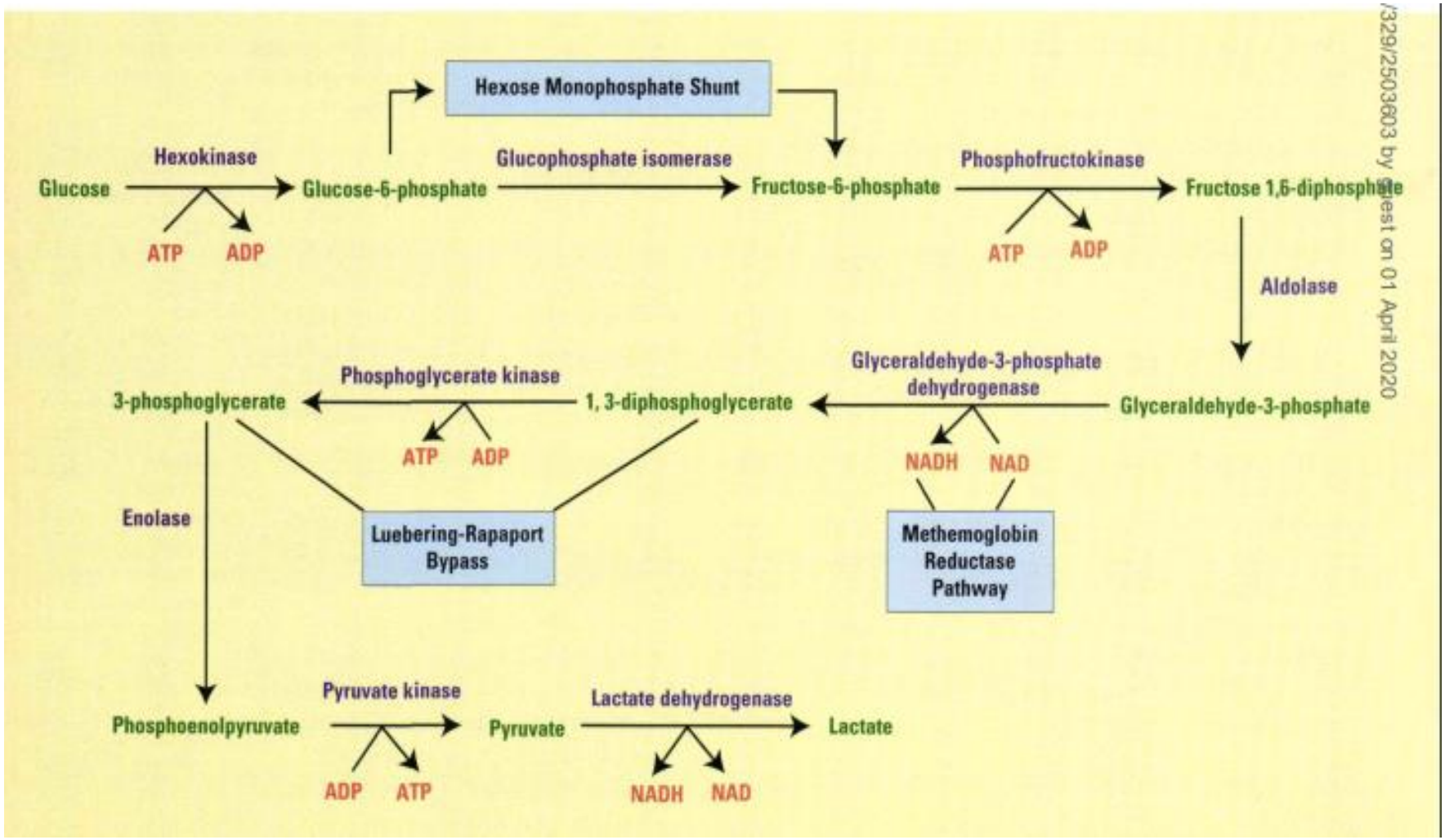
- **Energy production**: the only pathway that supplies the red cells with ATP.
- **Bisphosphoglycerate shunt** (BPG shunt).
- **Reduction of methemoglobin**: glycolysis provides NADH for reduction of met-Hb in red cells by the **NADH-cyt.b5-methemoglobin reductase system.**

- The **ferrous iron of hemoglobin** is susceptible to oxidation by superoxide and other oxidizing agents, forming **methemoglobin**, **which cannot transport oxygen.**
- Only a very small amount of methemoglobin is present in normal blood, as the red blood cell possesses an effective system **(the NADH-cytochrome b5 methemoglobin reductase system)** for reducing heme Fe3+ back to the Fe2+ state.

