Pentose Phosphate Pathway (PPP)

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Session learning objectives

- Explain the importance of HMP pathway
- Explain tissue specific nature of the pathway
- Describe the oxidative and non-oxidative phases of HMP
- Explain the biochemical function of NAPDH
- Show how the pentose phosphate pathway is related to other pathways
- Identify the rate-limiting step in the pentose phosphate pathway
- Explain how the rate-limiting enzyme is regulated
- Understand different mode of HMP pathway usage
- Understand the difference between transketolase and trans-aldolase
- Understand the biochemical explanation of glucose -6-P dehydrogenase deficiency in RBC
- Recognize the clinical function of transketolase
- Explain hemolytic anemia due to Glucose -6-P-Dehydrogenase

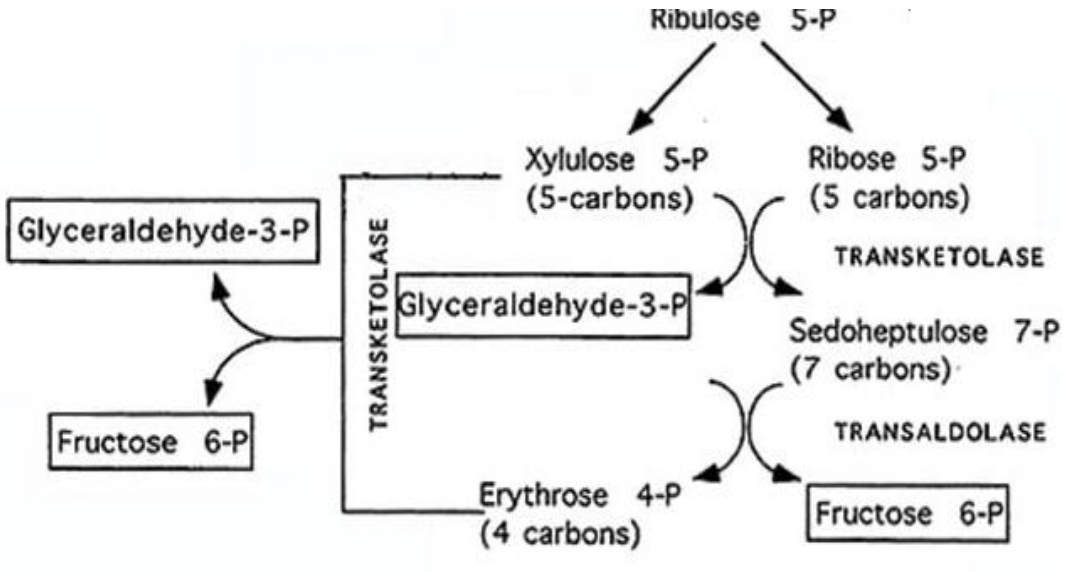
Pentose Phosphate Pathway

Other names: Hexose Monophosphate Pathway (HMP) and Phosphogluconate Pathway

- It is a cytosolic pathway present in all cells
- It is an alternative minor pathway for glucose oxidation
- It neither produce nor utilize ATP directly
- It aims at producing:
    - NADPH & Ribose 5-P
    - It produces intermediates of glycolysis Fructose-6-p and Glyceraldehydate -3-p

Pentose Phosphate Pathway

- The PP pathway is also described as a shunt when pentoses are not needed for biosynthetic rxn's
- **Pentose phosphate intermediates are**: Recycled to the mainstream of glycolysis by converting into **Fru-6-P & GA-3-P**
- This rerouting is especially important in **RBC & Non-dividing or quiescent cells**, b/c; there is limited need for synthesis of DNA & RNA



Tissues with active PP Pathway

Adrenal gland	Steroid synthesis
Liver	Fatty acid and cholesterol synthesis
Testes	Steroid synthesis
Adipose tissue	Fatty acid synthesis
Ovary	Steroid synthesis
Mammary gland	Fatty acid synthesis
Red blood cells	Maintenance of reduced glutathione

Pentose Phosphate Pathway

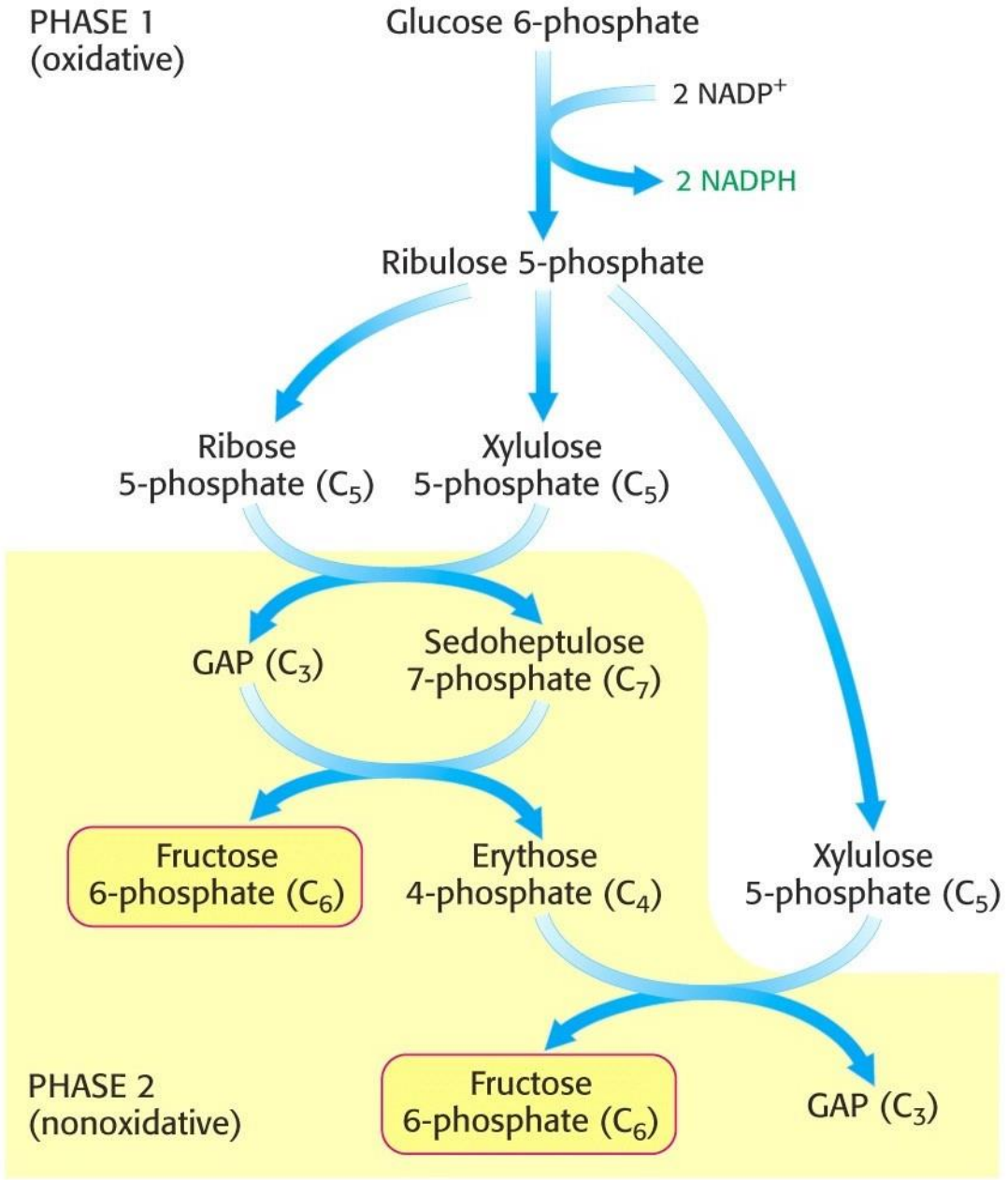
PP-Pathway is divided into:

- **Irreversible redox stage; Oxidative Phase**
  - Which yields both NADPH & Pentose phosphates
- **Reversible interconversion stage ; Nonoxidative Phase**

