Pentose Phosphate Pathway (PPP) also called Hexose Monophosphate Shunt (HMS)

Some basics

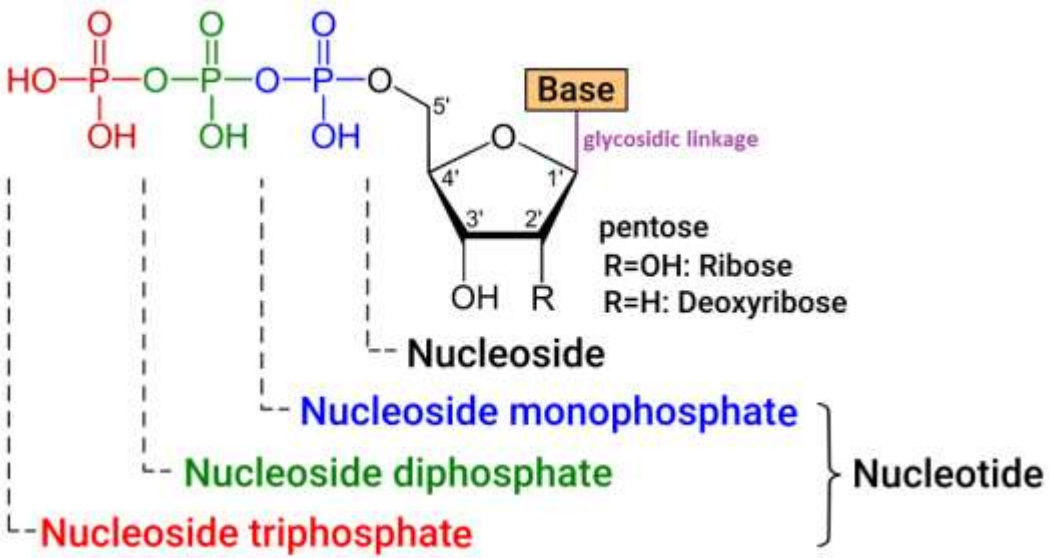
Remember from biology ***(Nucleotides)***

Each nucleotide consists of 3 components:

a. ***Pentose sugar*** (Ribose in RNA, Deoxyribose in DNA)

b. ***Nitrogenous base*** attached to C1' of the pentose (A,G,C,U in RNA; A,G,C,T in DNA)

c. ***Phosphate*** (1 or 2 or 3 phosphate groups attached to C5'



Pentose + N.base = ***Nucleoside***

Nucleoside + P (1 or 2 or 3) = ***Nucleotide***

RNA nucleotides called ***Ribonucleotides*** while DNA nucleotides called ***Deoxyribonucleotides***

RNA nucleotides:

Adenine nucleotides (AMP, ADP, ATP)

Guanine nucleotides (GMP, GDP, GTP)

Cytosine nucleotides (CMP, CDP, CTP)

Uracil nucleotides (UMP, UDP, UTP)

DNA nucleotides:

Adenine nucleotides (dAMP, dADP, dATP)

Guanine nucleotides (dGMP, dGDP, dGTP)

Cytosine nucleotides (dCMP, dCDP, dCTP)

Thymine nucleotides (dTMP, dTDP, dTTP)

Nucleotides are the building blocks of nucleic acids (DNA and RNA) also the are structural component of many coenzymes such as NAD^+, $NADP^+$, FAD, and CoA

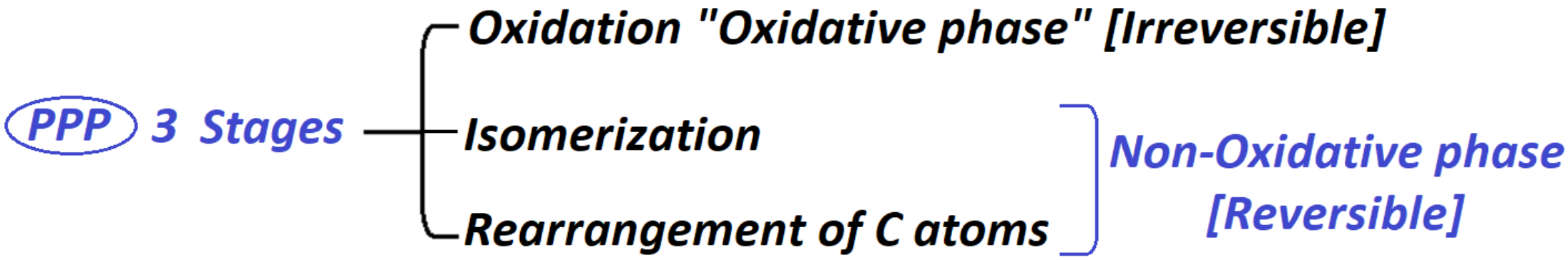
Pentose Phosphate Pathway (PPP) also called Hexose Monophosphate Shunt (HMS)

Occurs in the Cytosol

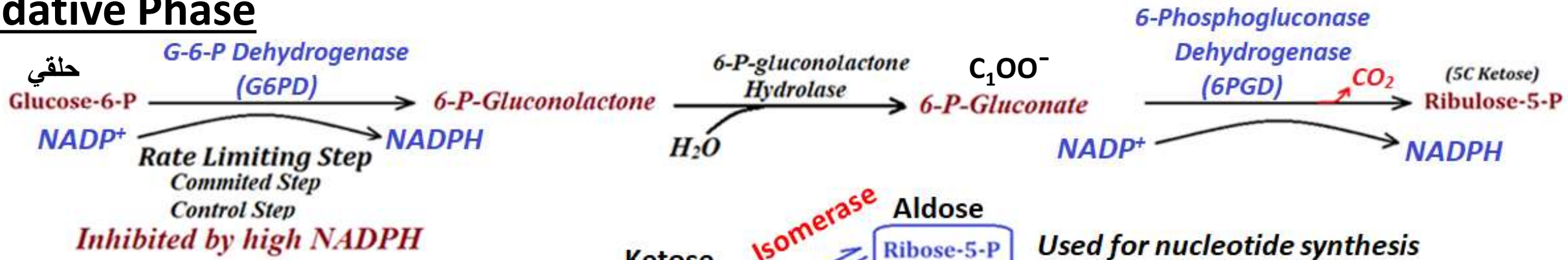
Aims: الاهداف

1. produce pentoses e.g. Ribose-5-P for nucleotide and nucleic acid synthesis
2. Produce NADPH [Reducing Agent عامل مختزل/مصدر الكترونات] important in lipid biosynthesis and detoxification process
3. Convert pentoses to Trioses and Hexoses for glycolysis or Gluconeogenesis
4. Provides intermediates for synthesis of Aromatic amino acids (Trp, Tyr, Phe)

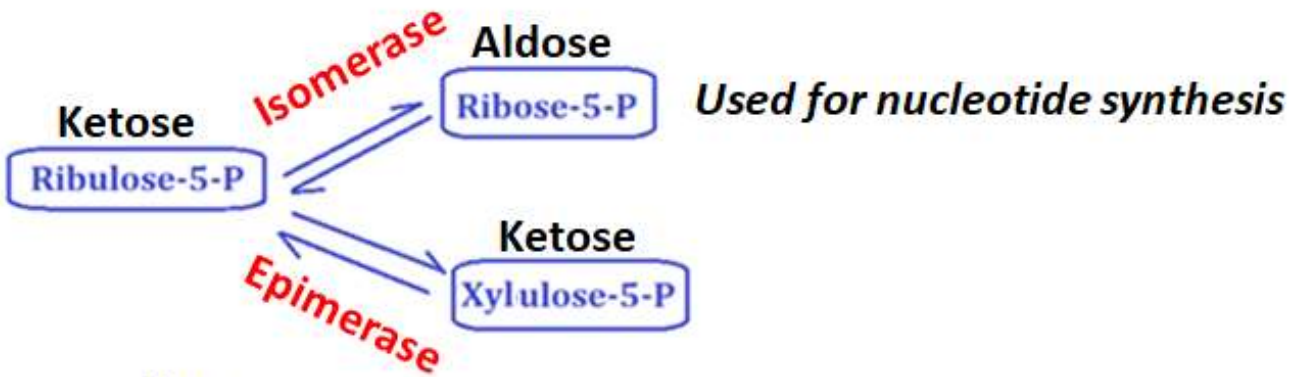
No ATP is directly consumed or produced in this pathway.



