DOCTOR 2020 | JU

METABOLISM

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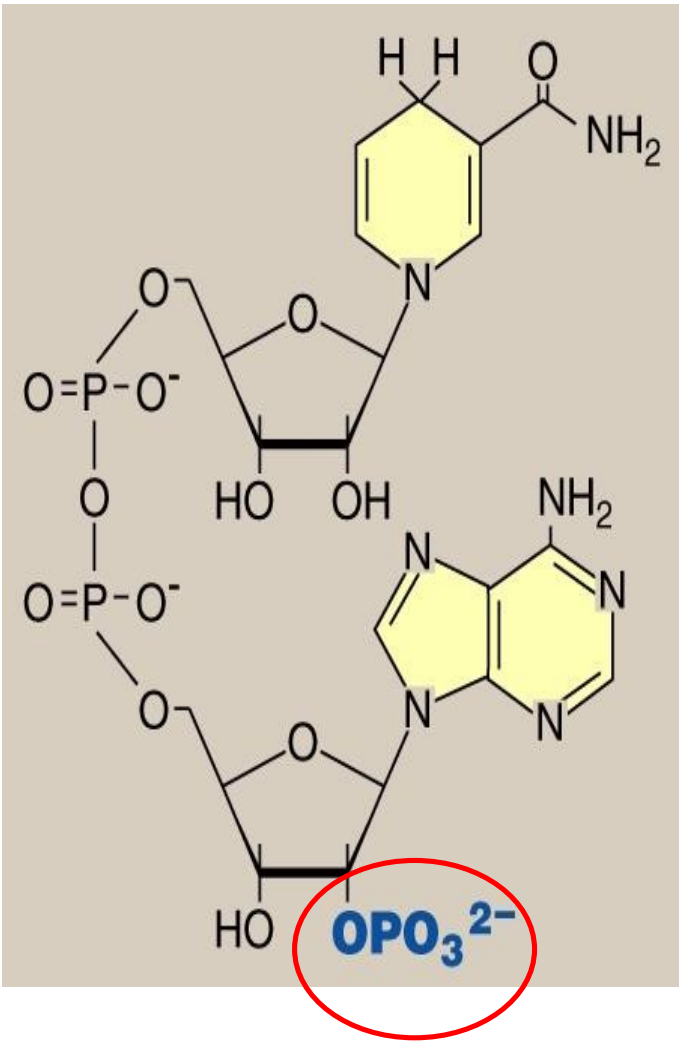
You're in the last sheet of carbohydrate metabolism ,discussing Pentose Phosphate Pathway (Hexose Monophosphate Shunt).

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

NADPH vs NADH:recall the structure of NADH(nicotinamide+adenine interconnected with their ribose sugars).On adenine's ribose specifically on C2,there is an OH group.Distinctly,NADPH has a phosphate group on this carbon



OH in NADH

- **The formation or production of NADPH,which acts as a coenzyme for many rxns,is one of the main fucntions of this pathway.**
- **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 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.**
- **Additionally,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.**

Another NADPH-dependent pathway: steroid hormones

Testes,ovaries,placenta and adrenal cortex,are the most common places with a high steroid hormone synthetic activity.

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.

Another rxn:maintenance and recycling of Glutathoine(GSH) in RBCs

Remember 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 gluthathoine.

