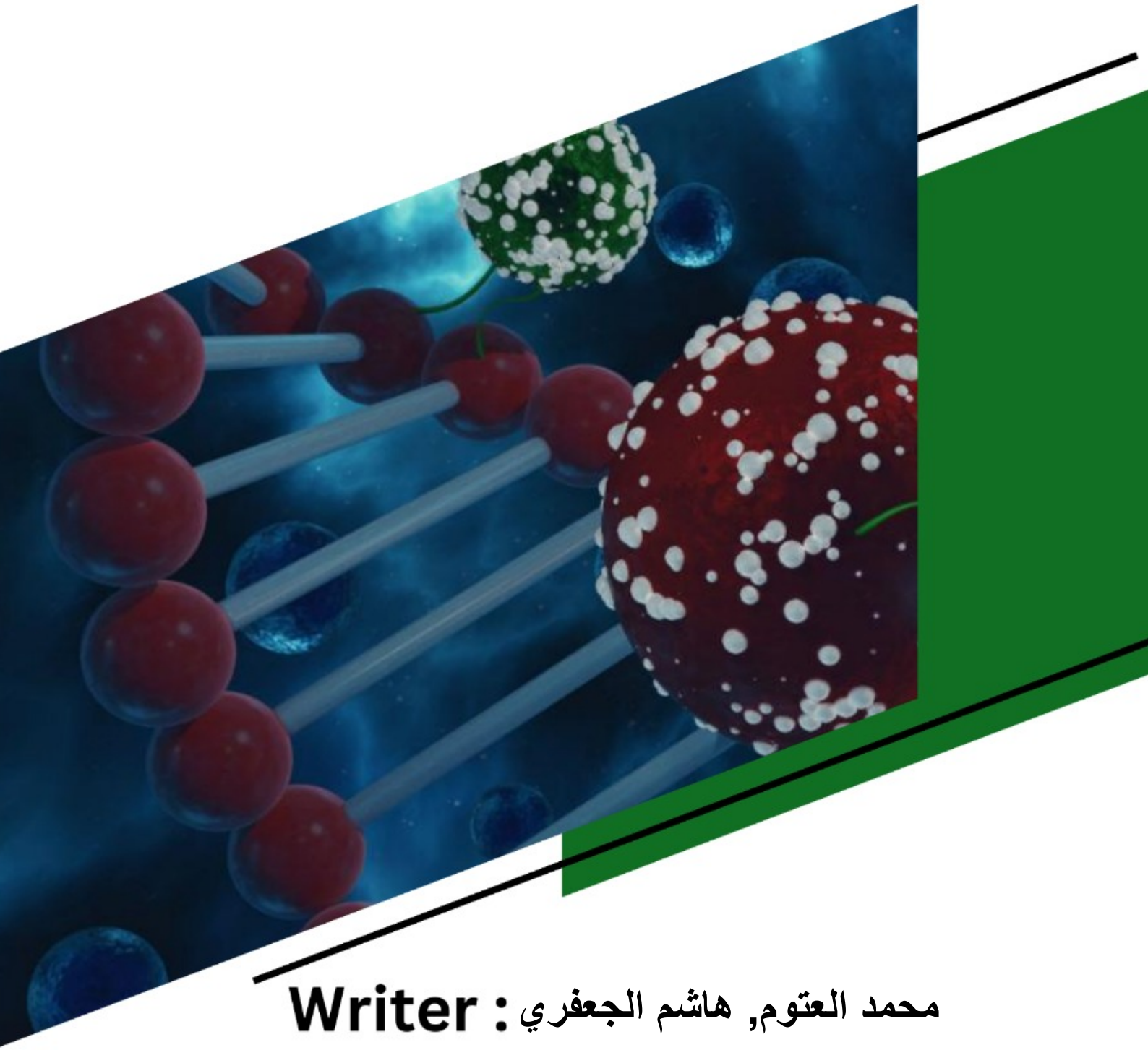
Doctor 021

METABOLISM

Sheet no. 17



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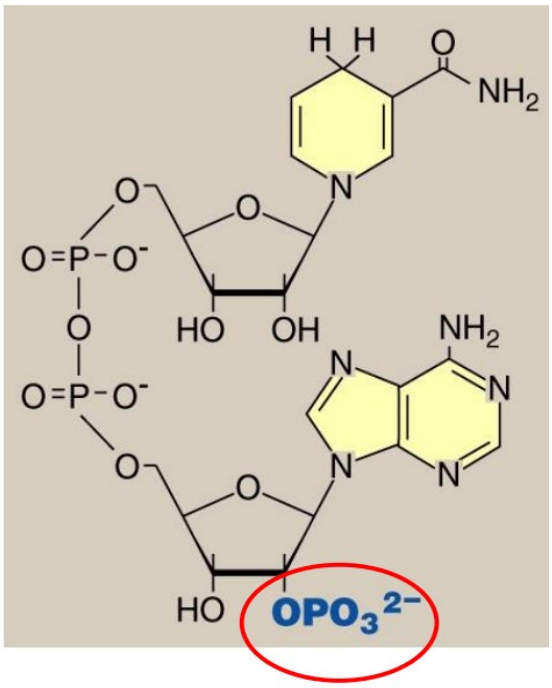
PENTOSE PHOSPHATE PATHWAY (PPP) OR HEXOSE MONOPHOSPHATE SHUNT

Firstly, let's analyze the name of it. Pentose refers to metabolism of 5C sugars, phosphate group that's present in (NADPHvs NADH).

