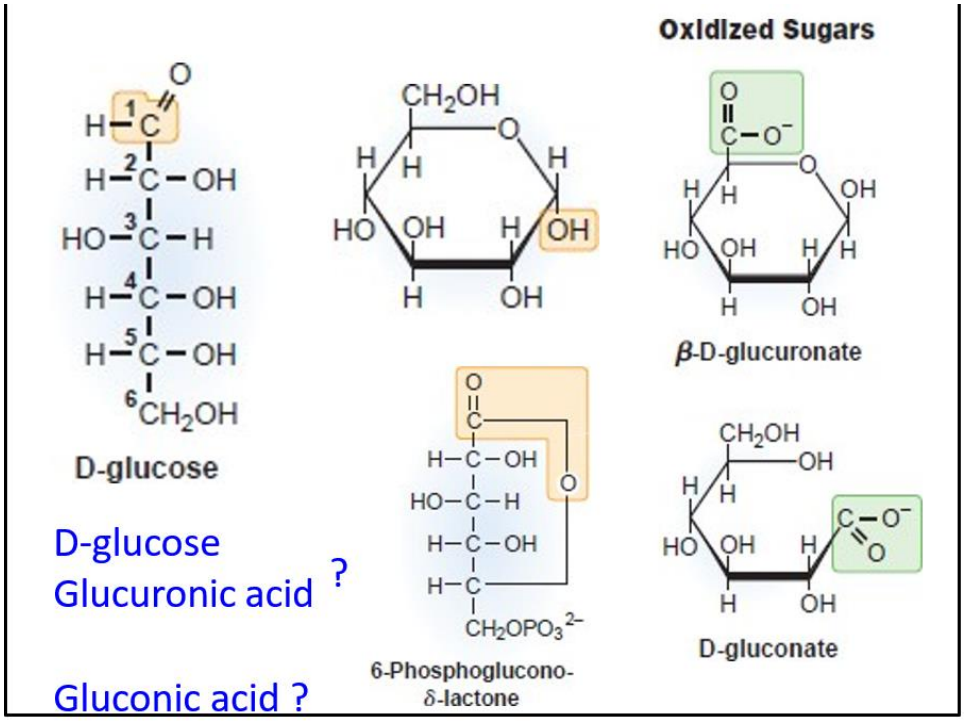
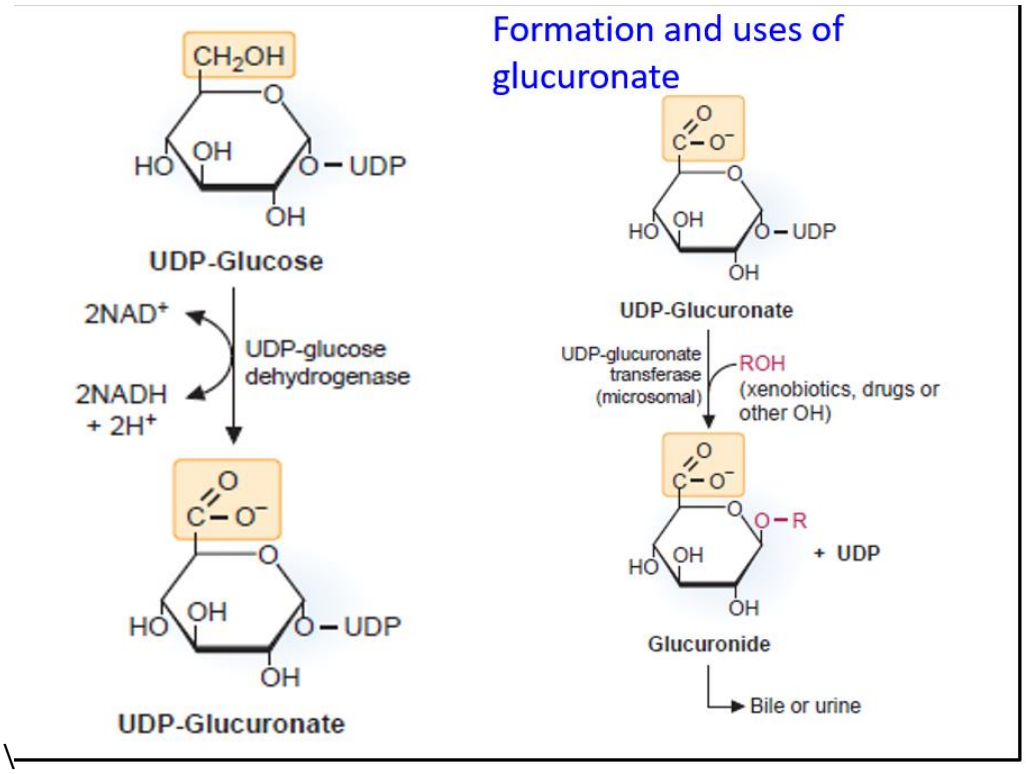


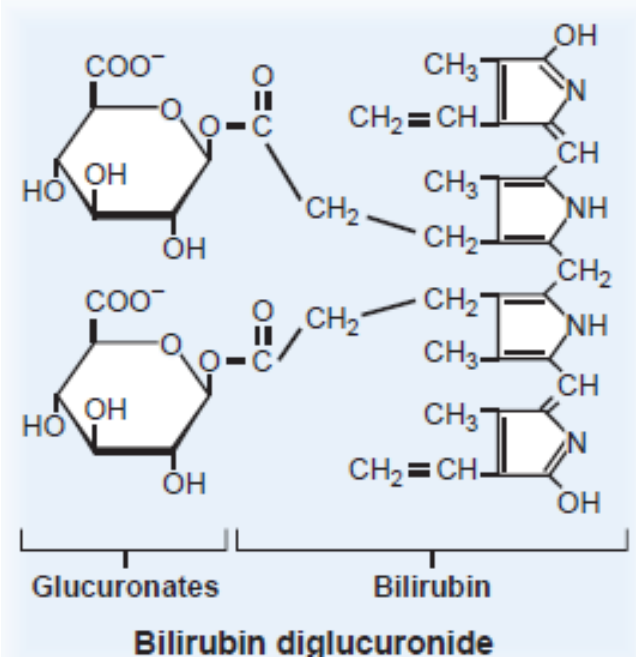
What was mentioned in the lecture:

pentose phosphate pathway is our last topic in carbohydrate metabolism, we would review some definitions before we start with the pentose phosphate pathway, the left upper corner depicts the structure of D-glucose in the ring formula and the chain formula, glucose contain two types of functional groups: one aldehyde group at carbon #1 and five hydroxyl groups, both aldehyde and hydroxyl groups can be oxidized to carboxyl groups, oxidation of carbon #6' hydroxyl group of glucose results in formation of glucuronic acid (glucoronate in the ionized form), glucuronic acid have a carboxyl group on carbon #6 instead of a hydroxyl group, glucuronic acid is extremely hydrophilic (even more than glucose) because it carry an ionizable group, the aldehyde group of carbon #1 of glucose also can be oxidized to a carboxyl group forming gluconic acid (gluconate in the ionized group), we also know that a carboxyl group can react with a hydroxyl group forming an ester group ($-COO^- + -OH \rightarrow -COO- + H_2O$), gluconic acid has a carboxyl group at carbon#1 and a hydroxyl group on carbon #5 which can react with each other forming an internal ester, a ring structure called lactone, the lactone formed from gluconic acid is called gluconolactone, to distinguish to which hydroxyl group the carboxyl group of gluconate has reacted, we use Greek letters, because it has reacted with the hydroxyl group on carbon #5, we call this lactone glucono-δ-lactone.