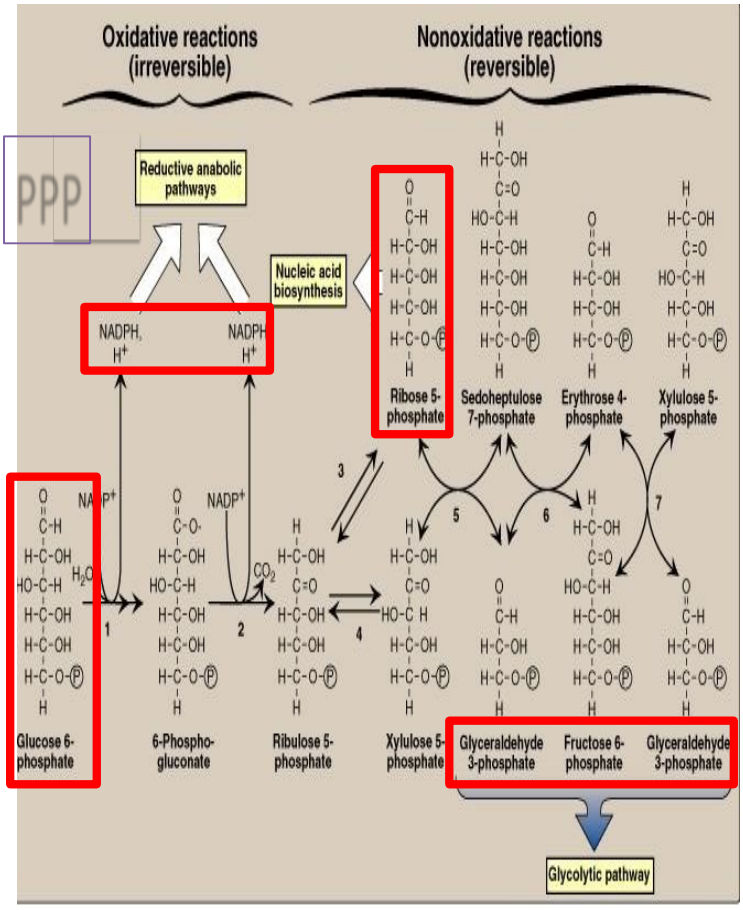
It acts as an antioxidant ,losing its hydrogen(donating an e^-) to ROS(reactive oxygen species),limiting their ability to damage the cell structure.

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 $NADP^+$.

- **Refer to the first page,we talked about one function of PPP,which is NADPH production(the main function),now the 2nd function is metabolism of 5C sugars(pentoses)**
- **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 is composed of two major phases,the first of which is oxidative reactions (irreversible),and the second is non-oxidative 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),this 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.

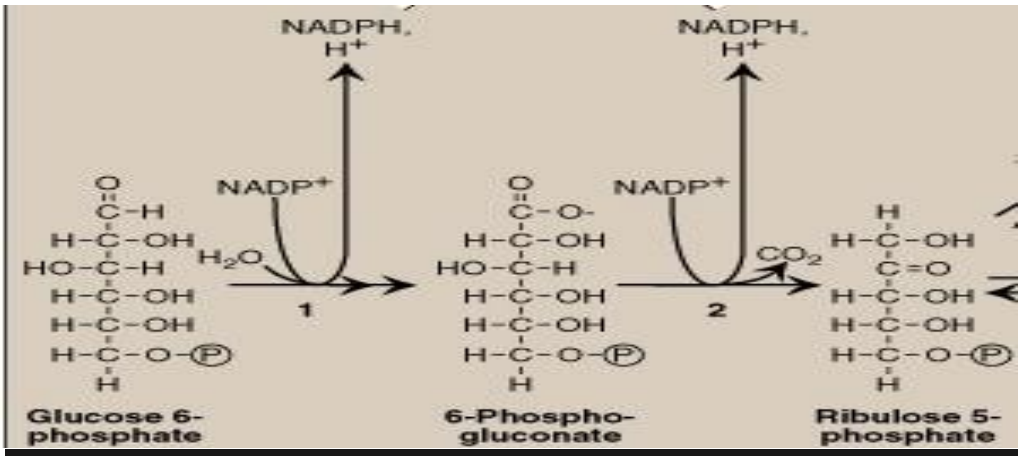


After that,once we enter the 2nd phase(reversible phase),we're going to produce ribose-5-phosphate(a pentose sugar that constitutes a part of the nucleotide),it can undergo further reduction producing 2-deoxyribose,a part of DNA structure particularly .Moreover,ribose-5-phosphate can be a part of other 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.