FUNCTIONS OF THE PPP

❖ Production of NADPH.

- NADPH dependent biosynthesis of fatty acids. (NADPH dependent biosynthesis of fatty acids)
- NADPH dependent biosynthesis of steroid hormones. (Testes, ovaries, placenta, and adrenal cortex and sex hormones such as estrogen, progesterone, testosterone, and adrenal gland hormonal products, all of them are made of cholesterol).



OH in NADH

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

❖ Metabolism of five-carbon sugars (Pentoses).

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

- The formation or production of NADPH,which acts as a coenzyme for many rxns,is one of the main fucntions of this pathway.
- **Side Note**: Many rxns such as alcohol metabolism ,Aldose reductase, Sorbitol dehydrogenase need NADPH as a part of their progress.

- Not only the aforementioned rxns necessitate NADPH, but also its needed in more important pathways such as

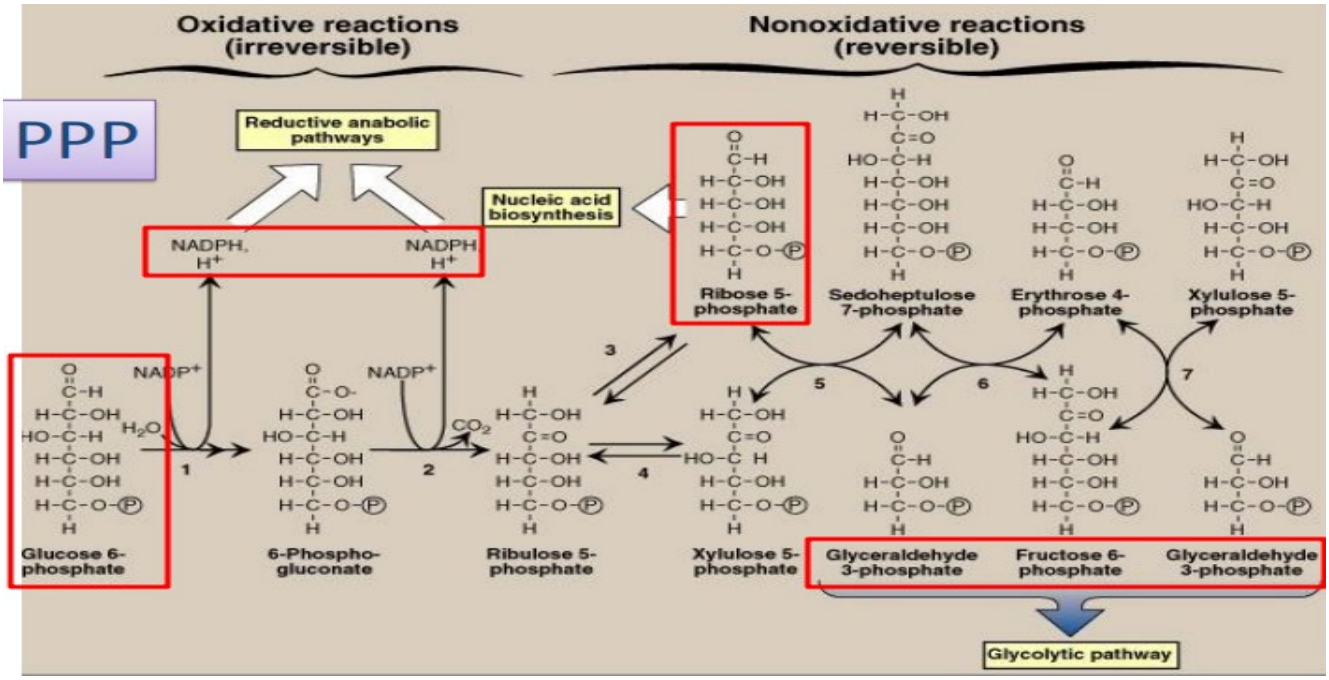
  i. fatty acids biosynthesis.
     - Fatty acids are necessary to the body in many functional tissues such as breast milk in lactating mammary glands (high amount of FA, which has a notable significance regarding the neural development of neonates.
     - Also FA are devoted to make triacylglycerols in the adipose tissue in order to store energy.
     - Fatty acids synthesis is directed to the liver, for many reasons, for example the formation of cholesterol esters (CE).
     - Fatty acids are a precursor of phospholipids and sphingolipids of membranes.