- This system consists of **NADH (generated by glycolysis),** a **flavoprotein named cytochrome b5 reductase** (also known as methemoglobin reductase), and **cytochrome b_5** (electron transport hemoprotein).
- The Fe^{3+} of methemoglobin is reduced back to the Fe^{2+} state by the action of **reduced cytochrome b_5**:

- Reduced cytochrome b_5 is then regenerated by the action of cytochrome b_5 reductase (NADH-dependent enzyme):

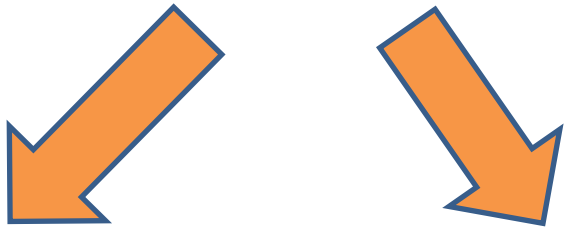

Anaerobic Glycolysis
Methemoglobin Reduction
G3P
PO4
GAPD
NAD
NADH
Cb5R
FAD
Cb5-Fe2+
Cb5-Fe3+
Hb-Fe+3
Hb-Fe+2
1,3DPG



Hemolytic anemia due to deficiency of glycolytic enzymes:

- Inherited deficiency of glycolytic enzymes produces hemolytic anemia because red cells are dependent on glycolysis for production of ATP.
- About 95% of these patients have deficiency of **pyruvate kinase** and 4% have deficiency of **phosphohexose isomerase**.

pentose phosphate pathway
(Hexose Monophosphate Shunt or HMP-shunt)



The source of ribose phosphate for synthesis of RNA and DNA

NADPH is a major product of the pentose phosphate pathway in all cells

- **The pentose phosphate pathway is a cytosolic pathway present in all cells**
- **This pathway is active in the cytosol of many cells e.g. liver, adipose tissues, adrenal cortex, ovaries, testis, red cells and retina.**

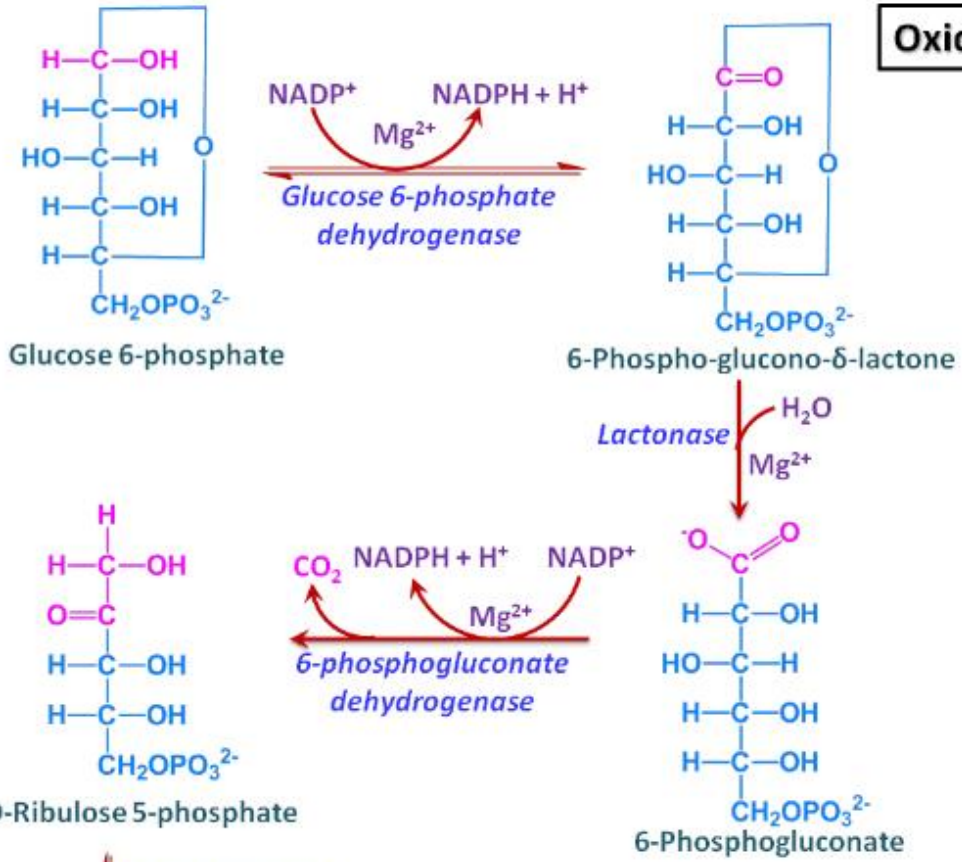
- **The pentose phosphate pathway is divided into:**

