Pentose Phosphate Pathway

(AKA Hexose Monophosphate Shunt)

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OUTLINE:

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    II. Tissue specificity
2. Pathway
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    II. Non-oxidative phase
3. Cellular Scenarios; When does the cell use the pentose phosphate pathway?
4. Glucose-6-phosphate dehydrogenase
    I. Mechanism
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5. Functions of NADPH
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    II. Generation of reduced glutathione
        A. Reaction
        B. Functions of reduced glutathione
            i. Protection against ROS
            ii. Maintain general reducing conditions
                a. Iron redox state (Methemoglobin)
                b. Proper folding of proteins (Heinz bodies)
                c. Hemolytic anemia and G6PD deficiency
            iii. Detoxification of drugs
    III. Production of ROS as protection against pathogens

LEARNING OBJECTIVES:

After studying this unit, you should be able to:

1. Compare and contrast the primary metabolic functions of glycolysis to the pentose phosphate pathway (PPP).
2. List molecules that require ribose-5-phosphate for their biosynthesis.
3. List molecules that require NADPH for their biosynthesis.
4. Compare and contrast the role of NAD+/NADH to NADP+/NADPH.
5. Name the two phases of the PPP.
6. Identify the gatekeeper of the PPP and know the reaction that it catalyzes.
7. Name the primary biomolecules generated from each of the phases of the PPP.

9. Identify points of convergence and related enzymes of the non-oxidative phase of the pentose phosphate pathway and glycolysis.
10. Understand how thiamine deficiency relates to the PPP and can lead to beri-beri disease.
11. Rationalize lab results from a thiamine deficiency test.
12. Describe two distinct scenarios where the cell would drive PPP and then feed back into glycolysis.
13. Describe a scenario where the cell would utilize the PPP and then choose not to feed back into glycolysis.
14. Describe a scenario where the cell would use the non-oxidative pathway without the oxidative phase.
15. Identify an evolutionary benefit to a deficiency in PPP enzymes, particularly G6PD.
16. Explain how the pentose phosphate pathway affects oxidative stress within the cell.
17. Draw the basic conversion of oxidized to reduced glutathione using NADPH.
18. Explain how oxidative conditions can lead to hemolytic anemia.
19. List four triggers that exacerbate hemolytic anemia for patients with G6PD deficiencies.
20. Describe the test and treatments for G6PD deficiency.
21. Link Covid-19 to the PPP.
22. Describe how the pentose phosphate pathway both intensifies and neutralizes harmful acetaminophen biproducts.
23. Identify treatments for acetaminophen overdose.
24. Explain the role of NADPH in chronic granulomatous disease.

READING REFERENCE:

1. Marks' Basic Medical Biochemistry: A Clinical Approach, 5th edition; Chapter 27 https://meded.lwwhealthlibrary.com/content.aspx?sectionid=249266913&bookid=2170

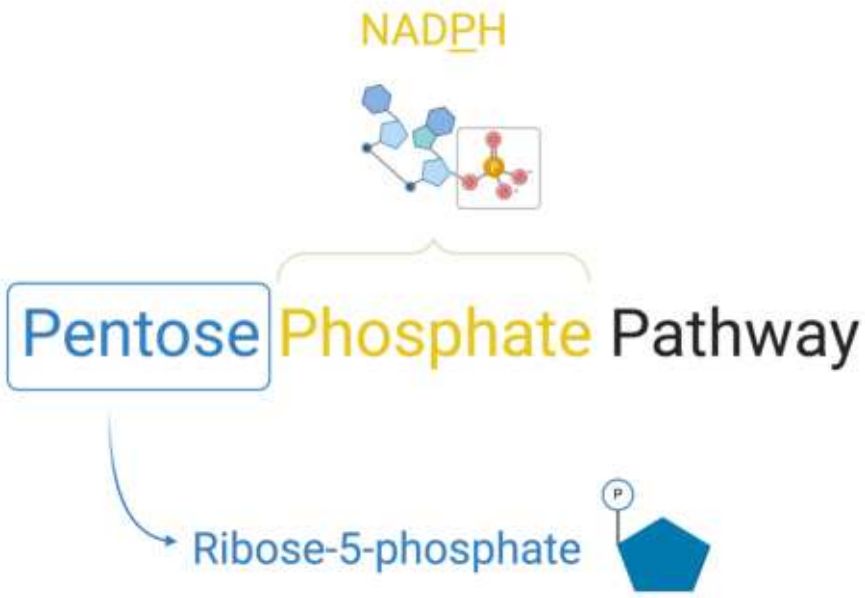
ACKNOWLEDGEMENTS:

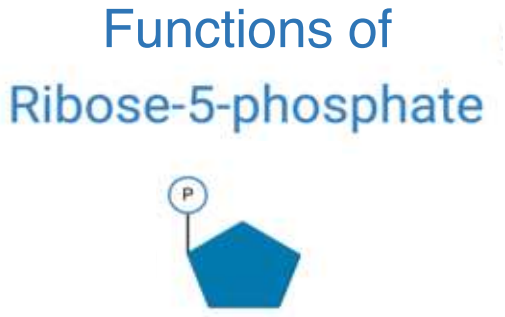
Special thanks to Dr. Chris Davies for providing the syllabus template. Many of the figures featured in this lecture were generated using Biorender.com.

1. Intro

I. Overview of primary products and their functions

The pentose phosphate pathway (also called the hexose monophosphate shunt) is a series of reactions that feeds off of glycolysis using glucose-6-phosphate as an initiating molecule and reenters glycolysis by generating fructose-6-phosphate and glyceraldehyde-3-phosphate. The pentose phosphate pathway becomes active for three primary reasons; the cell needs NADPH, the cell needs ribose-5-phosphate, or the body has ingested sugars that can use the pentose phosphate pathway to feed those sugars back into glycolysis to produce energy. Therefore, the primary products of the pentose phosphate pathway are NADPH and ribose-5-phosphate.





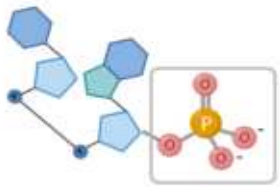
-Synthesis of nucleic acids: both DNA and RNA

-Synthesis of cofactors: ATP, NAD, NADP, FAD & CoA

-Interconversion of 3C, 4C, 5C, 6C & 7C sugars:
-Conversion of pentose sugars into glycolytic intermediates (including pentoses from diet)

Ribose-5-phosphate is one of the main products of the pentose phosphate pathway and is a required building block for nucleic acids, both ribonucleic acids (RNA) and deoxyribonucleic acids (DNA). Because nucleotides (particularly adenosine) are building blocks for many cofactors, ribose-5-phosphate is also a critical building block for the cofactors listed above.

Functions of NADPH



Nicotinamide Adenine Dinucleotide Phosphate

-Reductive biosynthesis of fatty acids, steroids, cholesterol, neurotransmitters, deoxynucleotides

-Production of reduced glutathione to protect against oxidative stress and for detoxification of drugs

-Production of ROS for host defense

NADPH is an important cofactor for many redox reactions in the cell. Various synthetic pathways of large, energy rich biomolecules require NADPH. Redox reactions within the synthesis pathways of fatty acids, steroids, cholesterol, neurotransmitters and deoxynucleotides all use NADPH. Additionally, NADPH is essential for maintenance of cellular reduced glutathione stores. Reduced glutathione is a primary antioxidant used to neutralize reactive oxygen species and combat oxidative stress. Furthermore, reduced glutathione can be used to neutralize a toxic byproduct of acetaminophen. Lastly, despite its role in neutralizing reactive oxygen species, NADPH can be used to generate ROS in specific situations such as phagocytes defending our bodies against incoming pathogens.

NAD+/NADH	NADP+/NADPH
Involved in catabolic reactions (degradation of larger biomolecules into smaller ones)	Involved in anabolic reactions (building smaller biomolecules into larger, energy rich ones)
NADH is less abundant in the cell than NAD+. The ratio: NAD+ > NADH (~700:1 in the cytoplasm)	NADPH is more abundant in the cell than NADP+. The ratio: NADP+ < NADPH (~0.005 in the cytoplasm)
-NAD+ is valued by the cell as an oxidizing agent (electron acceptor) particularly in glycolysis.	-NADPH is valued by the cell as a reducing agent (electron donor) for glutathione and other biosynthetic processes.

NAD+/NADH and NADP+/NADPH have nearly identical structures, apart from the addition of a phosphate group for NADP+, and yet these molecules are used in very different ways by the cell. The table above contrasts the cellular usages for NAD+/NADH versus NADP+/NADPH.

II. Tissue specificity

The pentose phosphate pathway is required in all cell types to generate NADPH and ribose-5-phosphate; however, some cell types require the pentose phosphate pathway more than others due to their specific functions as seen above. Erythrocytes (red blood cells) are the classic example of dependence on the PPP for NADPH due to their lack of mitochondria.

Tissues with highly active pentose phosphate pathways:

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

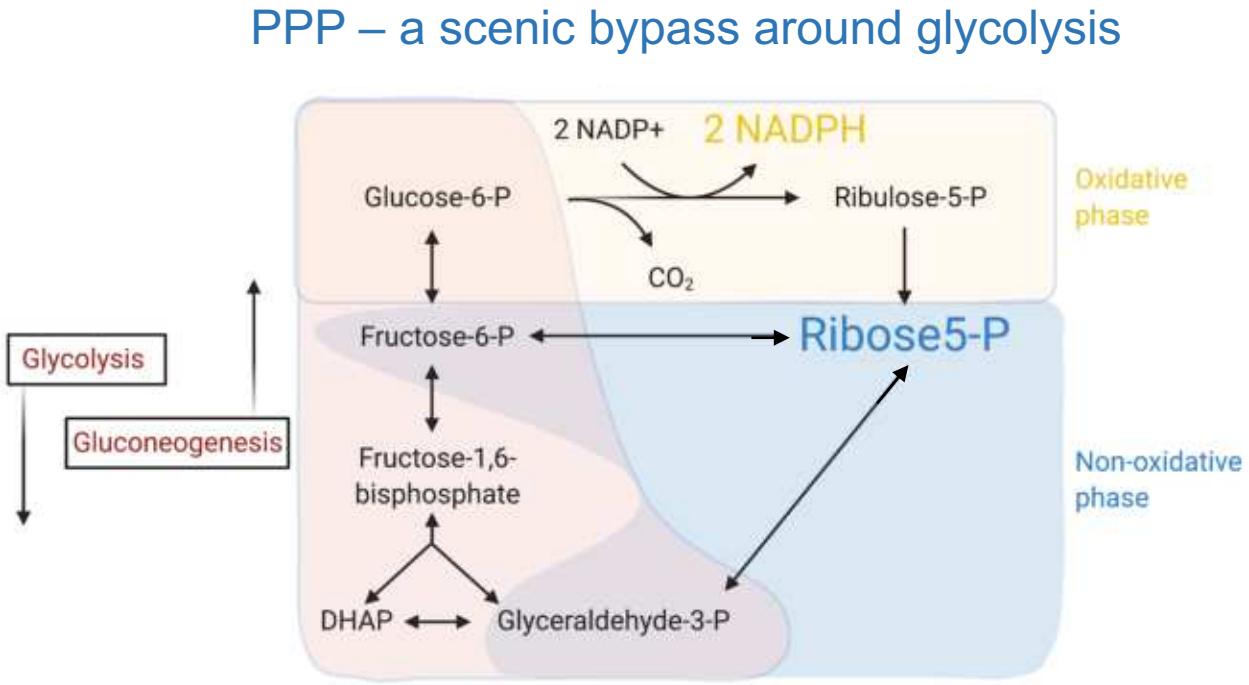
Modified from Marks; Biochemistry Ch 27

-NADPH can be generated by the PPP and select primarily mitochondrial reactions.
Red blood cells lack mitochondria and therefore rely heavily on the PPP for NADPH production.