It somehow sounds like I utilized glucose running its known pathways in an indirect way ((HEXOSE MONOPHOSPHATE SHUNT)).

Through this shunt ,glycolytic intermediates ,pentose sugars ,as well as the most important product(NADPH) are synthesized.

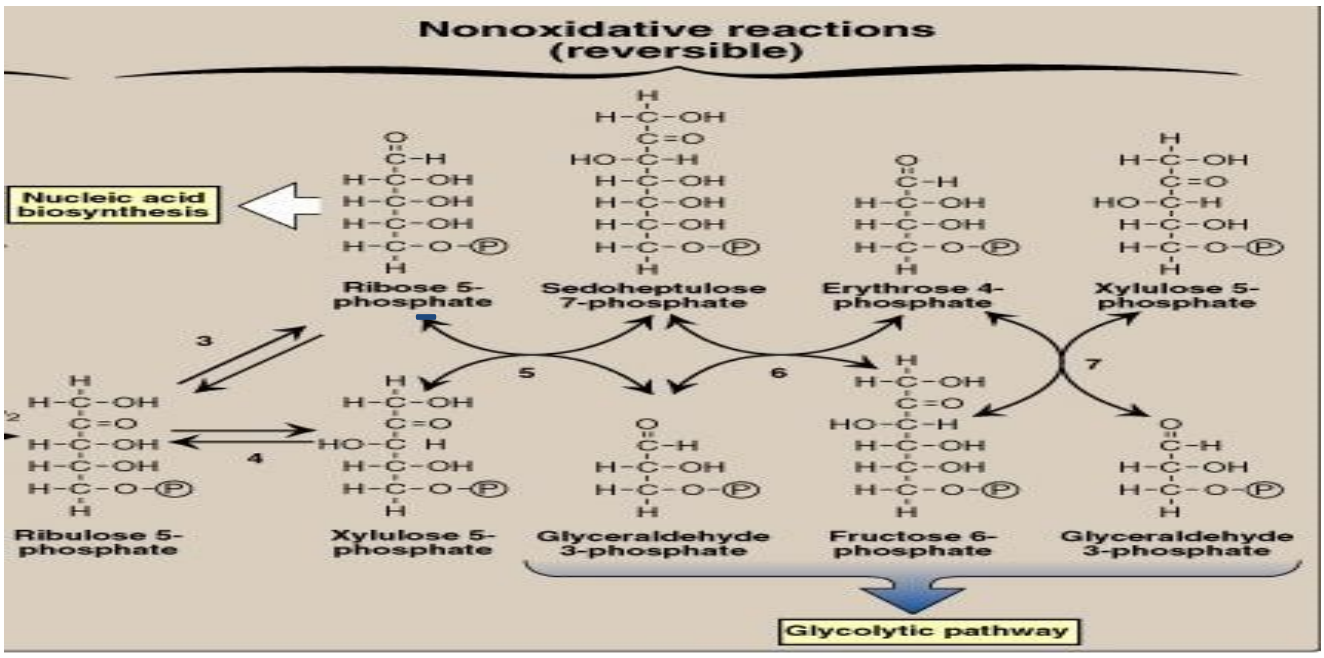


Irreversible phase:starts with glucose 6-phosphate that's going to be oxidized on C1 to produce 6-phospho-gluconate,catalyzed by glucose-6-phosphate dehydrogenase,and accompanied by $NADP^+$ reduction to NADPH. This is followed by oxidative decarboxylation of 6-phospho-gluconate(by the enzyme 6-phosphogluconate dehydrogenase,redox rxn) to produce a ketopentose ,ribulose 5-phosphate,as well as CO_2 resulting from carboxylic group removal.

NOTE 1::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.