What was mentioned in the lecture:

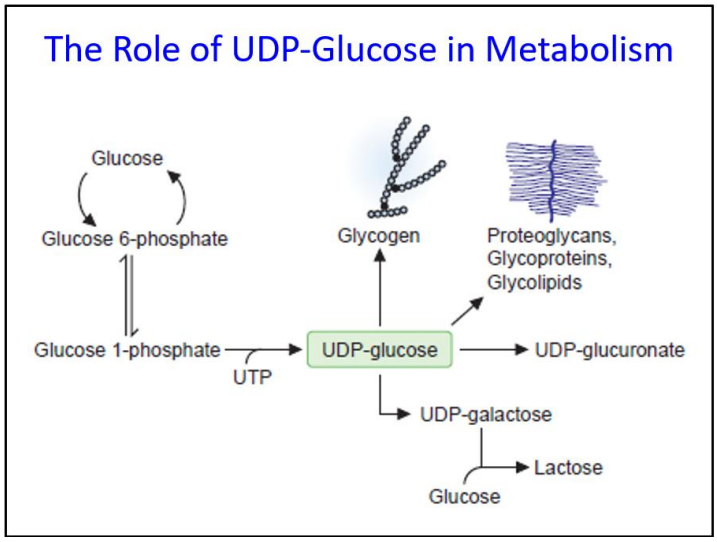
After we've discussed glucuronic acid and gluconic acid, we'll talk about their uses and how they are formed, glucoronate can be formed by oxidation of glucose, but to oxidize a glucose molecule, it must be conjugated to UDP, UDP-glucose dehydrogenase enzyme will catalyze the reaction of oxidation of carbon #6 of glucose conjugated to UDP with a conversion of NAD^+ to NADH forming UDP-glucuronate, what is the advantage of glucuronic acid? Because it is extremely hydrophilic, it can be conjugated to nonpolar (hydrophobic) molecules, to make them more water soluble, it can be transferred form UDP-glucuronate to other molecules.



Bilirubin diglucuronide

What was mentioned in the lecture:

Let's review this example, bilirubin is a molecule that results from degradation of heme, bilirubin is relatively water-insoluble, it is mostly hydrophilic except that it has two carboxyl groups, how can we make it water-soluble to make it possible to excrete it with urine or feces (it is mostly excreted with feces)? By conjugation of it to two glucuronic acid molecules, it cannot be excreted unless it was conjugated to two glucuronic acid molecules, this conjugation occurs in the liver.

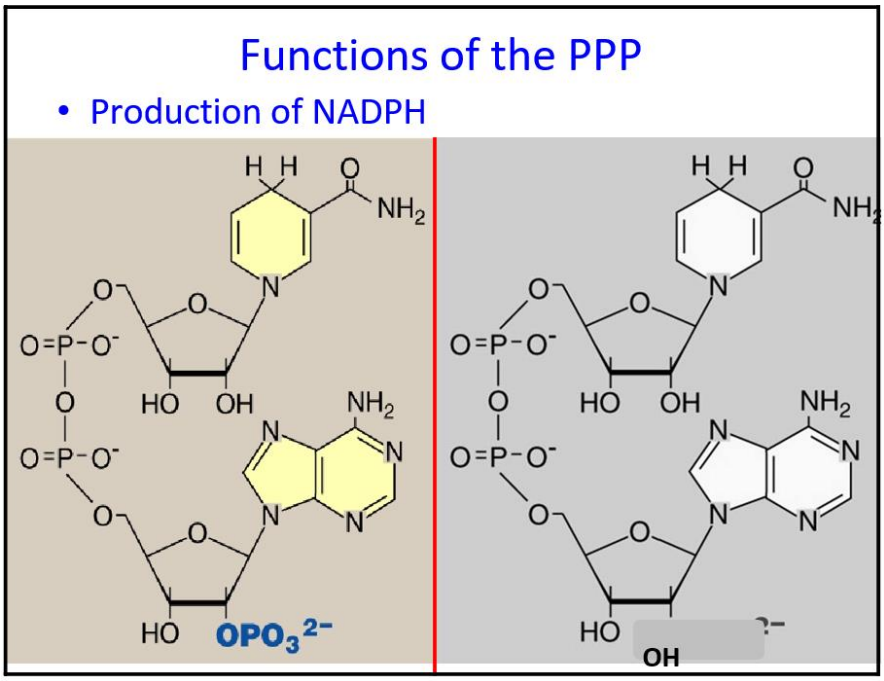


What was mentioned in the lecture:

UDP-glucose play a huge role in metabolism, it functions as glucose donor in glycogen synthesis, glycolipid and glycoprotein synthesis, and synthesis of proteoglycans, UDP-glucose can also be isomerized to UDP-galactose which is the galactose donor in lactose synthesis.

Pentose Phosphate Pathway (PPP)
or
Hexose Monophosphate Shunt

we are now done with the introduction, we'll now discuss the pentose phosphate pathway, which is also called hexose monophosphate shunt.



What was mentioned in the lecture:

The first function of the pentose phosphate pathway is producing of NADPH, NADPH structure is depicted on the left side of the figure, it differs from the NADH (on the right side of the figure) in that the one of the riboses carries a phosphate group instead of a hydroxyl group on carbon #2, (remember: NADH = nicotinamide adenine dinucleotide, this coenzyme is composed of: an adenine nitrogenous base, a ribose sugar, two phosphates, another ribose sugar, and the functional group which is the niacin ring), therefore, NADH has a total of two phosphate groups while NADPH has a total of three phosphate groups, however, because the reactive part which is the niacin ring (a six membered heterogenous aromatic ring) does not differ between NADH and NADPH, they both can do the same functions in catalyzing oxidation and reduction reactions, and addition of this phosphate group will not affect the reduction potential of NADH, then what is the difference between them? Why there is a phosphate group there? This phosphate group is a tag actually, this tag tells the enzyme that needs NADPH that I am here, enzymes that require NADPH rather than NADH can differentiate between the two using this phosphate.

Functions of the PPP

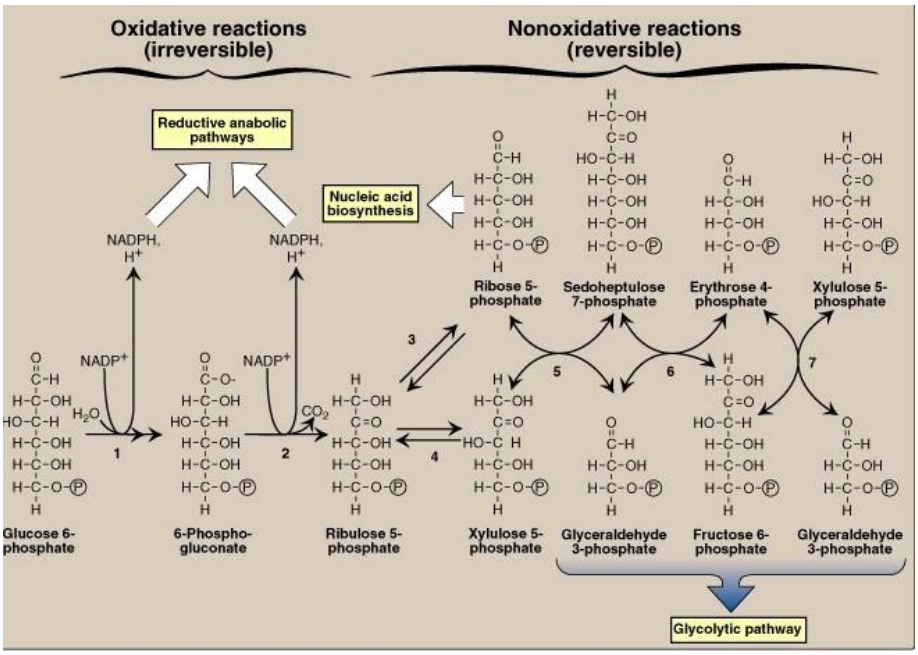
What was mentioned in the slide:

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

What was mentioned in the lecture:

Why we need NADPH? It is required for reductive biosynthesis, like the biosynthesis of fatty acids, biosynthesis of fatty acids take place in the liver, mammary glands and the adipose tissue, NADPH is also required for biosynthesis of steroid hormones (cholesterol derivatives) including testosterone, progesterone, aldosterone, estrogen, etc. that's why NADPH is needed in the testes, ovaries, placenta and adrenal cortex, all of this organs are active is synthesis of steroid hormones, also NADPH is needed for maintenance of glutathione in the reduced form (GSH) rather than the oxidized form (GS-SG, a disulfide bond) in the RBCs, we'll discuss all of these in detail later on.

The second function of pentose phosphate pathway is metabolism of five carbon sugars (pentoses), including both catabolism (breaking) and anabolism (building), we can synthesize pentoses or convert them into hexoses.