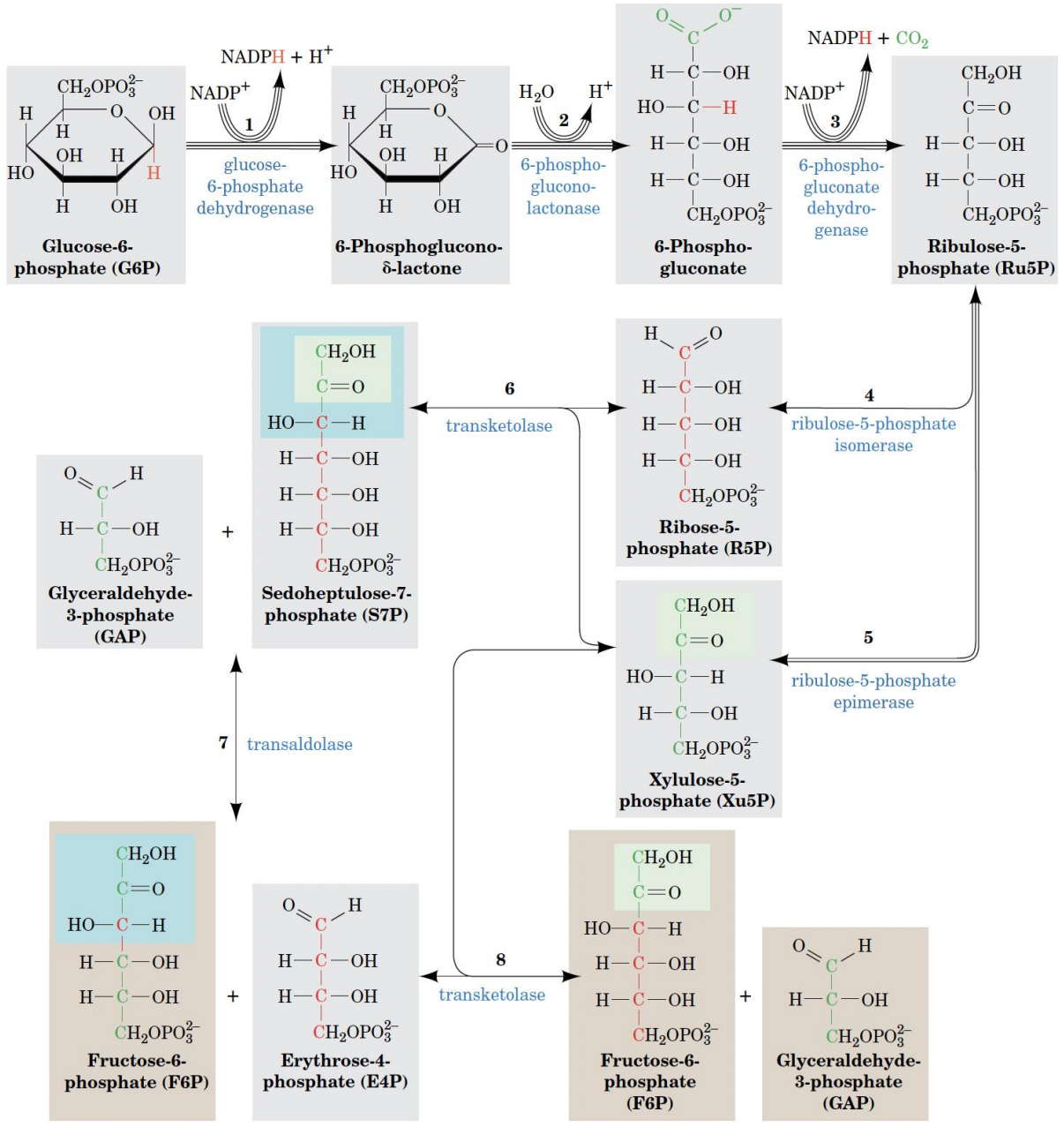
Excess pentose phosphates are converted into glycolytic intermediates

In cells that are not using ribose 5-phosphate :

- **Non-oxidative phase recycles**: 6 molecules of the Pentose into 5 molecules of the Hexose (**Glucose 6-phosphate)**
- **Allowing:** Continued production of NADPH & Converting Glc 6-phosphate to CO_2



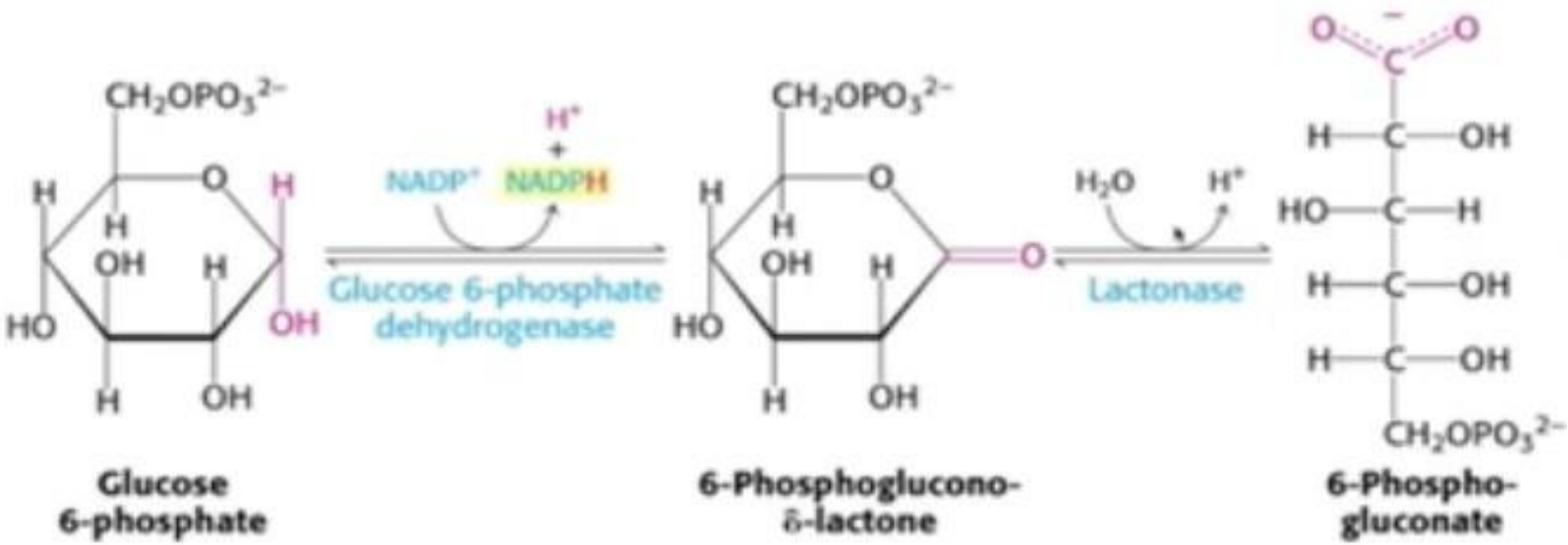
Pentose Phosphate Pathway



Steps of Pentose Phosphate Pathway

Glucose-6-phosphate Dehydrogenase catalyzes; Oxidation of the aldehyde (hemiacetal), at C_1 of Glc-6-P,to a carboxylic acid, in ester linkage (lactone)

- $NADP^+$ serves as electron acceptor

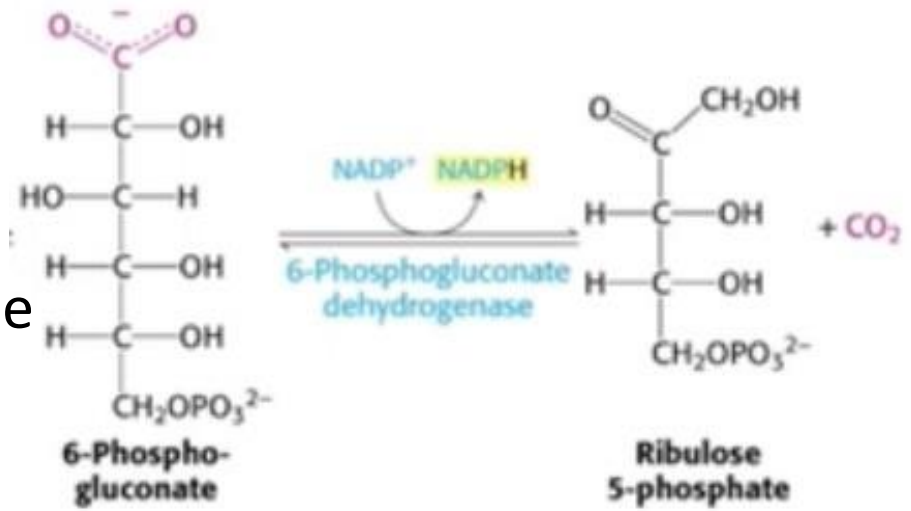


Pentose Phosphate Pathway

6-Phosphogluconolactonase catalyzes: Hydrolysis of the ester linkage, resulting in ring opening the product is 6-phosphogluconate

Although ring opening occurs in the absence of a catalyst, 6-Phosphogluconolactonase: Speeds up the rxn, decreasing the lifetime of the highly reactive, & thus potentially toxic, 6-phosphogluconolactone

- Oxidative decarboxylation of 6-phosphogluconate, to yield the 5-C ketose ribulose-5-phosphate
- The OH at C_3 (C_2 of product) is oxidized to a ketone
- This promotes loss of the carboxyl at C_1 as CO_2
- $NADP^+$ serves as oxidant