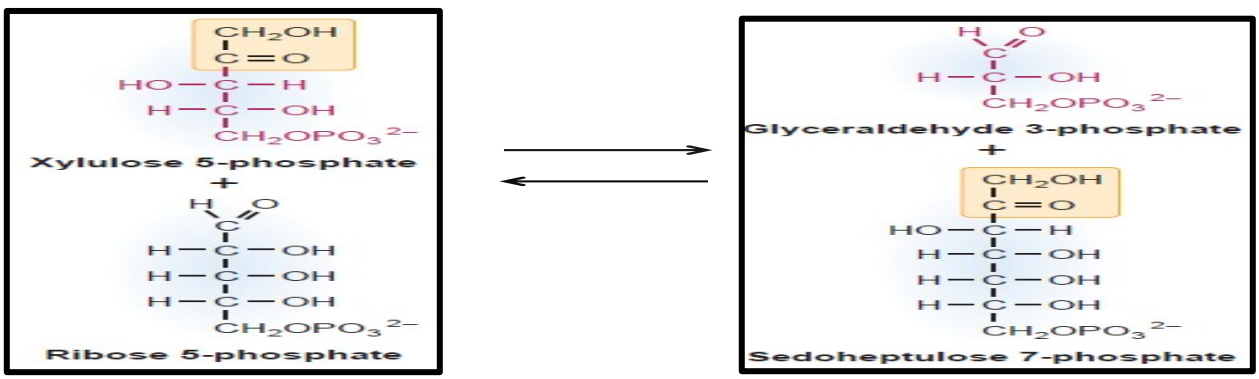
NOTE2::Glucose-6-phosphate dehydrogenase (G-6-PD) is commomly mutated ,we'll see the consequences when discussing clinical applications in the few following pages.

NOTE3::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 ,such as GLUT-4(increasing its gene expression),and the new one for us (G-6-PD,which definitely requires a well-fed state).



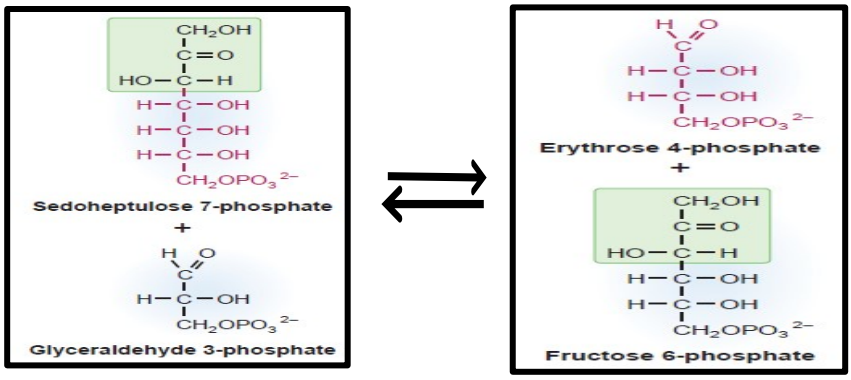
Reversible phase:Starts with ribulose 5-phosphate,look at step 3 in the picture,you see it has converted to ribose 5-phosphate(it's still a pentose sugar,but changed from ketose to aldose,catalyzed by the enzyme isomerase).The resultant ribose 5-phosphate may then by used for nucleic acid biosynthesis,or 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).