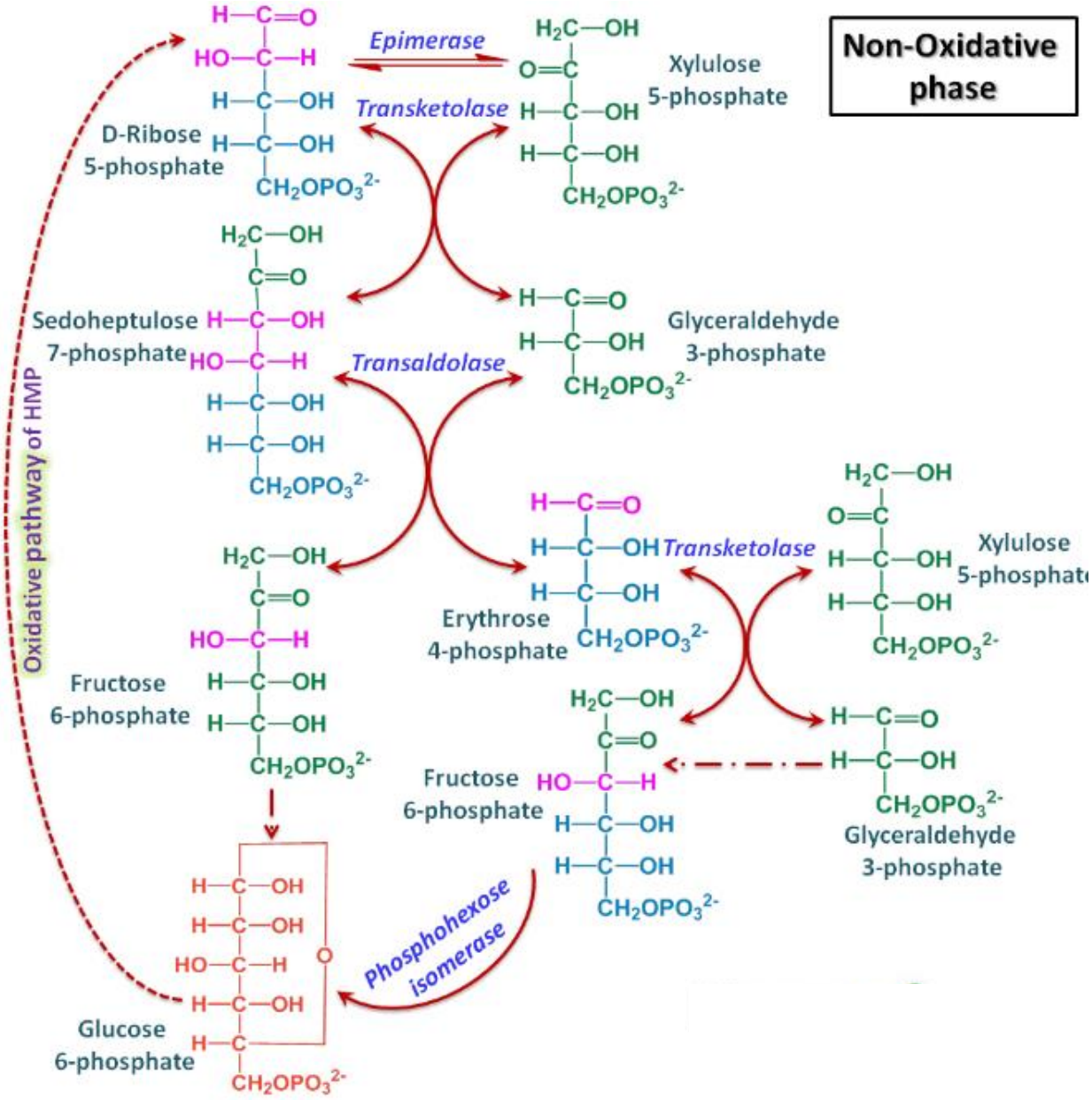
Irreversible redox stage, which yields both NADPH and pentose phosphates