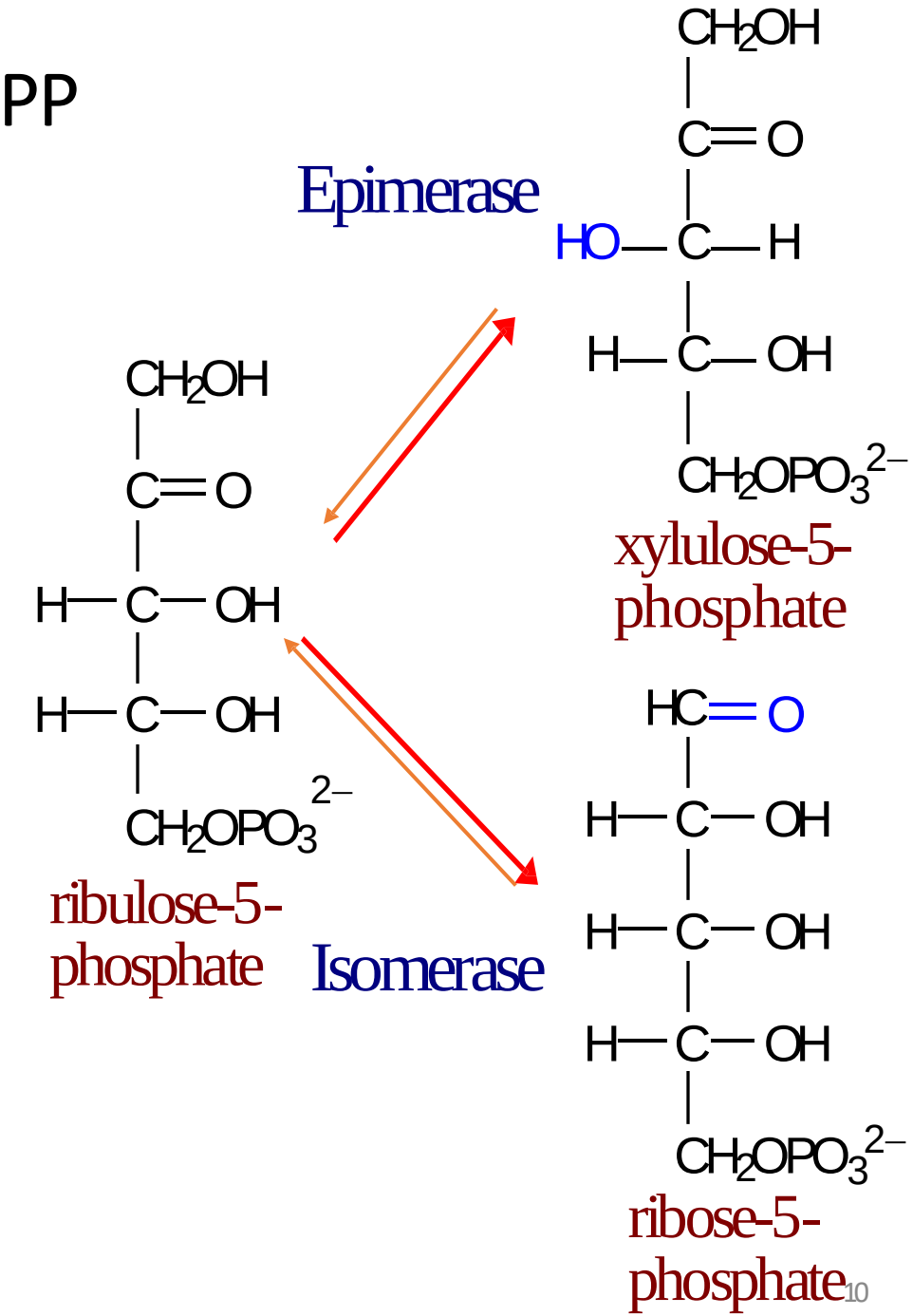


Non-oxidative Phase of PPP

- **Epimerase**: Inter-converts ribulose-5-P & xylulose-5-P
- **Isomerase:** Converts the ketose ribulose-5-P to the aldose ribose-5-P

Both rxn's involves:

- Deprotonation to an endiolate intermediate
- Followed by specific reprotonation to yield the product
- Both reactions are reversible



Non-oxidative Phase of PPP

Transketolase and Transaldolase

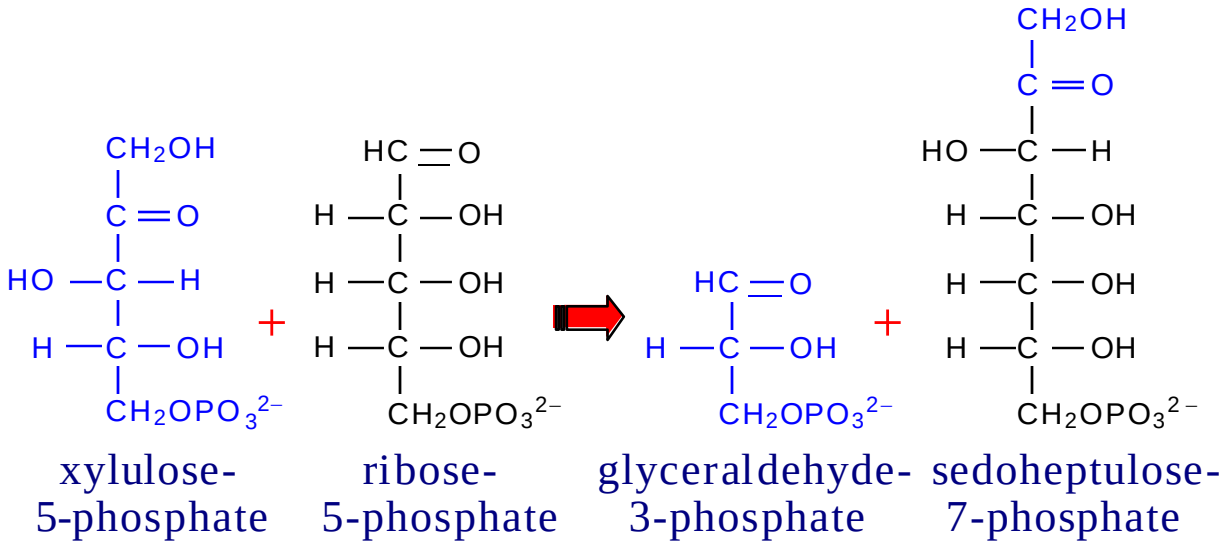
- **Catalyzes:** Transfer of 2-C or 3-C molecular fragments respectively, in each case from a **ketose donor** to **an aldose acceptor**
  - **D. E. Nicholson** has suggested that the names of these enzymes should be changed, since Transketolase actually transfers **an aldol moiety** (glyceraldehyde)
  - Transaldolase transfers **a ketol moiety** (dihydroxyacetone). However, the traditional enzyme names are used here.

Non-oxidative Phase of PPP

Transketolase

Transketolase transfers a 2-C fragment from **xylulose-5-P** to either **ribose-5-P or erythrose-4-P**

- Transketolase utilizes as prosthetic group **Thiamine pyrophosphate (TPP),** a derivative of Vit. B1



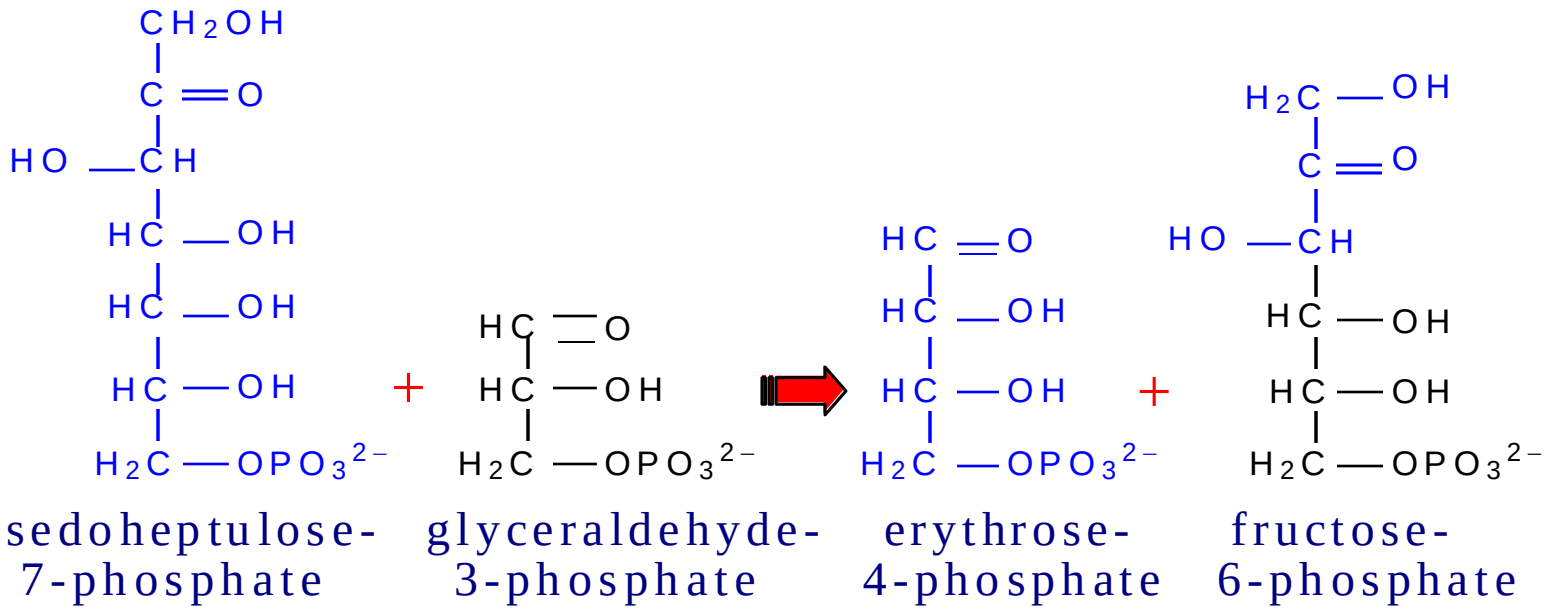
Thiamine Deficiency Diagnosis

Blood Transketolase Activity: One of the primary applications of transketolase lies in its ability to serve as an indicator of thiamine (vitamin B1) deficiency. Since transketolase requires thiamine as a cofactor for proper function, measuring its activity in red blood cells provides valuable insight into a patient's thiamine status. Reduced transketolase activity signifies a potential thiamine deficiency.