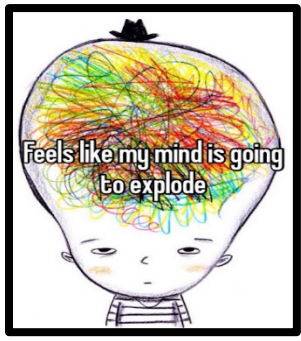


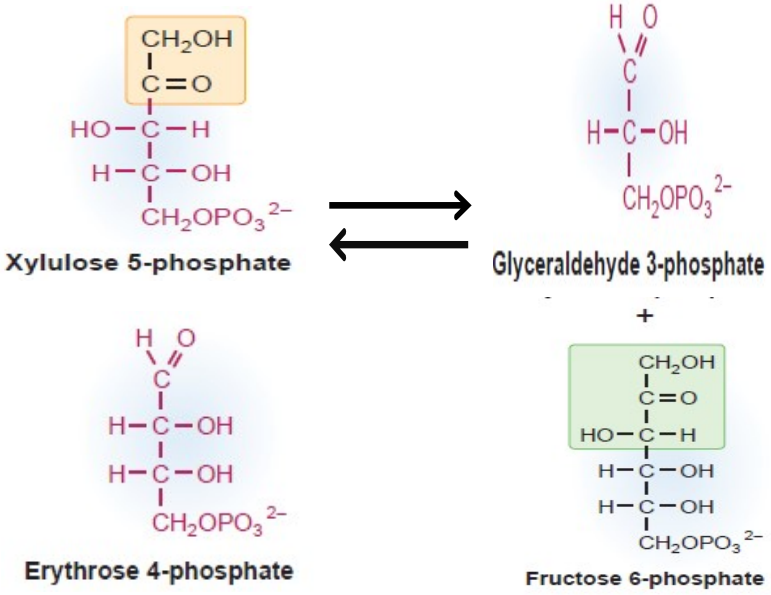
Note from the previous picture that the the each of the five-carbon sugars(xylulose and ribose) had their carbons rearranged to a three and seven-carbon sugar molecules,conserving the number of carbons.

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.





