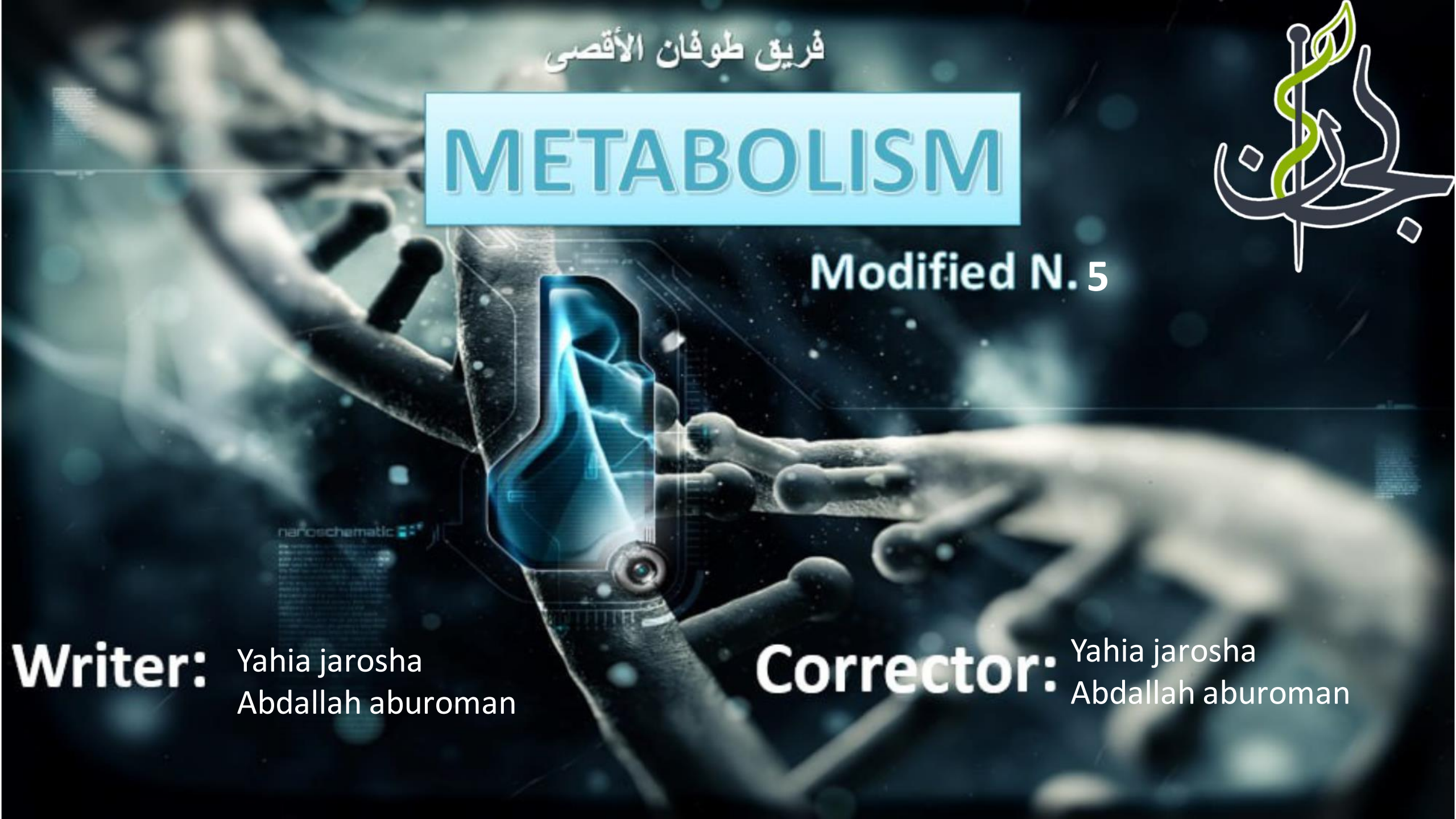
فريق طوفان الأقصى

# METABOLISM

## Modified N. 5




**Writer:** Yahia jarosha
Abdallah aburoman

**Corrector:** Yahia jarosha
Abdallah aburoman

# Pentose Phosphate Pathway (PPP) or Hexose Monophosphate Shunt

Dr Diala Abu-Hassan

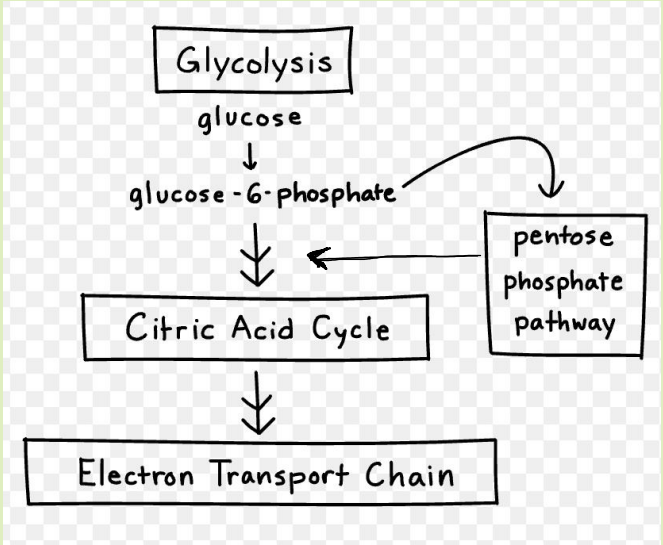
**NOTE:** the purpose of this pathway to :

**-1produce NADPH (mainly)** **sragus esotnep ecudorp -2**

- Occurs at well fed state

**NOTE:Shunt: تحويلةThe hexsoses pass alternative way rather than glycolysis, they undergo shunt then return back to glycolysis.**

**Attachment:**



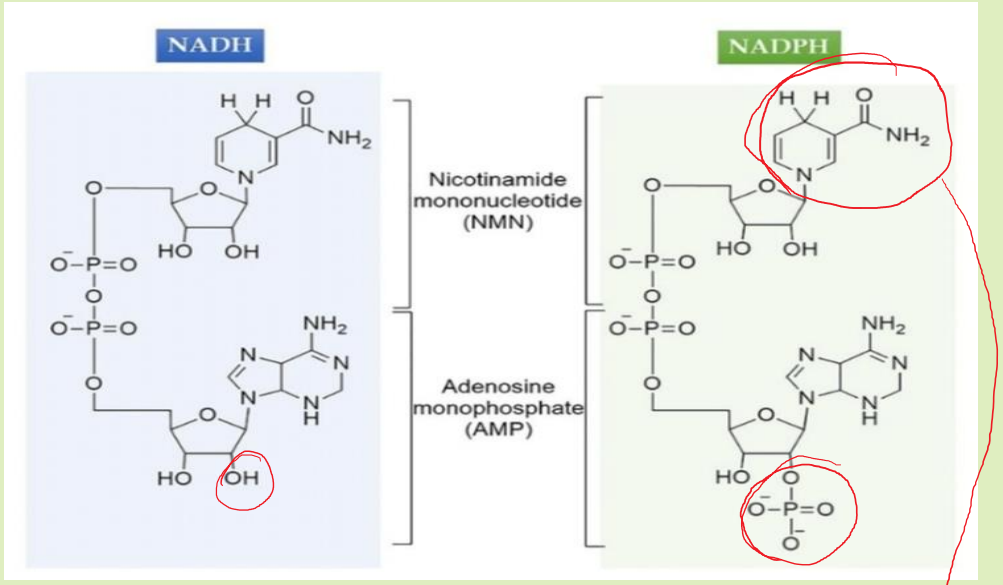
# Functions of the PPP

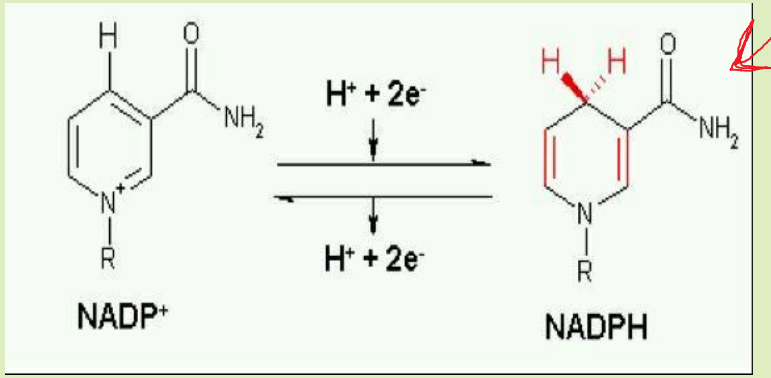
## 1. Production of NADPH Which is the most important