Oxidative Phase



Non-oxidative Phase



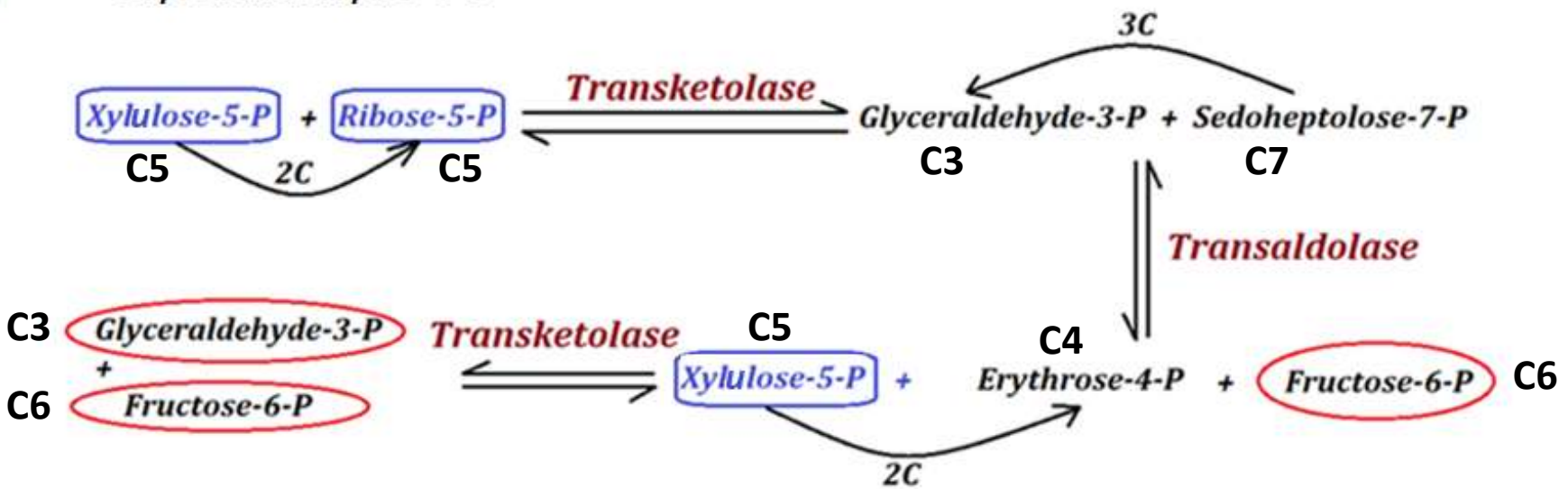
Now
Ribose-5-P
Xylulose-5-P
Xylulose-5-P

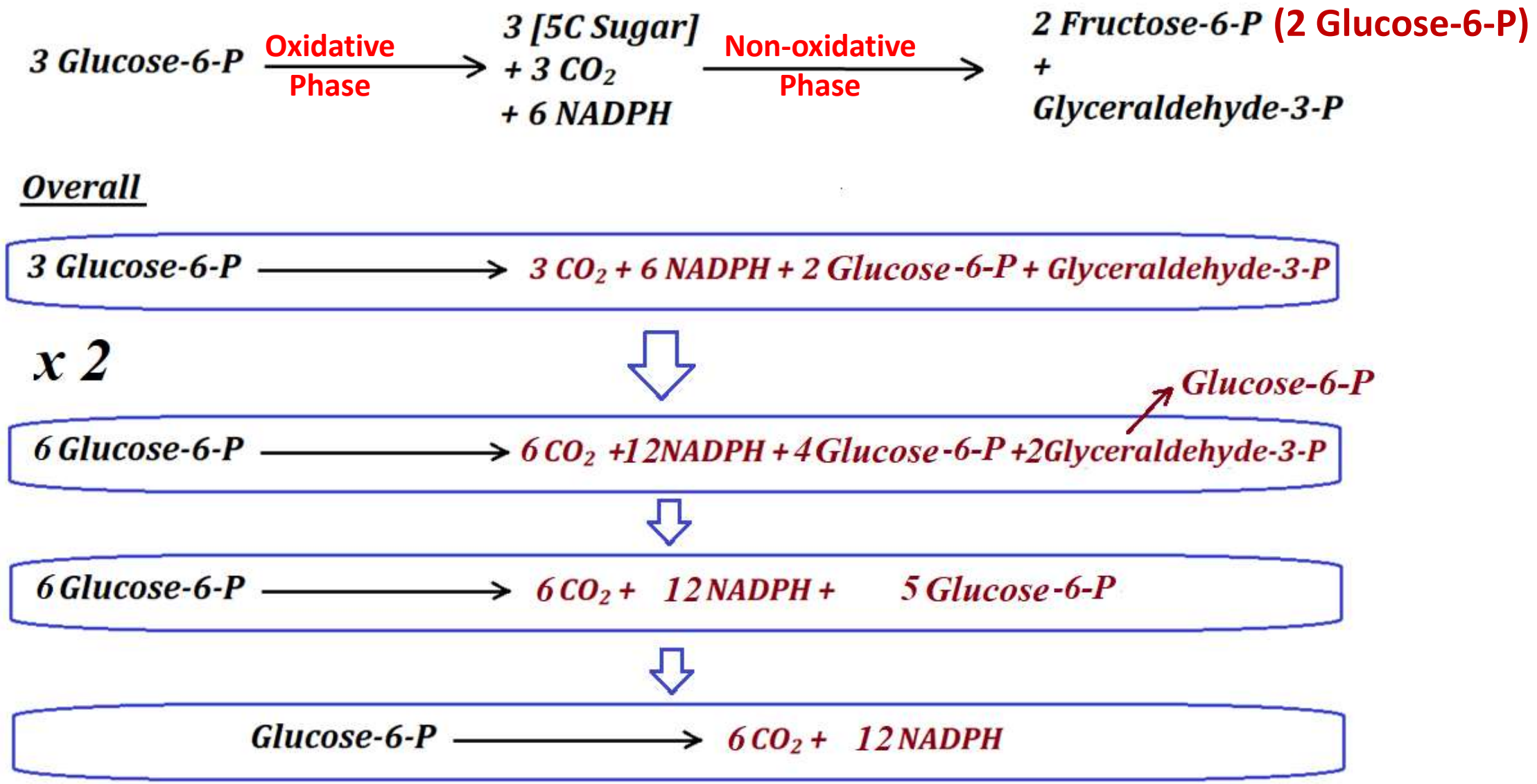
Fructose-6-P
Fructose-6-P
Glyceraldehyde-3-P

By:

Transketolase: need TPP, Transfer 2C

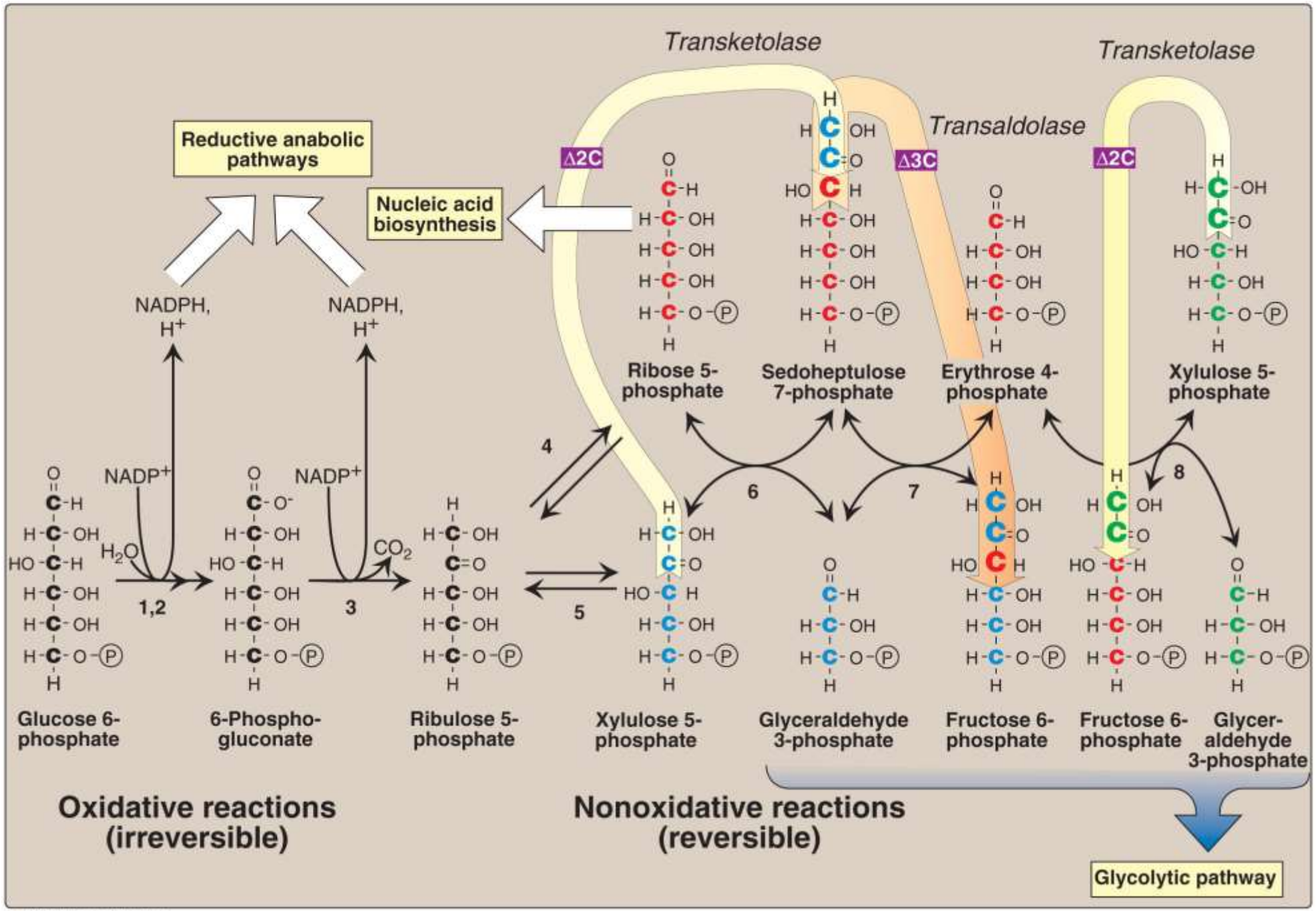
Transaldolase: Transfer 3C





لا يتم انتاج ATP في ال PPP

- **Transaldolase**: Transfer (swap, shuttle) 3C from ***Ketose to Aldose***
- **Transketolase**: Transfer 2C from ***Ketose to Aldose***


Transketolase
Transketolase
Transaldolase
Reductive anabolic pathways
Nucleic acid biosynthesis
Δ2C
Δ3C
Δ2C
NADPH, H+
NADPH, H+
NADP+
NADP+
H2O
CO2
1,2
3
4
5
6
7
8
Ribose 5-phosphate
Sedoheptulose 7-phosphate
Erythrose 4-phosphate
Xylulose 5-phosphate
Glucose 6-phosphate
6-Phospho-gluconate
Ribulose 5-phosphate
Xylulose 5-phosphate
Glyceraldehyde 3-phosphate
Fructose 6-phosphate
Fructose 6-phosphate
Glycer-aldehyde 3-phosphate
Oxidative reactions (irreversible)
Nonoxidative reactions (reversible)
Glycolytic pathway

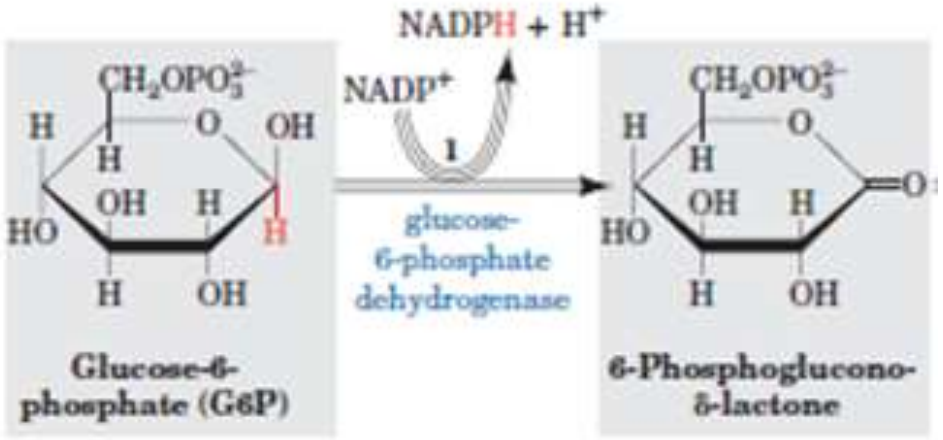
IRREVERSIBLE OXIDATIVE PHASE

- In this phase one CO_2 molecule and one pentose sugar-phosphate plus two NADPH are produced per each glucose 6-P molecule
- This phase is very important for the following tissues to get their need of NADPH

- This phase consists of three irreversible reactions:

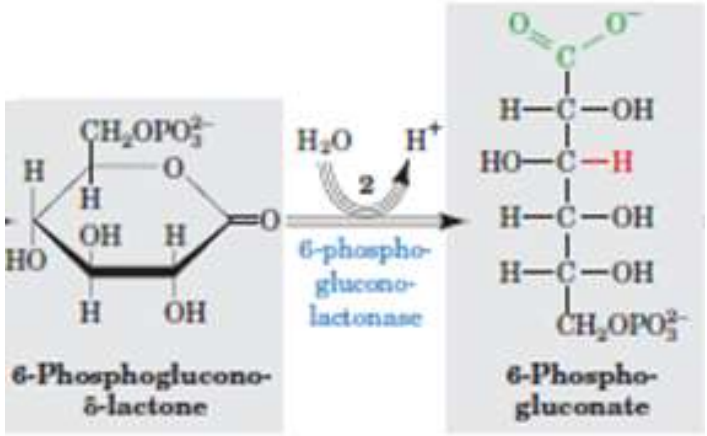
1. Glucose 6-phosphate **dehydrogenation (Oxidation)** to 6-phosphogluconolactone

- G6P come from Glycogen degradation, Gluconeogenesis or Diet
- The enzyme ***Glucose 6-phosphate dehydrogenase (G6PD)*** catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone by transferring a hydride ion ($2e + 1H^+$) from C1 of G6P to $NADP^+$ which is gets reduced to NADPH.