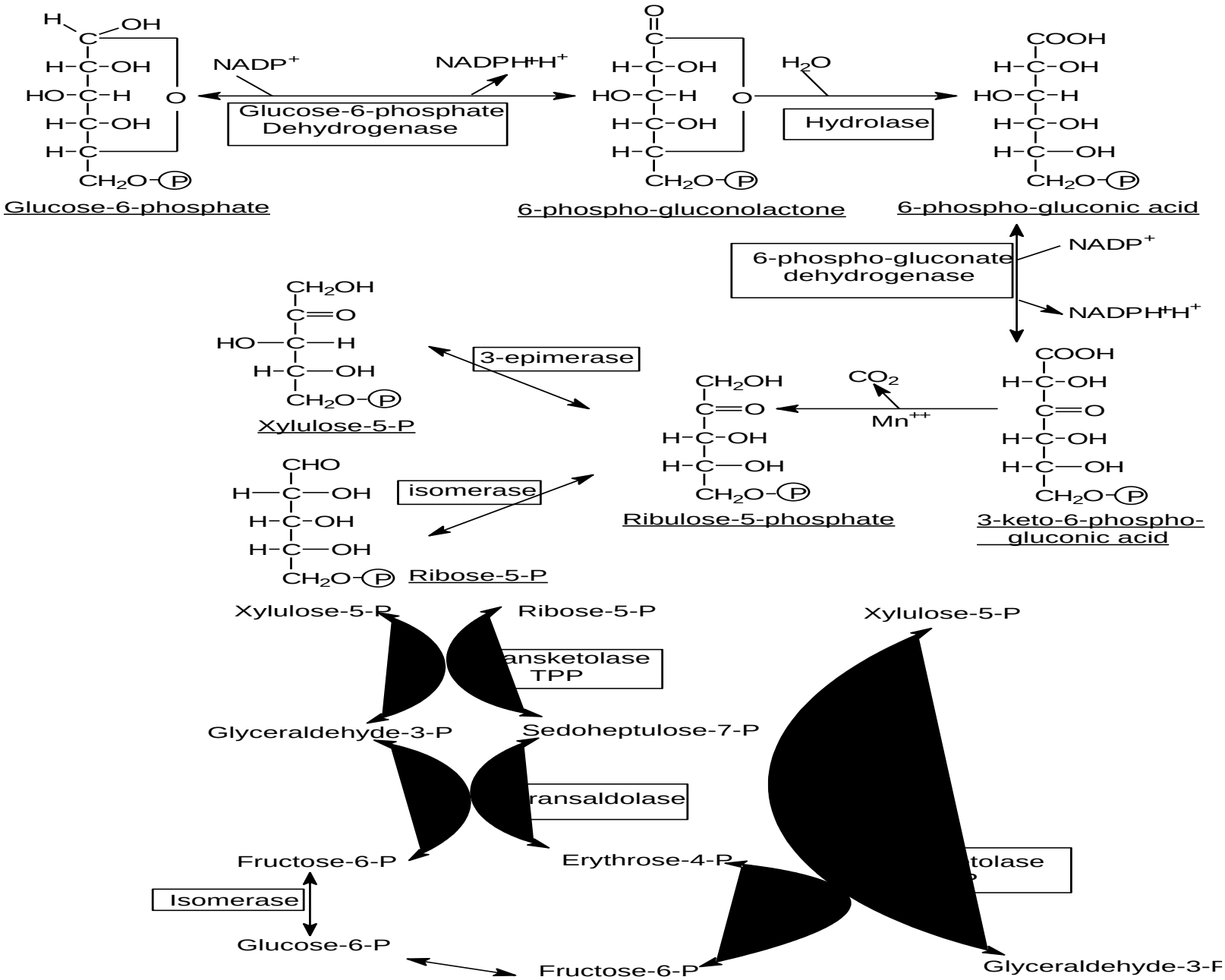
Reversible interconversion stage, in which excess pentose phosphates are converted into glycolytic intermediates.