Non-Oxidative Phase of PPP

Transaldolase

Transaldolase catalyzes transfer of a 3-C dihydroxyacetone moiety from Sedoheptulose-7-phosphate to GA-3-phosphate



Non-oxidative Phase of PPP

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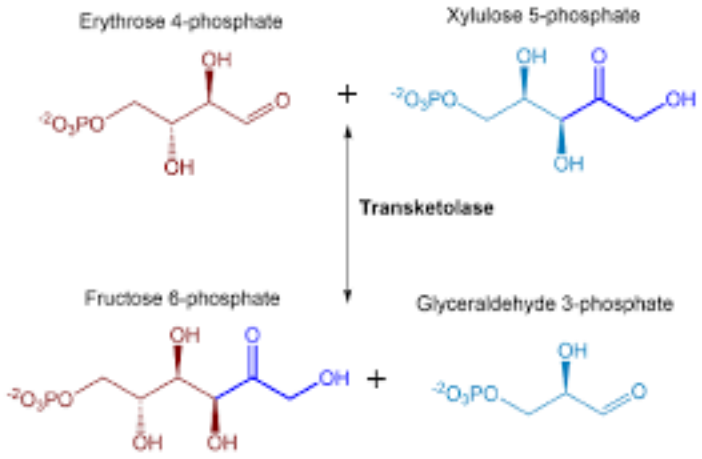
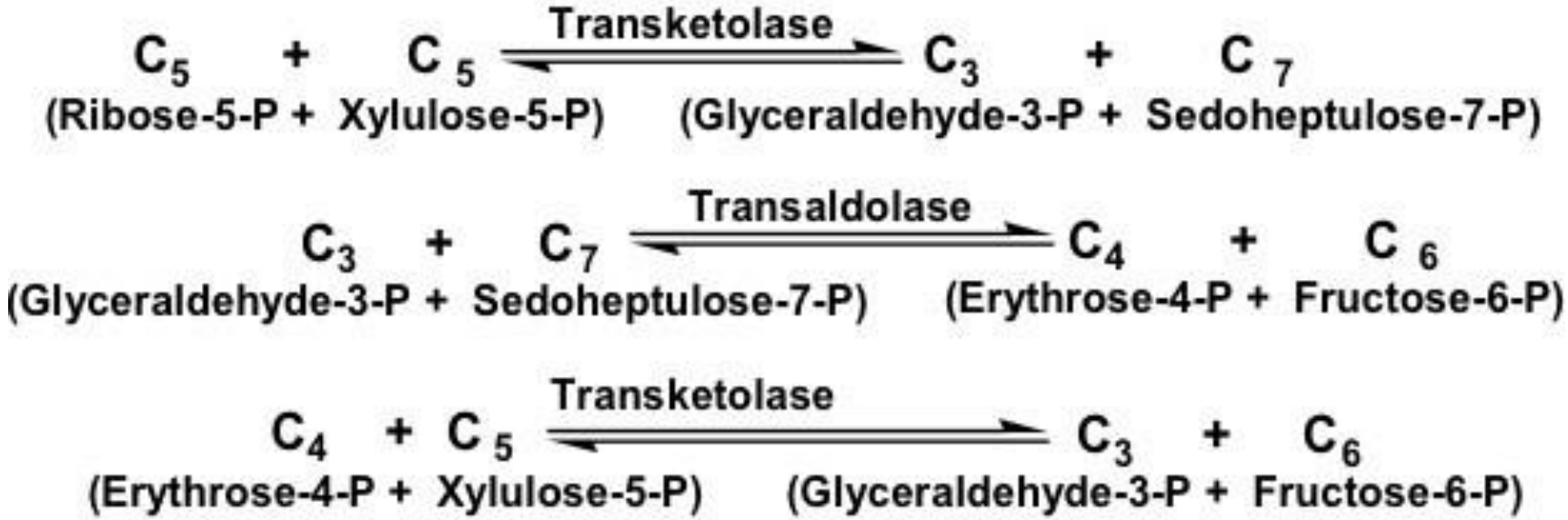


Balance sheet for flow of 15 C atoms through PP Shunt

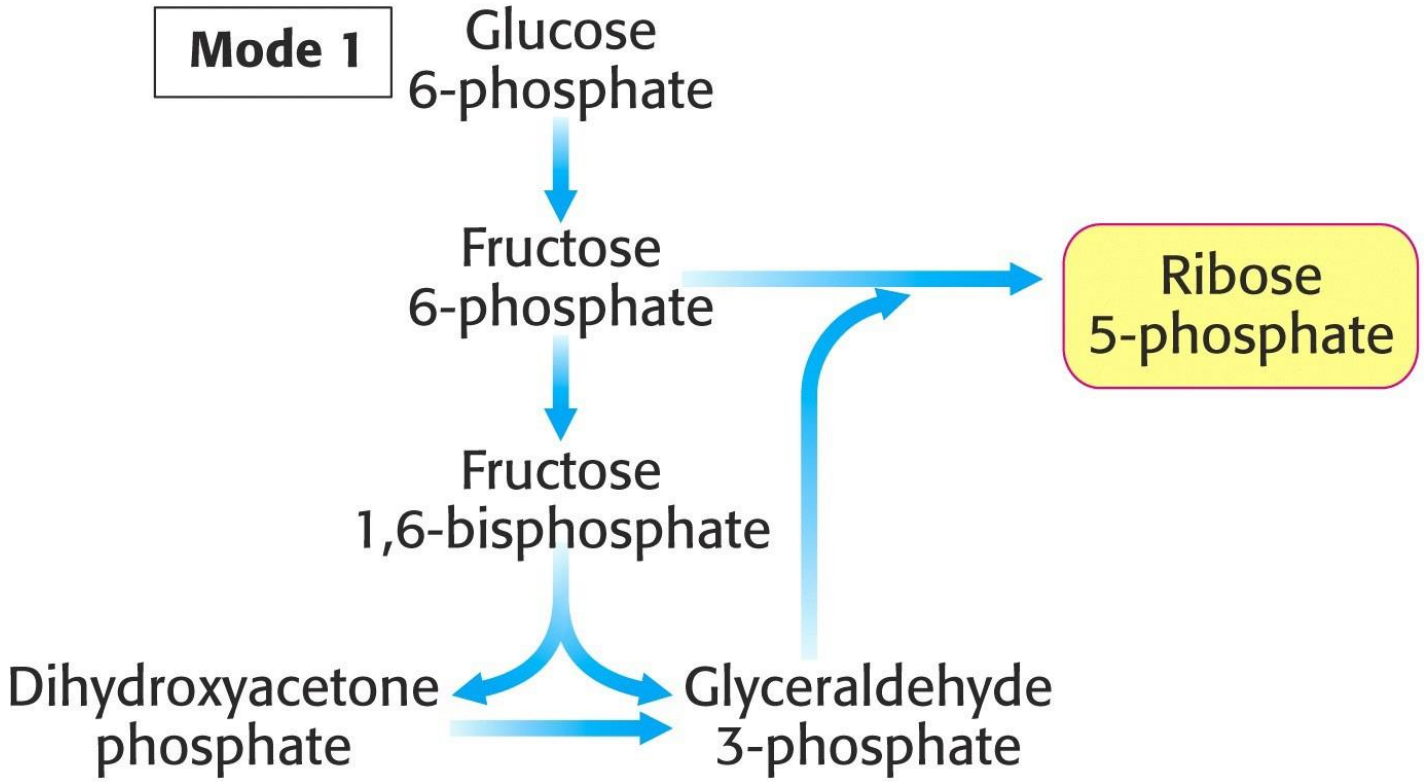


$3\ C_5 \longrightarrow 2\ C_6 + C_3$ (Overall)

Glc-6-phosphate may be regenerated from either the: 3-C GA-3-phosphate or 6-C Fru-6-phosphate via enzymes of Gluconeogenesis