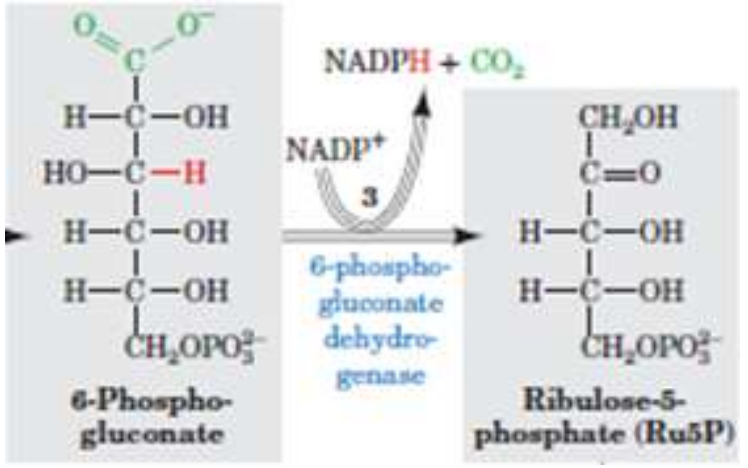


- **This reaction is the committed, rate limiting, and regulated step of the pathway.**
- **NADPH** is a potent ***competitive inhibitor*** of G6PD (High NADPH/$NADP^+$ ratio inhibit the Enzyme)
- If NADPH/NADP+ ratio is low → This enhances the activity of G6PD → flux of G6P through the pathway increases
- In addition, **Insulin** upregulates (يزيد) the expression (Transcription) of G6PD gene→ increase the rate of PPP in the absorptive state (after meal).

2. 6-Phosphogluconolactone is hydrolyzed to 6-phosphogluconate
- The nonenzymatic reaction occurs at a significant rate سرعته عالية حتى بدون انزيم
- ***6- phosphogluconolactone hydrolase*** increases the hydrolysis rate



3. The oxidative decarboxylation of 6-phosphogluconate to ribulose 5phosphate
- Catalyzed by ***6-phosphogluconate dehydrogenase***.
- Produces the Ribulose-5-P, CO_2 , and a second molecule of NADPH.



Reactions of Non-oxidative phase

- Isomerization and Epimerization of Ru5P

- Ru5P is converted to Ribose-5-P (R5P) by ***ribulose-5-phosphate isomerase*** or to Xylulose 5- phosphate (Xu5P) by ***ribulose-5-phosphate epimerase***

➢ Then, rearrangements of carbon atoms.

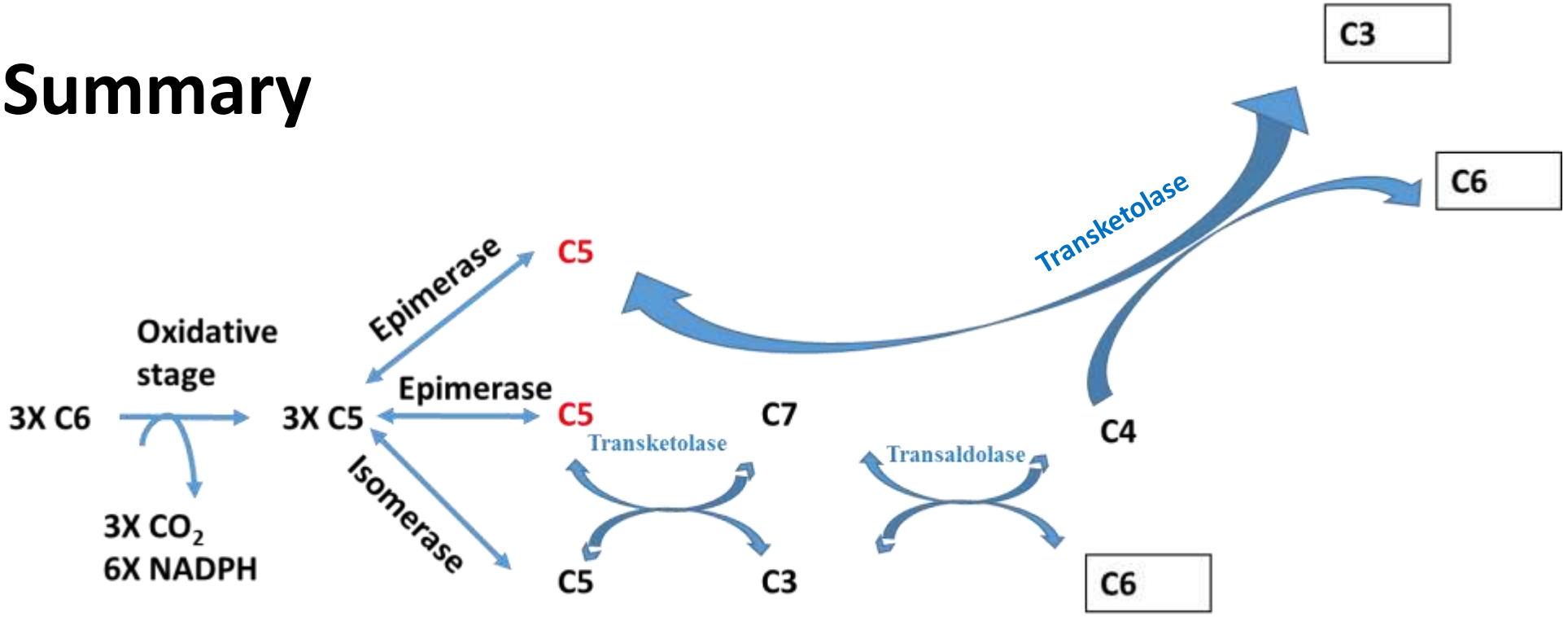
- three G6P molecules converted to **1 R5P and 2 Xu5P**
- The conversion of these three molecules of five carbon sugars (3 C5) to two molecules of six carbon sugars (2 C6) and one molecule of three carbon sugar (1 C3) is catalyzed by two enzymes, ***Transaldolase*** and ***Transketolase***.

- Transketolase catalyzes the transfer of two carbon units (C2 unit) and Transaldolase catalyzes the transfer of three carbon units (C3 unit)

1. Transketolase, which has a **Thiamine pyrophosphate cofactor (TPP)**, catalyzes the transfer of a C2 unit from Xu5P to R5P, yielding GAP and sedoheptulose-7-phosphate
2. Transaldolase catalyzes the transfer of a C3 unit from S7P to GAP yielding erythrose-4- phosphate (E4P) and F6P (note: Erythrose-4-P is precursor for aromatic amino acid synthesis Trp,Phe,Tyr)
3. In a second transketolase reaction, a C2 unit is transferred from a second molecule of Xu5P to E4P to form GAP and another molecule of F6P.

This phase of the PPP thus transforms two molecules of Xu5P and one of R5P to two molecules of F6P and one molecule of GAP.

Summary



Q: All of the following sugar arrangement are part of pentose phosphate pathway except:-

A. C5 + C5 → C7 + C3
B. C5 + C5 → C6 + C4
C. C7 + C3 → C6 + C4
D. C5 + C4 → C6 + C3
E. all of these arrangements occurs in pentose phosphate pathway

Q: in addition to pentoses , the pentose phosphate pathway involves sugars of these sizes except :-