Some PPP bullet points

- Common to all organisms
- Cytosolic
- Present in all cells
- Major source of NADPH in most cells
- Very active in tissues involved in biosynthesis (steroids, cholesterol, fatty acids)
- Very important for erythrocytes to maintain reducing conditions
- Important also for phagocytic cells where NADPH oxidase uses NADPH to generate superoxides

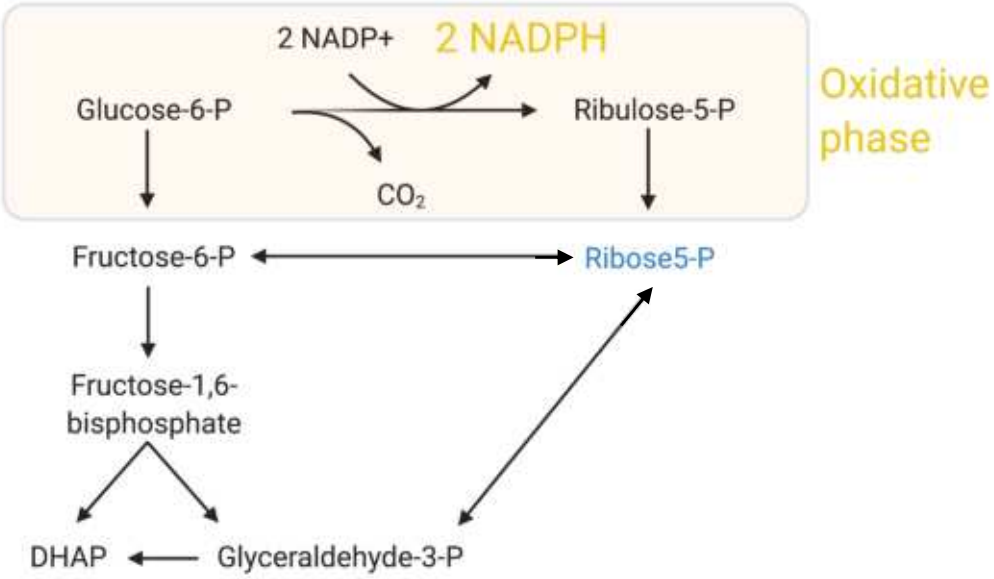
2. Pathway



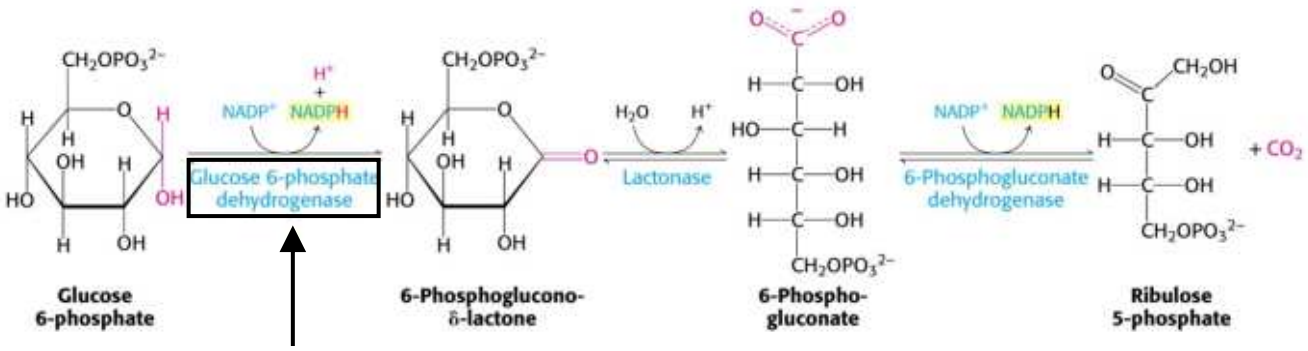
This is an overview of the two phases of the pentose phosphate pathway. The first is the oxidative phase that branches off of glycolysis from glucose-6-phosphate and generates 2 molecules of NADPH and CO_2 as the sugar is oxidized to ribulose-5-phosphate. Ribulose-5-phosphate is then converted to xylulose-5-phosphate or ribose-5-phosphate to initiate the non-oxidative phase. The non-oxidative phase shuffles carbons between different sugar molecules to regenerate the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate.

I. Oxidative phase

The oxidative phase highlighted below uses three reactions to convert glucose-6-phosphate into ribulose-5-phosphate. Two of these reactions are redox reactions, oxidizing the sugar molecules and in turn reducing two molecule of NADP+ to NADPH.



Oxidative phase: The oxidation of G6P to produce NADPH



G6PD deficiency occurs in ~7% of the world's population.

Net Yield: 1 G6P → 2 molecules of NADPH

-For all intents and purposes, this is an irreversible process.

Three reactions convert glucose-6-phosphate into ribulose-5-phosphate in the oxidative phase and create two molecules of NADPH and one molecule of CO_2 in the process.

STEP 1:
The first reaction is an oxidation reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PD), which is the key regulatory enzyme of the pathway. G6PD oxidizes the pink hydroxyl shown in glucose-6-phosphate to the carbonyl (C=O) in pink in 6-phosphoglucono-delta-lactone. The electrons from this oxidation process are transferred to NADP+ along with protons to generate the first molecule of NADPH.

Interestingly, G6PD is the most common enzyme deficiency found in the world's population with approximately 7% of humans having mutations in this enzyme.

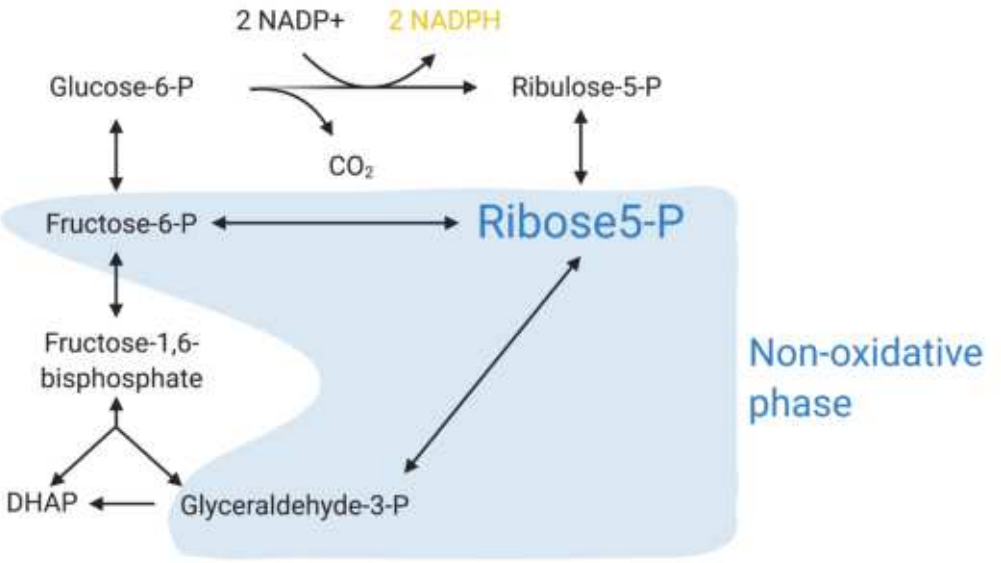
STEP 2:
Lactonase then opens the ring structure by hydrolyzing the ester bond between C5 and C1, generating 6-phosphogluconate.

STEP 3:
The last reaction is an oxidative decarboxylation reaction catalyzed by 6-phosphogluconate dehydrogenase. The sugar is oxidized to ribulose-5-phosphate and loses the carboxyl group highlighted in pink as carbon dioxide. The electrons harvested in this oxidative process are transferred to NADP+ to generate NADPH.

These steps are considered irreversible unlike the non-oxidative phase that we will talk about next.

II. Non-oxidative phase

Non-oxidative phase: Carbon shuffling



The non-oxidative phase is all about carbon shuffling to re-enter glycolysis. First, ribulose-5-phosphate must be converted to ribose-5-phosphate and xylulose-5-phosphate. Then, we use these two molecules as a starting point to shuffle carbons, generating various lengths of sugar molecules until we create 2 molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate. Both of these molecules can re-enter glycolysis for energy production.