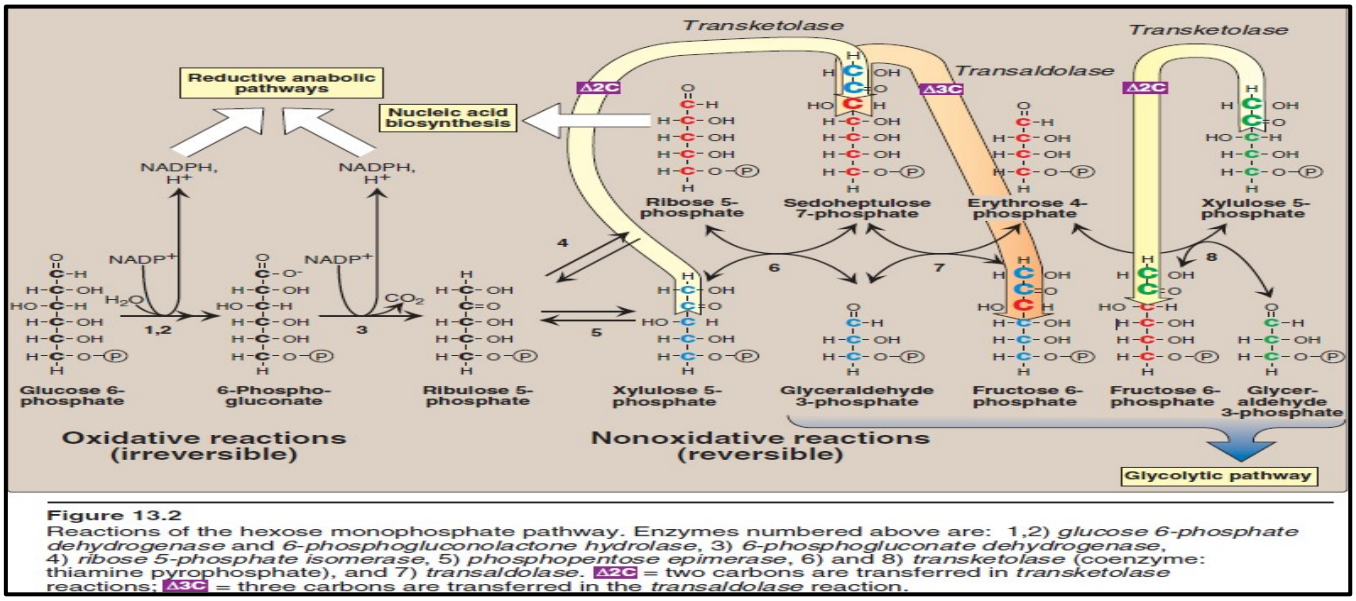


Figure 13.2
Reactions of the hexose monophosphate pathway. Enzymes numbered above are: 1,2) *glucose 6-phosphate dehydrogenase* and *6-phosphogluconolactone hydrolase*, 3) *6-phosphogluconate dehydrogenase*, 4) *ribose 5-phosphate isomerase*, 5) *phosphopentose epimerase*, 6) and 8) *transketolase* (coenzyme: thiamine pyrophosphate), and 7) *transaldolase*. Δ2C = two carbons are transferred in *transketolase* reactions; Δ3C = three carbons are transferred in the *transaldolase* reaction.

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:

Ketose + Aldose ⇌ Aldose + Ketose

C5 + C5 ⇌ C3 + C7
C7 + C3 ⇌ C4 + C6 → The reactants are the products of the first step
C5 + C4 ⇌ C3 + C6

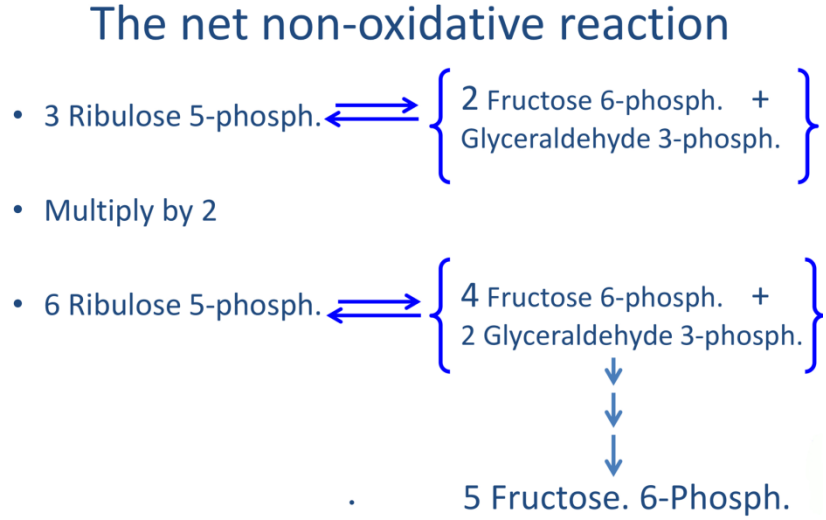
Reactants	Products
Xylulose + Ribose	Sedoheptulose+Glyceraldehyde 3-phosphate
Sedoheptulose+Glyceraldehyde 3-phosphate	Erythrose+Fructose 6-phosphate
Xylulose 2+Erythrose	Glyceraldehyde 3-phsophate+Fructose 6-phosphate

- Reversible reactions.
- Transfer 2 or 3 carbon fragment.
- Transketolase(2C)&Transaldolase(3C).
- From ketose to aldose.
- Rearrangement of sugars (3 pentose phosph.. to 2 hexose phosph+1 triose phosph.(2 F6P&1G3P). → Hexose monophosphate shunt

THE NET NON-OXIDATIVE REACTION:

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

When multiplying by 2……. 6 Ribulose-5-phosphate, then 4 Fructose-6-phosphate +2 Glyceraldehyde-3-phosphate → Every one of These 2 molecules contain 3C so I can consider that both of them form fructose-6-phosphate.