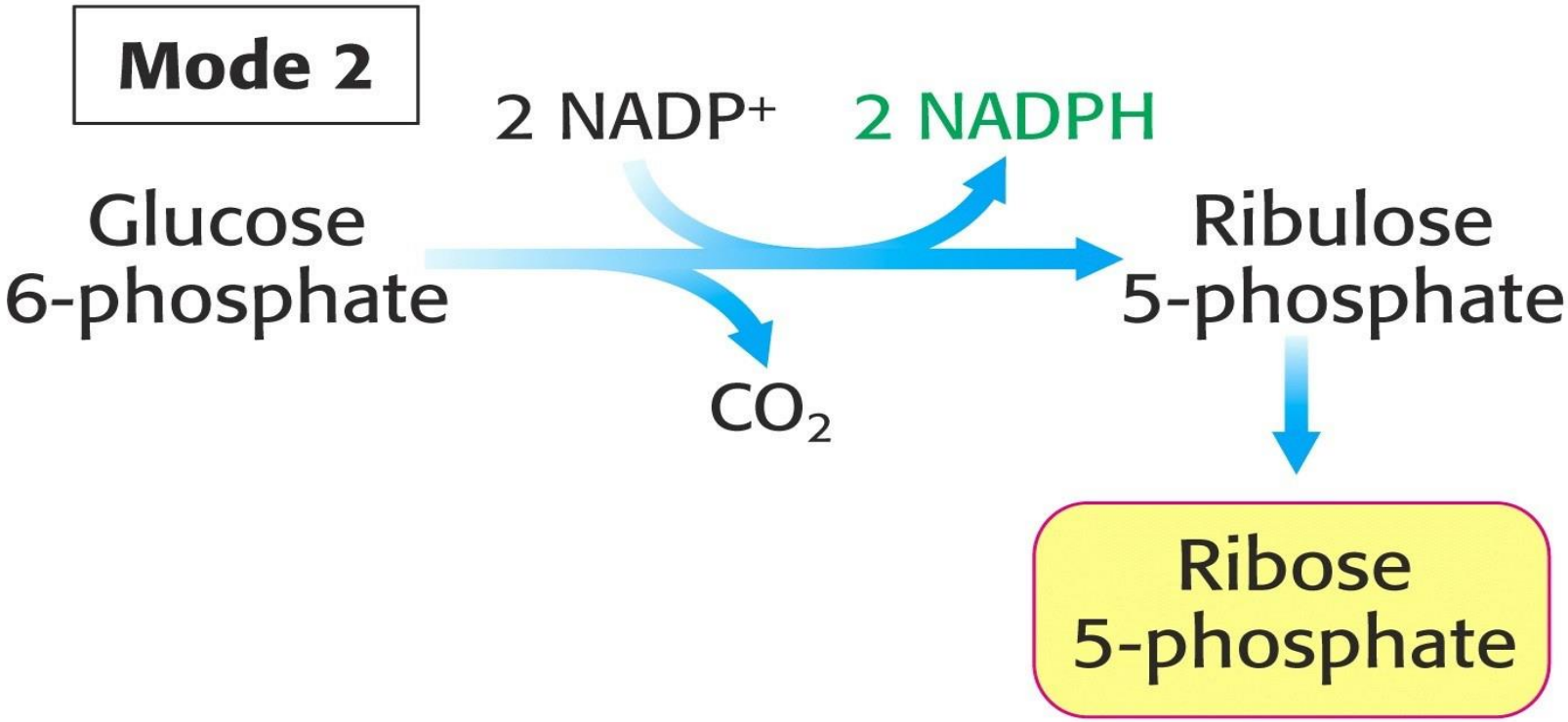
Cellular needs dictate the direction of PPP

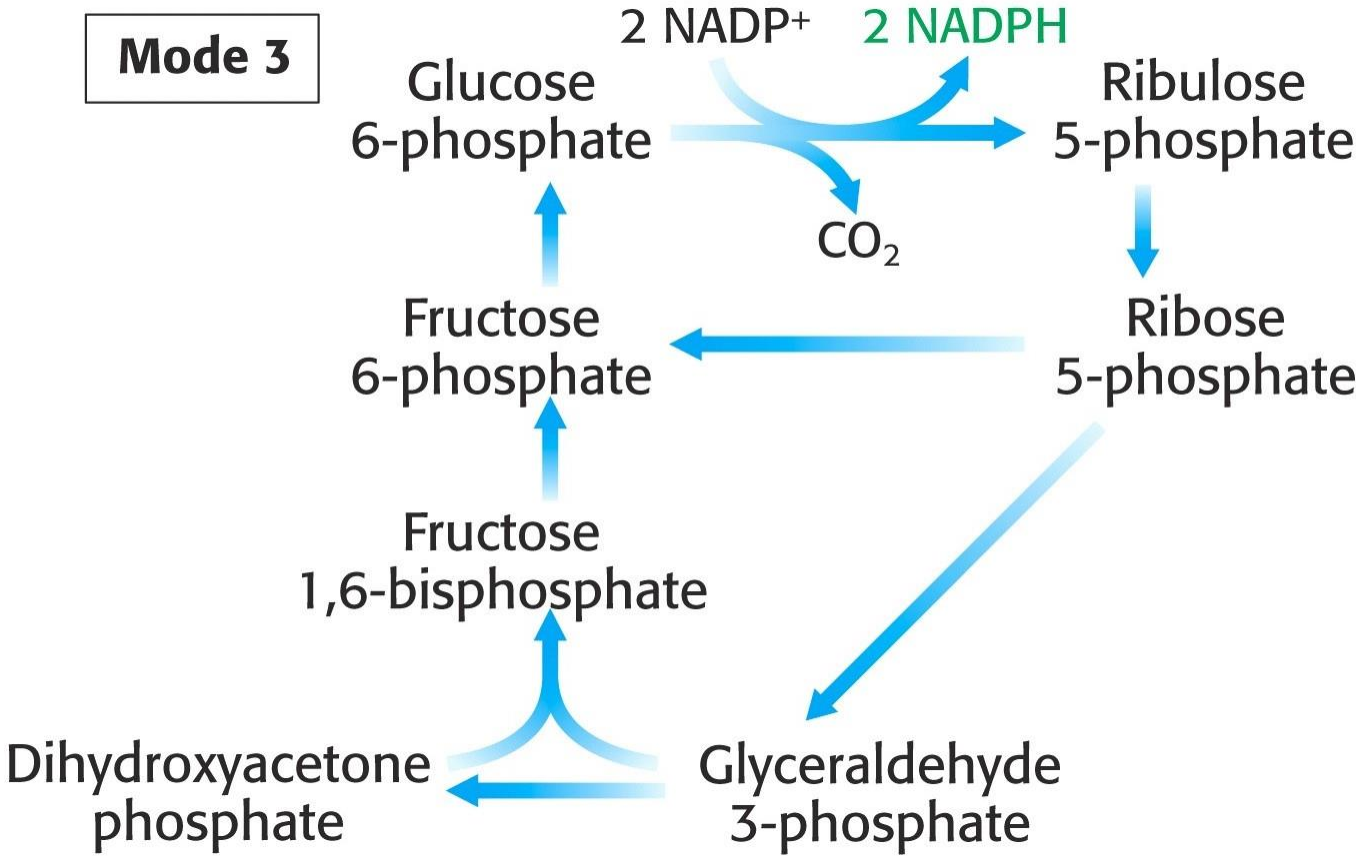
Depending on needs of a cell for **Ribose-5-phosphate, NADPH, & ATP, t**he PP Pathway can operate in various modes, to maximize different products



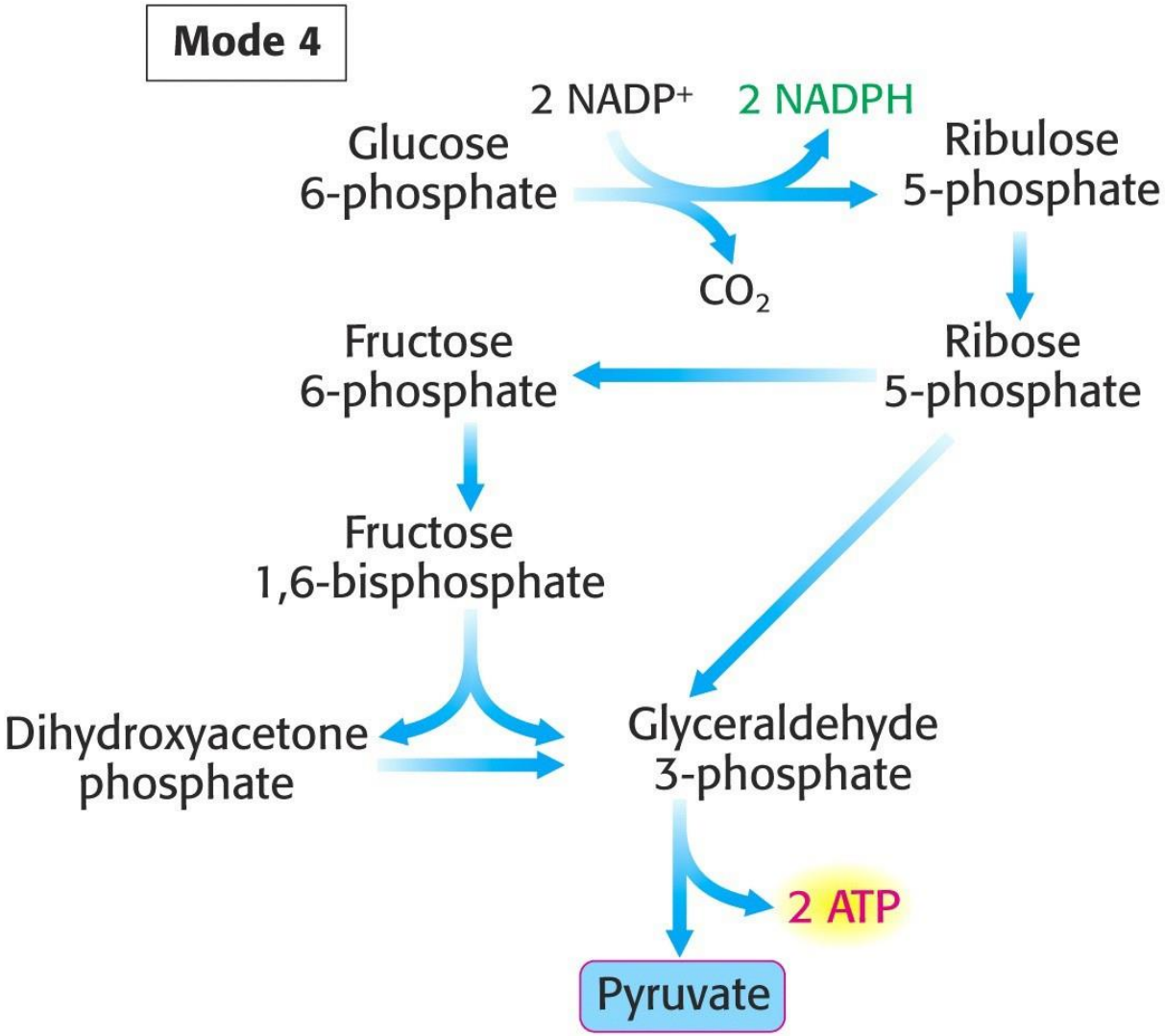
In tissues requiring **ribose-5-phosphate** only **(rapidly dividing cells):** by the **reversal of the non-oxidative** phase utilizing fru-6-P & GA-3-P from glycolysis the net equation is as follows: 5 Glc-6-P + ATP ↔6 Ribose-5-P + ADP

The need for NADPH and ribose 5-phosphate is balanced (liver cells).