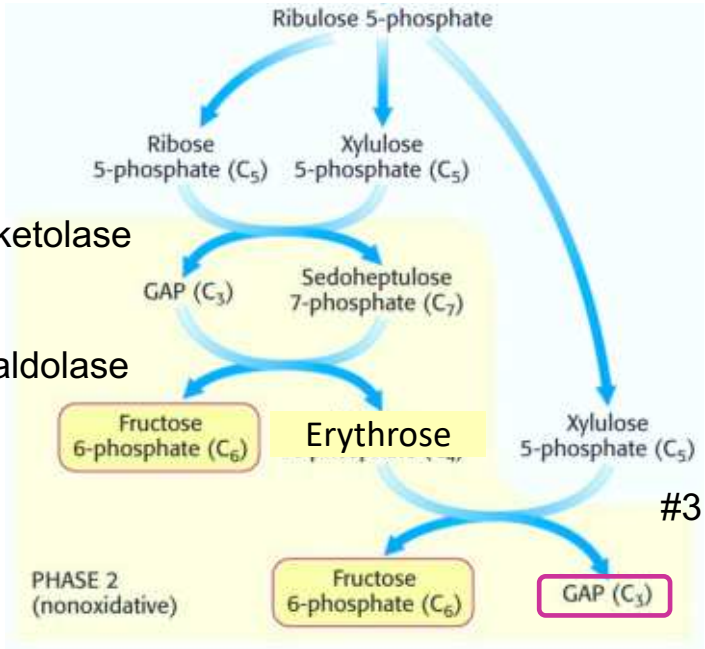
$C_5 + C_5$

⇩ #1 transketolase

$C_3 + C_7$

⇩ #2 transaldolase

$C_6 + C_4$



$C_4 + C_5$

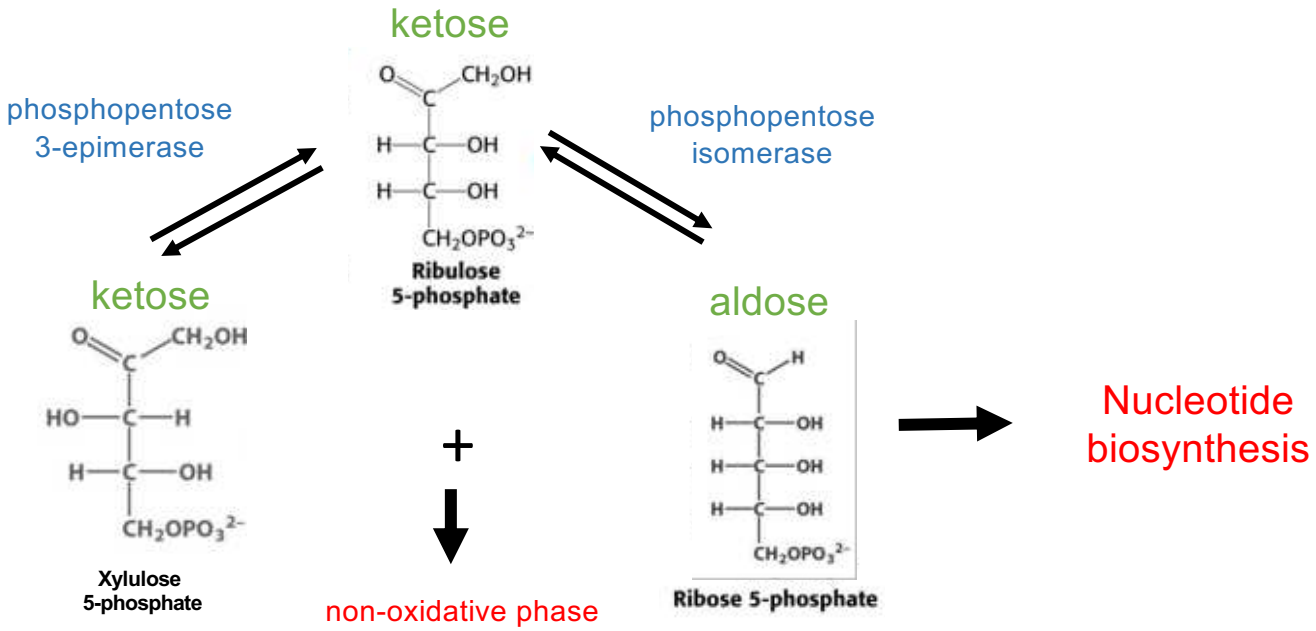
#3 transketolase ⇩

$C_6 + C_3$

A reversible link between the PPP and glycolysis

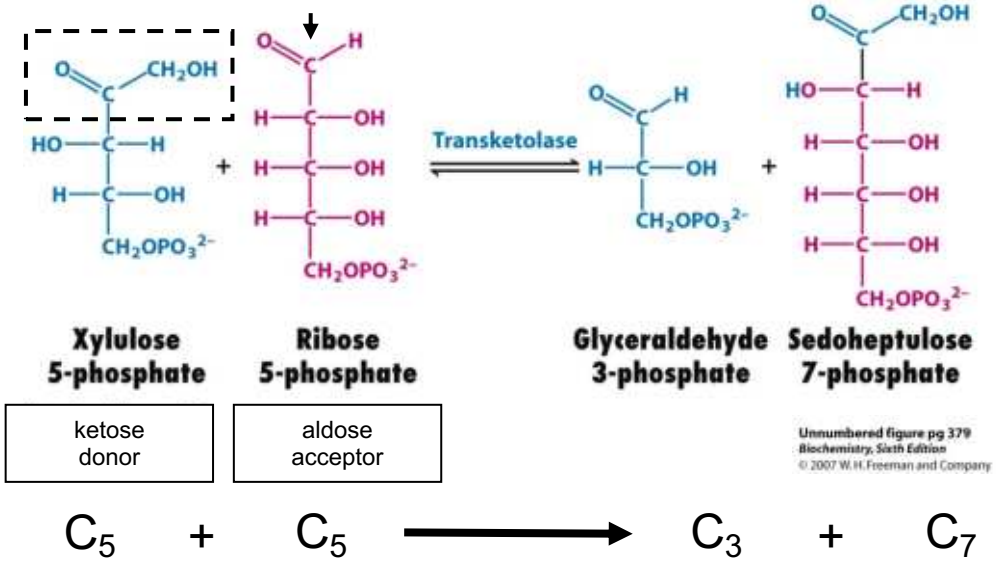
Two enzymes, transketolase and transaldolase, carry out the three reactions of the carbon shuffling phase (from ribose-5-phosphate and xylulose-5-phosphate forward). These carbon shuffling reactions are freely reversible. Therefore, fructose-6-phosphate and glyceraldehyde-3-phosphate can be converted back to ribose-5-phosphate if needed by the cell.

Ribulose 5-phosphate is converted to to ribose 5-phosphate and xylulose 5-phosphate



An intermediary step must occur between the oxidative and non-oxidative phases. These reactions are reversible and generate xylulose-5-phosphate (through an epimerization reaction) and ribose-5-phosphate (through an isomerization reaction) from ribulose-5-phosphate. Both of these 5 carbon sugars are required to begin the reactions of the non-oxidative phase.

#1. Transketolase transfers a 2C glycoaldehyde from a ketose to an aldose

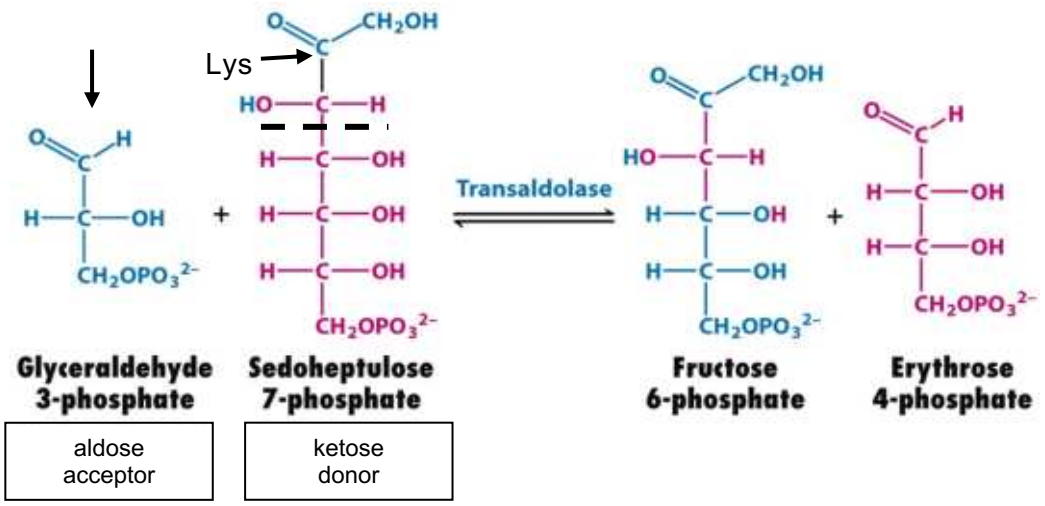


$C_5 + C_5 \longrightarrow C_3 + C_7$

Uses thiamine pyrophosphate (TPP) prosthetic group
(Recall pyruvate dehydrogenase and α-ketoglutarate dehydrogenase also used TPP)

Transketolase always performs the same chemistry, removing 2 carbons from a ketose and attaching them to the aldehyde group of an aldose as seen here in the first step of the non-oxidative phase. We generate one molecule of glyceraldehyde-3-phosphate in this step; however, we need this molecule to initiate the next reaction, along with sedoheptulose-7-phosphate, so we do not send it back to glycolysis yet.

#2. Transaldolase transfers a 3C unit from a ketose to an aldose

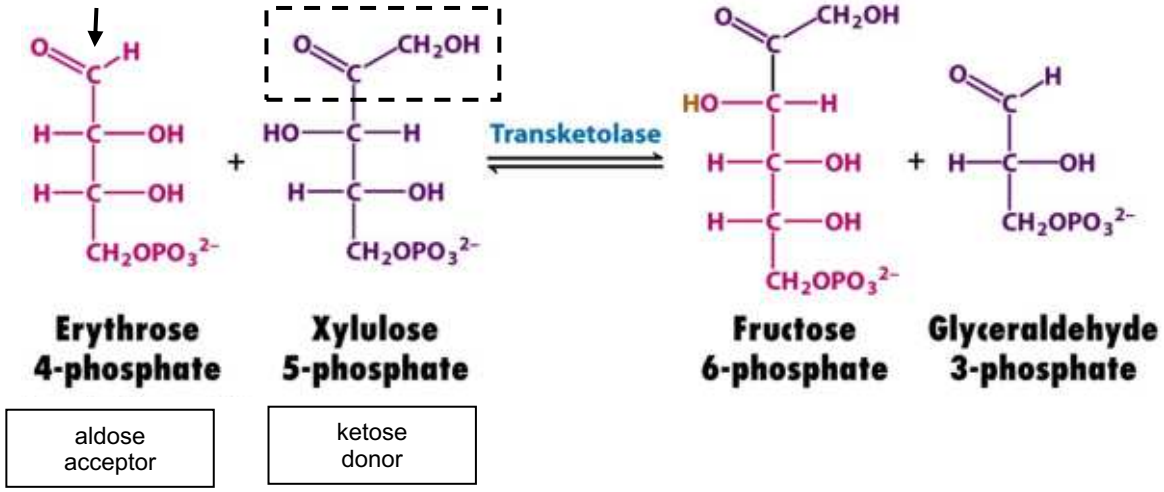


$C_3 + C_7 \longrightarrow C_6 + C_4$

Uses a Schiff's base between carbonyl of ketose and lysine

Both products from the previous reaction are required to initiate this reaction catalyzed by transaldolase. Transaldolase removes 3 carbons from sedoheptulose-7-phosphate and adds them to the aldehyde group of glyceraldehyde-3-phosphate. Similar chemistry to transketolase, but here transaldolase transfers three carbons instead of two. A lysine residue within transaldolase enzyme forms an intermediate covalent bond with the 3-carbon structure being transferred.