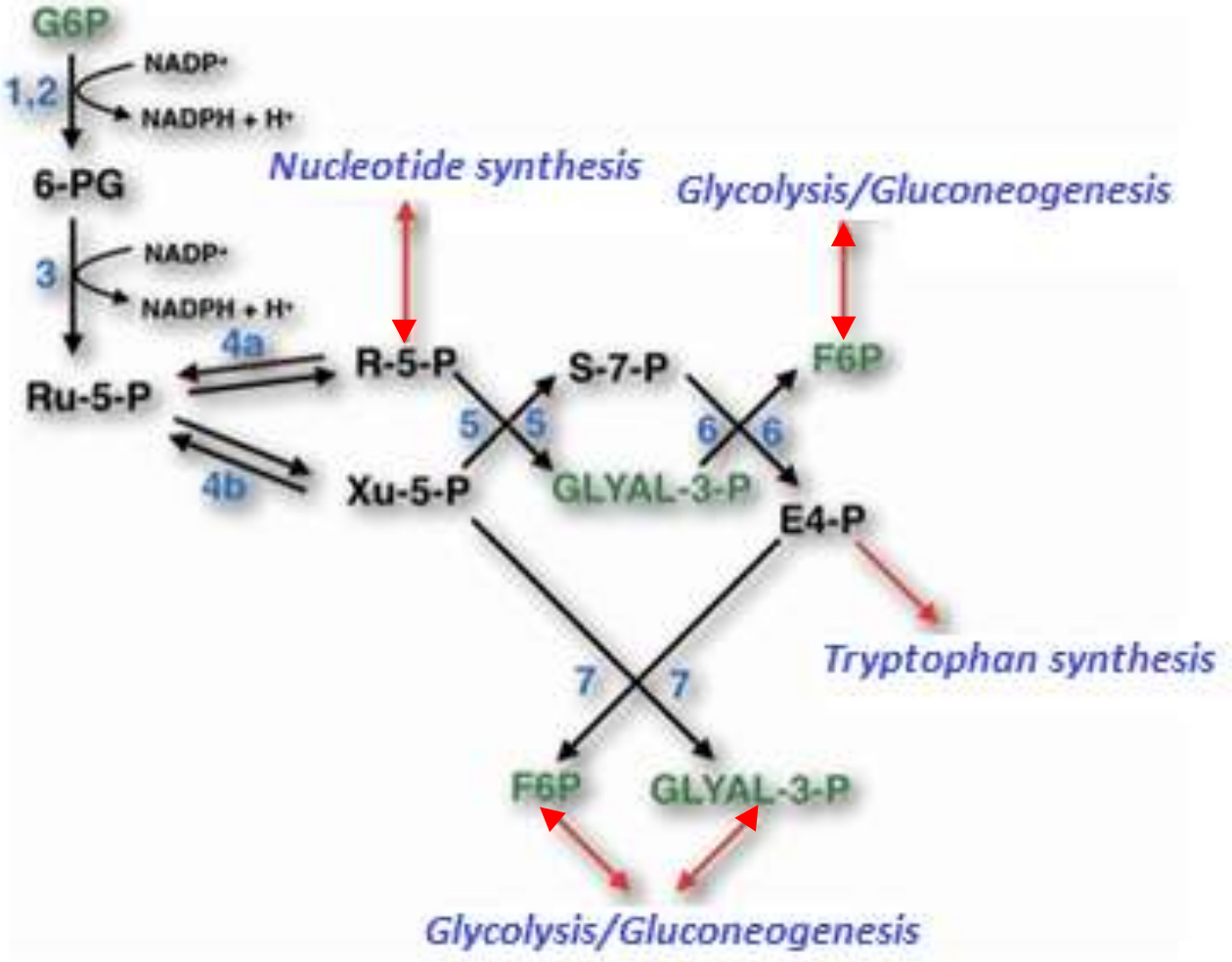
A. 3 carbons
B. 4 carbons
C. 6 carbons
D. 7 carbons
E. all of these sizes are used in this pathway

Notes:
Compared to other pathways PPP does not has specific starting molecule and specific end product
You may Start with G-6P or dietary pentoses or we can start with F6P and G3P
The products depends on the cell needs

PPP intersect with 4 other pathways:

- Glycolysis
- Gluconeogenesis
- Nucleotide synthesis
- Aromatic amino acid synthesis

PPP involve 9 sugars + 1 sugar derivative



How the PPP operate, from where we start and where we stop? This depends on the cell needs?

If the cell require NADPH but not Ribose-5-P

- We start from G6P through the Oxidative phase, then Isomerization and Epimerization and conversion of the pentoses by C-rearrangement to F6P and G3P

If the cell need Ribose-5-P but not NADPH

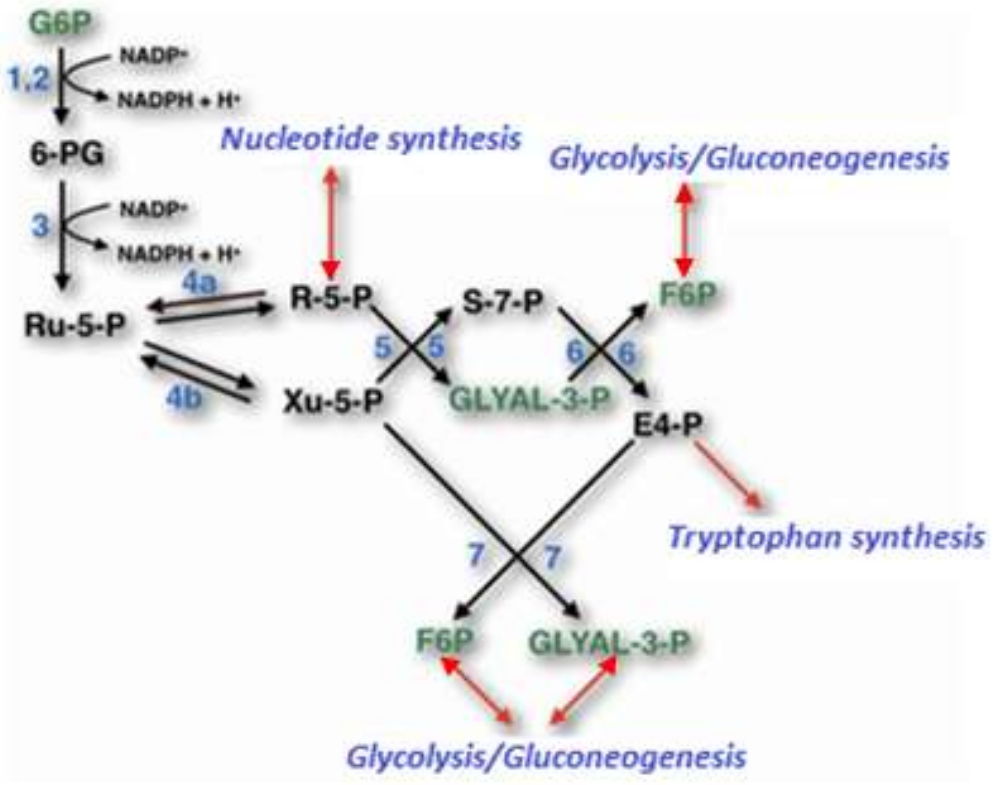
- We start from F6P and G3P through the the C-rearrangement to get Ribose-5-P without going through the oxidative steps

If the cell need both NADPH and Ribose-5-P

- We start from G6P through the oxidative phase, then the Ru5P is isomerized to Ribose-5-P

If the cell need E4P for synthesis of Aromatic amino acids not NADPH nor Ribose-5-P

-we start from F6P and G3P by C-rearrangement reactions to obtain E4P



Suppose we need NADPH and we don't have enough Glucose, what can we do?

- Nucleotide degradation to provide Ribose-5-P
- C-rearrangement to convert the pentoses to F6P and G3P
- Convert F6P and G3P to G6P then from G6P start through the oxidative phase

Important note about Pentose phosphate pathway

نقطة التقاء وتفرع

1. The pentose phosphate pathway (PPP) is an interchange of metabolic pathways.
2. It is important to cells as a) an important source of NADPH, b) an important source of ribose-5-P for nucleotide synthesis; c) an interchange; and d) a way to mix and match sugars (C-rearrangement) according to the needs of cells.
3. The pathway only has two oxidations, each of which produces NADPH. It has two isomerizations, and three sets of carbon rearrangements.
4. Carbon rearrangements are catalyzed by transaldolase (3 C swaps) and transketolase (2 C swaps).
5. The pathway doesn't really have a beginning or an end and the direction it moves depends on what is available and what the cell needs.
6. Molecules in the highest concentration will serve as input sources for the pathway and molecules in the least abundance will serve as outputs of the pathway.
7. Transketolase requires thiamine pyrophosphate (from the vitamin thiamine) as a coenzyme.
8. Pathways that can be input or output pathways include glycolysis/gluconeogenesis, aromatic amino acid metabolism, and nucleotide metabolism.