6 Ribulose-5-phosphate produces 5 Fructose-6-phosphate

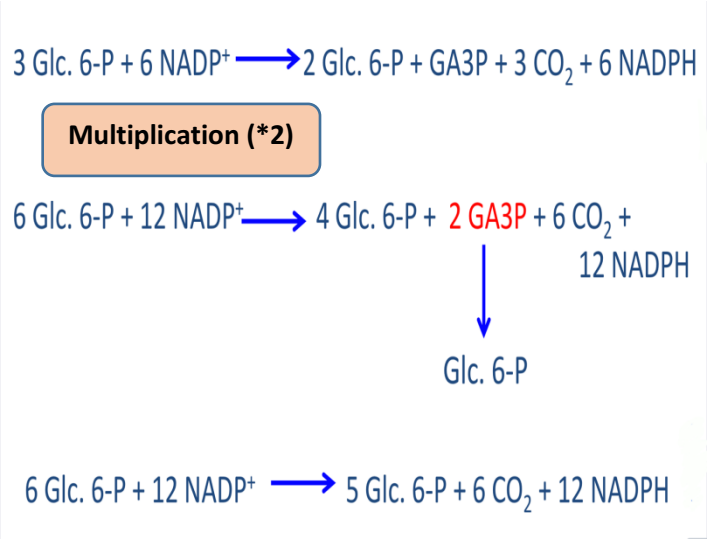
6 molecules with 5C enters…….. the product is 5 molecules with 6C

The net the product of the reactions (is the oxidative phase a non-oxidative phase)

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 2 fructose 6- phosphate, for every 6 molecules Glc. 6-P only one is lost on the shape of CO2 (as waste).

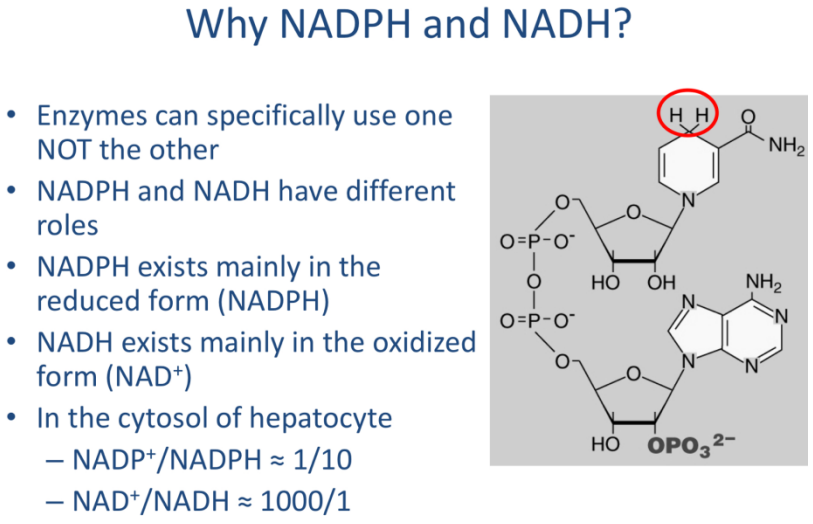
So, the produces Fructose 6-phosphate and GA3P are glycolytic intermediates we can get energy of them by completing the reactions.

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.



WHY NADPH AND NADH????

The main reason is that for regulation I should have two shapes because these molecules are mainly important in oxidation reduction reaction, so I must have got coenzyme that is found in higher amount in the oxidised form, and another one that is mostly found in the reduced form. So that the oxidised one will be reduced in the reaction which include oxidation of



another substrate, and the one that is found more in the reduced form will be oxidised in the other reactions that include reduction of the other substrate.
So NAD+/NADH and NADP+/NADPH is like having two poles , but one of them prefer the oxidised form and another prefer the reduced form.
NADH Prefer the oxidised form, so 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).
So they work opposite to each other.

The side where the oxidation reduction happens is on the hydrogen above and not near the phosphate group.

WHAT ARE THE USES OF NADPH????

1. **reductive biosynthesis**
   Some biosynthetic reactions require high energy electron donor to produce reduce the product.
   Examples: fatty acids ,steroids…
2. **Reduction of hydrogen peroxide (To get rid of reactive oxygen species) using glutathione which is anti-oxidant.**

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.

So I 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).

If the inflammation is rapidly occurring it may cause cancers (according to one of the hypotheses).

Antioxidants differ in their families and structures like vitamin C ,vitamin E, and sometimes vitamin A can sometimes work as an antioxidant (even if they are with different structures) for example the glutathione is tripeptide differ in structure than vitamins.