**Note:**

- **NADPH is an electron carrier has a role in oxidation reduction reaction**
- **NADPH stands for** nicotinamide adenine dinucleotide phosphate
- **NADH stands for** nicotinamide adenine dinucleotide
- The difference between them is the phosphate group on carbon number 2 in NADPH vs OH in NADH
- **In catabolic pathways the substrates are oxidized and coenzymes are reduced(NAD+ → NADH) while in anabolic pathways the substrates are reduced and coenzymes are oxidized(NADPH → NADP+)**

**Attachment:**

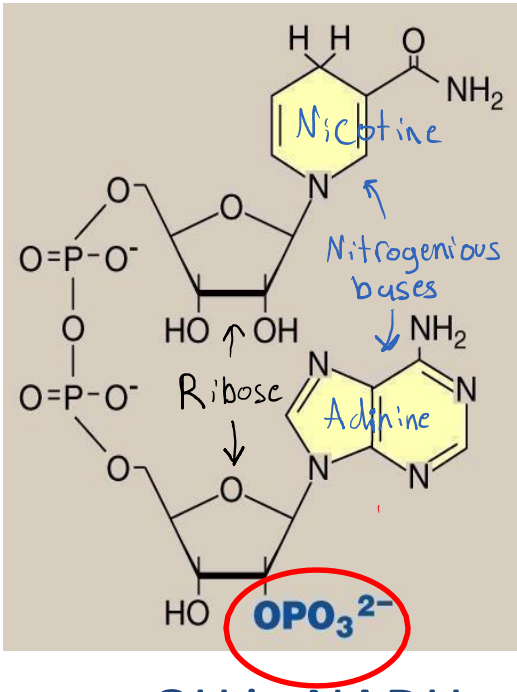



**oxidation reduction reaction occurs on Nicotine**

- NADPH dependent biosynthesis (anabolic pathway) of fatty acids
  - Liver, lactating mammary glands, adipose tissue
- NADPH dependent biosynthesis of steroid hormones
  - Testes, ovaries, placenta, and adrenal cortex

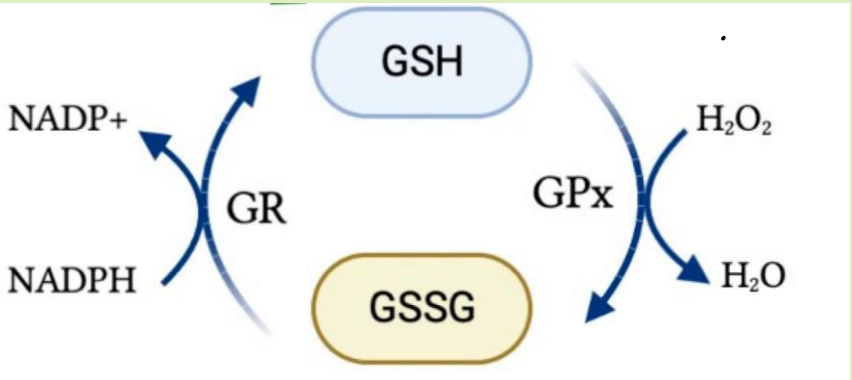
**NOTE:** synthesis of cholesterol which is used for sex hormones and other steroid hormones Cortisol aldosterone




- Maintenance of Glutathione (GSH) in the reduced form in the RBCs

**NOTE:** Recycling Glutathione ,tripeptide has a cystine in the middle which Oxidized by reactive oxygen species and make disulfied bridges, so it needs to be recycled to be used again. NADPH regenerate Glutathione again by reducing it , so we can get red of ROS due to the large amount of NADPH

**Attachment:**



# Functions of the PPP

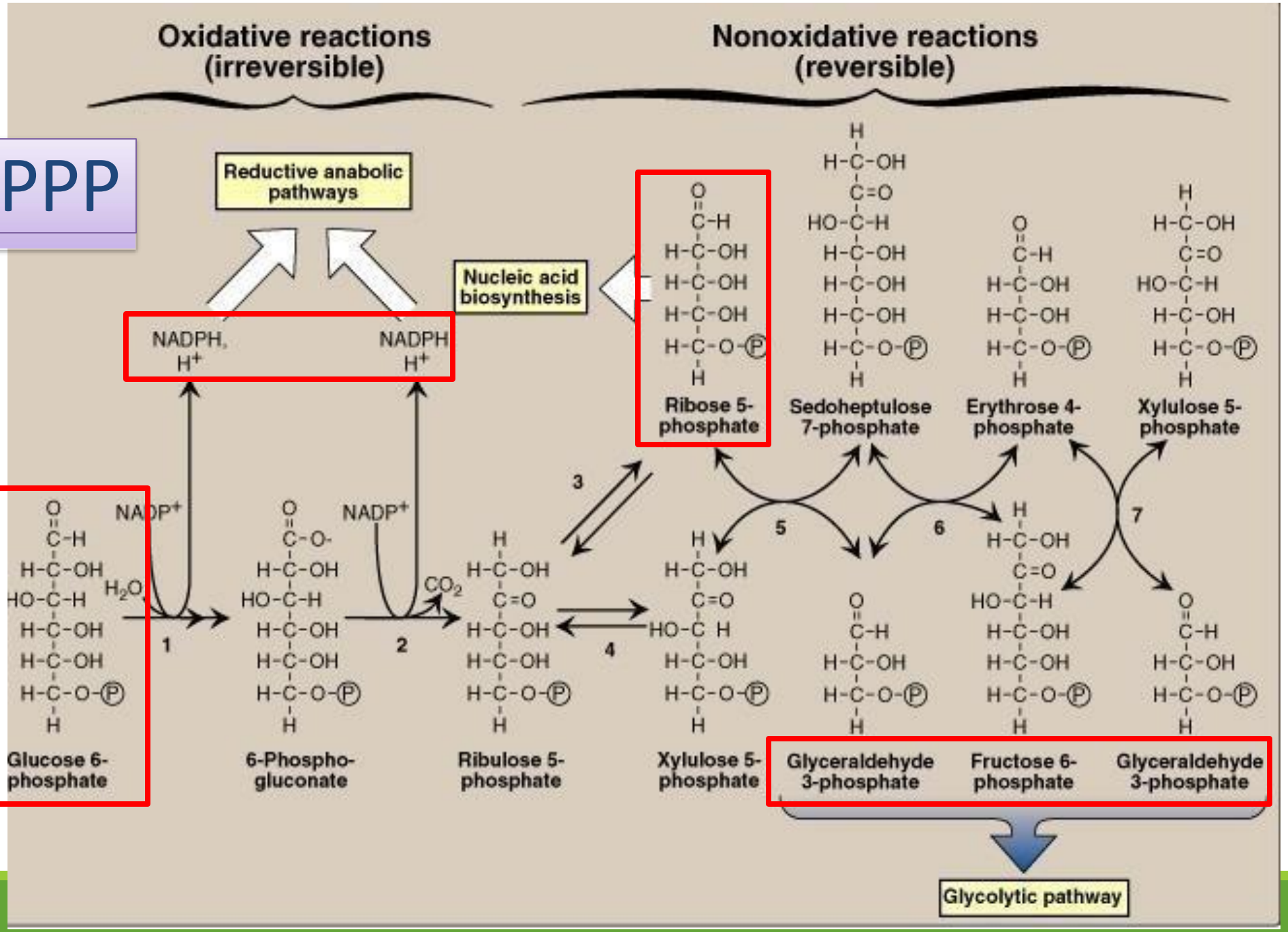
## 2. Metabolism of five-carbon sugars (Pentoses)

- Ribose 5-phosphate (nucleotide biosynthesis)
- Metabolism of pentoses

**NOTE:**

**Another use for PPP is to generate pentose sugars specially ribose which is used in nucleotides production. Nucleotides can be energy molecules , carriers of molecules during reactions , structure of DNA or RNA specially in DNA replication during S phase of cell cycle**