More **NADPH** is needed than ribose 5-phosphate; Fatty acid synthesis in adipose cells.

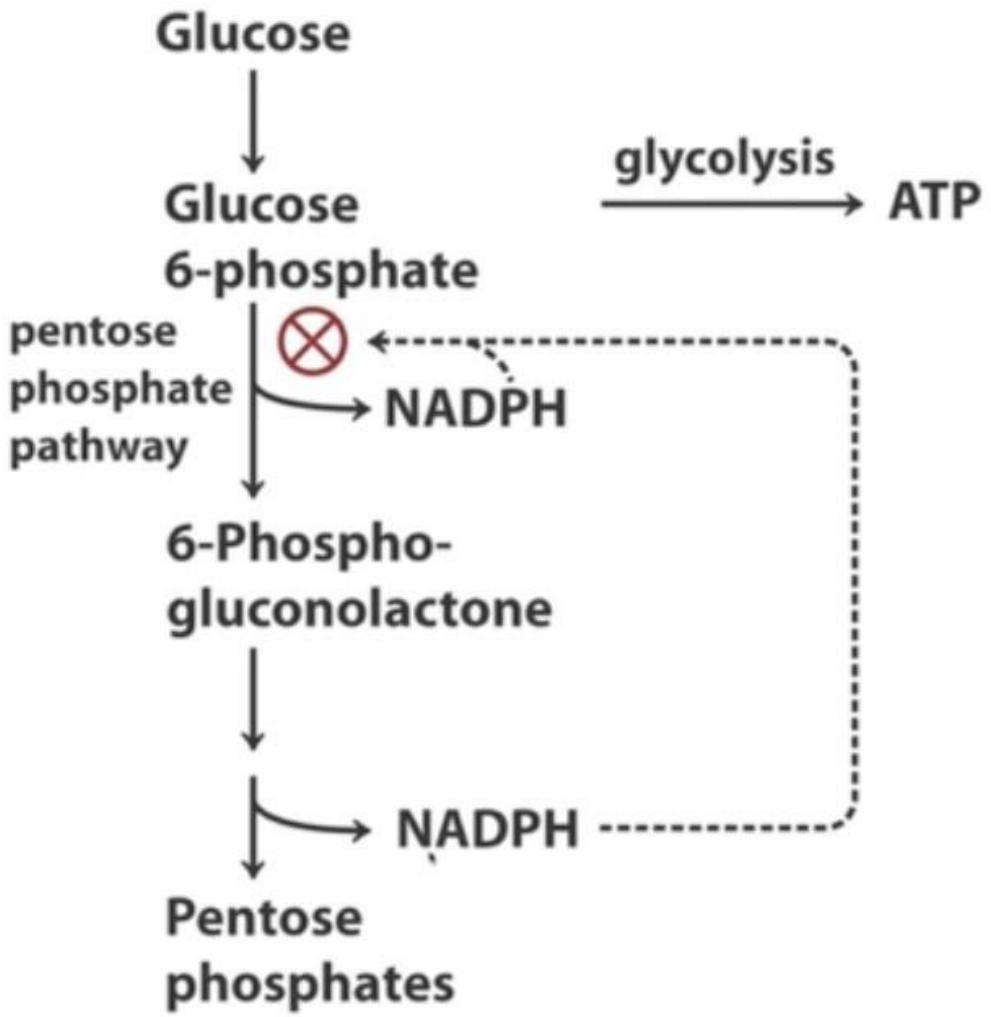


The cell needs both NADPH and ATP

Regulation of Glucose -6-Phosphate Dehydrogenase (G6PD)

G6PD is the committed step of the PP Pathway is regulated by availability of the substrate **$NADP^+$**

- As NADPH is utilized in reductive synthetic pathways, the increasing [$NADP^+$] stimulates the PP Pathway, to replenish NADPH
- The rest of the pathway converts Ribulose-5-P to: Ribose-5-P, or GA-3-P & Fru-6-P
- Additional enzymes include Isomerase, Epimerase, Transketolase, &Transaldolase



Regulation of Glucose -6-Phosphate Dehydrogenase (G6PD)

- Insulin (major activator in Liver & Adipose tissue)
  - Increases G6PD gene transcription .
  - Enhances NADPH availability for lipogenesis (fatty acid/cholesterol synthesis).
  - Stimulates G6PD during the fed state when glucose is abundant.
- Glucagon (Liver & Adipose tissue)
  - Suppresses G6PD gene expression via cAMP-PKA signaling.
  - During fasting, glucagon inhibits lipogenesis and shifts metabolism toward gluconeogenesis & glycogenolysis, reducing G6PD activity.
- Epinephrine (muscle & liver)
  - Activates PKA, which reduces G6PD expression.
  - Prioritizes glucose metabolism for energy production (glycolysis & glycogen breakdown) instead of NADPH production.
- Thyroxine (T4) & Triiodothyronine (T3) increase G6PD activity by stimulating lipogenesis and oxidative metabolism.
- Higher G6PD activity enhances NADPH production, supporting lipid synthesis & antioxidant defense.

NAPH as reducing agents

Reductive synthesis

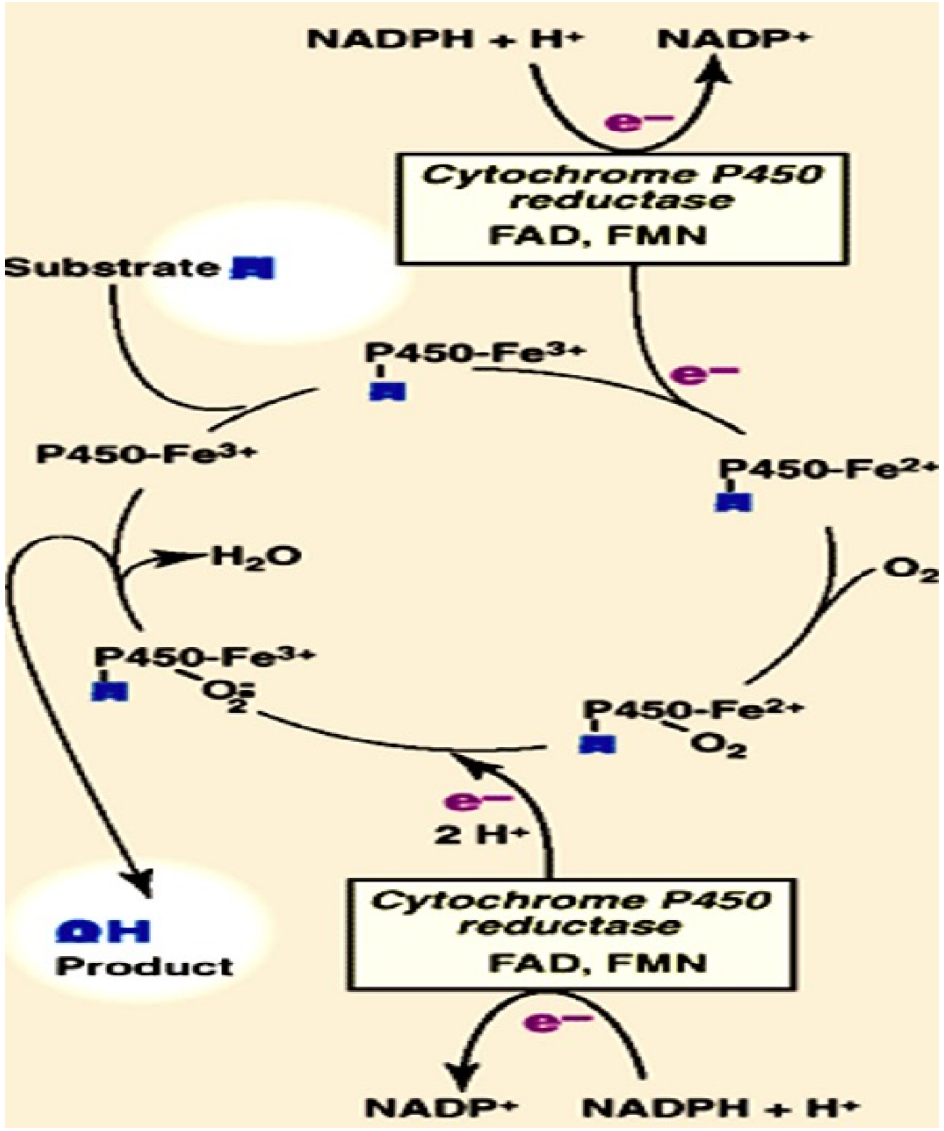
- Fatty acid synthesis
- Fatty acid chain elongation
- Cholesterol synthesis
- Neurotransmitters synthesis
- Nucleotide synthesis
- Superoxide synthesis

Pathways that require NADPH

Pathways that require NADPH

NADPH is used for Detoxification

- The Cytochrome P450 Monooxygenase System (CYP450) is a crucial enzyme system involved in detoxification, drug metabolism, and biosynthesis of hormones and lipids. It is found in the liver, intestines, lungs, and other tissues
- Cytochrome P450 enzymes require **NADPH** as an **electron donor** to drive oxidation reactions
  - Electrons move from NADPH to FAD to FMN then to the heme iron



Production of ROS During the Phagocytic Respiratory Burst by Activated Neutrophils.