  ii. steroid hormones biosynthesis:
     - Steroid hormones (e.x: sex hormones) include estrogen, progesterone and testosterone. In addition, adrenal gland hormonal products(cortisol, aldosterone). These are all made of cholesterol molecules, provided that their synthesis necessitates NADPH.

  iii. maintenance and recycling of Glutathione (GSH) in RBCs.
     - **Side note:** GSH, a tripeptide that consists of three amino acids: Gly-Cys-Glu.
     - The most important AA is cysteine, that has a distinct SH group through which the molecule can be oxidized, forming a disulfide bridge with another glutathione.
     - It acts as an antioxidant, losing its hydrogen (donating an e-) to ROS(reactive oxygen species),limiting their ability to damage the cell structure.
     - To recycle it and get it back to its reduced form, so it can interact with another ROS (e.x:H2O2), NADPH intervenes, by getting oxidized to NADP**+. More details will be discuss later in this sheet.**

- **The second function of PPP is metabolism of five-carbon sugars.**
- They undergo metabolism either to be synthesized as a part of nucleotide biosynthesis (ribose 5-phosphate),or to be metabolized(degraded) when nucleotide synthesis isn't needed to form other types of sugars.

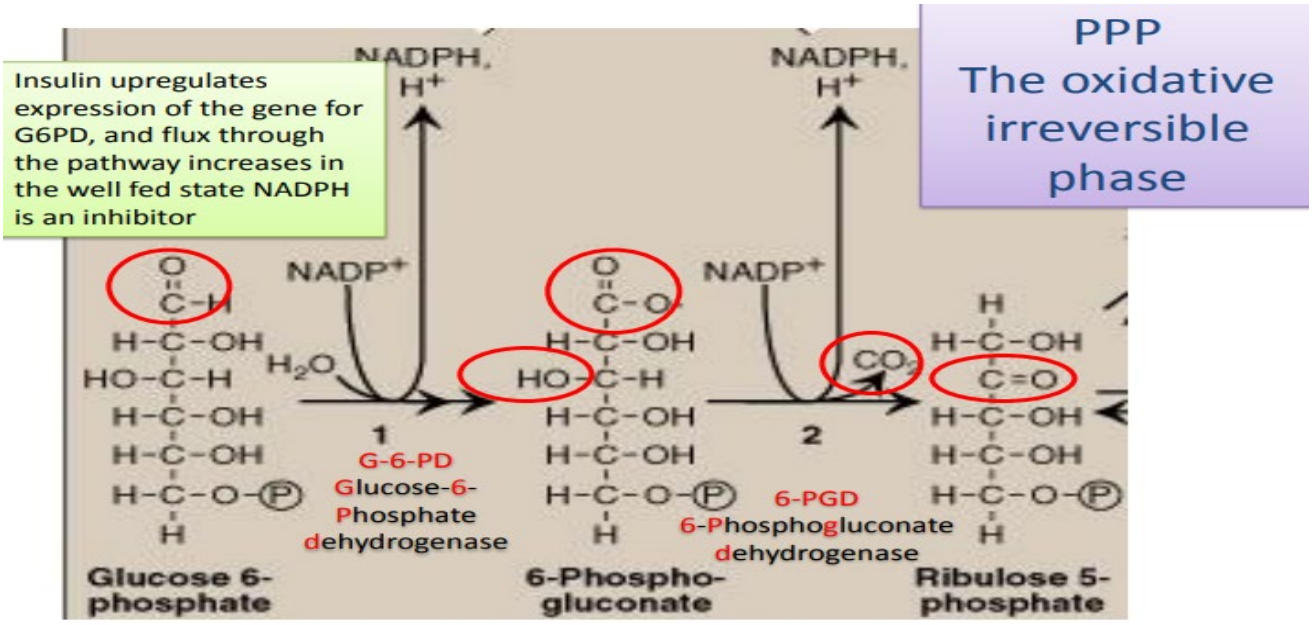
PPP PHASES



PONDER THE FIGURE

- PPP is composed of two major phases, the first of which is oxidative reactions (irreversible), and the second is nonoxidative reactions (reversible). The pathway starts with glucose-6- phosphate (remember that glucose is abundant in well-fed state, where insulin secretion is prominent as well as uptake of glucose into cells, it will then get phosphorylated forming glucose-6- phosphate, one fate of which is going through PPP), glucose-6-phosphate is going to be used to synthesize NADPH, so the first function of the pathway(production of NADPH) is achieved during the 1st phase.
- After that, once we enter the 2nd phase (reversible phase), we're going to produce ribose-5- phosphate (a pentose sugar that constitutes a part of the nucleotide), it can undergo further reduction producing 2-deoxyribose, a part of DNA structure. Moreover, ribose-5-phosphate can be a part of other nucleotides other in these in DNA or RNA, such as ATP, NADH, NADPH,…