# PPP

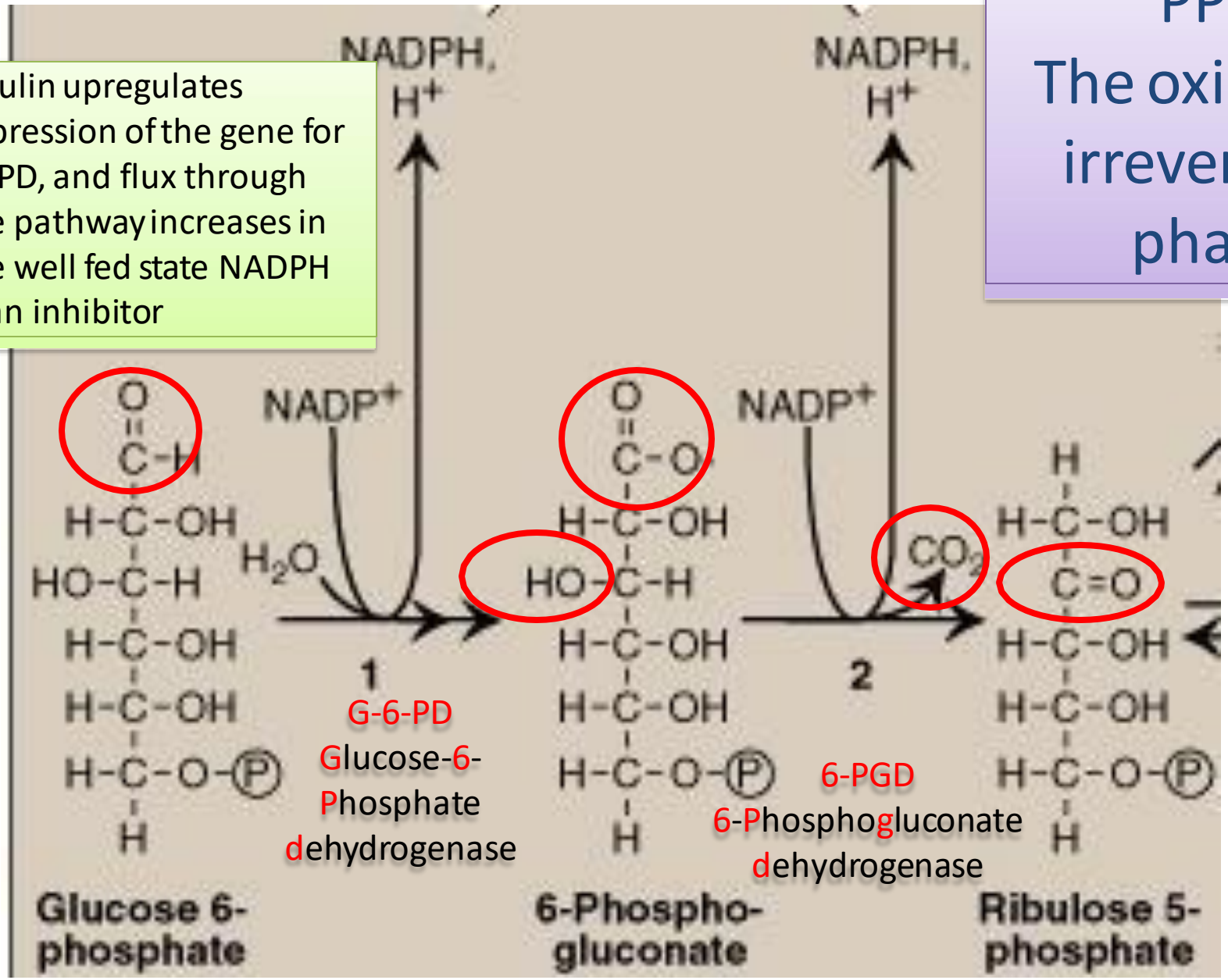

Oxidative reactions
(irreversible)
Nonoxidative reactions
(reversible)
Reductive anabolic pathways
Nucleic acid biosynthesis
NADPH, H+
NADPH H+
NADP+
NADP+
H2O
CO2
1
2
3
4
5
6
7
Glucose 6-phosphate
6-Phospho-gluconate
Ribulose 5-phosphate
Xylulose 5-phosphate
Ribose 5-phosphate
Sedoheptulose 7-phosphate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

- **The complement in this slide:** PPP pathway is composed of two phases:

1. the first phase is oxidative and irreversible it is composed of 2 steps where NADPH is produced
2. The second phase is reversible and nonoxidative , epimerisation, isommerisation and transfer of carbon units takes place in this phase to rearrange atoms. one of the products is Ribose-5-phosphate that is used in nucleotides synthesis

# PPP
# The oxidative irreversible phase

Insulin upregulates expression of the gene for G6PD, and flux through the pathway increases in the well fed state NADPH is an inhibitor




Glc. 6 Phosph. +2 $NADP^+$ ⟶ Ribulose 5-Phosph. + $CO_2$ + 2 NADPH

## The complement in this slide:

- First glucose enters the cell through the GLUT transporter then gets phospholyrated(through hexo- or gluco-kinase ) then converted to glucose-6-phosphate that will go maybe to glycolysis , PPP, interact with Aldose reductase to produce sorbitol …it Depends on the situation
- In PPP we are in the well Fed state ( high glucose )
- irreversible phase which composed of 2 irreversible step (oxidation):
- **First step :**

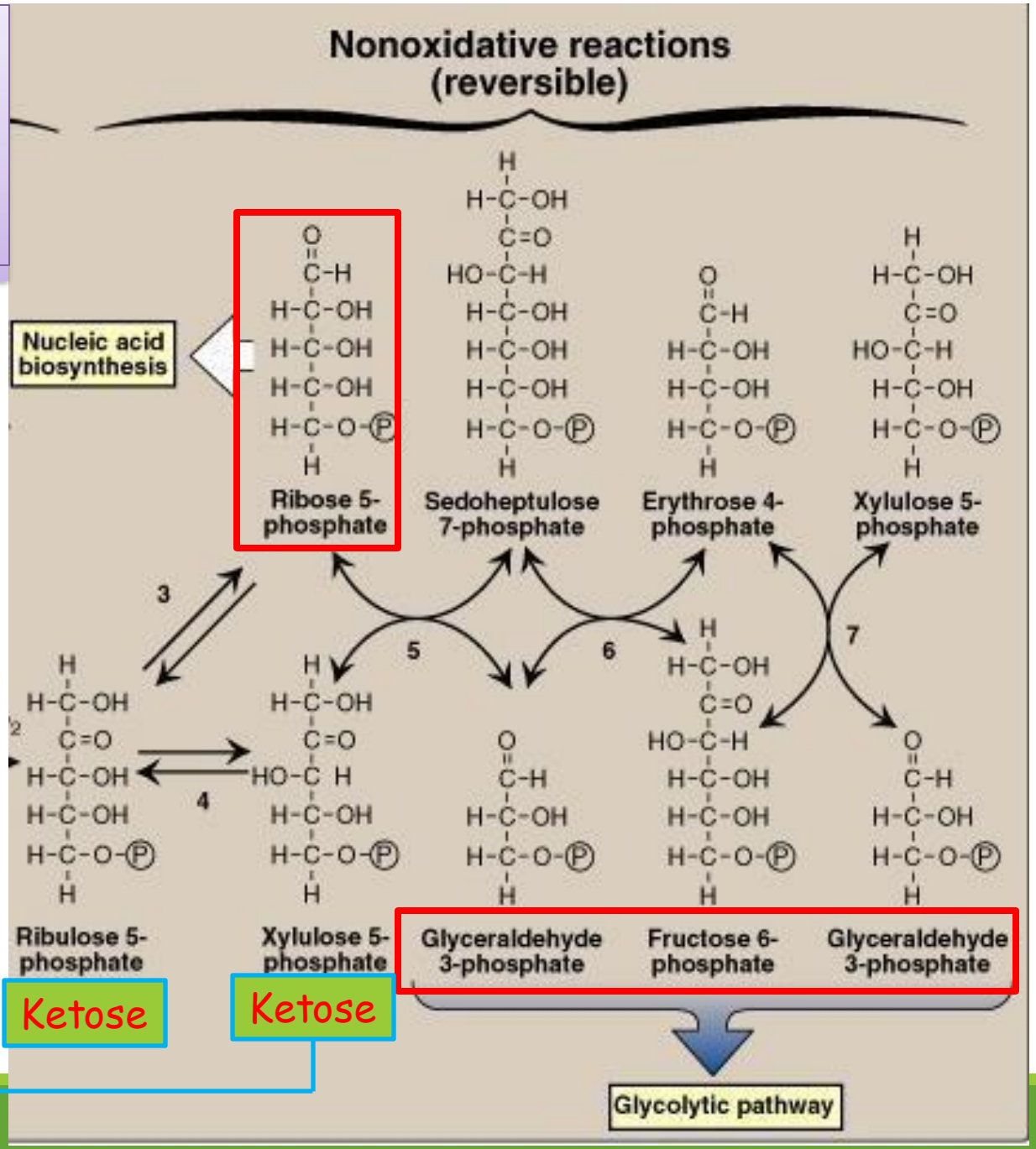
It starts with Glucose 6-P that will be oxidized to form 6-phosphogluconate
Enzyme : Glucose-6-phosphate dehydrogenase (G6PD) . This enzyme considered as target gene of insulin, as when insulin is high at well fed state, the activity of G6PD enzyme will increase