What are the uses of NADPH??

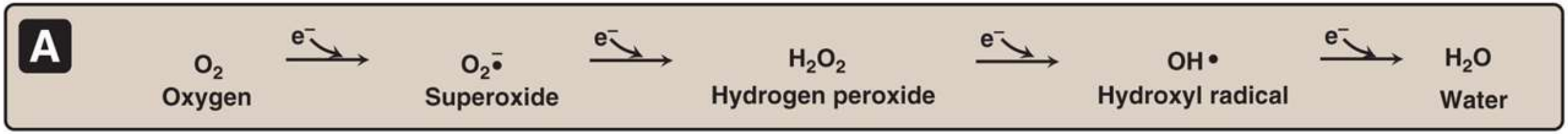
1. Reductive Biosynthesis (تصنيع) of Fatty acids and Steroids (cholesterol and steroid hormones).
2. NADPH required for reduction of Ribonucleotide to Deoxyribonucleotide for DNA synthesis
3. Important for Detoxification of harmful Oxidizing agents

E.g. Reactive Oxygen Species (ROS) (مركبات الاكسجين النشط) such as:

- **H_2O_2** Hydrogen Peroxide
- **$O_2^{\bullet -}$** Superoxide (Free radical) نظائر حرة تحتوي على الكترون منفرد
- **$OH^{\bullet}$** Hydroxyl radical (Free radical) اخطر واحد على الخلية

These oxidizing agents formed as by-products (نواتج ثانوية) of aerobic metabolism, Infections, drugs, toxins, alcohol, food…

ROS → oxidize and damage DNA, Lipids, proteins → cancer, Inflammation and cell death



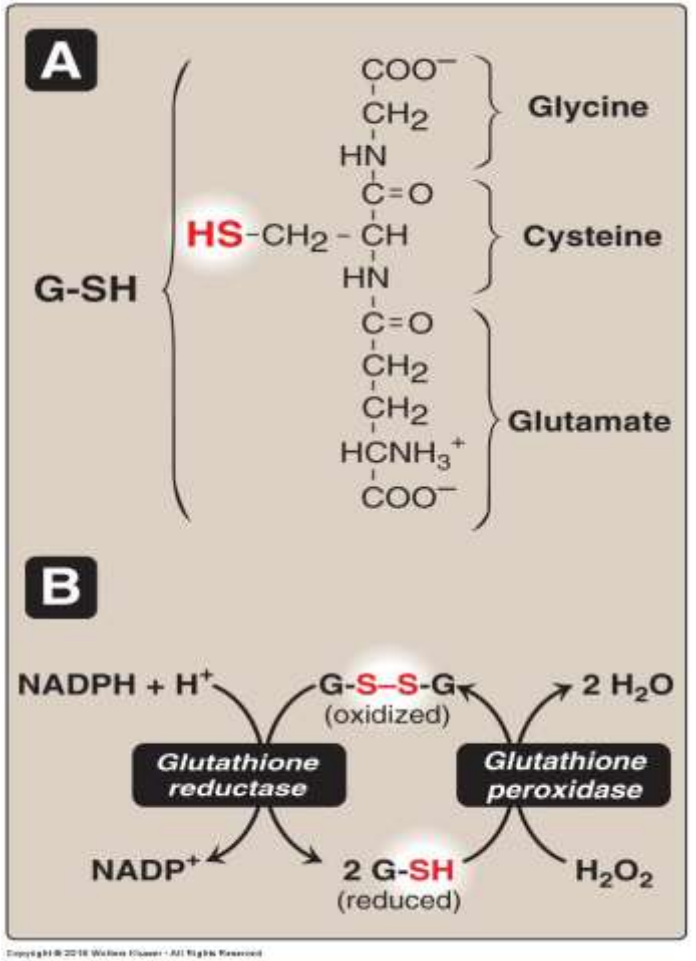
How can we detoxify H_2O_2??

This done mainly by ***Glutathione Peroxidase*** (Seleocysteine Containing Enzyme)

$$2\,GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$$

Reduced Glutathion → Oxidized Glutathion

Detoxification of H_2O_2 is catalyzed by a Selenoprotein called ***glutathione peroxidase***.
Glutathione is a tripeptide-thiol (γ-glutamyl-cysteinyl-glycine) present in most cells.
Glutathione peroxidase, forms oxidized glutathione (GS-SG), which no longer has protective properties

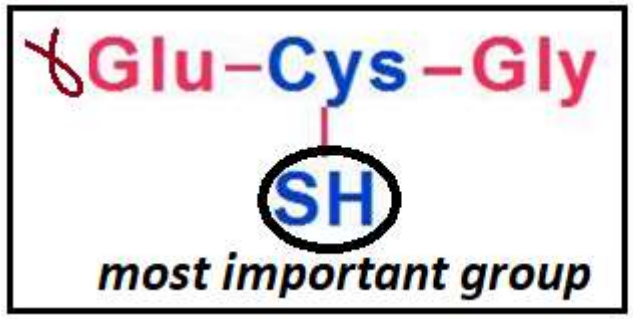


How to regenerate reduced Glutathione (GSH)

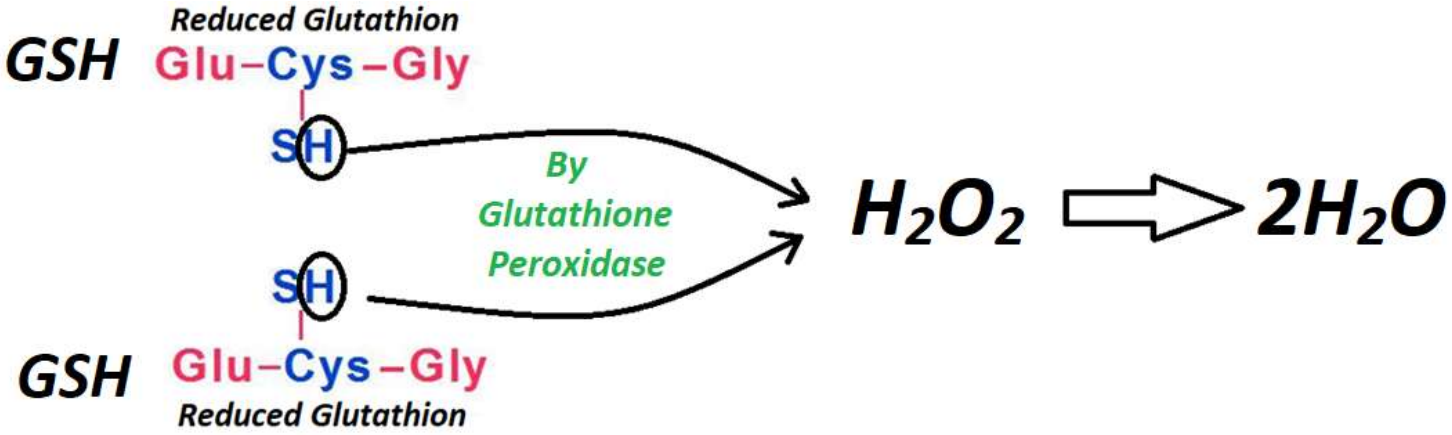
The cell regenerates GSH in a reaction catalyzed by ***glutathione reductase***, using NADPH as a source of reducing equivalents (2 electrons). Thus, NADPH indirectly provides electrons for the reduction of H_2O_2