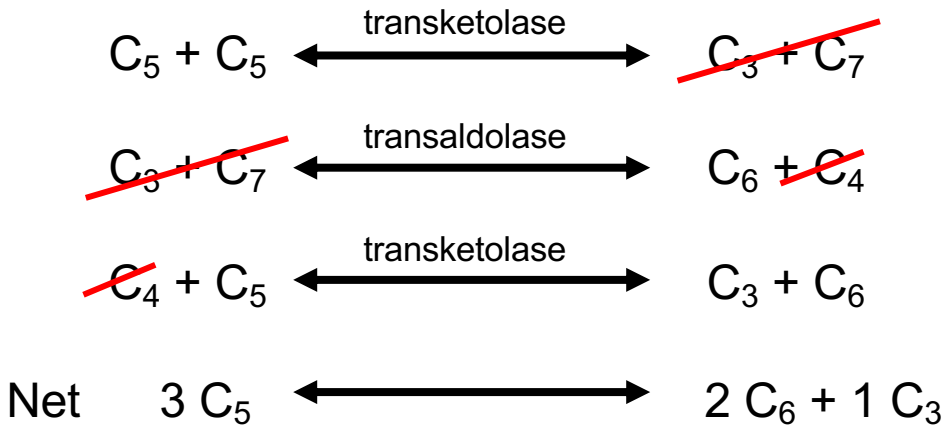
#3. Transketolase (again) transfers a 2C glycoaldehyde from a ketose to an aldose



$C_4 + C_5 \longrightarrow C_6 + C_3$

The last step of the non-oxidative phase is another reaction catalyzed by transketolase. Again, 2 carbons are transferred from the ketose donor to the aldose acceptor to give the final glycolytic intermediate products, fructose-6-phosphate and glyceraldehyde-3-phosphate.

Stoichiometry of the non-oxidative phase

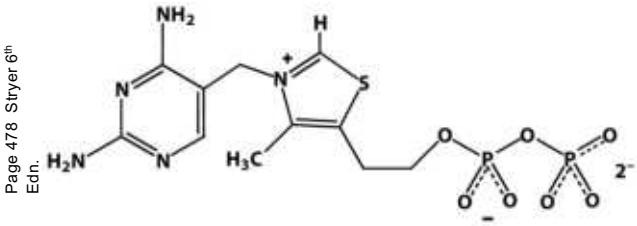


Overall, the non-oxidative phase uses three 5 carbon sugars (ribose-5-phosphate and xylulose-5-phosphate initially, and then another molecule of xylulose-5-phosphate in the third reaction) and generates two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate.

Transketolase transfers two carbons at a time. Transaldolase transfers three carbons. Both enzymes are moving carbons from a ketose donor to an aldose acceptor.

Clinical aside: Thiamine deficiency

Thiamine pyrophosphate is prosthetic group of several enzymes:



Thiamine is vitamin B_1 - obtained from the diet
TPP is synthesized from thiamine and pyrophosphate

- Pyruvate dehydrogenase (E1)
- α-Ketoglutarate dehydrogenase
- Branched chain α-keto acid dehydrogenase complex
- **Transketolase (pentose phosphate pathway)**

Thiamine deficiency leads to beri-beri (a problem in some populations with rice heavy diets because "polished" rice is low in thiamine)

In Western societies, thiamine deficiency is most likely caused by alcoholism because alcohol can interfere with absorption of thiamine – beri-beri and Wernicke-Korsakoff syndrome

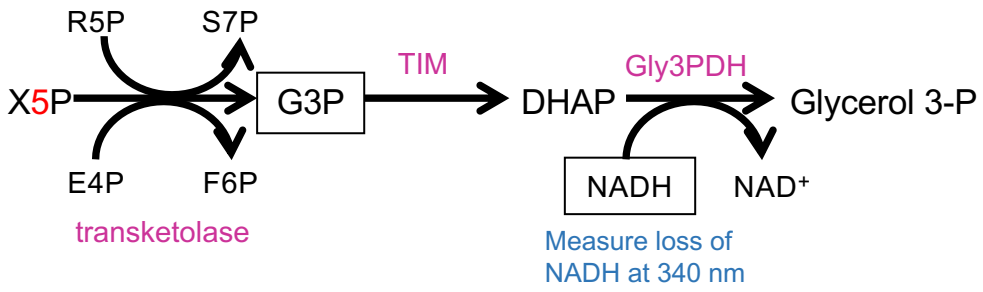
Causes dysfunction in heart, muscle and nervous tissue because these have high requirement for the TCA cycle

Treatment is IV or IM thiamine. For hypoglycemic alcoholics, thiamine is administered before glucose to avoid the onset of Wernicke's encephalopathy.

Diagnosing thiamine deficiency

- The transketolase activity of red blood cells is used to measure thiamine nutritional status and diagnose deficiency
- Activity measured in presence and absence of added thiamine
- If thiamine levels are inadequate, transketolase activity rises upon addition
- Detected by loss of NADH

Enzyme-linked assay:



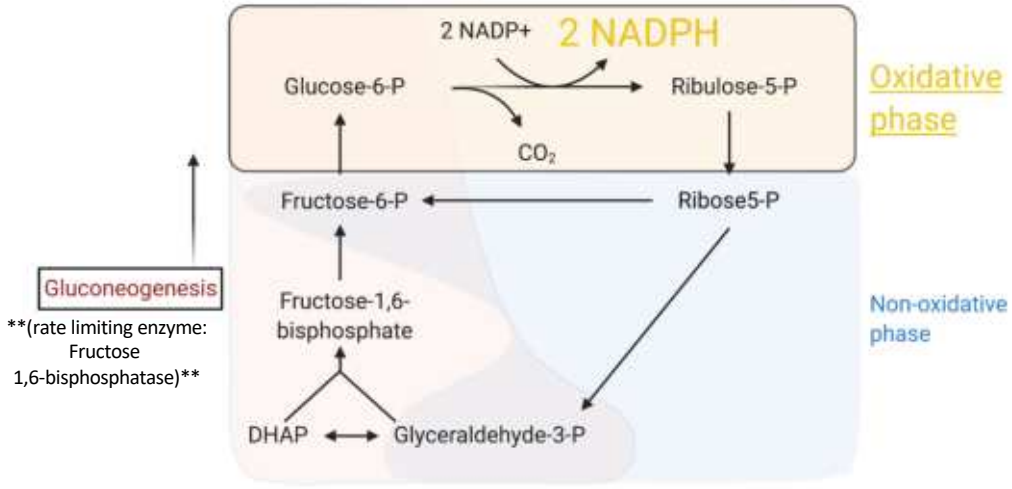
A simple blood test diagnoses thiamine deficiency by measuring transketolase activity in red blood cells. Thiamine is required for the transketolase reaction shown above converting xylulose-5-phosphate to glyceraldehyde-3-phosphate (it completes this same conversion in two separate reactions designated above and below the arrows). The readout for this blood test measures a downstream reaction where after glyceraldehyde-3-phosphate is converted to DHAP, it can further be reacted on by glycerol-3-phosphate dehydrogenase, an enzyme that uses NADH to make glycerol-3-phosphate. NADH absorbs light at 340nm and the absorbance at 340nm is what will be measured for this assay.

After a blood draw, part of the sample is spiked with additional thiamine. If there is sufficient thiamine in the diet, NADH levels will not change dramatically upon addition of excess thiamine as the reaction cascade shown above was already occurring appropriately. A portion of the sample is left without additional thiamine for comparison. If the patient is thiamine deficient, adding exogenous thiamine will activate this enzymatic cascade and lead to a detectible change in NADH conversion to NAD+. NAD+ is not visible at 340nm. Therefore, a patient that is thiamine deficient will have a positive test as assessed by a decrease in NADH and a decrease in light absorbed at 340nm.

3. Cellular Scenarios: When does the cell use the pentose phosphate pathway?

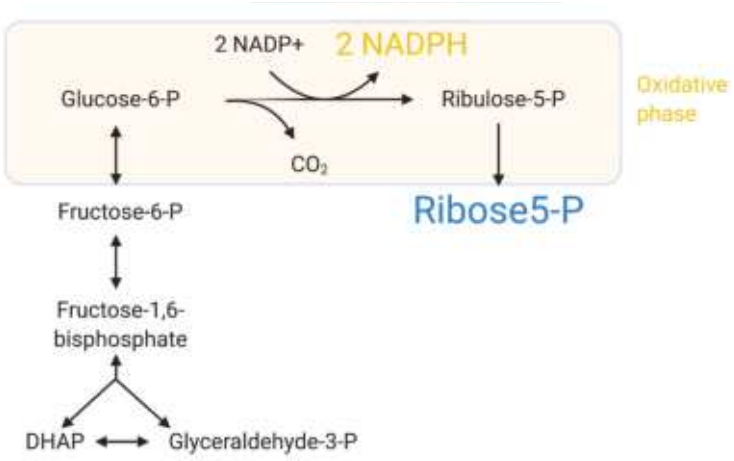
- The cell can use the two phases of the PPP in different ways depending on the biomolecular requirements at the time.

Scenario 1: The cell needs to fight against oxidative stress or pathogens; NADPH only



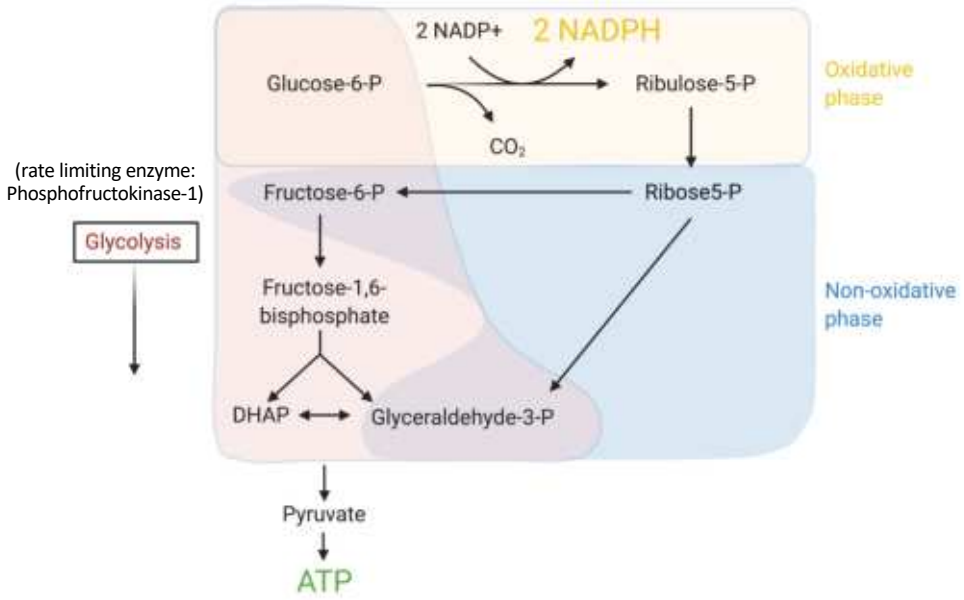
When the cell is fighting against oxidative stress or incoming pathogens, it requires very high levels of NADPH, but does not require the sugars such as ribose-5-phosphate. In this scenario, the cell shuttles glucose-6-phosphate into the oxidative phase of the PPP to generate NADPH. The sugars can then be converted back to glycolytic intermediates. Conveniently, these intermediates can use gluconeogenesis, whose rate limiting enzyme is fructose-1,6-bisphosphatase, to regenerate glucose-6-phosphate and reenter the PPP as many times as needed until sufficient quantities of NADPH have been generated.