How : oxidation on C1 ( from carbonyl to carboxylic acid)
Reduction of NADP+ —> NADPH

- **Second step :**

6-phosphogluconate will be oxidized (on C3) and decarboxylated (on C1) so it will lose its carboxyl group to form Ribulose 5-phosohate
Enzyme : 6-Phosphogluconate dehydrogenase (6PGD). How : decarboxylation & oxidation
Reduction of NADP+ —> NADPH
So far, we finish the first phase with production of 2 molecules of NADPH for each glucose 6-P.

# PPP
# The non-oxidative reversible phase



- The complement in this slide:

Phase 2 :reversible phase (Non-oxidative)

**3rd step** : Ribulose 5-P (ketose) will be isomerized to Ribose 5-P (aldose) catalyzed by an isomerase enzyme

Ribose 5-p may exit the pathway to be used in nucleotides synthesis if we need it, however it will continue the rxns.

Then another Glucose molecule will enter( because no nucleic acid synthesis is required )and go through the irreversible step that we mentioned and it will go through:

**step 4:** it will convert Ribulose-5-phosphate to xylulose-5-phosphate (ketose also) by epimerase enzyme on C3 ( note: it won't go through step 3)

Epimerization : change the orientation of the OH group of one carbon only

**Step 5 :**

Ribose-5-phosphate will take 2 carbons from xylulose-5-phosphate , result in sedoheptulose7-phosphate ( A 7 carbon ketone sugar ), the lift over of xylulose 5-P turned to Glyceraldehyde-3-phosphate (3 carbon molecule)

BE ATTENTION: There are no loss of carbon in the form of CO2

**Step 6:**

Removed 3 carbons from sedoheptulose7-phosphate , result in Erythrose-4-phosphate( a 4 carbon aldose, 7-3=4)

Now where is the 3 carbons left ?? I added them to Glyceraldehyde-3-phosphate (3+3=6 carbon molecule) so it turns to Fructose-6-phosphate.

- The complement in this slide:

until step 6 we used 2 glucose molecules. Now we need another glucose molecule ! The **3rd** Glucose molecule will undergo ALL STEPS THAT WE MENTIONED until we reach the ribulose-5-phosphate, it will go through **step 4** and epimerized to xylulose-5-phosphate and STOPS then go to step 7.

**Step 7 :**

Xylulose-5-phosphate( 5 carbons ) from the third glucose lose 2 carbons and donate them to Erythrose-4-phosphate (4 carbons ) to form Fructose-6-phosphate .(6 carbons ), the lift over of it will make Glyceraldehyde-3-phosphate (3 carbons) .

So we generate 2 Fructose 6-p and 1 glyceraldehyde 3-P.

Go back to the figure and make sure you understand every single step :)

You need to know that :

Transketolase : transferring 2 carbons .

Transaldolsae : transferring 3 carbons .

NOTE :

Ketoses always donate carbons to aldoses

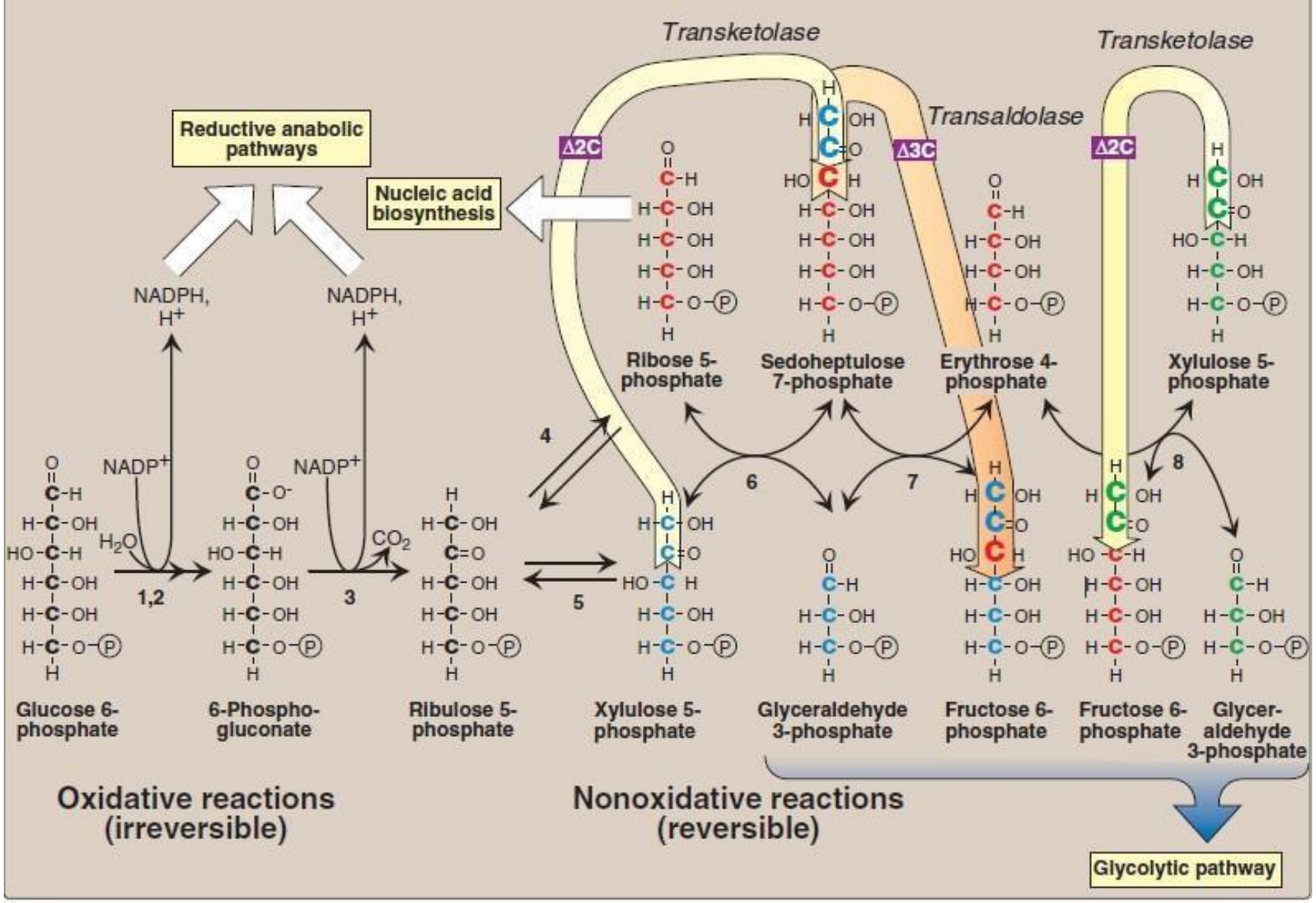
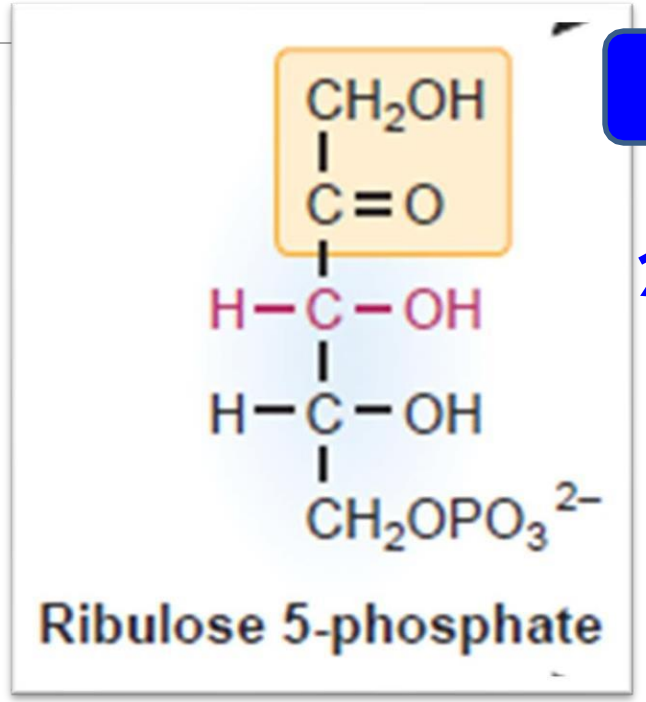