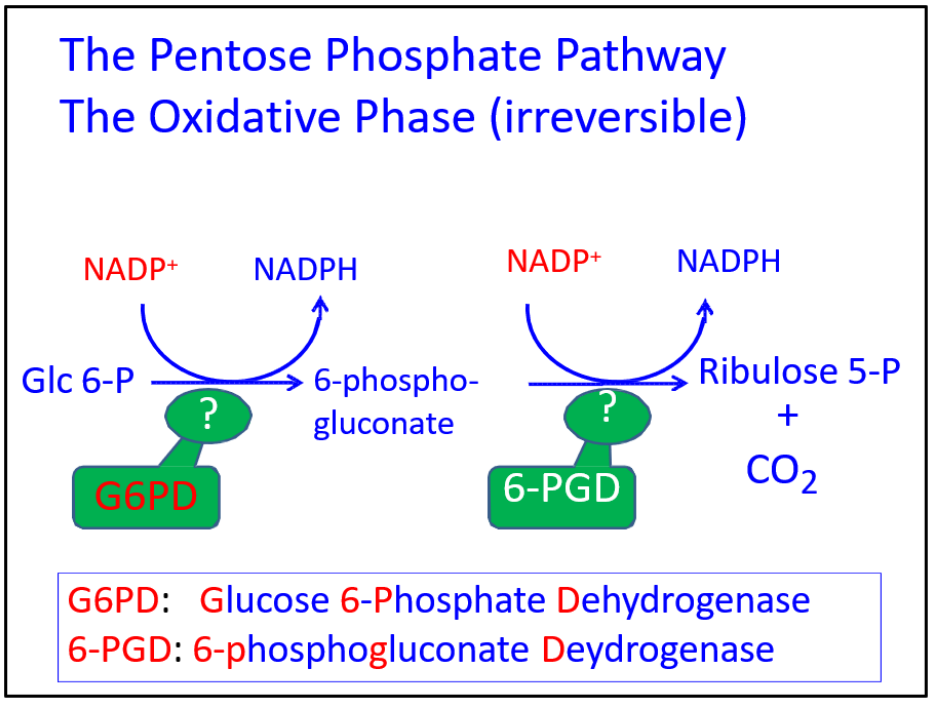


What was mentioned in the lecture:

This figure depicts the pentose phosphate pathway with all its enzymes and steps, it looks very difficult when you see it for the first time, but if you pay attention, you'll be able to understand it and memorize it very well.

The pentose phosphate pathway is composed of two parts: the irreversible oxidative reactions followed by reversible nonoxidative reactions, the starting material of this pathway is glucose 6-phosphate, in the irreversible oxidative reactions, by two steps, the glucose 6-phosphate is converted to a pentose ketose called ribulose biphosphate, after that (in the reversible nonoxidative reactions) ribulose 6-phosphate can be converted to one of two sugars: ribose 6-phosphate which present in RNA or another isomer called xylulose 6-phosphate (xylulose is an epimer for ribulose and a constitutional isomer of ribose), form these two, we can go into further reactions which can reproduce fructose 6-phosphate or glyceraldehyde 3-phosphate (remember: those two are intermediates of glycolysis).

In the conversion of glucose 6-phosphate to ribulose 5- phosphate, 2 NADPH molecules and one CO_2 molecule will be produced, therefore, this conversion must involve two oxidation reactions and one decarboxylation reaction, the CO_2 came from the carbon #1of glucose 6-phosphate which has been oxidized to carry carboxyl group and then was decarboxylated forming a pentose, what makes this conversion irreversible is that the second step is an oxidative decarboxylation, oxidative decarboxylation reactions in general are extremely exergonic and cannot be easily reversed (we've talked about that in TCA cycle), so decarboxylation makes the oxidative reactions irreversible, after producing of ribulose 5-phosphate, it can be isomerized to ribose 5-phosphate, which can in turn proceed in a series of reactions where the end products will be fructose 6-phophate and glyceraldehyde 3-phosphate, those are intermediates of glycolysis which can proceed into the glycolytic pathway or gluconeogenesis.

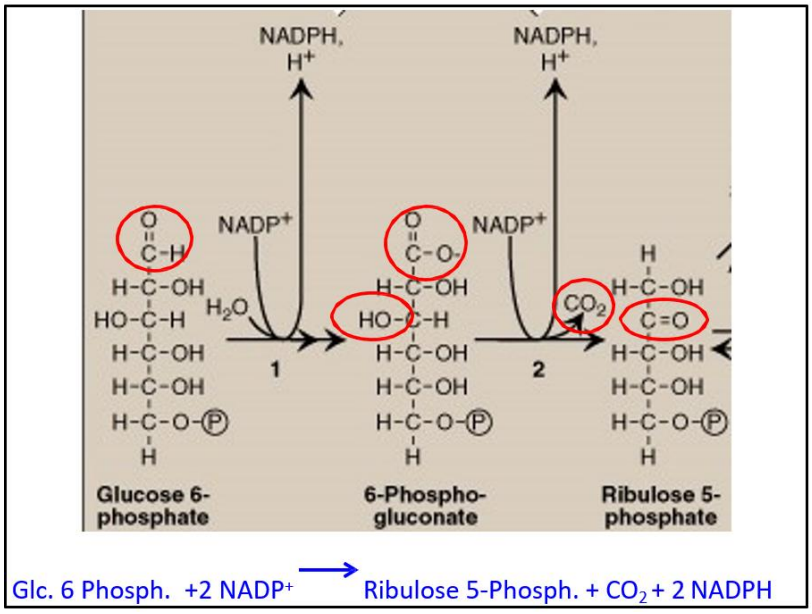


What was mentioned in the lecture:

In the first step of the oxidative phase of pentose phosphate pathway, glucose 6-phosphate is oxidized into 6-phosphogluconate, this step (oxidation of glucose 6-phosphate) actually involves two reactions but consider then as one step, in this step, carbon#1 of glucose is the one that is oxidized, NADPH is produced in this step.

In the second step, also oxidation occurs accompanied with release of CO_2, it is oxidative decarboxylation of 6-phosphogluconate forming ribulose 5-phosphate

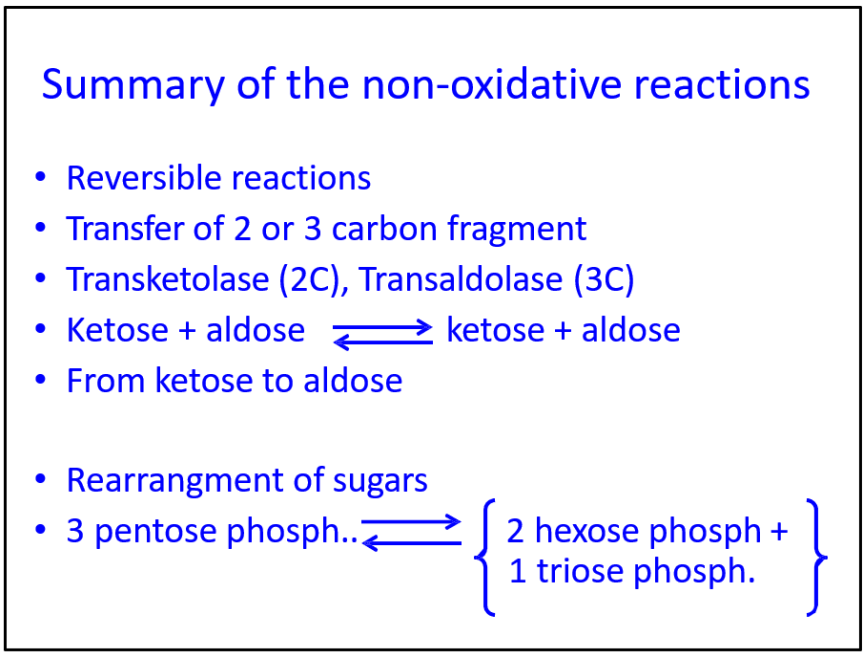
The name of the enzyme that catalyzes the first step is glucose 6-phosphate dehydrogenase while the one that catalyzes the second step is 6-phosphogluconate dehydrogenase.



What was mentioned in the lecture:

This figure depicts the structures of intermediated formed in the irreversible oxidative steps of pentose phosphate pathway, in the first step, carbon #1 of glucose 6-phosphate is oxidized to 6-phosphogluronate, after that, carbon #1 of 6-phospogluconate is decarboxylated, and the hydroxyl group of carbon number #2 is oxidized to a ketone group, forming ribulose 5-phosphate, ribulose is a ketose, that what characterizes it from ribose which is an aldose, just like fructose and glucose, if you see 'ul' in the name of the sugar, then this must be a ketose, like ribulose, xylulose, etc.