Scenario 2: Replicate DNA; need NADPH and Ribose-5-P



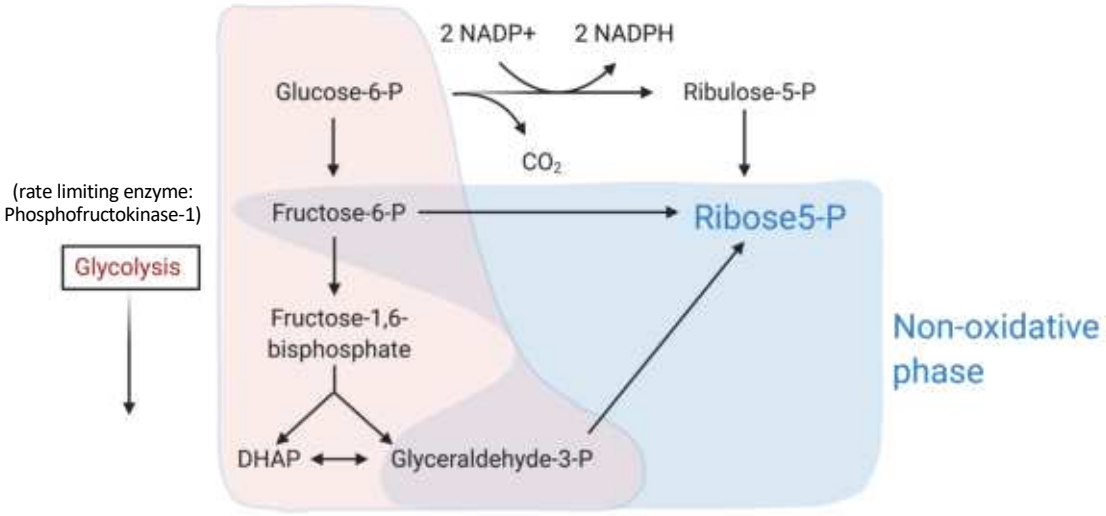
When replicating DNA, the cell requires both NADPH and ribose-5-phosphate because reactions within the DNA synthesis pathway require NADPH. In this case, G6P enters the oxidative phase to generate NADPH, the ribulose-5-phosphate is converted to ribose-5-phosphate and the ribose-5-phosphate is directly used for DNA synthesis.

Scenario 3: Biosynthesis of cholesterol or FAs; need NADPH and ATP energy



When building large, energetically-rich biomolecules like cholesterol and fatty acids, both NADPH and ATP are required. Therefore, the oxidative phase is used to generate NADPH and the sugars are converted back to glycolytic intermediates to continue through carbohydrate metabolism to create ATP.

Scenario 4: RNA synthesis; only need Ribose-5-P



SIDENOTE: Introduction of ribose or other 5 carbon sugar from your diet can be fed in through the PPP non-oxidative pathway and into glycolysis for ATP generation.

The last scenario highlighted here is RNA synthesis. Unlike DNA synthesis, the RNA synthetic pathways do not have a direct requirement for NADPH. Therefore, only ribose-5-phosphate is required from the PPP for this process. Because the reactions of the non-oxidative phase are reversible, fructose-6-phosphate and glyceraldehyde-3-phosphate can be converted back to ribose-5-phosphate with no need to run the oxidative phase.

A little more detail on scenario 1: The cell needs NADPH, but not Ribose-5-P.

Why would the cell need NADPH?

-biosynthesis

-combat free radical crisis from oxidative stress

-need reducing power to neutralize free radicals that cause major cellular damage to membranes, proteins, DNA, etc.

-detoxification of drugs

Three major medical consequences of imbalanced NADPH:

-inability to fight infections

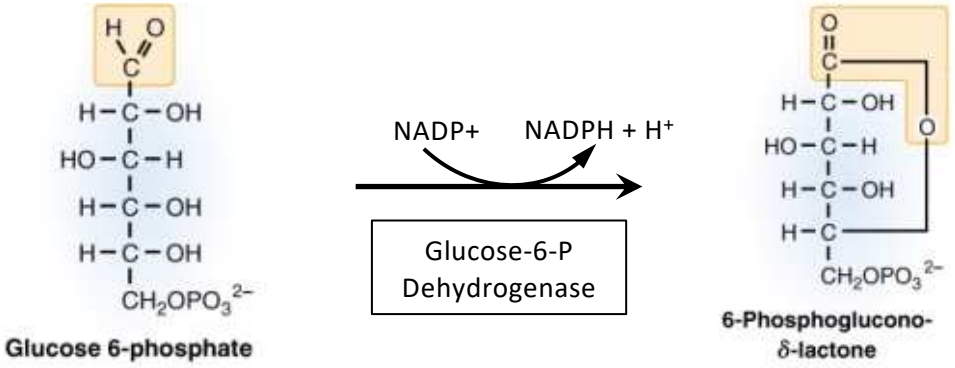
-hemolytic anemia as a result of oxidative damage

-acetaminophen drug tolerance vs overdose

4. Glucose-6-phosphate dehydrogenase

I. Mechanism

Important first step of the PPP

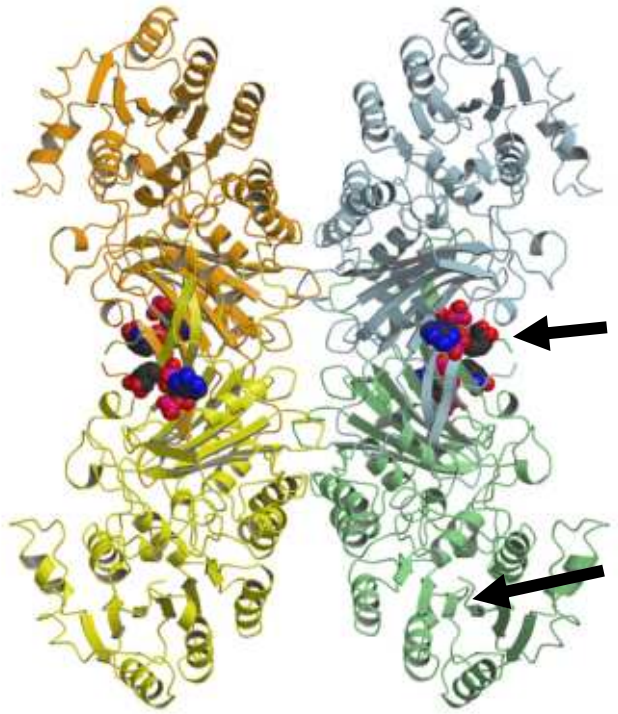


Entry of G6P into PPP is determined by [$NADP^+$]

Glucose-6-phosphate dehydrogenase (G6PD) is the rate limiting enzyme and controls entry of glucose-6-phosphate into the pentose phosphate pathway. The concentration of NADP+ regulates G6PD. If the concentration of NADP+ increases in the cell, G6PD will activate to generate more NADPH.

Regulation of glucose 6-phosphate dehydrogenase

- Enzyme exists as a monomer, dimer or tetramer
- Dimer and tetramer forms are more active
- Two binding sites for $NADP^+$:
  - Coenzyme & structural sites
- Binding of $NADP^+$ to structural site increases stability
- Mutations associated with G6PDH deficiency tend to cluster around structural site.



The crystal structure of G6PD shown above is a tetramer with each monomeric subunit shown in a different color, but the enzyme can be active as a monomer, dimer or a tetramer. The dimeric and tetrameric structures display increased activity when compared to the monomeric form. NADP+ has two binding sites within the G6PD complex; one binding site exists within the active site to participate in catalysis, and another site plays a structural role. The structural site is close to the dimer interface and helps to stabilize the higher order structures, thereby increasing the activity of the enzyme overall.

II. Deficiency

G6PD Mutations

There are several mutations responsible for G6PD deficiencies around the world.

- Severity of disease varies according to specific mutations
- Mutations tend to cluster around the structural site and affect stability of higher order structures (dimer/tetramer)

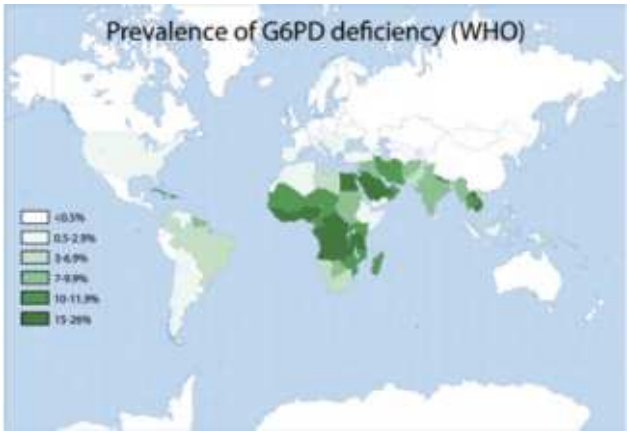
Common Genetic Variants of Glucose-6-Phosphate Dehydrogenase (G6PD)	
Variant	Description
B (non-mutated)	Biologic: Normal activity Clinical: No hemolysis
A− (G202A and A376G)	Accounts for most cases of G6PD deficiency in persons of African descent; found in 10% to 15% of black Americans and Africans from West and Central Africa
Biologic:	Unstable G6PD; G6PD levels decrease as red cells age
Clinical:	Mild-to-moderate hemolysis, only older red cells affected (moderate enzyme deficiency, 10–60% of normal); favism uncommon
Mediterranean (C563T)	Most common variant in whites
Biologic:	G6PD synthesized at reduced rate, with reduced catalytic activity; low G6PD levels in all red cells
Clinical:	May cause marked hemolysis with all red cells affected (severe enzyme deficiency, 1–10% of normal); favism common

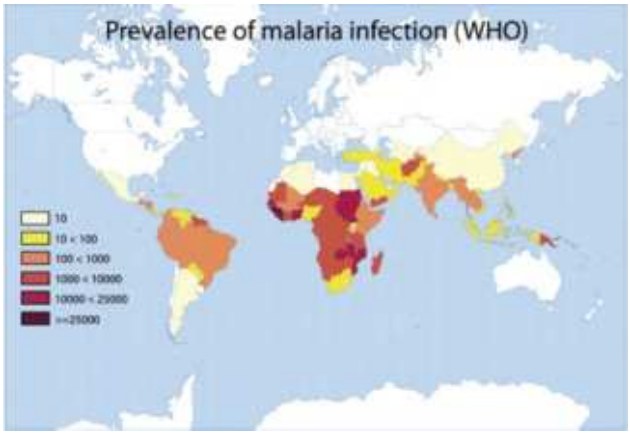
Deficiency of glucose 6-phosphate dehydrogenase

- X-linked disorder
- The most common enzymopathy - 400 million cases worldwide
- ~7% of the world's population, ~2% in the U.S.
- Most prevalent in Africa, the Mediterranean and South Asia
- Major cause of neonatal jaundice (1/3 of cases)
- Mostly asymptomatic, but increased susceptibility of hemolytic anemia due to inadequate protection against ROS

Why has there been an extreme selection for G6PD deficiencies?