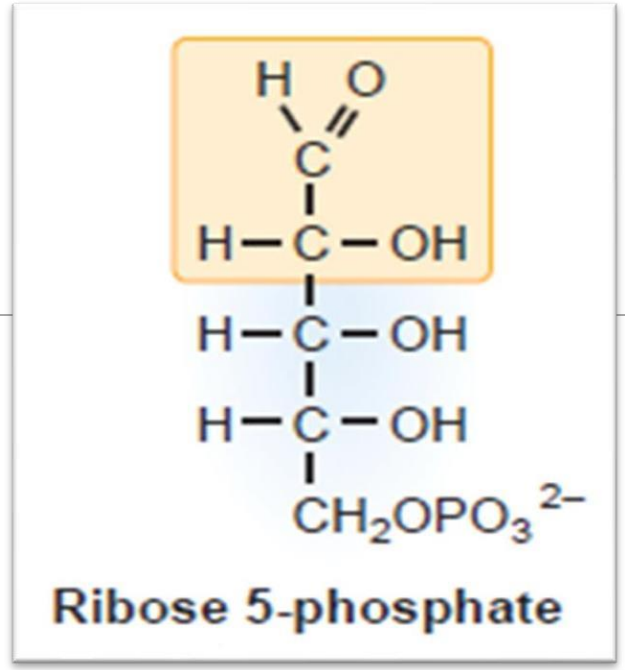

**Figure 13.2**

Reactions of the hexose monophosphate pathway. Enzymes numbered above are: 1,2) *glucose 6-phosphate dehydrogenase* and *6-phosphogluconolactone hydrolase*, 3) *6-phosphogluconate dehydrogenase*, 4) *ribose 5-phosphate isomerase*, 5) *phosphopentose epimerase*, 6) and 8) *transketolase* (coenzyme: thiamine pyrophosphate), and 7) *transaldolase*. Δ2C = two carbons are transferred in *transketolase* reactions; Δ3C = three carbons are transferred in the *transaldolase* reaction.

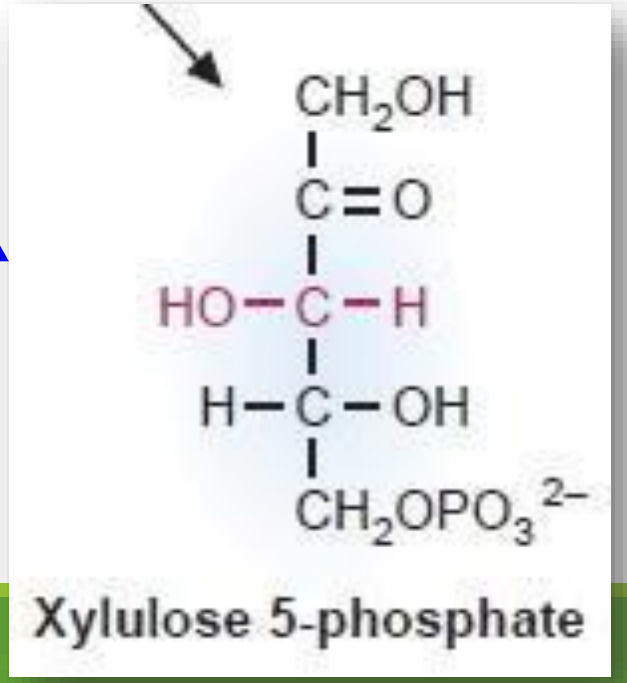
**NOTE:** next four slides is just review