(1)Activation of NADPH oxidase on the outer side of the plasma membrane initiates the respiratory burst with the generation of superoxide.

During phagocytosis, the plasma membrane invaginates, so superoxide is released into the vacuole space.

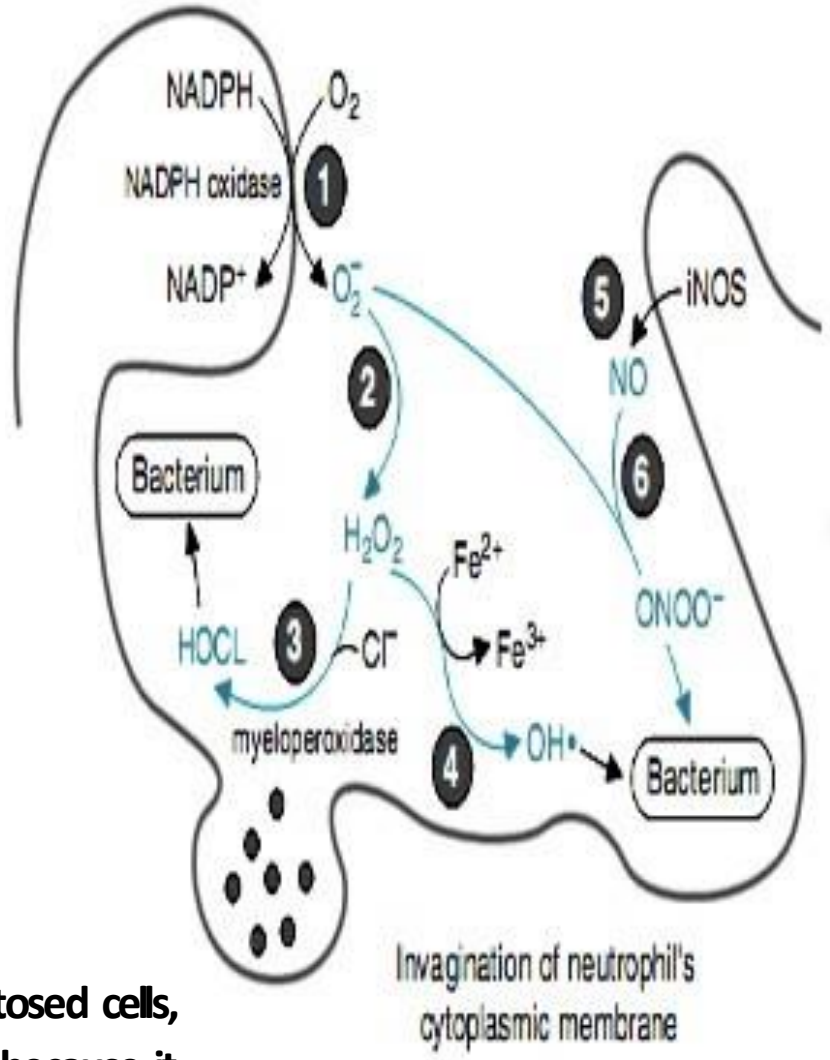
(2)Superoxide (either spontaneously or enzymatically via superoxide dismutase [SOD]) generates H_2O_2.

(3)Granules containing myeloperoxidase are secreted into the phagosome, where myeloperoxidase generates HOCl and other halides.

(4)H_2O_2 can also generate the hydroxyl radical from the Fenton reaction.

(5)Inducible nitric oxide synthase may be activated and generate NO.

(6)Nitric oxide combines with superoxide to form peroxynitrite, which may generate additional RNOS.



The result is an attack on the membranes and other components of phagocytosed cells, and eventual lysis. The whole process is referred to as the respiratory burst because it lasts only 30 to 60 minutes and consumes O2.

Glutathione and Erythrocytes

- GSH is extremely important particularly in the highly oxidizing environment of the red blood cell.
- Mature RBCs have no mitochondria and are totally dependent on NADPH from the pentose phosphate pathway to regenerate GSH from GSSG via glutathione reductase.
- In fact, as much as 10% of glucose consumption, by erythrocytes, is mediated by the pentose pathway.
- The reduced form of glutathione serves as a sulfhydryl buffer.
- It maintains cysteine residues in hemoglobin and other proteins in a reduced state.
- GSH is essential for normal RBC structure and keeping hemoglobin in Fe^{++} state

- Reduced glutathione also detoxifies peroxides.

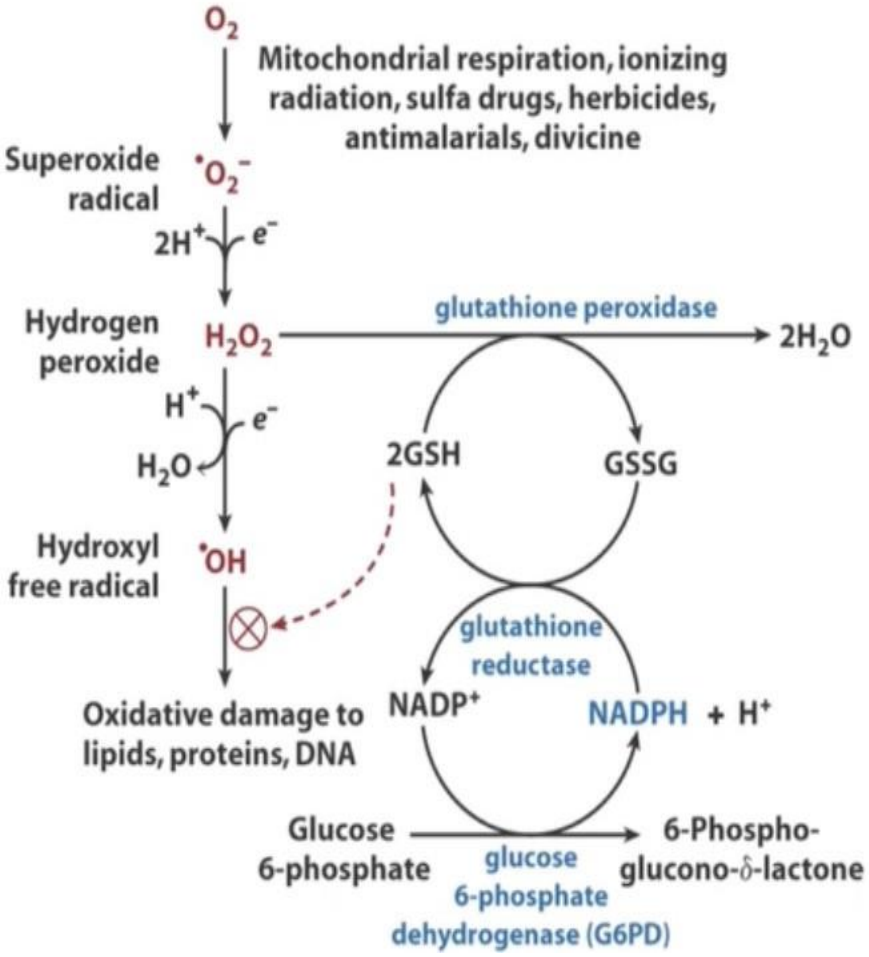
  $2GSH + ROOH \rightarrow GSSG + H_2O + ROH$
- Cells with low levels of GSH are susceptible hemolysis.
- Individuals with reduced level of GSH are subject to hemolysis.
- This is often clinically seen as black urine under certain conditions.

Pathways that require NADPH

NADPH as antioxidants

- **Reduction of hydrogen peroxide**
  - Formation of reactive intermediates from molecular oxygen
  - Actions of antioxidant enzymes

✓G-SH = reduced glutathione; G-S-S-G = oxidized glutathione



So, what happens if glucose 6-phosphate DH is defective?

Insufficient production of NADPH.

Which translates into insufficient glutathione.