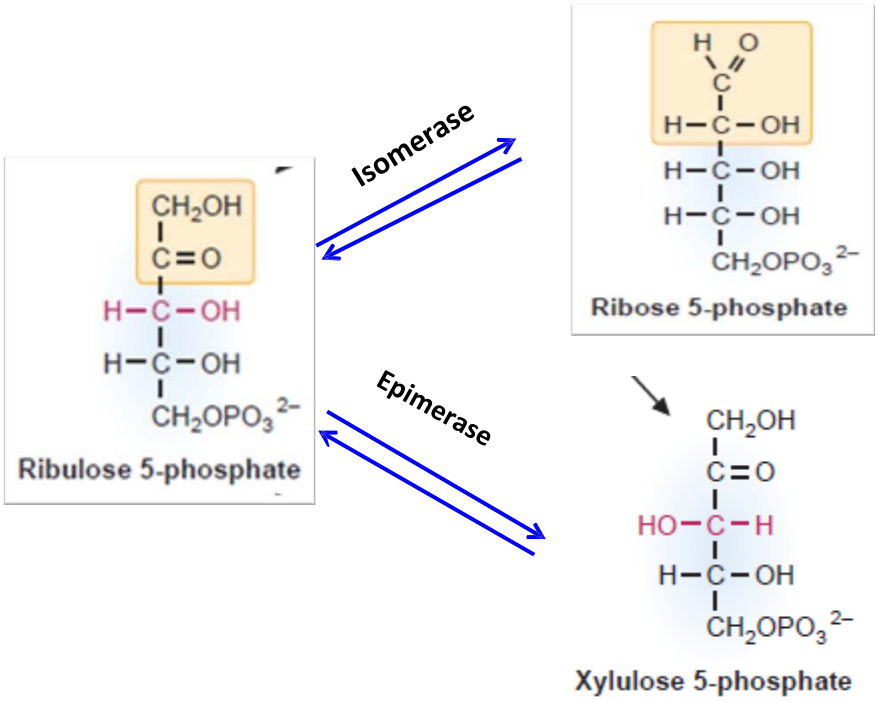
In total, the irreversible oxidative reactions of pentose phosphate pathway results in production of two NADPH molecules for each glucose 6-phosphate molecules and one molecule of CO_2 and a molecule of ribulose 5-phosphate.



What was mentioned in the lecture:

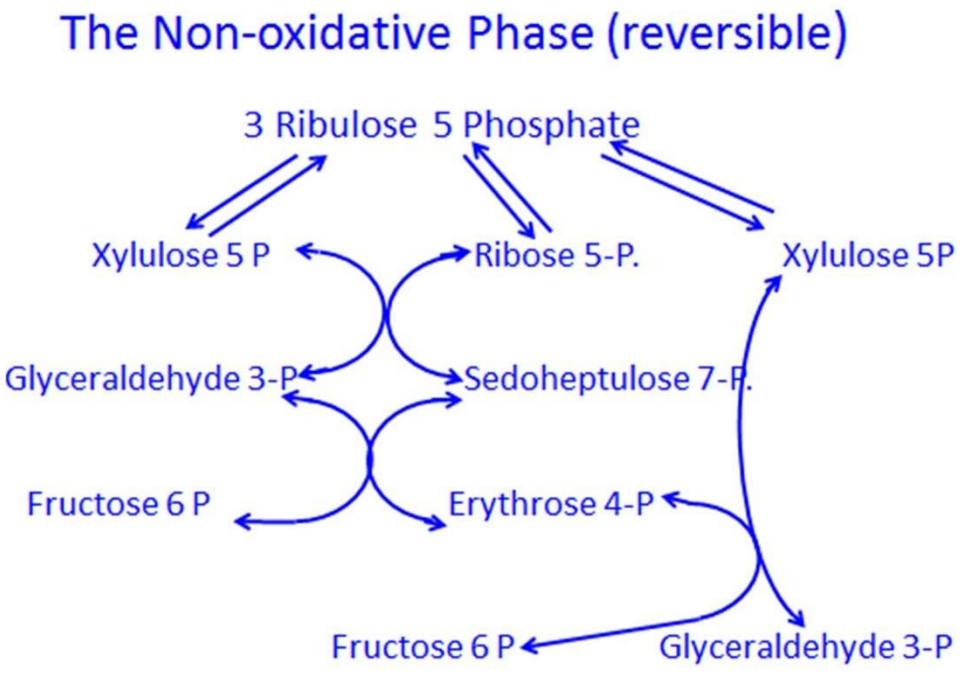
Regarding the nonoxidative part of pentose phosphate pathway, all reactions in this part are reversible, the point behind these reactions is to reuse ribulose, these reactions involve transfer of 2 or 3 carbon fragments, the enzymes that catalyze transfer of these fragments are called transketolase (for transfer of two carbon fragments) or transaldolase (for transfer of three carbon fragments), those transfer reactions are reversible, and always the donor of carbon fragments is the ketose and the acceptor is the aldose, it is like rearrangement of sugars, this reactions in total consumes two pentose molecules to produce two hexose molecule and one triose molecule, when we want to convert a pentose into a hexose, we cannot do that by carboxylation.

If we compared the total number of carbons proceeding into the reversible reactions and the number of carbons in the outcome, the number is 15 of both sides, it is equal, then those reactions are just rearrangement and they we do not use or lose any carboxyl groups (there is no carboxylation or decarboxylation in these reactions). (notice that also the number of phosphate groups is equal)



What was mentioned in the lecture:

How can we reuse ribulose 5-phosphate which has resulted from the oxidative reactions? It can be isomerized into ribose 5-phosphate or can be epimerized into xylulose 5-phosphate, those are catalyzed by an isomerase and an epimerase respectively. Epimerases interconvert epimers, ribulose and xylulose are epimers, they only differ in the orientation of hydroxyl group on carbon #3, while sugar isomerases convert aldoses into ketoses and vice versa.



What was mentioned in the lecture:

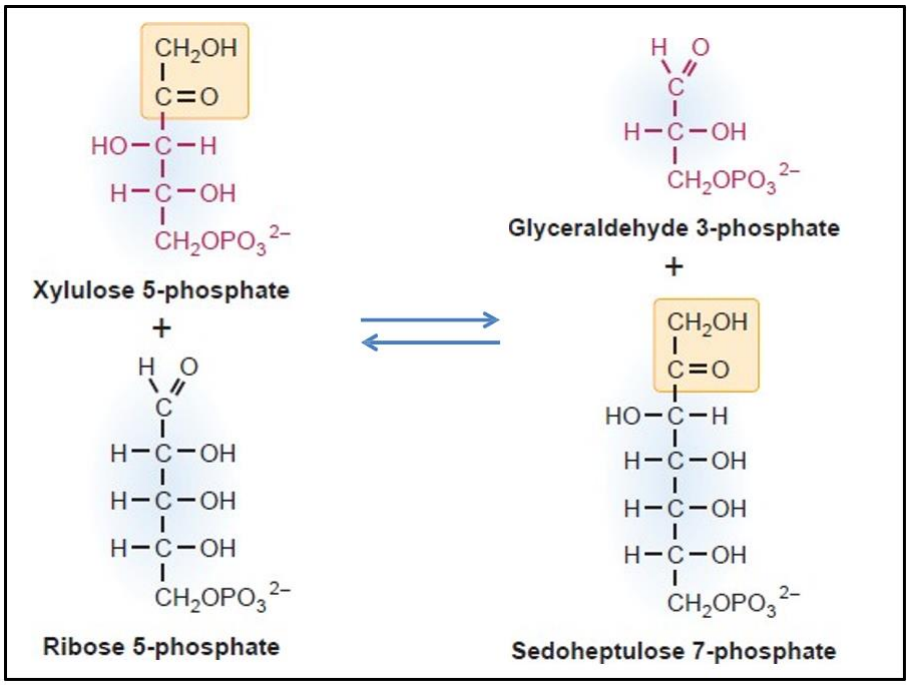
Now we'll discuss the nonoxidative part of pentose phosphate pathway step by step, if we suppose that 3 ribulose 5-phosphate molecules proceeded into these reactions, what will happen? Let's suppose that two of them will be epimerized into xylulose 5-phosphate, and the remaining one will be isomerized into ribose 5-phosphate, then one molecule of xylulose 5-phosphate and one molecule of ribose 5-phospate react with each other where two carbons are transferred from xylulose 5-phsophate to ribose 5-phosphate forming glyceraldehyde 3-phosphate and sedoheptulose 7- phosphate (" hepto" contains seven carbons and "ul" it is a ketose), the name of the enzyme that catalyzes this transfer is transketolase, then these two products (glyceraldehyde 3-phsophate and sedoheptulose 7-phosphate) react again where 3 carbons are transferred from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate forming fructose 6-phosphate and erythrose 4-phosphate (erythrose is a tetroaldose), the enzyme that catalyzes this reaction is called transaldolase, notice that is each of these transfers, and aldose react with a ketose forming a different aldose and a different ketose, notice also that always the donor is a ketose (like xylulose 5-P and sedoheptulose 7-P) while the acceptor is always an aldose (like glyceraldehyde 3-phosphate and ribose 5-P), in the third reaction, the other xylulose 5-phosphate which have entered those reactions firstly react with the erythrose 4-phosphate that resulted from the second transfer where two carbons of xylulose 5-P are transferred to erythrose 4-P forming fructose 6-phosphate and glyceraldehyde 3- phosphate again, the enzyme here again is transketolase.