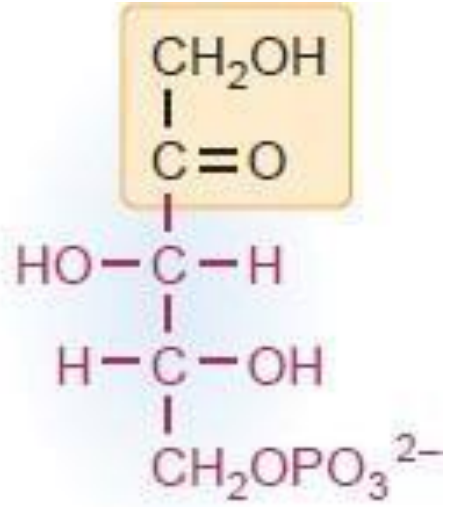



Isom erase



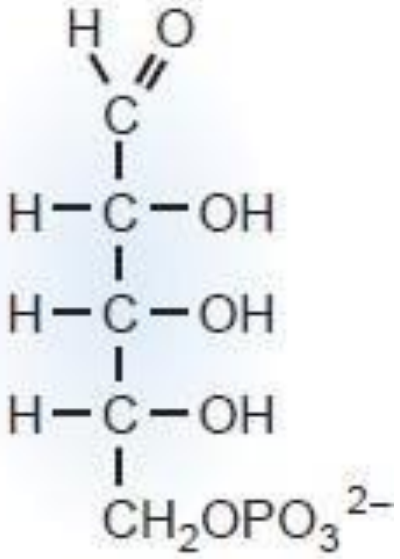

epim erase





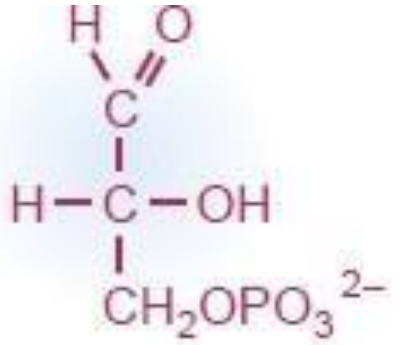

**Xylulose 5-phosphate**

+



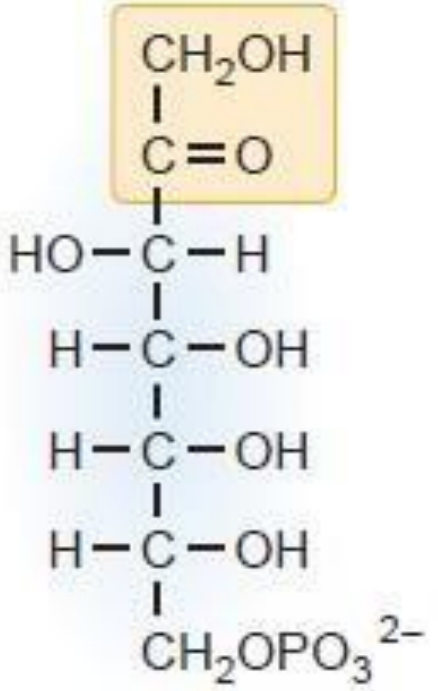

**Ribose 5-phosphate**

Transketolase

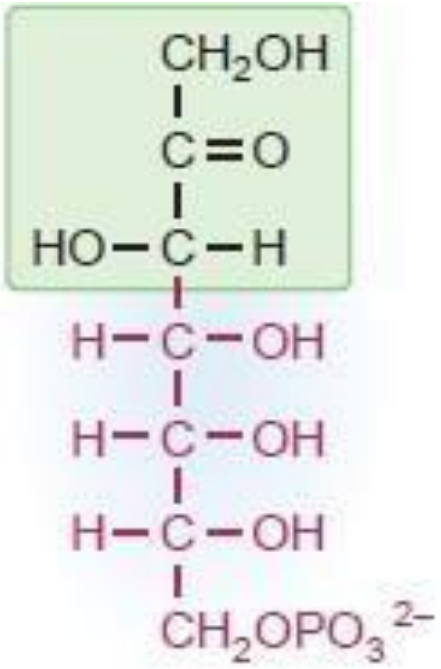



**Glyceraldehyde 3-phosphate**

+

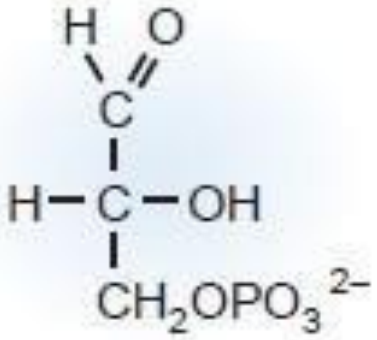



**Sedoheptulose 7-phosphate**



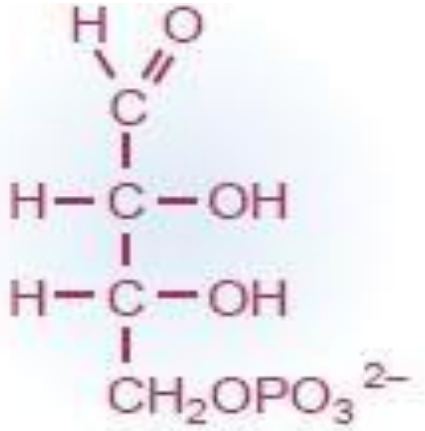
Sedoheptulose 7-phosphate

+



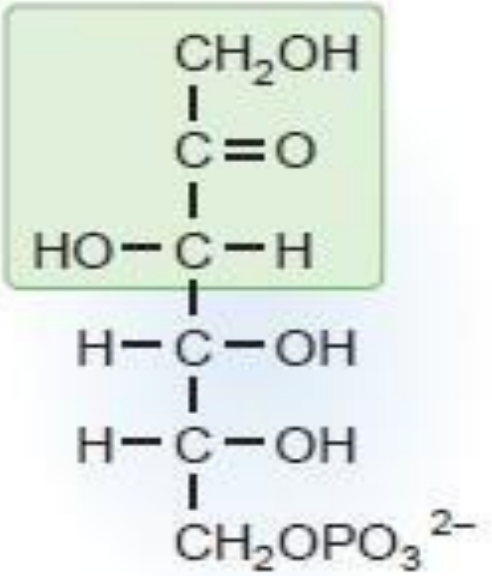
Glyceraldehyde 3-phosphate

Transaldolase

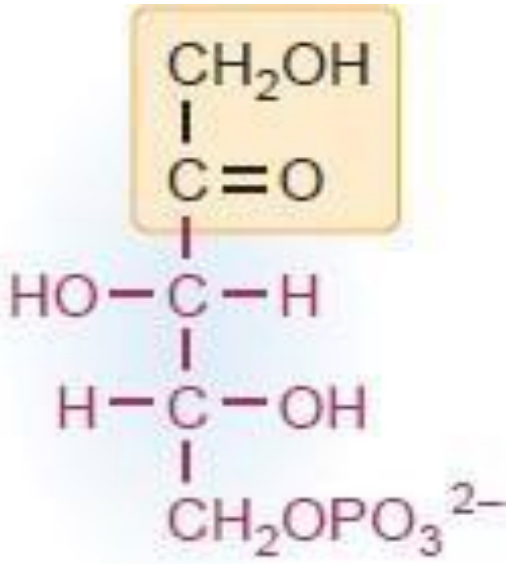


Erythrose 4-phosphate

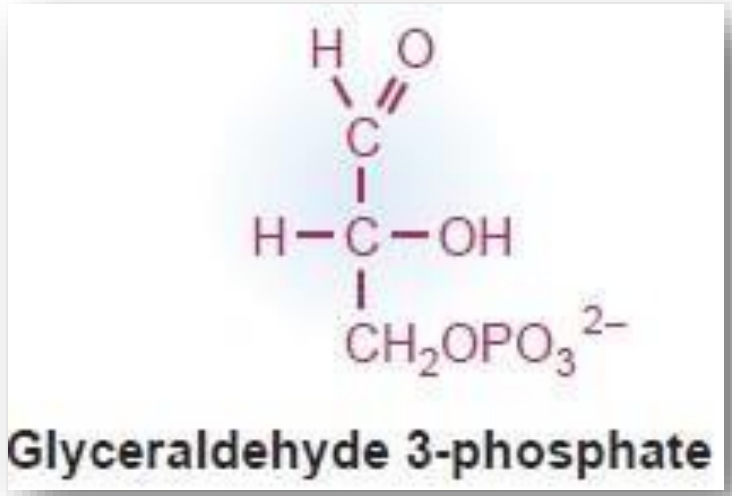
+



Fructose 6-phosphate



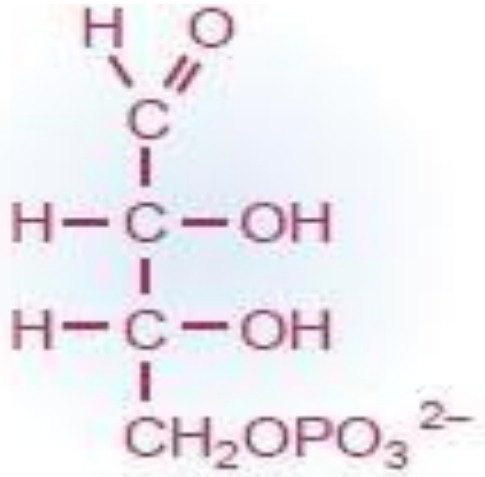
Xylulose 5-phosphate



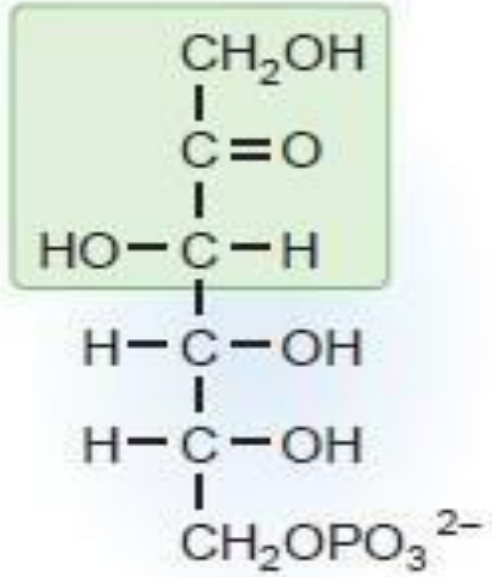
Glyceraldehyde 3-phosphate

Transketolase

+



Erythrose 4-phosphate



Fructose 6-phosphate

# Carbon movements in non-oxidative reactions

the whole idea of this slide is to make you know that every aldose will convert to ketose and vice versa

Ketose + Aldose ⇌ Aldose + Ketose

**C5** + **C5** ⇌ **C3** + **C7**

Ribose 5-P Xylulose-5-P Glyceraldehyde 3-P Sedoheptulose 7-P

**C7** + **C3** ⇌ **C4** + **C6**

Sedoheptulose 6-P Glyceraldehyde 3-P Erythrose 4-P Fructose 6-P

**C5** + **C4** ⇌ **C3** + **C6**

Xylulose 5-P Erythrose 4-P Glyceraldehyde 3-P Fructose P-6

# Summary of the non-oxidative reactions

- Reversible reactions
- Transfer of 2 or 3 carbon fragment
- Transketolase (2C), Transaldolase (3C)
- Ketose + aldose ⇌ ketose + aldose
- From ketose to aldose

- Rearrangment of sugars
- 3 pentose phosph.. ⇌ { 2 hexose phosph + 1 triose phosph. }

■ The complement in this slide:

In each step in **non oxidative reactions**: ketose and aldose react to give us other ketose and aldose, while transferring and rearrangement of carbons.

*Note* that these non oxidative reactions were ONLY about rearrangement of sugars. There wasn't any energy used or produced!