Is this a medical problem?
YES

Glutathione and Erythrocytes

- Regeneration of reduced glutathione requires; NADPH, produced within RBCs in the PP shunt
- Glutathione Reductase catalyzes:
  - $GSSG + NADPH + H^+ \longrightarrow 2\ G\text{-}SH + NADP^+$
  - Genetic deficiency of G6PD can lead to; **Hemolytic anemia** , due to inadequate [NADPH] within RBCs
  - The effect of partial deficiency of G6PD is exacerbated by substances that lead to increased production of reactive oxygen species (ROS) e.g., the antimalarial drug **(Primaquine)**

Glutathione and Erythrocytes

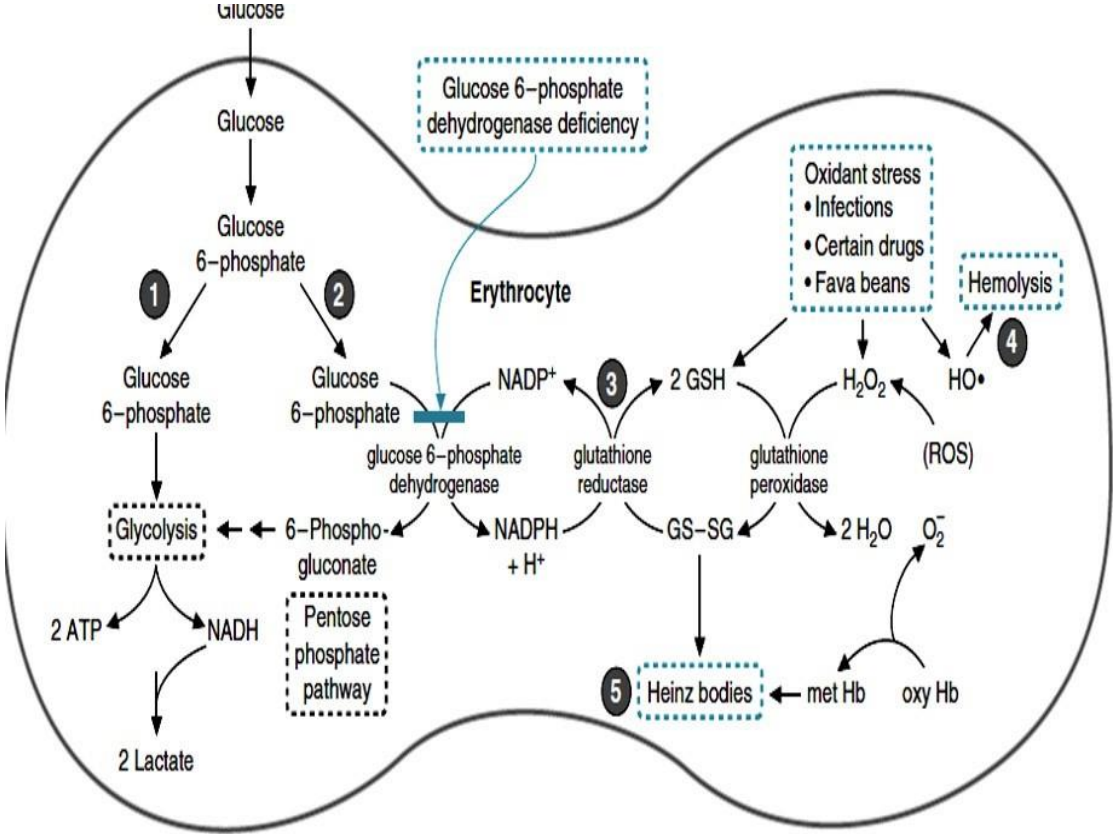
The glutathione defense system is compromised by

- G6PD deficiency
- Infections
- Certain drugs
- The purine glycosides of fava beans

As a consequence,

Heinz bodies, aggregates of cross-linked hemoglobin, form on the cell membranes and subject the cell to mechanical stress as it tries to go through small capillaries

- The action of the ROS on the cell membrane as well as mechanical stress from the lack of deformability , result in **Hemolysis**

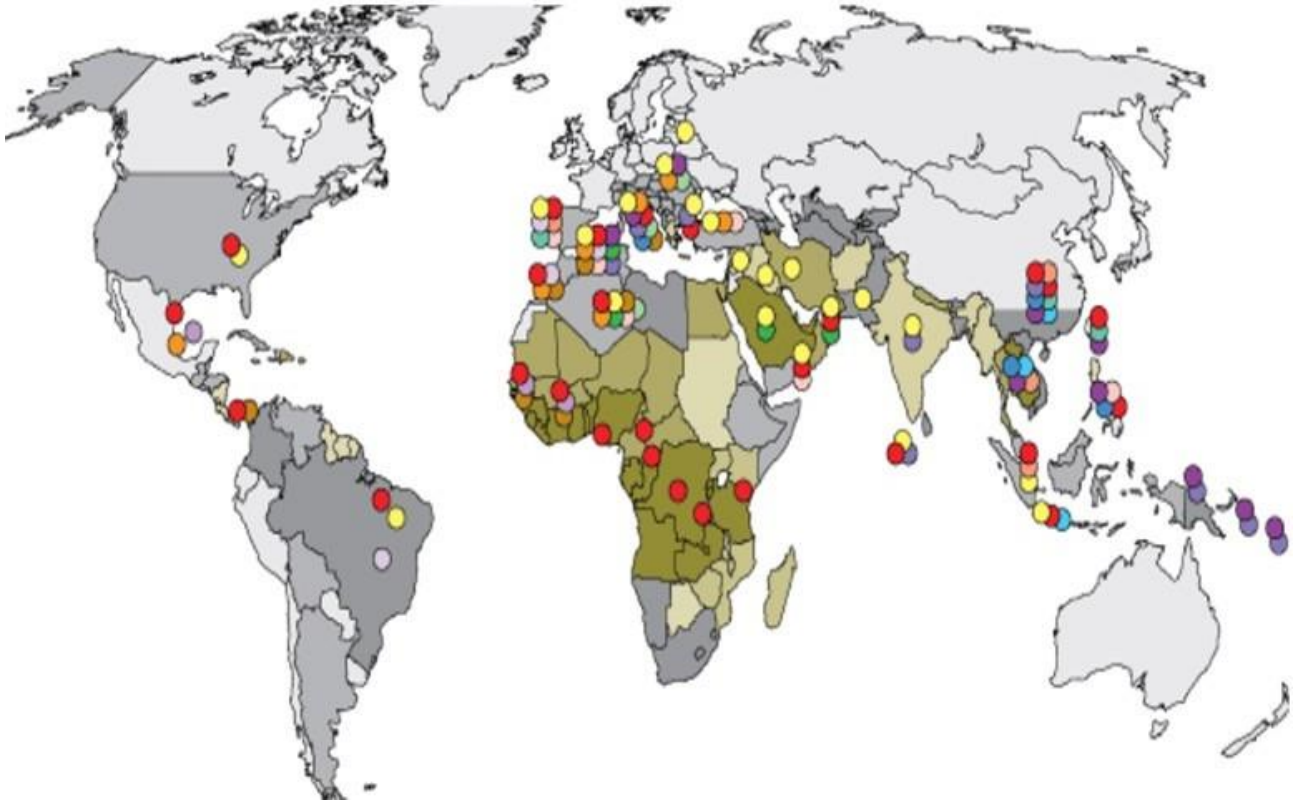


Hemolysis caused by reactive oxygen species

Glucose 6—phosphate deficiency

- The most common disease-producing enzyme abnormality in humans, affecting more than 200 million individuals worldwide
- This deficiency has the highest prevalence in the: Middle East, tropical Africa & Asia, & parts of the Mediterranean
- **G6PD deficiency is X-linked**, and is, in fact, a family of deficiencies caused by more than 400 different mutations in the gene coding for G6PD, only some of these mutations cause clinical symptoms
- The life span of many individuals with G6PD deficiency is somewhat shortened as a result of complications arising from chronic hemolysis

Glucose 6—phosphate deficiency



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- The different shadings indicate increasingly high levels of prevalence, up to about 20%;
- The different colored symbols indicate individual genetic variants of G6PD, each one having a different mutation.

Glucose 6—phosphate deficiency

- Almost all G6PD variants are caused by; point mutations in the G6PD gene
- Some mutations do not disrupt the structure of the enzyme's active site &, hence, do not affect enzymic activity

However, many mutant enzymes show altered kinetic properties. For e.g., variant enzymes may show:

- Decreased catalytic activity,
- Decreased stability, or
- An alteration of binding affinity for $NADP^+$, NADPH, or glucose 6-phosphate

I (Severe)	Chronic non-spherocytic hemolytic anemia	Chronic fatigue, weakness, jaundice
II (Variants with variable expression)	Spectrum of variants with varying severity	May not experience symptoms, or experience acute hemolytic anemia triggered by specific factors
III (Favism)	Primarily associated with fava bean consumption	Acute hemolytic anemia after ingesting fava beans or inhaling its pollen
IV (African-American variant)	Prevalent in individuals of African descent	Less severe acute hemolytic anemia compared to other variants
V (Mediterranean variant)	Common in people of Mediterranean ancestry	Moderate acute hemolytic anemia

Decline of RBC G6PD activity with cell age for the three most commonly encountered forms of the enzyme.

- The RBCs contain an unstable, but kinetically normal G6PD, with most of the enzyme activity present in the reticulocytes & younger erythrocytes
- The oldest cells, therefore, have the lowest level of enzyme activity, are preferentially removed in a hemolytic episode

Glucose 6—phosphate deficiency

Acute HA can develop as a result of 3 types of triggers:

- Oxidant drugs
- Infections
- Fava beans

In response to infectious agents and other stimuli, phagocytic cells of the immune system: neutrophils, eosinophils, & monocytes/macrophages exhibit a rapid consumption of O_2 called the respiratory burst
The respiratory burst is a major source of superoxide, hydrogen peroxide, the hydroxyl radical, hypochlorous acid (HOCl), & RNOS
The generation of free radicals is part of the human antimicrobial defense system intended to destroy invading microorganisms; tumor cells, & other cells targeted for removal

Oxidant drugs: commonly used drugs that produce HA in patients with G6PD deficiency are best remembered from the mnemonic AAA:

- Antibiotics (sulfamethoxazole & chloramphenicol),
- Antimalarials (primaquine but not quinine),
- Antipyretics (acetanilid but not acetaminophen)

The Mediterranean plant Vicia faba is a source of fava beans that contain the purine glycoside vicine.

Thank you