Both stages are important in the RBC, since it needs NADPH for reduction of glutathione, but has limited need for de novo synthesis of nucleotides.

Oxidative phase




Non-Oxidative phase
Oxidative pathway of HMP
Epimerase
Transketolase
Transaldolase
Transketolase
Phosphohexose isomerase
D-Ribose 5-phosphate
Xylulose 5-phosphate
Sedoheptulose 7-phosphate
Glyceraldehyde 3-phosphate
Fructose 6-phosphate
Erythrose 4-phosphate
Xylulose 5-phosphate
Fructose 6-phosphate
Glyceraldehyde 3-phosphate
Glucose 6-phosphate


Glucose-6-phosphate
NADP+
NADPH+H+
Glucose-6-phosphate Dehydrogenase
6-phospho-gluconolactone
H2O
Hydrolase
6-phospho-gluconic acid
6-phospho-gluconate dehydrogenase
NADP+
NADPH+H+
Xylulose-5-P
3-epimerase
CO2
Mn++
Ribulose-5-phosphate
3-keto-6-phospho-gluconic acid
isomerase
Ribose-5-P
Xylulose-5-P
Ribose-5-P
ansketolase TPP
Glyceraldehyde-3-P
Sedoheptulose-7-P
ransaldolase
Fructose-6-P
Erythrose-4-P
tolase
Isomerase
Glucose-6-P
Fructose-6-P
Xylulose-5-P
Glyceraldehyde-3-P

- Transketolase transfers two-carbon unit (carbons 1 & 2) of a ketose onto the aldehyde carbon of an aldose sugar (TPP is needed).

- Transaldolase transfers three-carbon dihydroxyacetone moiety (carbons 1–3) of a ketose onto the aldehyde carbon of an aldose sugar (No TPP is needed).

- This pathway branches from glycolysis at the level of Glc-6-P: thus, its alternative designation, the hexose monophosphate shunt.

- The pentose phosphate pathway is sometimes described as a shunt, rather than a pathway, because **when pentoses are not needed for biosynthetic reactions, the pentose phosphate intermediates are recycled back into the mainstream of glycolysis by conversion into Fru-6-P and glyceraldehyde-3-phosphate.**

- This rerouting is especially important in the RBC and in nondividing or quiescent cells, where there is limited need for synthesis of DNA and RNA.

- In tissues with active lipid biosynthesis, e.g. liver, adrenal cortex or lactating mammary glands, the NADPH is used in redox reactions required for biosynthesis of cholesterol, bile salts, steroid hormones and triglycerides.

- The liver also uses NADPH for hydroxylation reactions involved in the detoxification and excretion of drugs.

- The RBC has little biosynthetic activity, but still shunts about 10% of glucose through the pentose phosphate pathway, in this case almost exclusively for the production of NADPH.

- The NADPH is used primarily for the reduction of a cysteine-containing tripeptide, glutathione (GSH), an essential cofactor for antioxidant protection.