Then what was the PURPOSE of this second phase?
To supply us with glyceraldehyde-3-phosphate and 2 fructose-6-phosphate, which can go into glycolysis.

NOTICE that we use the first phase three times and the second phase one times to complete the pathway.

# The net non-oxidative reaction

- 3 Ribulose 5-phosph. ⇌ 2 Fructose 6-phosph. + Glyceraldehyde 3-phosph.

**NOTE:** that 3 ribulose-5-phosphate in total means 15C, and as we mentioned before there's no decarboxylation for the carbon number to decrease. Only transferring carbons, so the carbon number stays the same. We arranged the 3 ribulose-5-phosphate to:

2 fructose-6-phosphate ( since fructose is 6 carbon, so 2x6=12)

1 glyceraldehyde-3-phosphate ( 3 carbons)

- Multiply by 2
- 6 Ribulose 5-phosph. ⇌ 4 Fructose 6-phosph. + 2 Glyceraldehyde 3-phosph.

**NOTE**: If we multiply by 2, then we use 6 of **ribulose-5-phosphate**, resulting in:

- 4 **fructose-6-phosphate**
- 2 **glyceraldehyde-3-phosphate**

↓

5 Fructose. 6-Phosph.

- The complement in this slide:

Here the 2 **glyceraldehyde-3-phosphate** can give us 1 **fructose 6-phosphate** in addition to the 4. So 5 fructose-6-phosphate in total. 6 pentoses produce 5 hexoses (this is not the real products )

# Net Products of the Reactions

**NOTE:** this must be fructose 6-P but we consider its as glucose to make them all similar

3 Glc. 6-P + 6 $NADP^+$ ⟶ 2 Glc. 6-P + GA3P + 3 $CO_2$ + 6 NADPH

**NOTE:** 3CO2 from 3 6-phosphogluteranate decarboxylation

6 Glc. 6-P + 12 $NADP^+$ ⟶ 4 Glc. 6-P + 2 GA3P + 6 $CO_2$ +12 NADPH

↓

Glc. 6-P

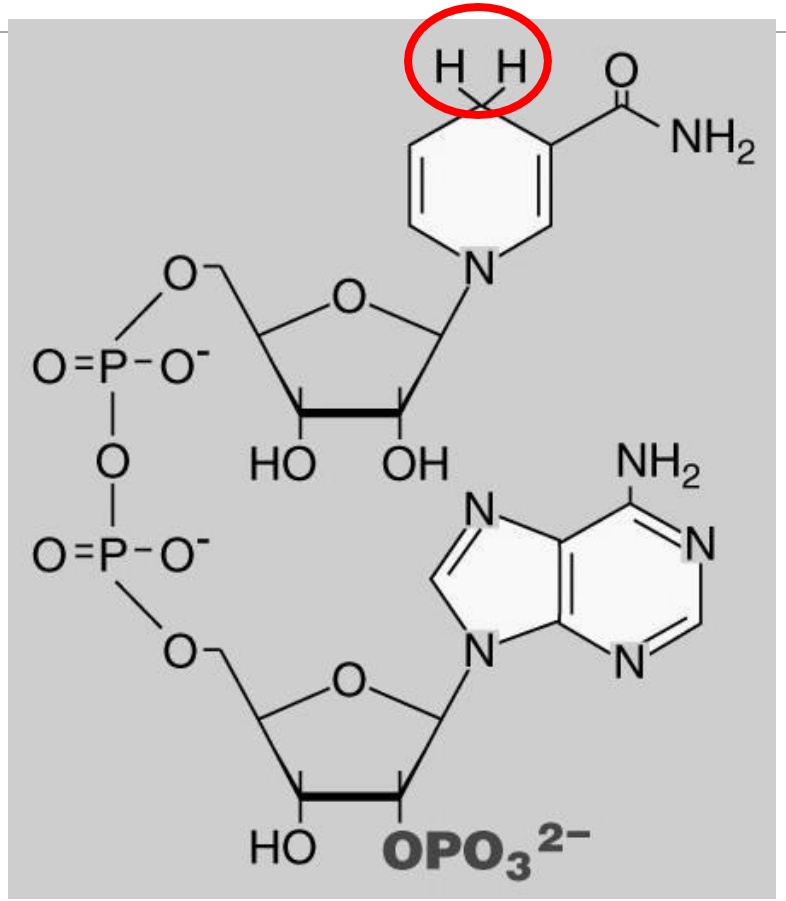
**NOTE:** the next slide explain the last equation

6 Glc. 6-P + 12 $NADP^+$ ⟶ 5 Glc. 6-P + 6 $CO_2$ + 12 NADPH

- **The complement in this slide:**
- Again this is not the real equation, the massage we can get it that when 6 GLUCOSE 6-P molecules enter the PPP they will lose one glucose in the form of CO2 and conserve the atoms of five molecules. And we generate NADPH .

# Why NADPH and NADH?

- Enzymes can specifically use one NOT the other
- NADPH and NADH have different roles
- NADPH exists mainly in the reduced form (NADPH)
- NADH exists mainly in the oxidized form ($NAD^+$)
- In the cytosol of hepatocyte
  - $NADP^+/NADPH \approx 1/10$
  - $NAD^+/NADH \approx 1000/1$



- **The complement in this slide:** NAD+/NADH and NADP+/NADPH are like having two poles, but one of them prefer the oxidized form while the other prefer the reduced form.

- • NADH Prefer the oxidized form, it is found in NAD+ more than NADH(inside the sole anti patricides the ratio between them is 1000/1).

- NADPH are found more in the reduced form (NADP+/NADPH = 1/10).As a result, they work opposite to each other.

# What are the uses of NADPH?

## 1. Reductive Biosynthesis

- Some biosynthetic reactions require high energy electron donor to produce reduced product
- Examples: Fatty acids, Steroids …

- **The complement in this slide:** NADPH is very important specifically in anabolic pathway, because anabolic pathways usually involve reduction of the main substrate and oxidation of the co-enzyme (NADPH in this case).

- ROS are produced in all states (health/ non-health), but the difference is in the response of the body to those ROS and how it can deal with them.

- ROS are highly reactive substances, they react randomly with any molecule like DNA, proteins etc. when ROS react with proteins, they increase or decrease their function, so we need to control them by some mechanisms.

# What are the uses of NADPH?

## 2. Reduction of Hydrogen Peroxide

- $H_2O_2$ one of a family of compounds known as Reactive Oxygen Species (**ROS**)
- Other: Super oxide, hydroxyl radical,
- Formed continuously
  - As by products of aerobic metabolism
  - Interaction with drugs and environmental toxins
- Can cause chemical damage to proteins, lipids and DNA ➔ cancer, inflammatory disease, cell death

**NOTE: ROS are highly reactive substances that contain oxygen.**

- **The complement in this slide:** The ROS are produced continuously from all the metabolic pathways producing side products which maybe these ROS (like super oxide ion, Hydrogen peroxide and Hydroxyl radical), even sometimes they have to be produced in order to defend our body against microorganisms. Also ROS can be produced by toxins.

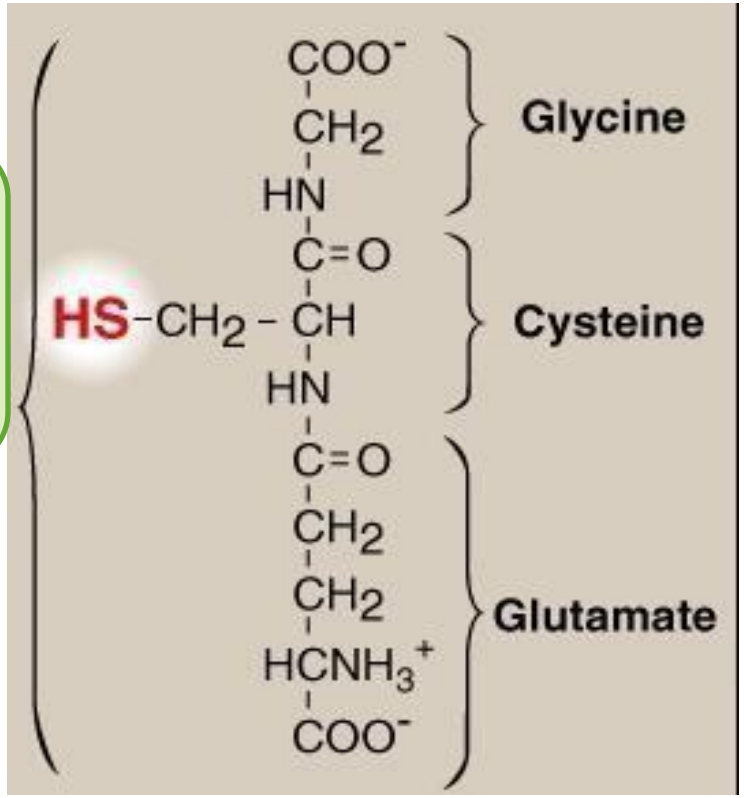
- We want them to react with another specific substances that can get rid of this function. (Prevent them from reacting with DNA or another protein disrupting their function and structure and even may cause some diseases like cancer and inflammatory diseases).