Those transfer reactions require a specific coenzyme, it is thiamine pyrophosphate which is derived from vitamin B_1.

In total, three glucose 6-phosphate molecules will produce three ribulose 5-phosphate molecules after passing the oxidative reactions, which in turn will produce two fructose 6-phosphate molecules and one glyceraldehyde 3-phosphate molecule after passing the non-oxidative reactions.

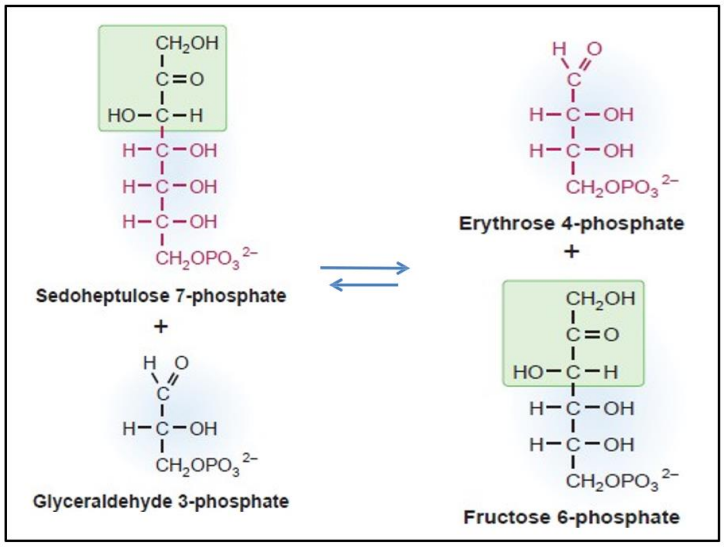
What was mentioned in the lecture:

This is a summary of the nonoxidative reversible reactions, notice that these reactions are always a transfer of carbon fragments from an aldose to a ketose forming a different aldose and a different ketose.



What was mentioned in the lecture:

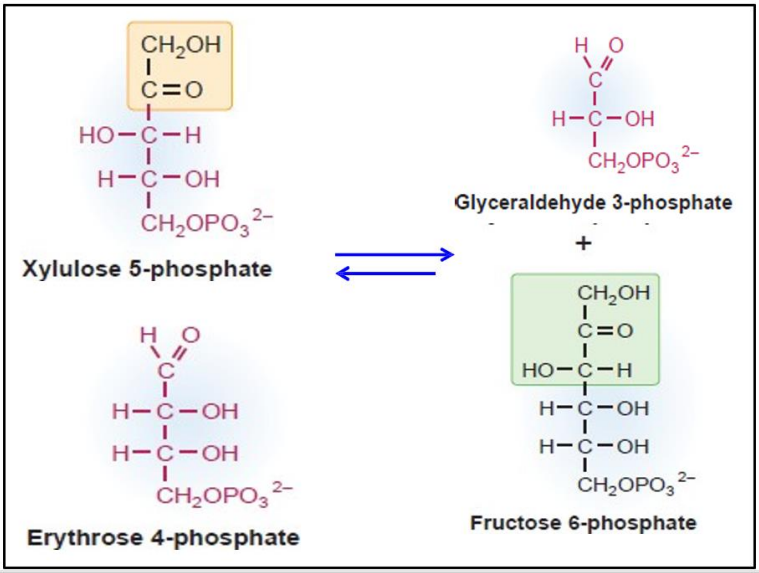
This figure depicts the first two reactions from the irreversible reactions with the structure, in the first reaction, two carbon fragment is transferred from xylulose 5-P to ribose 5-P forming glyceraldehyde 3-P and sedoheptulose 7-P, the enzyme that catalyzes this transfer is a transketolase.



What was mentioned in the lecture:

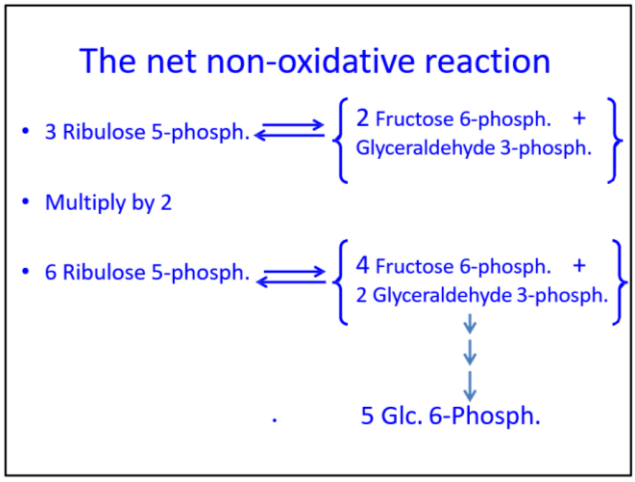
In the second reaction of the reversible reactions, 3-carbon fragment is transferred from sedoheptulose 7-P to glyceraldehyde 3-P forming erythrose 4-P and fructose 6-P, this reaction is somewhat opposite to the aldol cleavage reaction of glycolysis (cleavage of fructose 1,6-bisphosphate to dihydroxyacetone

phosphate and glyceraldehyde 3- phosphate) that's why we call the enzyme that catalyzes this reaction transaldolase.



What was mentioned in the lecture:

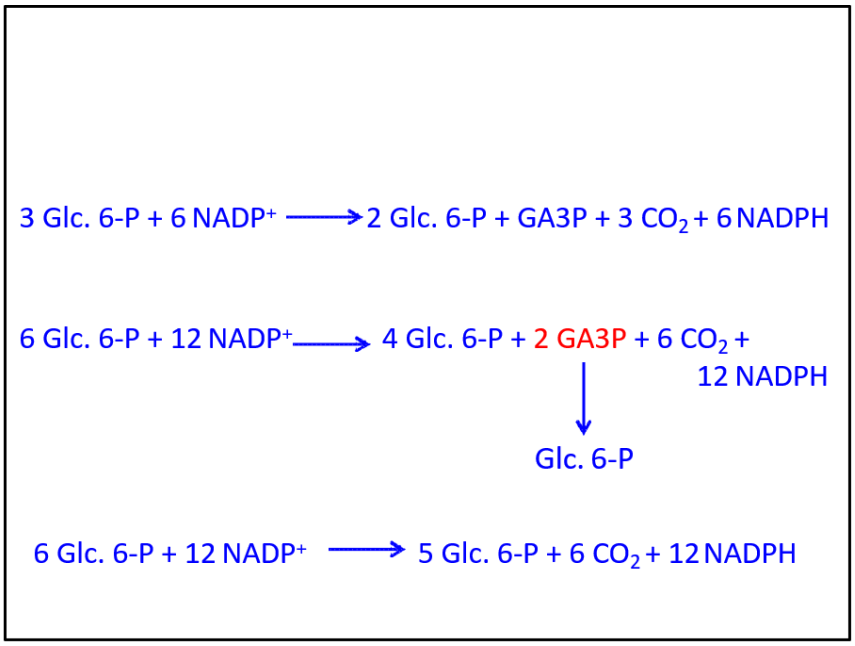
In third reaction of the reversible reactions, two-carbon fragment is transferred from a xylulose 5-P to erythrose 4-P forming glyceraldehyde 3-P and fructose 6-P, this reaction is catalyzed by a transketolase.