- If nucleotide synthesis isn't needed, these sugars are going to be converted to other forms of sugars (glyceraldehyde 3-phosphate and fructose 6-phosphate), which are glycolytic intermediates!! AGAIN, we're talking about a well-fed state (back to the first lines above). Otherwise, the pathway won't get activated.
- **NOTE: the most important function of this pathway is producing NADPH.**



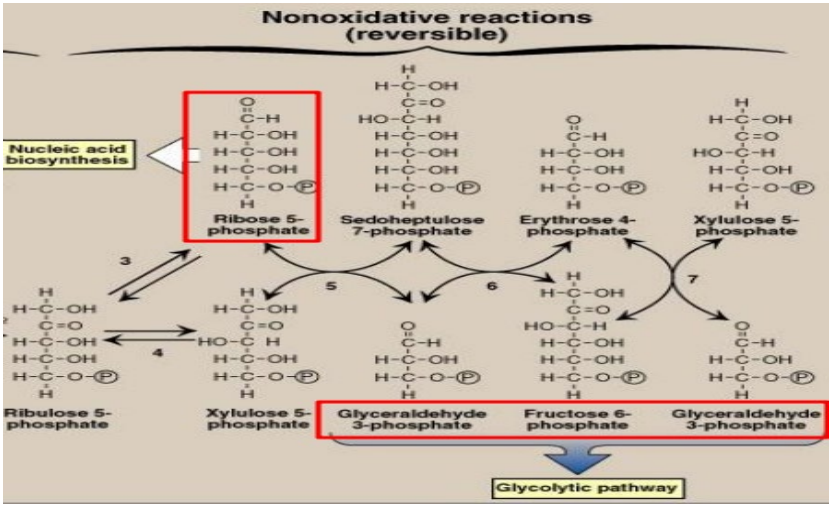
Glc. 6 Phosph. +2 $NADP^+$ ⟶ Ribulose 5-Phosph. + CO_2 + 2 NADPH

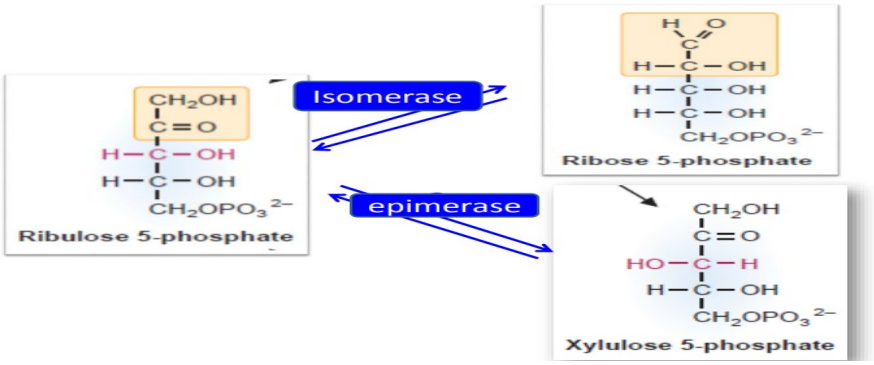
- Irreversible phase: starts with glucose 6-phosphate that's going to be oxidized on C1 to produce 6-phospho-gluconate, catalyzed by glucose-6-phosphate dehydrogenase, and accompanied by NADP+ reduction to NADPH. This is followed by oxidative decarboxylation of 6-phosphogluconate (by the enzyme 6-phosphogluconate dehydrogenase, redox rxn) to produce a ketopentose, ribulose 5-phosphate, as well as CO2 resulting from carboxylic group removal.

- **NOTE: A second molecule of NADPH is produced in the latter rxn, which means that one glucose has given me two NADPH. Logically, when produced in high amounts it will inhibit this pathway.**

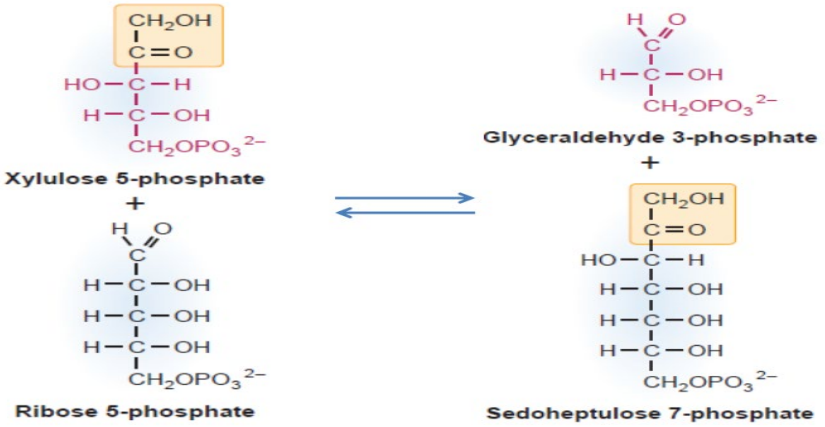
- **NOTE: G-6-PD is also a target gene for Insulin. When secreted, it activates the receptors to initiate the signaling pathway–mentioned before- through which target genes are activated.**