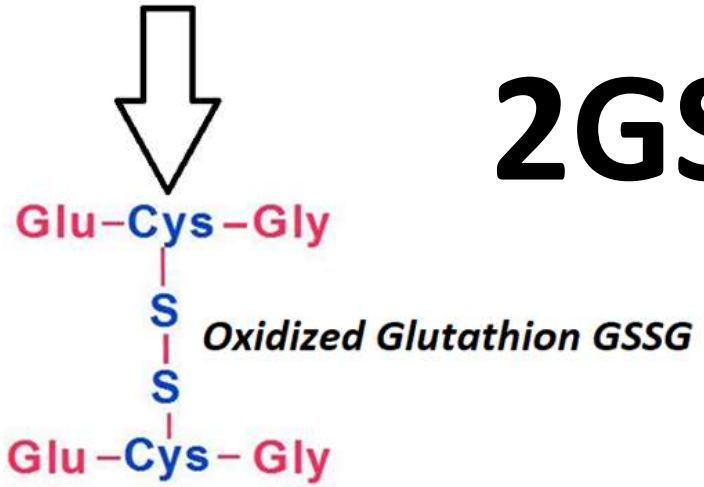
$$GSSG + NADPH \xrightarrow{Glutathion\ Reductase} 2\,GSH + NADP^+$$

Glutathione is an Antioxidant tripeptide-thiol (γ-glutamyl-cysteinyl-glycine)



GSH **Reduced Glutathione GSH**





2GSH → GSSG

بلعمة

4. NADPH help White blood cell phagocytosis and microbe killing

Neutrophils and monocytes are have both oxygen-independent and oxygen-dependent mechanisms for killing bacteria.

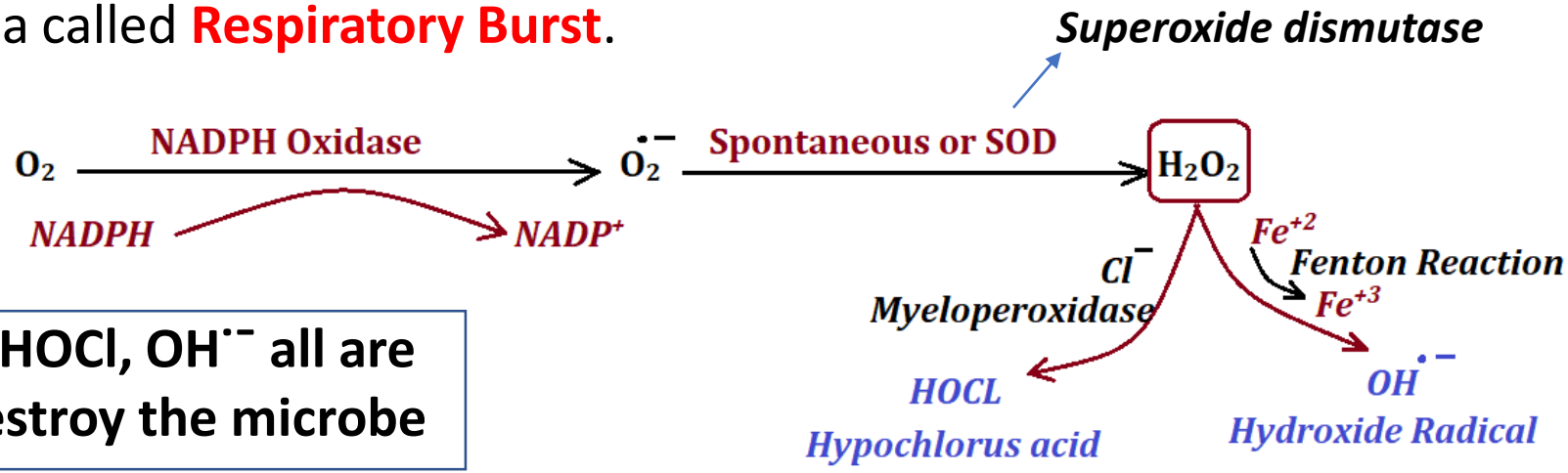
Oxygen-independent mechanisms: use pH changes in phagolysosomes and lysosomal enzymes to destroy pathogens.

Oxygen-dependent mechanisms: include the enzymes **NADPH oxidase** and **myeloperoxidase (MPO)** that work together in killing bacteria.

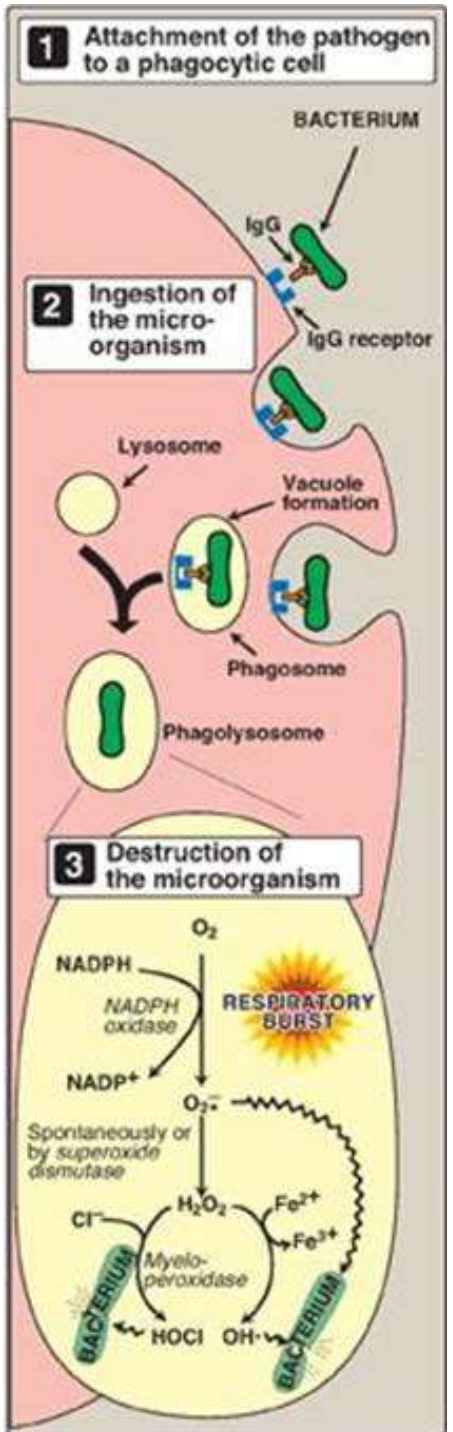
An invading bacterium is recognized by the immune system and attacked by antibodies (IgG) that bind it to a receptor on a phagocytic cell.

After internalization (ادخال) of the microorganism has occurred, NADPH oxidase, located in the leukocyte cell membrane, is activated and reduces O_2 to superoxide ($O_2^{\bullet -}$), a free radical ROS, as NADPH is oxidized.

This phenomena called **Respiratory Burst**.