What was mentioned in the lecture:

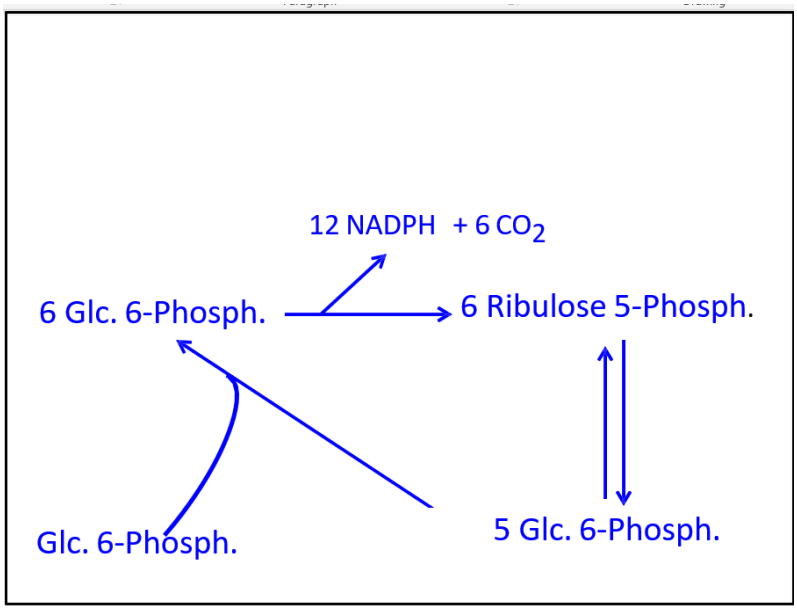
In the net nonoxidative reactions, 3 ribulose 5-phosphate molecules give two fructose 6-phosphate molecule and one glyceraldehyde 3-phosphate molecules, if we multiply that by 2, 6 ribulose 5-phosphate molecules will give 4 fructose 6-phosphate molecules and 2 glyceraldehyde 3-phosphate molecule, if you remember form gluconeogenesis lecture, the 2 glyceraldehyde 3-phosphate molecules can be converted into one fructose 6-phosphate molecules, we can do that if we isomerized one of them into

dihydroxyacetone phosphate which will react with the remaining glyceraldehyde 3-phosphate in a reaction that is reverse to the aldol cleavage of glycolysis forming fructose 1,6-bisphosphate, which then can be converted to fructose 6-phosphate, therefore, we can say that 6 ribulose 6-P molecules can produce 5 fructose 5-P molecules after passing the nonoxidative branch of pentose phosphate pathway, notice that in the both sides, the number of carbons is 30 (6 ribulose 5-P gave fructose 6-P), so the whole nonoxidative branch of pentose phosphate pathway is just a rearrangement of sugars to produce fructose 6-P which can easily be converted into glucose 6-P.



What was mentioned in the lecture:

If we summarized the whole pentose phosphate pathway, we'll see that it is just like if 6 glucose 6-phosphate molecules entered the oxidative part, 5 glucose 6-phopshate molecules will leave the nonoxidative part while 6 CO_2 molecules and 12 NADPH molecules will leave the oxidative part.



What was mentioned in the lecture:

If 6 glucose 6-phosphate molecules entered the oxidative part of PPP, they'll leave as 6 ribulose 5-phosphate molecules, 6 CO_2 and 12 NADPH molecules, we can convert the 6 ribose 5-phosphate molecules back into 5 glucose 6-phsophate molecules.

If we suppose that the cell only needs NADPH, it makes sense that the cell will use pentose phosphate pathway to spend 1 molecule of glucose 6-phosphate and get back 12 NADPH molecules, maybe you'll think that the cell needs only the oxidative part of PPT, but that is wrong, because without the nonoxidative part, ribulose 5-phosphate will accumulate in the cell and the cell doesn't want that, it will give 6 glucose 6-P molecules, which -after the oxidative part- will give 6 ribulose 5-P molecules, 6 CO_2 molecules and 12 NADPH molecules, the 6 ribulose 5-P can be converted back to 5 glucose 6-phosphate molecules using the nonoxidative reversible reaction, so it is like the cell had spent only one molecule of glucose.

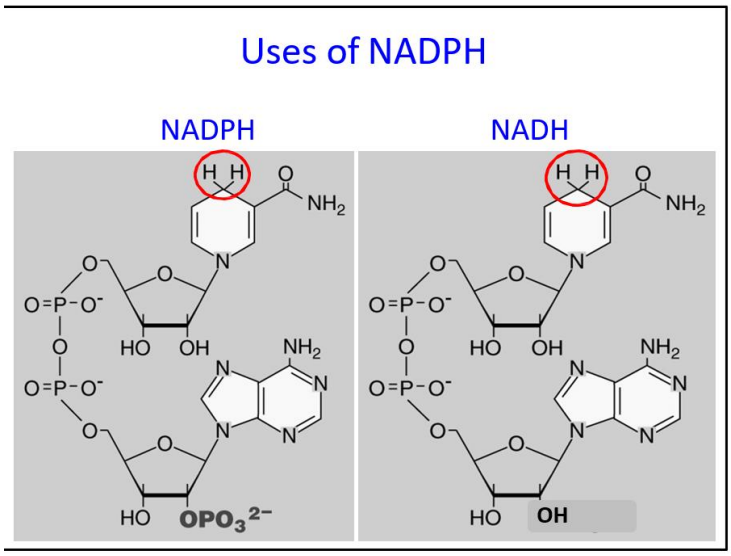
But if we suppose that the cell needs only pentoses (like ribose for RNA synthesis), we can produce them without the need of production of NADPH (remember: we mustn't allow for all $NADP^+$ to be reduced to NADPH, because the amount of $NADP^+$ in the cell is limited and we need it for other pathways), how can we do that, by letting glucose 6-phosphate enter the pentose phosphate pathway in the reverse direction, by isomerizing it into fructose 6-phosphate and then transfer of 2-carbon fragment from fructose 6-P into glyceraldehyde 3-P forming erythrose 4-P and xylulose 5-P, xylulose 5-P can then be epimerized into ribulose 5-P which in turn can be isomerized into ribose 5-P, and erythrose 4-P can then react with another fructose 6-P with a transfer of 3-carbon fragment from F6P to E4P forming sedoheptulose 7-P and glyceraldehyde 3-P, after that, sedoheptulose 7-P can donate a two-carbon fragment to glyceraldehyde 3-P forming ribose 5-P and xylulose 5-P, the later can be epimerized and isomerized to ribose 5-P. therefore, if the cell want pentoses, it can play with pentose phosphate pathway to produce pentoses from hexoses without consuming of $NADP^+$, by the same idea, glucose can be produced form ribose.

What if we need both pentoses and NADPH? In this case, we must activate the oxidative part only, that will give us NADPH, CO_2 and ribulose 5-phosphate from which pentoses can be produced, but we'll not need to activate the whole non-oxidative part, that will produces hexoses and not pentoses.

باختصار, البنتوز فوسفات باثواي الو أهميتين : انتاج البنتوز و انتاج NADPH , اذا الخلية بدها بس NADPH لازم تستخدم الباثواي كامل لحتى تنتج ال NADPH بال oxidative part و تعمل اعادة تدوير للجلوكوز الي استخدمته بال nonoxidative part اما اذا بدها تنتج pentoses

بس, بإمكانها تتحايل على الباثواي, هسا المنتج النهائي تبع الnonoxidative part هو glucose 6-phsophate بينما المنتج البدائي هو ribulose 5- phosphate و بما أنو هاض الجزء reversible, بتقدر الخلية تستخدم الاتجاه العكسي لأي تفاعل فيه لحتى تنتج أي بنتوز ضمن الباثواي و بدون ما تضطر تستهلك طاقة و تدخل جلوكوز عال oxidative part هسا صحيح انو ال oxidative part ممكن ينتجلنا pentoses, بس الخلية اقتصادية دايمًا, و اذا انها بتقدر تنتج بنتوز بدون طاقة باستخدام ال nonoxidative part بالعكس, فليش لحتى تصرف طاقة ؟؟؟؟

باختصار, اذا بدنا ننتج pentose من hexose او hexose من pentose او heptose من pentose او شو ما كان, بنستخدم ال irreversible part of pentose phosphate pathway، و اذا بدنا ننتج NADPH لازم نستخدم الباثواي كامل .