- **Reversible phase**: Starts with ribulose 5-phosphate, look at step 3 in the picture, you see it has converted to ribose 5-phosphate (it's still a pentose sugar, but changed from ketose to aldose, catalyzed by the enzyme isomerase).The resultant ribose 5-phosphate may then by used for nucleic acid biosynthesis, or if not needed, the pathway will proceed(how??) In this case(no need for nucleic acids) ribose 5-phosphate can't continue the pathway on its own, therefore, another glucose in the form of glucose 6-phosphate will enter the irreversible phase again, producing ribulose 5-phosphate,that will be destined to form xylulose 5-phosphate (rather than ribose 5-phosphate,step 4).As you see, xylulose 5-phosphate is an epimer to ribulose 5-phosphate (with the orientation of OH group changed on C3,the enzyme catalyzing the rxn is epimerase),but both of them are ketoses for sure.

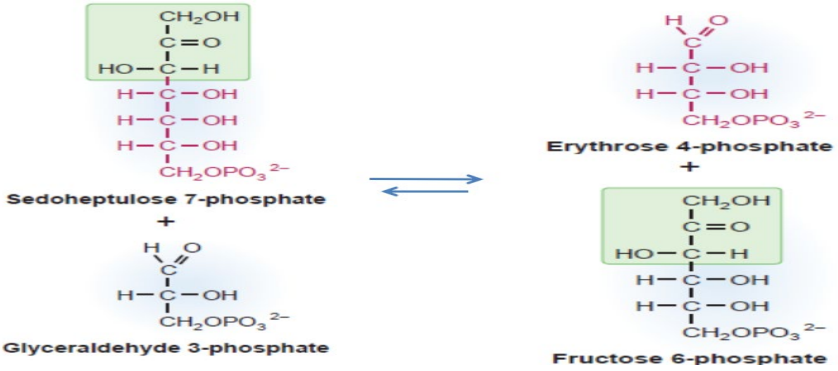




- Note that the second molecule of glucose has produced xylulose 5-phosphate. Assuming that the first one was used to synthesize ribose 5-phosphate but it wasn't needed to make nucleic acids ,this ribose 5-phosphate will interact with xylulose 5-phosphate (both have 5 carbons) so these will be rearranged to yield different types of sugars. The first step occurs by xylulose 5-phosphate giving two of its carbons to ribose 5- phosphate(aldose),so the latter will have seven carbons instead of five (sedoheptulose 7- phosphate,ketose).The remenant three carbons of xylulose(ketose) will form glyceraldehyde 3-phosphate(aldose).

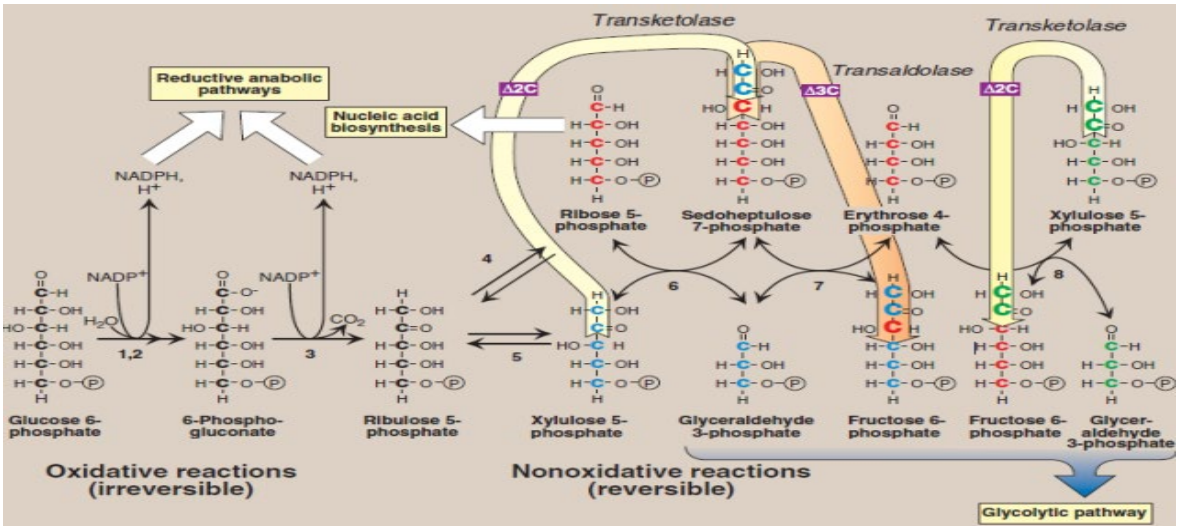


- Note from the previous picture that the the each of the five-carbon sugars(xylulose and ribose) had their carbons rearranged to a three and seven-carbon sugar molecules, conserving the number of carbons. We're still not done!! Now another arrangement will take place, sedoheptulose 7- phosphate(ketose) will lose three of its carbons to form erythrose 4- phosphate(aldose), these carbons will go for glyceraldehyde 3-phosphate(aldose) to produce fructose 6-phosphate(ketose).