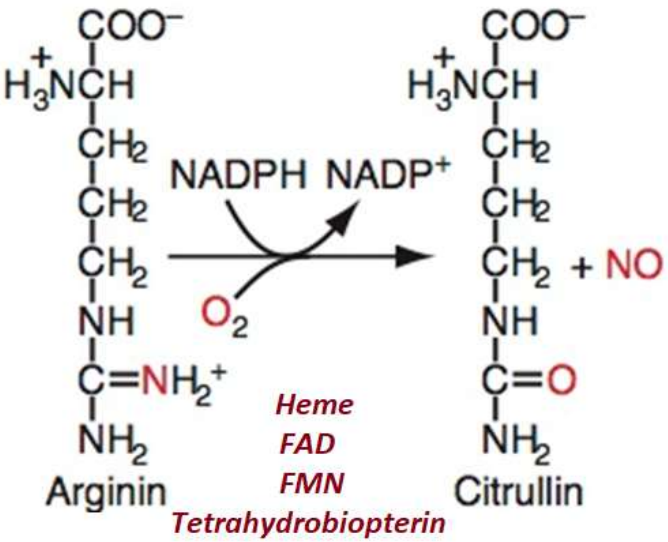


$O_2^{\bullet -}$, H_2O_2, HOCl, $OH^{\bullet -}$ all are ROS that destroy the microbe



5. Nitric oxide synthesis

- Arginine, O_2, and NADPH are substrates for cytosolic NO synthase [NOS].
- Flavin mononucleotide (FMN), FAD, heme, and tetrahydrobiopterin are coenzymes
- **NO** and citrulline are products of the reaction.
- Enzyme: Nitric Oxide Synthase (NOS)
- **Three NOS isozymes**.



a. **eNOS (Endothelium)** synthesize NO for Vasodilation and inhibition of platelet aggregation
b. **nNOS (Neuronal)** synthesize NO as Neurotransmitter
c. **iNOS (Inducible)** found in Macrophage, Monocytes, Neutrophil to generate Reactive Nitrogen species to kill microbes during infection

Note:

In addition of Glutathione and antioxidant enzymes some vitamins and metabolic intermediates can act as antioxidants fro example Vitamin C, Vitamin E, Carotenoid, and Uric acid

Glucose-6-P Dehydrogenase (G6PD) Deficiency الانزيم المسؤول عن الخطوة الاولى

- Most common ***Intracellular*** Enzyme Deficiency
- 200-400 Million Individuals (شخص) are affected (مصاب), Mainly in the Middle East, South East Asia, Mediterranean, and Tropical Africa
- Recessive (متنحي) X-linked (more common in Males XY) جين هذا الانزيم موجود على الكروموسوم X
- Patients of G6PD deficiency suffer (يعانو من) from attacks (نوبات) Hemolytic anemia (تكسر خلايا الدم الحمراء); Why???

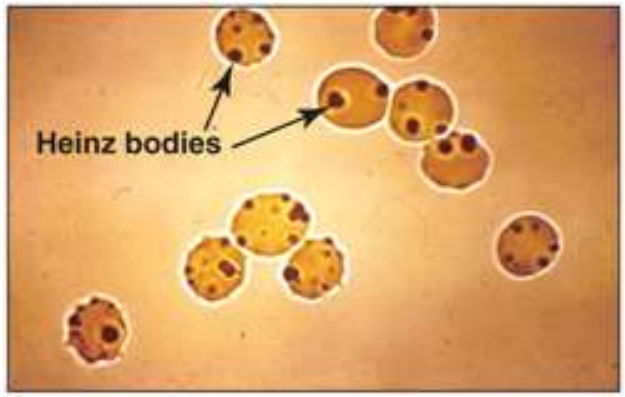
بعض الطفرات في جين G6PD لا تؤدي لنقص حاد بنشاط الانزيم بالتالي لا تسبب اي اعراض لحاملها

Also other cells can increase the synthesis of G6PD to compensate low activity (RBCs Cannot)

PPP is the Only source of NADPH in RBCs, Other cells have other source (Malate Dehydrogenase System "Malic Enzyme")

So, if G6PD is deficient, RBCs can NOT synthesize adequate (كافية) amount of NADPH

- NADPH is required for regeneration of GSH
- Low NADPH in RBCs means Low GSH, Consequently (بالتالي) RBCs will be weak against oxidizing agents
- So, if oxidative stress (Oxidizing agents اي حالة بتزيد) occurs → oxidation of lipids and Hemoglobin → Oxidized Cysteine SH group of hemoglobin accumulate in the cell forming insoluble structures called ***Heinz bodies*** → Hemolysis (تكسر خلايا الدم الحمراء) → Hemolytic anemia (فقر دم)

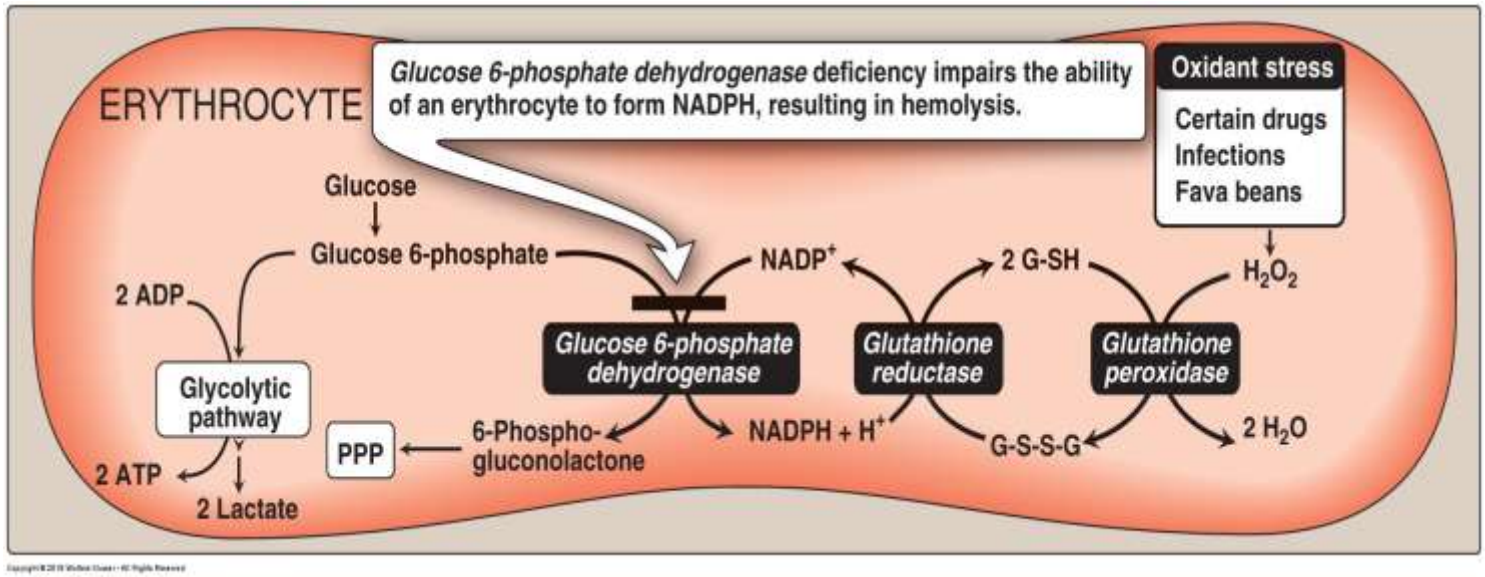


Accumulation of Bilirubin → Jaundice

In Words

1. Glutathione Peroxidase consume (يستهلك) GSH to GSSG for detoxification of H_2O_2
2. Glutathione Reductase regenerate (يعيد انتاج) GSH using NADPH
3. G6PD (PPP) is the only source of NADPH

If G6PD is deficient (نقص) then GSH can NOT be regenerated then the RBC can NOT detoxify oxidizing agents and eventually will have shorter life span→ Lyse



Note: G6PD deficiency provide resistance against Falciparum Malaria (the parasite that cause Malaria)

Oxidative Stress ac result from:

1. Drugs
   - Antibiotics (for example, sulfamethoxazole) بعض المضادات الحيوية
   - Antimalarial (for example, primaquine) بعض ادوية الملاريا
   - Antipyretics (for example, acetanilide) بعض خافضات الحرارة
2. Fava beans (Favism) الفول الاخضر
3. Infections (الالتهابات) which is the most common factor

بعض المصابين باكلو فول بدون ما يصير عندهم تكسر في خلايا الدم الحمراء لانو نقص الانزيم ما بكون عندهم حاد. لكن اي شخص باكل فول وبصير عندو تكسر في خلايا الدم الحمراء هاد اكيد مصاب ب G6PD deficiency