What was mentioned in the lecture:

What are the uses of NADPH? And what is the difference between NADPH and NADH? As we said, the reactive group which is the niacin ring is the same in both $NADP^+$ and NAD^+, therefore, they both can do the same function in oxidation-reduction reactions, as you see carbon #4 of the niacin ring (the one labeled in red) is the reactive group, this carbon is the one that carries and donate electrons (in the figure it is in the reduced form), both NAD^+ and NADPH have the same reduction potential, the only structural difference between NADPH and NADH is that one ribose on NADPH have a phosphate group instead of a hydroxyl group on its carbon #2, this phosphate functions as a tag, when enzymes recognize this tag, they prevent shuttling of NADPH into the electron transport chain (therefore, NADH and $FADH_2$ can be used for the electron transport chain but not NADPH), NADH formed in the cytosol in glycolysis can be transferred to the mitochondria for the electron transport chain using the mitochondrial shuttling systems, but NADPH cannot and must stay in the cytosol, because NADPH is used in the reductive biosynthesis and not for production of ATP.

Why NADPH and NADH?

What was mentioned in the slide:

- 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 ≈ 1/10
  - NAD^+/NADH ≈ 1000/1

What was mentioned in the lecture:

There is no enzyme that can use both NADH and NADPH, an enzyme can only use one of them and not the other, the total sum of the amount of NADH and NADPH remain almost constant in the cell, but NADH exist in the cell mainly in the oxidized form (as NAD^+ and not NADH), while NADPH mainly exist mainly in the reduced form (as NADPH and not $NADP^+$), we need NADPH to be always reduced because we utilize it usually in biosynthesis, while we need NAD^+ to be always oxidized to make it available for glycolysis and glycolysis will always go on (remember: glycolysis requires NAD^+ to oxidize glyceraldehyde 3-phosphate into 1,3- bisphosphoglycerate), when the ratio of NADH/NAD^+ becomes high, glycolysis will discontinue.

Uses of NADPH (Reductive Biosynthesis)

What was mentioned in the slide:

- Some biosynthetic reactions require high energy electron donor to produce reduced product
- Examples: Fatty acids, Steroids …
- 8 $CH_3COO \rightarrow C_{15}H_{33}COO$

What was mentioned in the lecture:

We need NADPH for reductive biosynthesis, like fatty acid synthesis, fatty acid synthesis involve conversion of 8 molecules of acetyl CoA (acetate) into palmitic acid, you can notice that palmitic acid is much more reduced that acetate (the ratio of oxygens to carbons is lower (lower oxygen content), 2 per 2 in acetate versus 2 per 16 in palmitic acid), always and always, reduction reactions are endergonic, they need energy to happen, while oxidation reactions are exergonic and produce energy, the energy needed for biosynthesis of fatty acids case comes from oxidation of NADPH to $NADP^+$ which an exergonic reaction (we use energy from the exergonic reaction to power the endergonic reaction, the principle of coupling).

Uses of NADPH/ Reduction of Hydrogen Peroxide

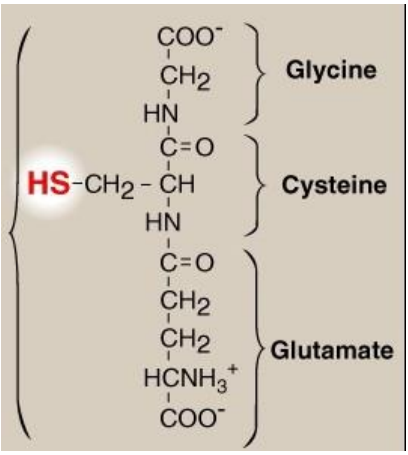
What was mentioned in the slide:

- H_2O_2 one of a family of compounds known as Reactive Oxygen Species (**ROS**)
- Other: Super oxide, hydroxyl radical,
- 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

What was mentioned in the lecture:

The other important use of NADPH is reduction of reactive oxygen species, mainly hydrogen peroxide (H_2O_2), H_2O_2 is produced continuously in our cells as a byproduct of oxidation reactions, it is harmful to our cells, therefore it must be reduced into H_2O, other examples on ROS include superoxide (O_2^-) and hydroxyl radical (·OH), those also are continuously being produced due to the oxidative reactions, as byproducts of aerobic metabolism and interaction with drugs and environmental toxins (like sun exposure), we can reduce the amount of ROS if we eat an adequate supply of antioxidants, the problem with ROS is that they are very reactive, therefore, they can cause damage to cell's proteins, lipids, and DNA, damage of DNA can cause cancer, or it can cause cell death and inflammation, ROS can be utilized for the cell benefit, some cells like macrophages and neutrophils use reactive oxygen species to kill microbes.

Enzymes that catalyze antioxidant reactions



What was mentioned in the slide:

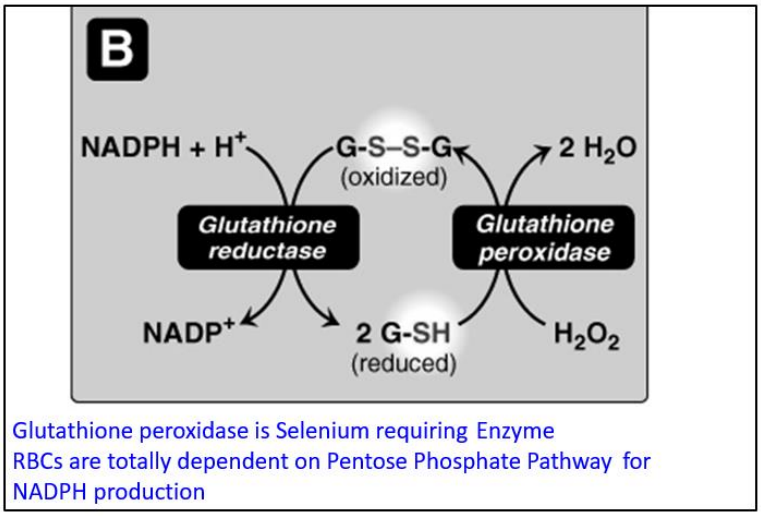
- Glutathione peroxidase
- Glutathione is a reducing agent
- Tripeptide
- GSH is the reduced form
- Oxidation ⟶ two molecules joined by disulfide (GSSG)
- 2 GSH → GSSG

What was mentioned in the lecture:

What are the enzymes that catalyze reduction of ROS? And what is the role of NADPH is that? Glutathione is an important reducing agent of cells for ROS, from a biochemical point of view, glutathione is tripeptide

composed glycine, cysteine, and glutamate, (the name indicates the components: gluta= glutamate thione= sulfur=cysteine) its reactive group is the thiol group of cystine, this group is oxidized to reduce reactive oxygen species, -SH is the reduced functional form (the one that can be oxidized) of the thiol group, when two thiol groups are oxidized to reduce a reactive oxygen species molecule, a disulfide bridge (-S-S-) forms between two glutathione molecules. Glutathione is antioxidant that prevents oxidation damaging reactions of ROS with DNA and protein by oxidizing itself.

Glutathione in its oxidized form (two molecules joined by a disulfide bridge) is not functional, it cannot function as an antioxidant when it is oxidized, therefore, it must be reduced again to its active form to be reused, here comes the role of NADPH, NADPH reacts with the oxidized form of glutathione (GS-SG + NADPH → GSH + $NADP^+$), reducing it back again to its functional form.



Glutathione peroxidase is Selenium requiring Enzyme
RBCs are totally dependent on Pentose Phosphate Pathway for NADPH production

What was mentioned in the lecture:

The oxidized form of glutathione is composed of two glutathione molecules joined by a disulfide bridge, it is produced when the reduced form (GSH) reduces H_2O_2 forming H_2O and GS-SG, tis reaction reaction is catalyzed by an enzyme called glutathione peroxidase, how can we get the oxidized form back to the reduced form? This is done by an enzyme called glutathione reductase, this enzyme reduces glutathione back to its functional form using NADPH as a reducing agent, this is another important usage of NADPH.