- Go back to the non-oxidative reactions pic. Until step 6,we have erythrose 4-phosphate and fructose 6-phosphate(4+6=10,still the number of carbons is conserved).Now a third glucose molecule ,enters as glucose 6-phosphate To the irreversible phase making ribulose 5-phosphate,which will proceed as xylulose 5-phosphate –rather than ribose-.The latter xylulose will perform the last step we stopped at(step 7),together with erythrose 4- phosphate(5+4=nine carbons).Xylulose will lose two of its carbons for erythrose, which will become the six-carbon sugar fructose 6-phosphate. The three carbons left from xylulose are going to become glyceraldehyde 3-phosphate.

- Remember: Glyceraldehyde-3-phosphate and fructose-6-phosphate are glycolytic intermediates.



- As we said, 1 glucose 6-phosphate will initiate the irreversible phase yielding 2NADPH and ribulose 5-phosphate(steps 1-3),which will isomerize to ribose 5-phosphate(step 4).
- A second glucose will carry out the same steps,rather,it will proceed through step 5,where ribulose 5-phosphate will epimerize to xylulose 5-phosphate.If not utilized for nucleotide synthesis,ribose will interact with xylulose (initiating step 6,where products of the previous two steps become reactants),this interaction will result in sedoheptulose(by the two carbons transferred from xylulose) and glyceraldehyde 3-phosphate. Consequently,three carbons are going to be transferred from sedoheptulose(which will become erythrose) to glyceraldehyde 3-phosphate(which will become fructose 6- phosphate).
- We stopped here(step 6),waiting for a third glucose to join,it repeats the same steps,proceeding through step 5,and forming that uppermost xylulose 5-phosphate on the right side,which will interact with erythrose only from the previous step (fructose here will be a final product),so the new xylulose will transfer two of its carbons to erythrose(to form another fructose 6-phosphate as a final product), the remaining three carbons will produce glyceraldehyde 3-phosphate(a final product).

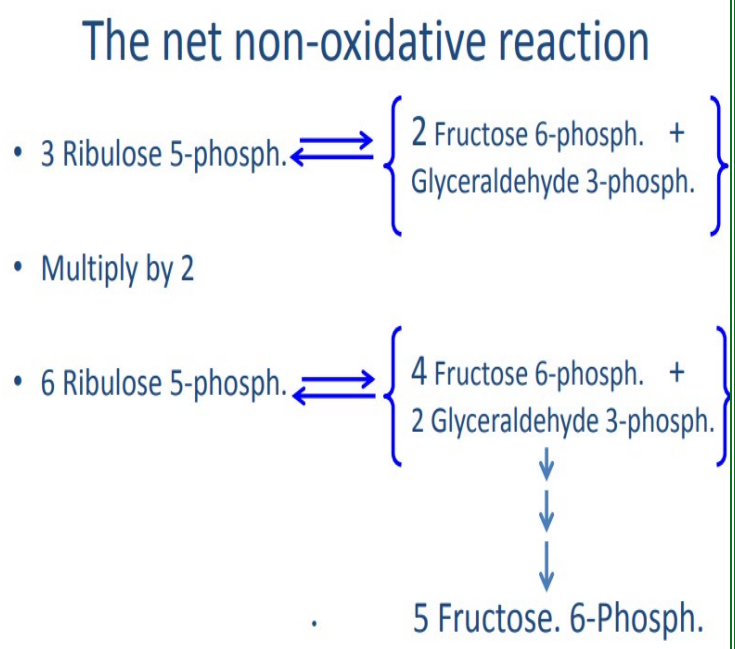
SUMMARY OF THE NON-OXIDATIVE REACTIONS

Reactant	Products
Xylulose + Ribose	Sedoheptulose + Glyceraldehye3-phosphate
Sedoheptulose + Glyceraldehyde 3- phosphate	Erythrose + Fructose 6-phosphate
Xylulose 2 + Erythrose	Glyceraldehyde3-phsophate +Fructose 6-phosphate

- ❖ Reversible reactions
- ❖ Transfer of 2 or 3 carbon fragment
- ❖ Transketolase (2C), Transaldolase (3C).
- ❖ Ketose + aldose ⇌ ketose + aldose.
- ❖ • From ketose to aldose
- ❖ Rearrangement t of sugar
- ❖ 3 pentose phosphate... (2 hexose phosph + 1 triose phosph.)