# Enzymes that catalyze antioxidant reactions

1. Glutathione peroxidase

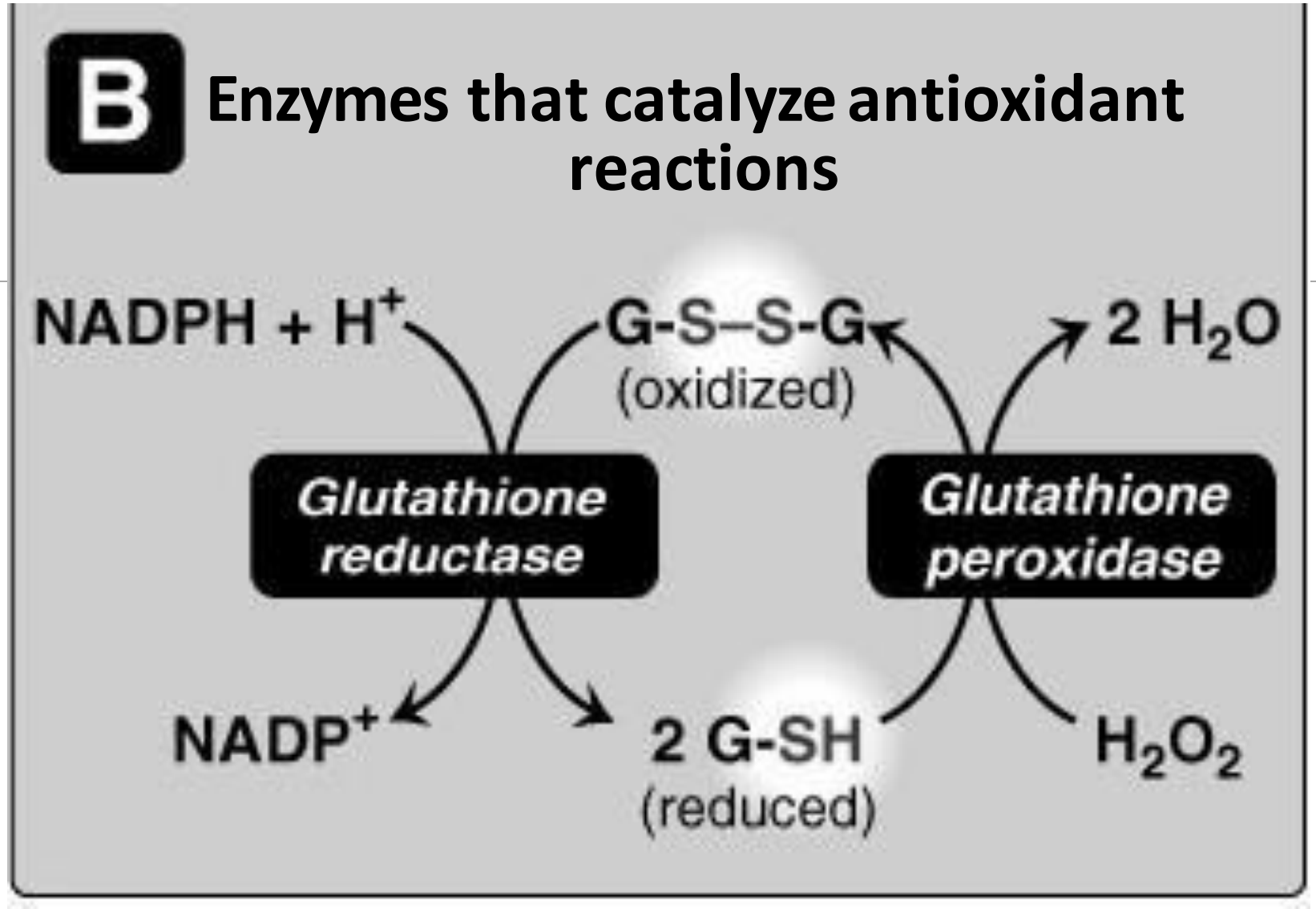
- Glutathione is a reducing agent
- Tripeptide
- GSH is the reduced form
- Oxidation ➔ two molecules joined by disulfide ( GSSG )
- 2 GSH ⟶ GSSG

**NOTE:** Glutathione peroxidase is considered the internal defense mechanism against ROS



- **The complement in this slide:** Glutathione as we remember is a tri peptide made of glycine, cysteine, and glutamate. The most important one is cysteine because of the thiol group that can alternate between oxidized and reduced state.
- If it reduced it will be in the form of GSH but if it is oxidized then two glutathione molecules will join together by a disulfide Bridge, and it will be named as GSSG.

- For example, $H_2O_2$ is produced as an ROS and we need to get rid of it, so we need to get to hydrogens for the extra oxygen in the molecule to form $H_2O$, and these two extra hydrogens are from the glutathione so it will be oxidized in the form of GSSG and $2H_2O$ molecules will be produced.
- The enzyme that is involved in this reaction is the glutathione peroxidase. And now I get rid of the ROS, but we also need to recycle the glutathione to reuse it again by reducing it using glutathione reductase. In this point NADPH is oxidized providing 2H for the purpose of reducing Glutathione.




Glutathione peroxidase is Selenium requiring Enzyme

RBCs are totally dependent on PPP for NADPH production

# Clinical Hint: G6PD Deficiency

- Common disease
- characterized by hemolytic anemia
- 200 – 400 millions individuals worldwide
- Highest prevalence in Middle East, S.E. Asia, Mediterranean
- X-linked inheritance
- > 400 different mutations
- Deficiency provides resistance to falciparum malaria

- **The complement in this slide:** G6PD deficiency is a common disease that is inherited on the X chromosome (autosomal recessive) ,So males are more susceptible to it.

- Many mutations can happen not all patients have the same mutations but all of these mutations are on the same gene (>400 mutation) so there are a differences between patients in the severity. This deficiency means that the pathway is going down because this is the first enzyme in the whole pathway so there is no NADPH production ,so they build oxidative stress and a metabolic pathways that depend on the NADPH will be compromised.

- And as a result the cells that will be mostly affected are the RBCs because of accumulation of oxidative stress: they like NADPH to work with them so it will cause hemolysis, these patients suffer from hemolytic anaemia(RBC's half life become less than normal-120 days-). Those patients are more resistant to malaria (the reason still unknown).

- The limit of the bodies of these patients to cope with oxidative stress is less than normal bodies Depending on the deficiency.

# Precipitating Factors in G6PD Deficiency

- Oxidant drugs
  - Antibiotics e.g. Sulfomethxazole
  - Antimalaria Primaquine
  - Antipyretics Acetanalid
- Favism due to vicine and covicine in fava beans in some G6PD deficient patients
- Infection
- Neonatal Jaundice

**NOTE:** those factors increase the oxidative stress.

**NOTE:** infection (bacteria or viruses) ROS are produced by the body to kill the microbes

**NOTE:** Bilirubin is produced as a result of heme group metabolism

اللهم انصر أهل غزة وثبت اقدامهم ،اللهم احفظهم بعينك التي لا تنام ، اللهم استر عوراتهم وآمن روعاتهم

V2: SLIDE 17

Sedoheptulose 7-P INSTEAD OF Sedoheptulose 6-P

Xylulose-5-P INSTEAD OF Ribulose-5-p

V3: Slide 7

**Ribose-5-phosphate instead Ribose-6-phosphate**