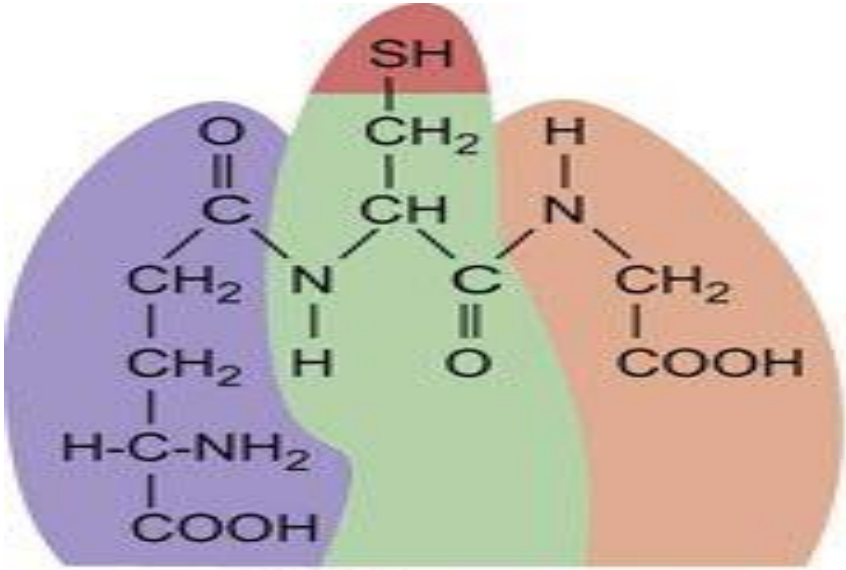
Importance of HMP in red cells:

- Red cells are liable for oxidative damage by H_2O_2 due to their role in oxygen transport.
- H2O2 is a powerful oxidant that produces damage of cellular DNA, proteins and phospholipids.
- H_2O_2 in red cells oxidizes the iron of Hb to form methemoglobin. In addition H_2O_2 produces lipid peroxidation, which increases the cell membrane fragility.
- **Glutathione reductase** and **glutathione peroxidase** are important for removal of H2O2.

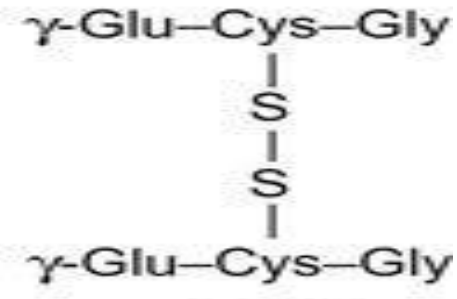
- The major role of HMP in red cells, is the production of NADPH, which protect these cells from oxidative damage by providing **reduced glutathione** for removal of H_2O_2 .

So what is glutathion? And what is its function?

Glutathione (GSH) is a tripeptide γ-glutamyl-cysteinyl-glycine. It is present in cells, 99% in the reduced (thiol) form, and is an essential coenzyme for protection of the cell against a range of oxidative and chemical insults. **Most of the NADPH formed in the red cell is used by glutathione reductase which is a flavoprotein enzyme (contains FAD) to maintain GSH in the reduced state.**