THE NET NON-OXIDATIVE REACTION:

- So we used the three glucose molecules and converted them into three glucose-6-phosphate then we loss a carbon from each one of them in the shape of **CO2,** as a result, the remaining is **3 Ribulose-5-phosphate** (in the whole reaction 15 atom of C entered). Then by rearranging them we will have **2 Fructose-6-phosphate** (6c) and **Glyceraldehyde-3-phosphate** (**3c**). 2*6 + 3 = 15C.



- When **multiplying by 2**, the total will **be 4 fructose-6-phosphate** + **2 glyceraldehyde-3-phosphate** ⟹ each one of these 2 molecule contain 3C so we consider them both as a form of **fructose-6-phosphate**.

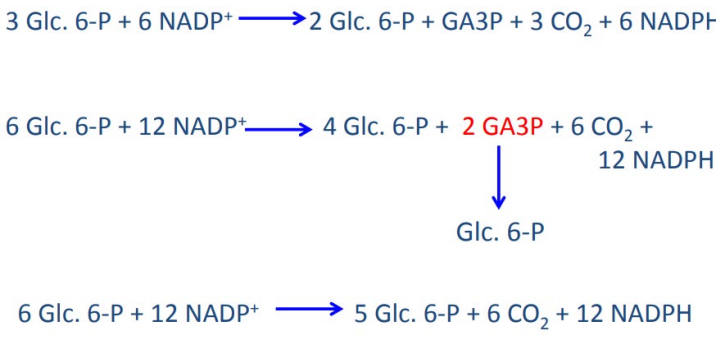
- **6 Ribulose-5-phosphate** produces **5 Fructose-6-phosphat.** 6 molecules with 5C enters……. the product is 5 molecules with 6C**.**

THE NET PRODUCTS OF THE REACTIONS (OXIDATIVE AND NON-OXIDATIVE)

- **3 Glc.6-P** enters (EACH MOLECULE **PRODUCES 2 NADPH**) and **reduce 6 NADP+** so **6 NADPH are produced**. Also each one of them produces 1 CO2. So 3 CO2 molecules out. The three molecules produce **1 Glyceraldehyde-3-phosphate** and **2fructose 6- phosphate**, for every 6 molecules Glc. 6-P only one is lost on the shape of CO2 (as waste).
- So, the produces **Fructose 6-phosphate and GA3P are glycolytic** intermediates we can get energy of them by completing the reactions.

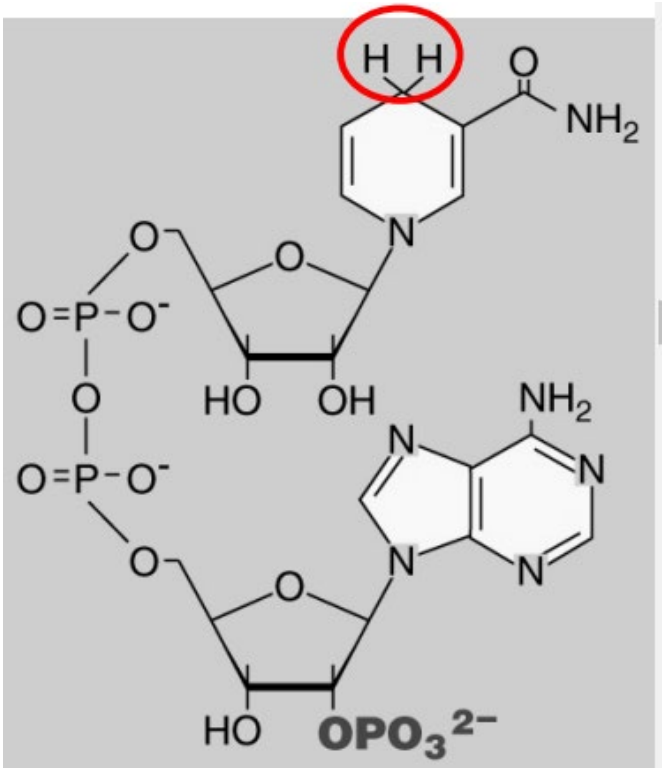
- Most sugars that are need to build structures and a source of energy are not the sedoheptolose or erythrose instead most of them are pento or hexo sugars. These sugars are even not common to be stored in the body, and it is not considered as standard forms of sugar that can be used for many pathways.

Net Products of the Reactions



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).
- ❖ In the cytosol of hepatocyte
  - NADP+ /NADPH ≈ 1/10
  - NAD+ /NADH ≈ 1000/1



- The main reason for regulation is that we should have two shapes because these molecules are mainly important in oxidation reduction reaction, so we must have got coenzyme that is found in higher amount in the oxidized form, and another one that is mostly found in the reduced form.
- So that **the oxidized one will be reduced in the reaction which include oxidation of 6 Ribulose-5-phosphate produces 5 Fructose-6-phosphate** Multiplication (*2) another substrate, and the one that is found more in the reduced form will be oxidized in the other reactions that include reduction of the other substrate.