The ROS are produced continuously all the metabolic pathways Produce side products which maybe these ROS , even sometimes I need to produce them specially to defend our body against microorganisms (I will increase the ROS production in the body so that it will kill the microorganism).

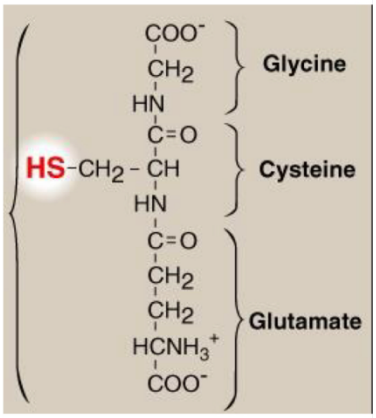
Glutathione as we remember is it tripeptide made of glycine ,cysteine ,and glutamate. The most important one is cysteine because of the thiol group that can alternate between oxidised and reduced state.

It is reduced it will be in the form of GSH but if it is oxidised then two glutathione molecules will join together by a disulfide Bridge ,and it will be named as GSSG.

Enzymes that catalyze antioxidant reactions

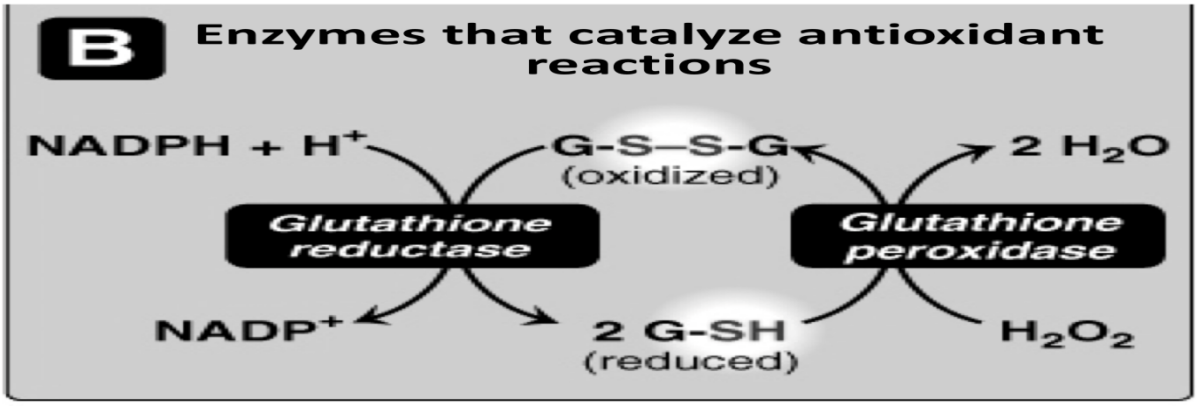
1. Glutathione peroxidase

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



For example H2O2 is produced as an ROS and we need to get rid of it, so I need to get to hydrogens for the extra oxygen in the molecule to form H2O, and I will get these two extra hydrogens are from the glutathione so it will be oxidised in the form of GSSG and we will produce 2H2O molecules.

The enzyme that is involved in this reaction is the glutathione peroxidase. And now I get rid of the ROS ,but I need to recycle the glutathione to use it again by reducing it using glutathione reductase. So something else have to be oxidised which would be NADPH to NADP+.



Glutathione peroxidase is Selenium requiring Enzyme
RBCs are totally dependent on PPP for NADPH production

CLINICAL HINT: G6PD DEFICIENCY

It 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 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 streso they like NADPH to work with them so it will cause haemolysis ,so 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. some antibiotics like Sulfomethxazole
  II. Anti-malaria like Primaquine
  III. Antipyretics like Acetanalid .(خوافض الحرارة)

- Favism due to vicine and covicine in fava beans in some G6PD deficiency patients (التفول)
- Infection
- Neonatal jaundice (الصفار)

All of these cause increase of oxidative stress in these patients.

The advantage for this patients is that they have more resistance to falciparum malaria compared with normal people.

GOOD LUCK