G6PD deficiency is protective against malaria caused by *Plasmodium falciparum*
-this parasite requires highly reducing conditions to thrive



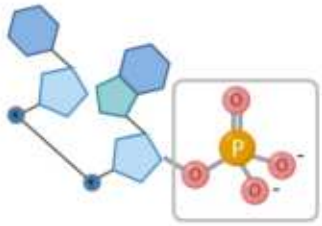


http://education.med.nyu.edu/mbm/carbohydrates/pentosePathway.shtml

G6PD deficiency results in a slightly oxidized environment within the cell due to reduced levels of NADPH. Plasmodium falciparum prefers a reduced environment and therefore is growth restricted in G6PD deficient individuals. G6PD deficiency has become relatively common in the world's population because it is protective against malaria.

5. Functions of NADPH

Functions of NADPH



-Reductive biosynthesis of fatty acids, steroids, cholesterol, neurotransmitters, deoxynucleotides

-Production of reduced glutathione to protect against oxidative stress and for detoxification of drugs

-Production of ROS for host defense

I. Biosynthetic reactions

NADPH for biosynthesis

-Reductive biosynthesis of fatty acids, steroids, cholesterol, neurotransmitters, deoxynucleotides

-Required when the cell is mass producing lipids (e.g. adipose tissue, mammary glands and in the liver)

-Also required in rapidly dividing cells which need to create new lipid rich membranes to encapsulate daughter cells and their intracellular components (e.g. nucleus, mitochondria, ER, golgi, etc.)

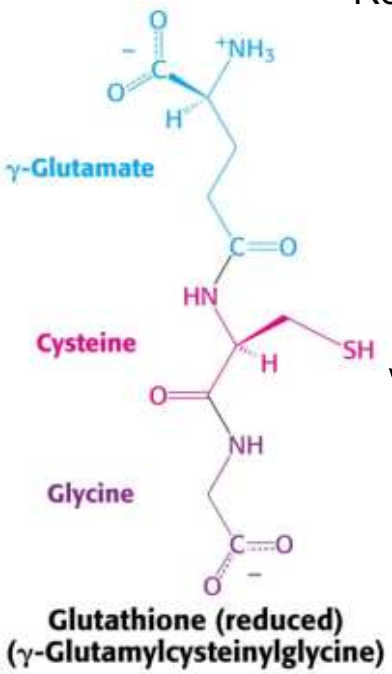
-Therefore, this pathway is upregulated in cancers and a current target for chemotherapeutics.

II. Generation of reduced glutathione

A. Reaction

NADPH is required to maintain reducing power within the cell through glutathione

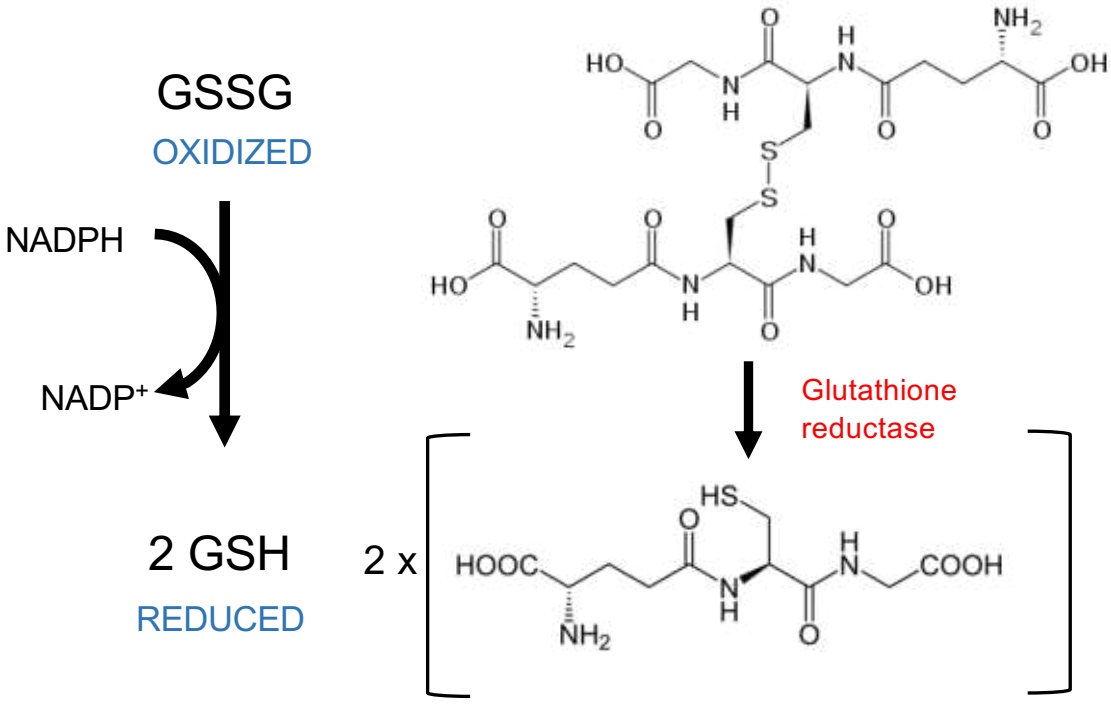
Reduced glutathione



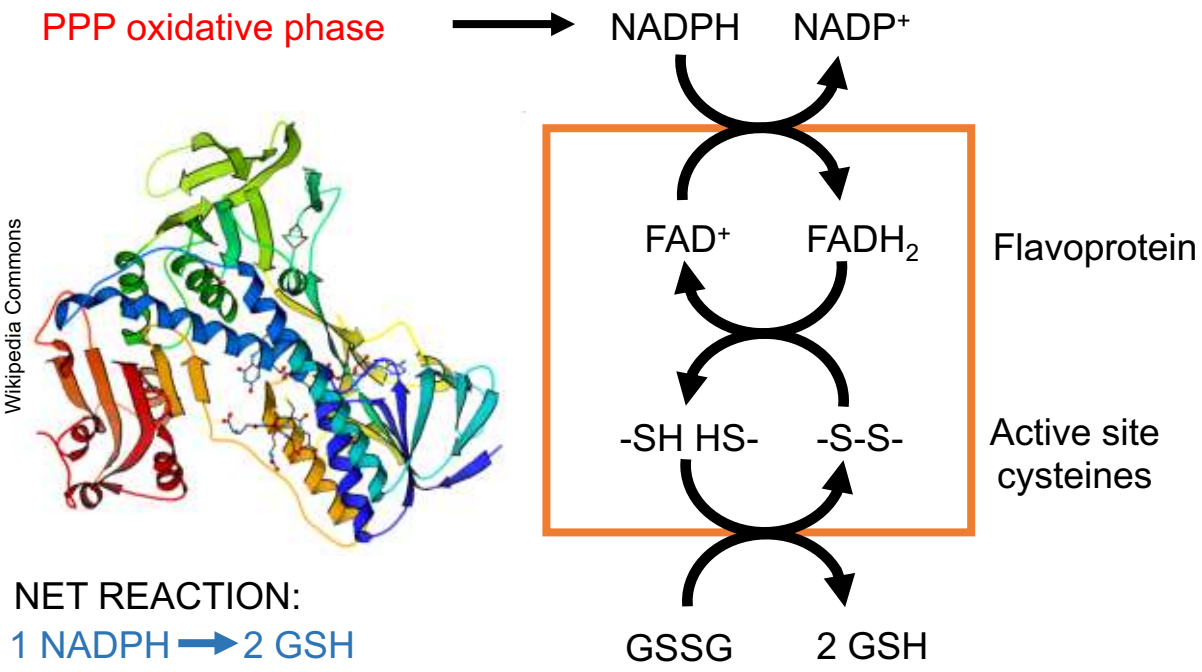
- Major defense against oxidative stress
- Essential in aerobic systems
- Antioxidant (electron donor)
- 5 mM in animal cells
- 500:1 ratio, reduced:oxidized (used as a measure of cell toxicity)

Reduced form has free sulfhydryl – used to reduce species (and becomes oxidized)

Synthesis of reduced glutathione by glutathione reductase requires NADPH



Glutathione reductase



Glutathione reductase is a flavoprotein (covalently coordinates a FAD+ molecule) that uses active site cysteine residues to reduce one molecule of oxidized glutathione to two molecules of reduced glutathione. The resulting disulfide within the active site is reduced by the internal $FADH_2$, which is in turn reduced using NADPH; hence the requirement for NADPH in maintenance of our reduced glutathione stores.

B. Functions of reduced glutathione

•<u>Protects against ROS generated in oxidative metabolism</u>

e.g. Peroxides form readily in the oxygen-rich environment of red blood cells, but RBCs do not have mitochondria and so lack reducing power. Up to 10% of glucose in RBCs is directed to PPP to make NADPH and then glutathione.

- Defective PPP leads to cell lysis and anemia.

•<u>Maintains general reducing conditions</u>

e.g. in red blood cells:

(a) Cysteine residues of hemoglobin as sulfhydryls

(b) Iron in ferrous state (Fe^{2+})

- Defective PPP leads to MetHb and Heinz bodies

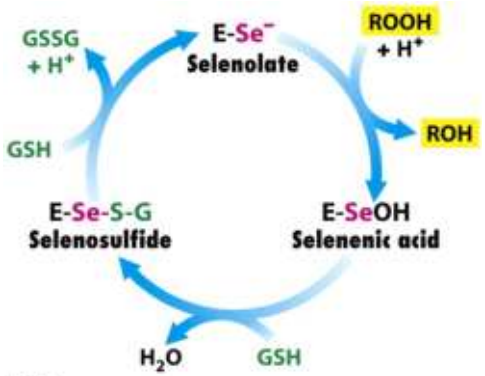
•<u>Detoxification</u>

e.g. protection against drug overdose

i. Protection against ROS

Glutathione peroxidases protect against ROS

- Family of enzymes
- Require reduced glutathione (GSH) to regenerate selenolate
- Convert reduce lipid hydroperoxides to corresponding alcohols
- Also reduce hydrogen peroxide to water



$$2\ GSH + ROOH \longrightarrow GSSG + H_2O + ROH$$

ii. Maintain general reducing conditions

a. Iron redox state/ Methemoglobin

Methemoglobin (MetHb)

Typically, iron centers within hemoglobin exist in the ferrous form (Fe^{2+} form). Methemoglobin is the hemoglobin state when iron is in the ferric form (Fe^{3+}). Methemoglobin does not transport oxygen as efficiently as hemoglobin and general reducing conditions are required to maintain iron within hemoglobin in the ferrous form for efficient oxygen transport.