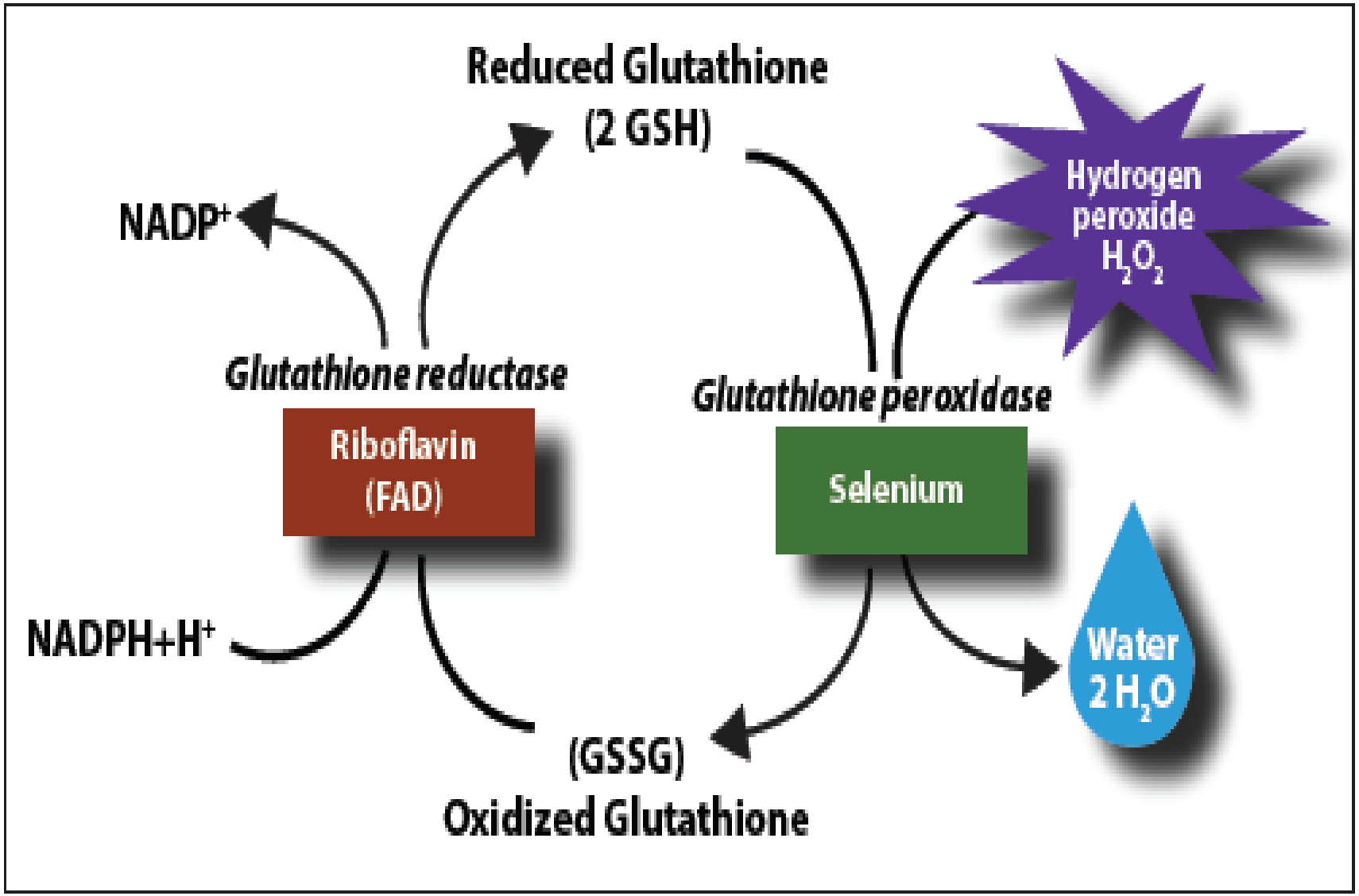
GSH



GSSG

- Reduced glutathione (G-SH) is a coenzyme for the enzyme **glutathione peroxidase** (contains selenium) that reduces hydrogen peroxide to water, protecting cells from its toxic effects.

- During its function as a coenzyme for antioxidant activities, GSH is oxidized to the disulfide form, GSSG, which is then regenerated by the action of **glutathione reductase .**


Reduced Glutathione
(2 GSH)
NADP+
Hydrogen
peroxide
H2O2
Glutathione reductase
Glutathione peroxidase
Riboflavin
(FAD)
Selenium
NADPH+H+
Water
2 H2O
(GSSG)
Oxidized Glutathione

FAVISM

- It is a genetic deficiency of glucose-6-phosphate dehydrogenase (G6PD).
- The red cell capacity to protect itself from oxidative damage is markedly decreased (due to decreased concentration of NADPH).
- Administration of certain drugs (premaquine, asprin or sulfonamides), which stimulate the production of H_2O_2 or eating fava beans (contain oxidizing agents as divicine and isouramil) produce lysis of the fragile red cells.
- **Treatment :** Avoid fava beans & oxidizing drugs. Blood transfusion is done after crisis.

- Although G6PD deficiency occurs in all cells of the affected individual, it is **most sever in erythrocytes** where **HMP pathway provides the only means of generating NADPH** – other tissues have alternative sources of NADPH production. The RBCs has no nucleus or ribosomes and cannot renew its supply of the enzyme.
- Deficiency of G6PD in the red blood cells is the most common enzymopathy (diseases caused by abnormalities of enzymes)
- G6PD gene is located on X-chromosome so the abnormal genes in affected males are of maternal origin (It is an X-linked recessive disorder)