- 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 …

2. REDUCTION OF HYDROGEN PEROXIDE

To remove any reactive oxygen species, one of these one anti-oxidant is Glutathione, we will discuss it later on.

❖ H2O2 one of a family of compounds known as Reactive Oxygen Species (ROS).

- The ROS are produced continuously from all the metabolic pathways producing side products which maybe these ROS, even sometimes they have to be produced in order to defend our body against microorganisms.
  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).

❖ Other: Super oxide, hydroxyl radical.

- H2O2 One of the reactive oxygen species and others are superoxide (O2 with an extra electron) and hydroxyl radical (OH also with extra electron) because they contain extra electrons they are highly reactive so they form covalent bonds and they donor there two electrons.

❖ Formed continuously:
  - As byproducts of aerobic metabolism.
  - Interaction with drugs and environmental toxins.

❖ Can cause chemical damage to proteins, lipids and DNA ➔ cancer, inflammatory disease, cell death

ENZYMES THAT CATALYZE ANTIOXIDANT REACTIONS

1. Glutathione peroxidase

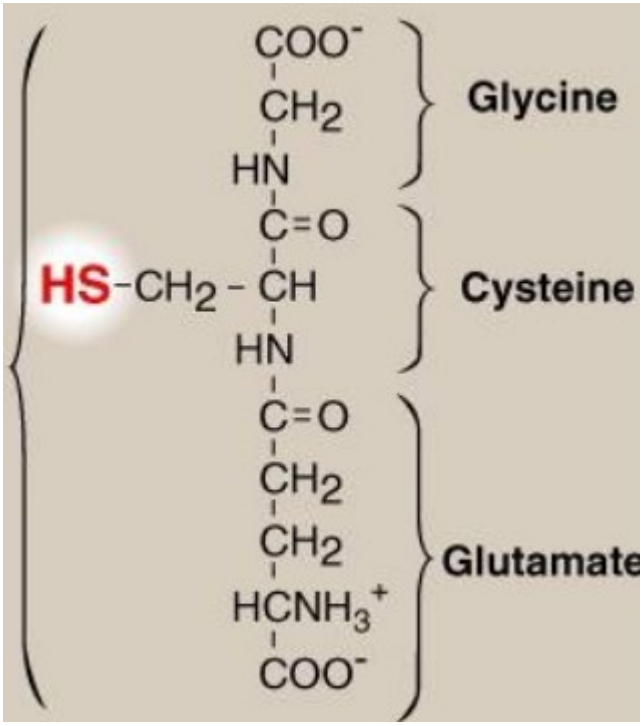
❖ Glutathione is a reducing agent.

❖ Tripeptide.

❖ GSH is the reduced form.

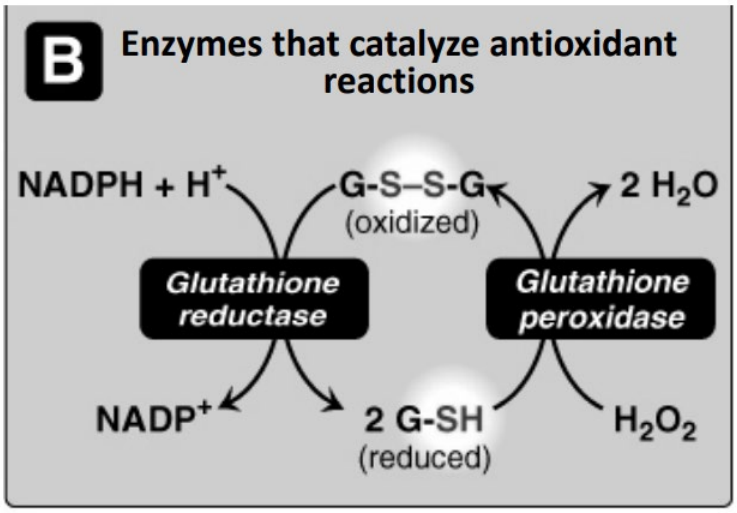
❖ Oxidation ➔ two molecules joined by disulfide (GSSG).

❖ 2 GSH ➔ GSSG



- Glutathione as we remember is a tripeptide 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, H2O2 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 H2O, and these two extra hydrogens are from the glutathione so it will be oxidized in the form of GSSG and 2H2O 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.

- t is a common disease that is inherited on the X chromosome (autosomal recessive) ,So males are more susceptible to it, o 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 is a differ 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 haemolysis, these patients suffer from haemolytic anaemia.

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

- Conditions can lead to increase in oxidative stress: increase the production of ROS, or deficiency of oxygen like hypoxia.

PRECIPITATING FACTORS IN G6PD DEFICIENCY

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

(و الحمد لله رب العالمين)