- The Fe^{3+} (ferric) form of hemoglobin has very low affinity for oxygen

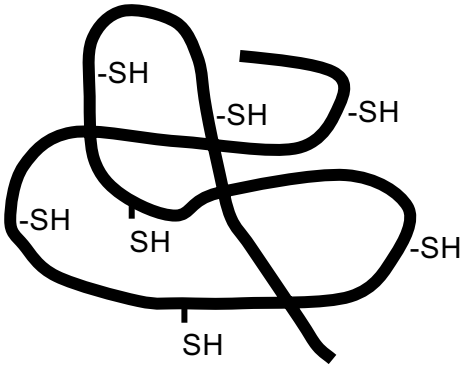
- There is more Fe^{3+} when mechanisms that protect against oxidative stress are overwhelmed

- [Congenital methemoglobinemia – autosomal recessive disease caused by deficiency of NADH methemoglobin reductase (diaphorase I)]

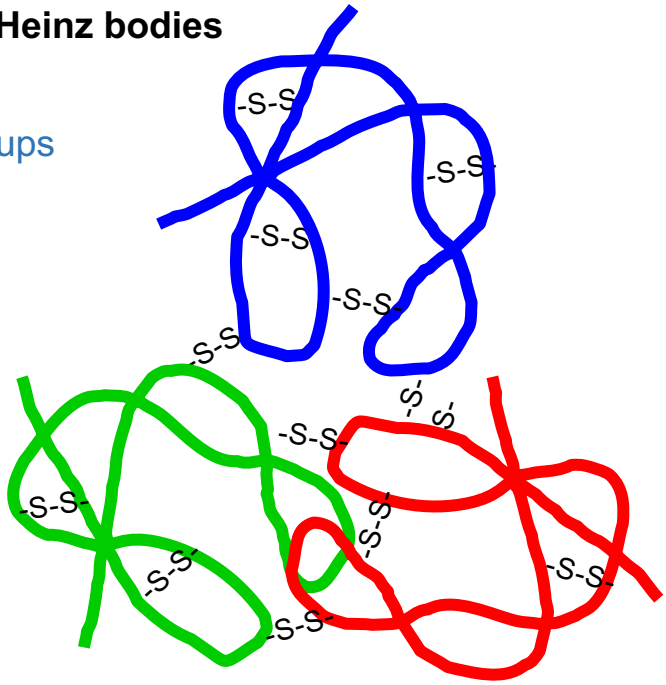
NADH methemoglobin reductase is the enzyme responsible for converting methemoglobin back into hemoglobin. If this enzyme is mutated, patients have a chronic deficiency in oxygen transport.

b. Proper folding of proteins and Heinz bodies

General reducing conditions:
Maintenance of free sulfhydryl groups



Correctly folded protein



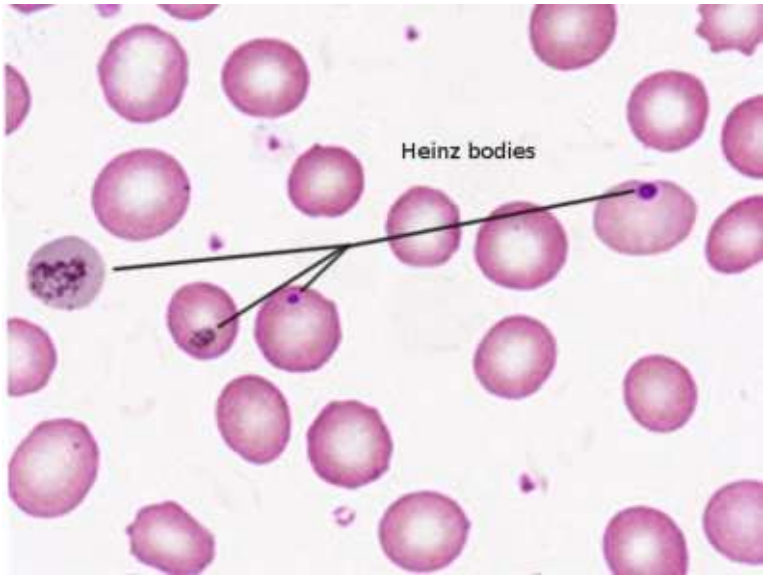
Misfolded and aggregated protein

Another reason to maintain general reducing conditions in the cell is for proper folding of proteins. The cellular environment has evolved to be in a reduced state and therefore our intracellular proteins have evolved to properly fold under reduced conditions. This primarily affects cysteine residues, which will be in a free sulfhydryl form when reduced and can form disulfide bonds (covalent bonds between two cysteine residues) when oxidized. If the environment in the cell becomes oxidized, cysteine residues can form unintended disulfide bonds, leading to misfolding and aggregation of proteins. The extracellular environment is not reduced like the intracellular environment and therefore secreted proteins frequently employ disulfide bonds for proper folding.

Heinz bodies in red blood cells

Heinz bodies are clumps of denatured/ aggregated hemoglobin.

Increases susceptibility to cell lysis

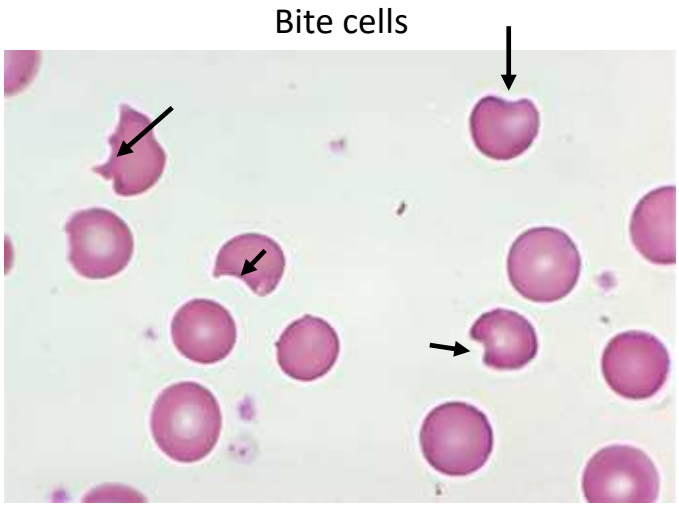


Heinz bodies. Image courtesy S Bhimji MD. Treasure Island (FL): StatPearls Publishing; 2020 Jan

Heinz bodies are visible with crystal violet staining under a microscope if red blood cells are under oxidative stress and high levels of denatured hemoglobin protein aggregates.

NADPH is required to maintain reducing power within the cell through glutathione

Bite cells appear in blood smears as macrophages in the spleen "take bites" out of red blood cells in an attempt to clear Heinz body build up.



Red blood cells don't have mitochondria, which is the other major cellular source of NADPH.

If the PPP isn't functional, then RBCs are damaged and can lyse!

a. Hemolytic anemia and G6PD deficiency

Haemolytic anaemia (HA) is characterized by the destruction of the red blood cells.

Causes:

•Defects in the RBC Membrane
e.g. *Hereditary spherocytosis*

•Abnormal Hemoglobin
e.g. *Thalassaemia*

•Abnormal glycolytic enzymes
i.e. *non-spherocytic hemolytic anemia*

Defective enzymes: Hexokinase, pyruvate kinase, glucose 6-phosphate isomerase and phosphoglycerate kinase, and glucose-6-phosphate dehydrogenase of pentose phosphate pathway.

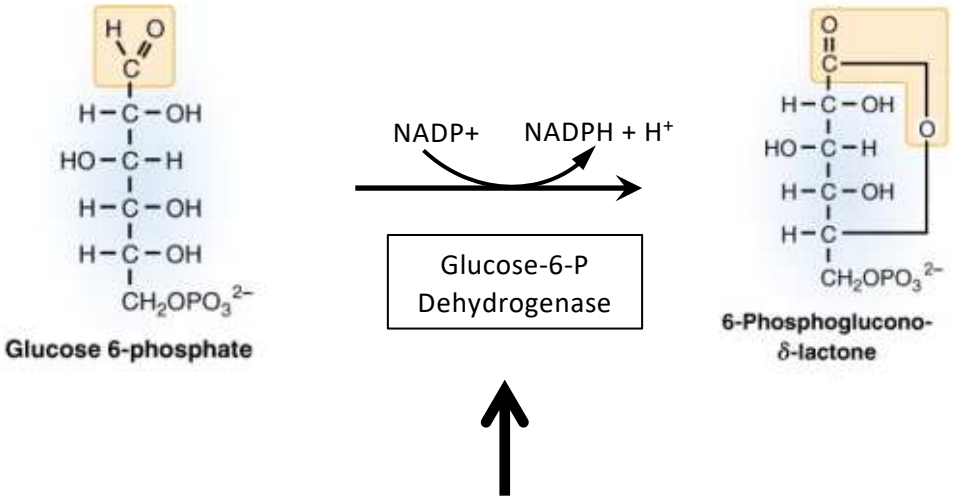
Defects in PPP enzymes cause disease

G6PD deficiency causes:

-less NADPH

-less reduced glutathione

-less protection against oxidative damage



Most common deficiency

Most serious

G6PD deficiency is generally asymptomatic until triggered

Examples of triggers for hemolytic crises in G6PD deficient patients:

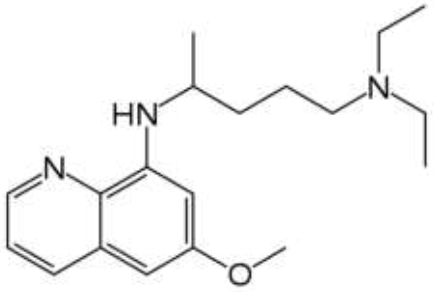
-infections (free radicals produced by macrophages diffuse into RBCs)

-antimalarial drugs *e.g.* primaquine, hydroxychloroquine

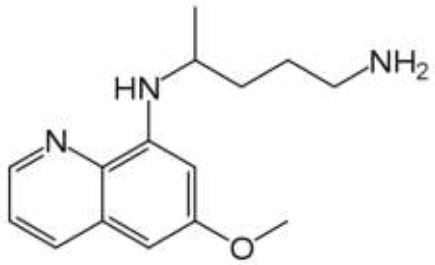
-sulfa drugs

-fava beans

Antimalarial drugs



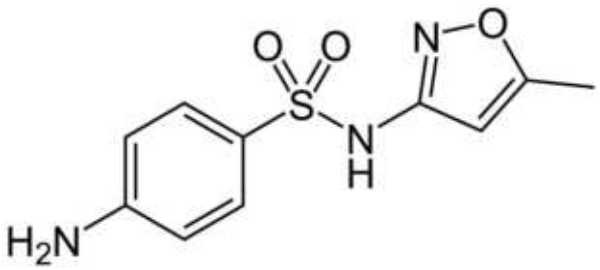
Pamaquine



Primaquine

- 8-aminoquinolones
- Pamaquine was first synthetic antimalarial
- Drugs are oxidizing and can cause methemoglobinemia in all patients who take it, but seldom causes problems unless patients have G6PD deficiency
- For patients with G6PD deficiency, it can cause hemolytic anemia – patients should be screened before prescribing

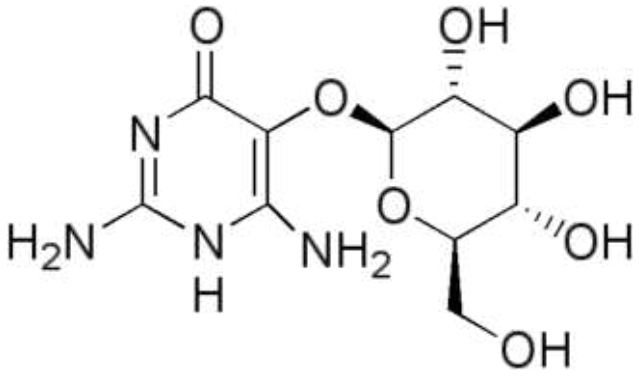
Sulfa drugs



sulfamethoxazole

- Sulphonamides - the first class of antibiotics discovered
- Inhibits dihydropteroate synthase (DHPS)
- *e.g.* sulfamethoxazole (Component of Bactrim) and dapsone
- Oxidizing - causes MetHb
- Should also be avoided by patients with G6PD deficiency

Fava beans → Vicine



Favism - a hemolytic reaction due to consumption of broad beans (or Fava beans - Italian name for broad bean is *fava*).

Vicine (a component of fava beans) causes HA either by direct action on RBC membranes or by producing hydrogen peroxide, which breaks down red blood cell membranes and can't be neutralized in G6PD deficient patients

Beutler fluorescent spot test

•Detects deficiency of glucose 6-phosphate dehydrogenase.

•Rapid and inexpensive

•Measures production of NADPH in blood using UV (365 nm).

•Normal samples fluoresce brightly (negative test), whereas samples from patients with deficiency show little or no fluorescence (positive test)

•False negatives occur in patients who are actively hemolysing – test should therefore be conducted several weeks after a hemolytic episode

•Less sensitive for heterozygous females – therefore more sensitive, but also more expensive, cytochemical assay is required (uses a stain that changes color when reduced by NADPH)

Ordered before administration of any chloroquines or high dose sulfa drugs (e.g. Bactrim).

Treatments for G6PDH deficiency

•Most cases are self limiting

•Vaccination to reduce risk of infection *e.g.* Hep B

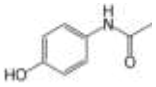
•Avoidance of trigger drugs

•Blood transfusions

•Splenectomy or bone marrow transplantation in most severe cases

•Gene therapy in the future?

iii. Detoxification of drugs

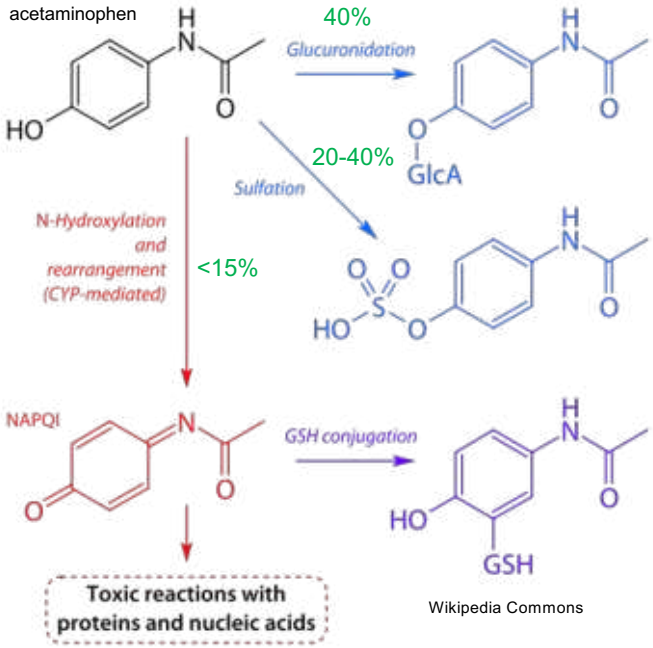


Toxicity of acetaminophen ("Tylenol")

•Over-the-counter analgesic (aka Paracetamol)
•In 2006, 56,000 emergency room visits and 26,000 hospitalizations were due to overdoses of acetaminophen (Source - FDA).
•Symptoms – irritability, abdominal pain, appetite loss, nausea, diarrhea, jaundice, sweating, coma, convulsions
•Acetaminophen hepatotoxicity is most common cause of acute liver failure in the United States
•Excess causes formation of *N*-acetyl-*p*-benzoquinone imine (NAPQI), which causes free radical damage to liver cells
•NAPQI is detoxified by glutathione, but levels of latter are exhausted with overdose.
•Treatment is *N*-acetylcysteine (NAC) – restores reduced glutathione, is a precursor to glutathione and itself can scavenge free radicals.

Metabolism of acetaminophen

%'s for normal dose



Wikipedia Commons

Cytochrome P450 system – uses NADPH

Products of glucuronidation and sulfate are excreted

NAPQI is detoxified by conjugation with glutathione

and lipid peroxidation of cell membranes

Glucuronidation and sulfation products from processing of acetaminophen are non-toxic and excreted. NAPQI is a toxic biproduct of acetaminophen that contains an unstable ring structure that will undergo toxic reactions with proteins, nucleic acids and cell membranes causing damage. Cytochrome P450 (using NADPH) converts acetaminophen to NAPQI. Glutathione is used to neutralize the ring through a covalent bond shown above. Once conjugated to glutathione, the product is non-toxic and excreted. However, because this is a covalent linkage, the glutathione is lost to the reaction and can not be recovered. Therefore, if you overdose on acetaminophen, you will quickly deplete your stores of reduced glutathione in an attempt to neutralize NAPQI. Remember, reduced glutathione is the cells primary antioxidant and depleting these stores will leave the cells vulnerable to oxidative stress and cellular damage.

Treatment with N-Acetyl Cysteine

The treatment for Tylenol overdose is administration of N-Acetyl Cysteine which can restore hepatic glutathione. N-Acetyl cysteine can be used as a precursor to glutathione. It can also substitute for glutathione by directly conjugating to NAPQI.

Mechanism of action- Source: UpToDate

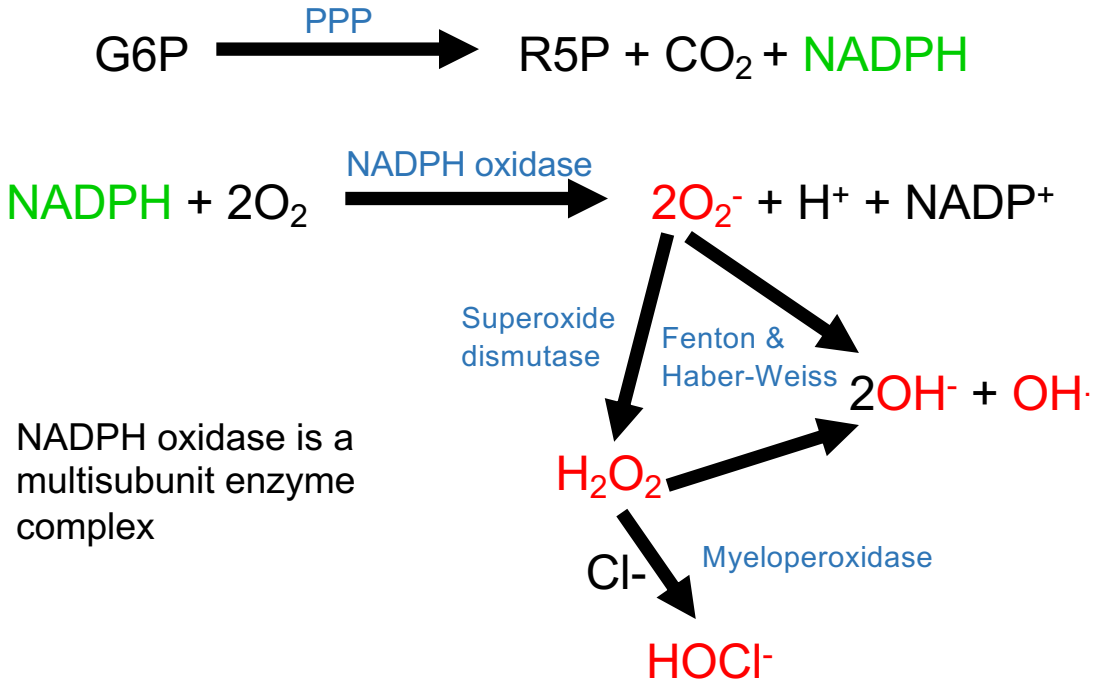
"Acetylcysteine acts as a hepatoprotective agent by restoring hepatic glutathione, serving as a glutathione substitute, and enhancing the nontoxic sulfate conjugation of acetaminophen."

-Contraindications listed are hypersensitivity. Therefore, NAC is generally considered non-toxic and will be administered if there is a suspicion of overdose.

III. Production of ROS as protection against pathogens

Production of ROS as protection against pathogens

- The highest concentrations of G6PD are found in phagocytic cells
- In phagocytes, NADPH oxidase uses NADPH to form superoxide from molecular oxygen.
- The superoxide is used to generate hydrogen peroxide and other reactive oxygen species to kill microorganisms taken up by the phagocytic cells



The pathway above shows how the cell can use NADPH and molecular oxygen to generate 5 different reactive oxygen species through the initial reaction catalyzed by NADPH oxidase. Each of these species can be used by phagocytes to damage pathogens.

Chronic granulomatous disease

- Hereditary disease affecting phagocytes
- Caused by defects in NADPH oxidase
- Inability to fight infection
- Incidence 1 in 200,000 in US
- Early diagnosis important → typically suspected with recurrent infections and abscesses
- Treatment is antibiotic prophylaxis or interferon
- Survivability is increasing

Summary of the pentose phosphate pathway

•Comprises oxidative and non-oxidative phases

•Oxidative phase generates NADPH and a 5C sugar

•Non-oxidative phase interconverts 3C, 4C, 5C, 6C and 7C sugars to link with glycolysis

•NADPH used for biosynthetic reductions, respiratory bursts, and to make reduced glutathione

•Ribose 5-phosphate used for nucleotide biosynthesis

•Glutathione maintains reducing conditions and is protective against oxidative stress

•Deficiency in glucose 6-phosphate dehydrogenase can cause hemolytic anemia in response to various triggers, but is also protective against malaria

•Glutathione is needed to detoxify certain drugs, *e.g.* acetaminophen