Glutathione peroxide enzyme requires a metal called selenium, the is the only reaction in our body that requires selenium, therefore selenium is a trace element that is required in a very small amount.

Red blood cells are totally dependent on pentose phosphate pathway for production of NADPH, actually there are other methods of producing NADPH, but not in RBCs.

G6PD Deficiency

What was mentioned in the slide:

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

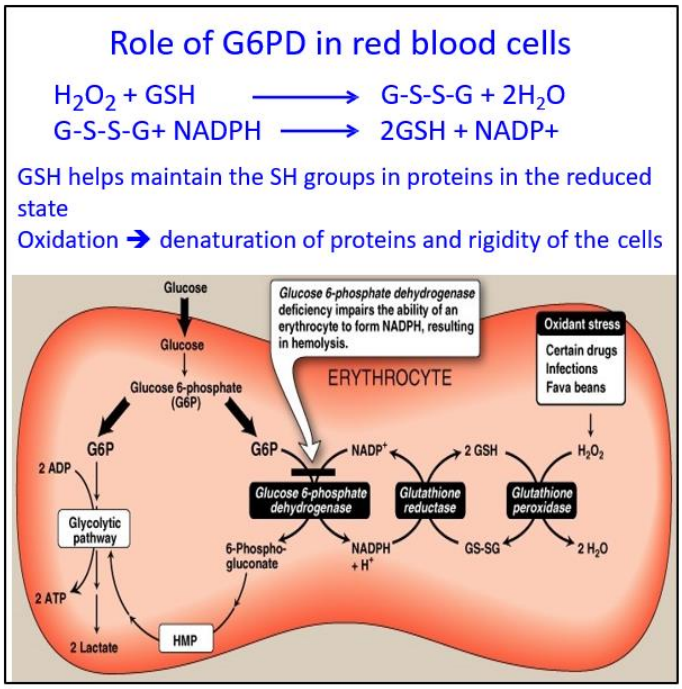
What was mentioned in the lecture:

There are diseases that result from deficiency of glucose 6-phosphate dehydrogenase, the first enzyme of pentose phosphate pathway, in any pathway, a deficiency of an enzyme due to mutation must result in a disease, or this mutation can be not compatible with life causing early death, glucose 6-phosphate dehydrogenase deficiency is a common disease that is characterized by hemolytic anemia (because RBCs are totally dependent on pentose phosphate pathway for producing of NADPH, without it, RBCs will die, causing hemolytic anemia), there are 200-400 million person that have this disease worldwide, the highest prevalence of this disease is in the middle east, south east Asia, and the Mediterranean region.

This disease's inheritance is X-linked, meaning that females need two mutated genes to develop this disease, while males only need one mutated gene for this disease to appear, therefore, this disease is more common in males, because males have only one X chromosome while females have two X chromosomes, but a female can be carrier of the disease (has the abnormal gene but do not develop symptoms), and she can inherit the disease for her children even if their dad doesn't have the disease.

This disease is compatible with life, patients with this disease do not die very young.

Plasmodium falciparum is the causative agent of malaria, it is a parasite that causes intracellular infection of Red blood cells, if RBCs has a short life span, this parasite would not find a proper environment to live, therefore, people with glucose 6-phosphate dehydrogenase deficiency are resistant to malaria.



What was mentioned in the lecture:

This figure depicts the reactions that happen in erythrocytes (Red blood cells), in the normal case, there must be glutathione in its reduced form (GSH), if it is not present, some proteins (like the hemoglobin) present inside the cytosol of Red blood cells can be oxidized, causing accumulation (precipitation) of denatured hemoglobin (proteins) inside the red blood cells, so the RBCs mast maintain an adequate reservoir of reduced glutathione in order to combat oxidation by ROS, unless, the denatured proteins will accumulate causing deformity of red blood cells and death of them, if a person was infected, or had ate beans or certain drugs, that will cause oxidative stress in the red blood cells where there is overproduction of H_2O_2, glutathione will reduce H_2O_2 and therefore it will be consumed rapidly, that will cause a higher demand of NADPH, if NADPH production is impaired, glutathione will remain in the oxidized form, it will not function as an antioxidant any more, causing damage to proteins of RBCs (RBCs do not contain DNA), and deformity and death of RBCs, that's why any cells must have a continuous supply of NADPH, if we are talking about an RBC which depend totally on PPP for NADPH production, if PPP was interrupted like (G6-P deficiency), that will cause great damage to RBCs, another thing is that RBCs do not contain ribosomes and cannot carry protein synthesis, therefore, they cannot compensate this deficient enzyme, but if the cell was a hepatocyte, it can easily produce more and more of this deficient enzyme.

Glutathione helps in maintaining the thiol groups of proteins in their reduced form, it prevents denaturing of proteins and deformation of RBCs, RBCs in general are very flexible, if they become rigid, that would result in hemolytic anemia.

Precipitating Factors in G6PD Deficiency

What was mentioned in the slide:

- Oxidant drugs
  - Antibiotics e.g. Sulfamethoxazole
  - Antimalaria Primaquine
  - Antipyretics Acetanalid
- Favism
- Infection
- Neonatal Jaundice

What was mentioned in the lecture:

What are the factors that trigger the oxidative stress in RBCs promoting hemolytic anemia? Firstly, administration of oxidant drugs, those include some antibiotics like sulfamethoxazole, sulfamethoxazole is used to treat typhoid fever and other bacterial infections, some antimalarial drugs like primaquine (but not Chloroquine) an some antipyretics like acetanilide, acetanilide have a similar effect to that of paracetamol.

The other cause is favism, مرض التفوّل أو الفُوال, this disease is characterized by hemolytic anemia upon eating of beans, patients with disease must avoid eating beans, other causes of oxidative stress in RBCs is infections and neonatal jaundice الصَفار الي بصير مع حديثي الولادة.

G6PD Deficiency Variants

What was mentioned in the slide:

- Wild type B
- Mediterranean Variant B^- (Class II): 563C T
- African Variant A^- (Class III); two-point mutation
- African Variant A; Normal activity 80%
- Very severe deficiency (Class I)
- Majority missense mutation point mutation
- Large deletions or frame shift; Not Observed

What was mentioned in the lecture:

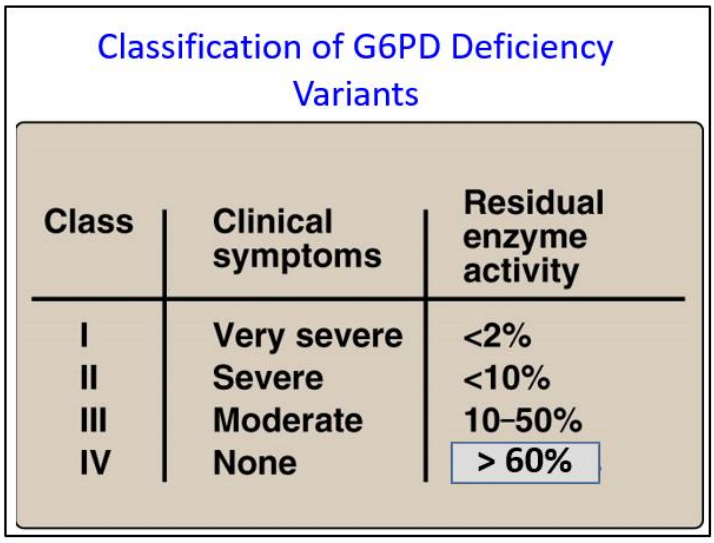
There are multiple types of glucose 6-phophate dehydrogenase deficiency, as we said, there are 400 mutations that were observed in the gene that codes for glucose 6-phosphate dehydrogenase, those mutations result in expressing four different isoforms of glucose 6-phosphate dehydrogenase, each person might have a different isoform of these four, those are: G6PD B, G6PD B^-, G6PD A^-, and G6PD A, G6PD B is the wild type (the one that functions normally), people that have the G6PD B^- isoform have the

Mediterranean variant of glucose 6-phosphate dehydrogenase deficiency or class II G6PD deficiency, this isoform is produced when the cytosine residue number 563 in gene that codes for G6PD is substituted with a thymine residue.

People with the A^- isoform of G6PD have the African variant A^- of G6PD deficiency (class III G6PD deficiency), this isoform is produced when there is two point mutations طفرات موضعية in the gene while people with A isoform of G6PD have the African variant A of G6PD deficiency

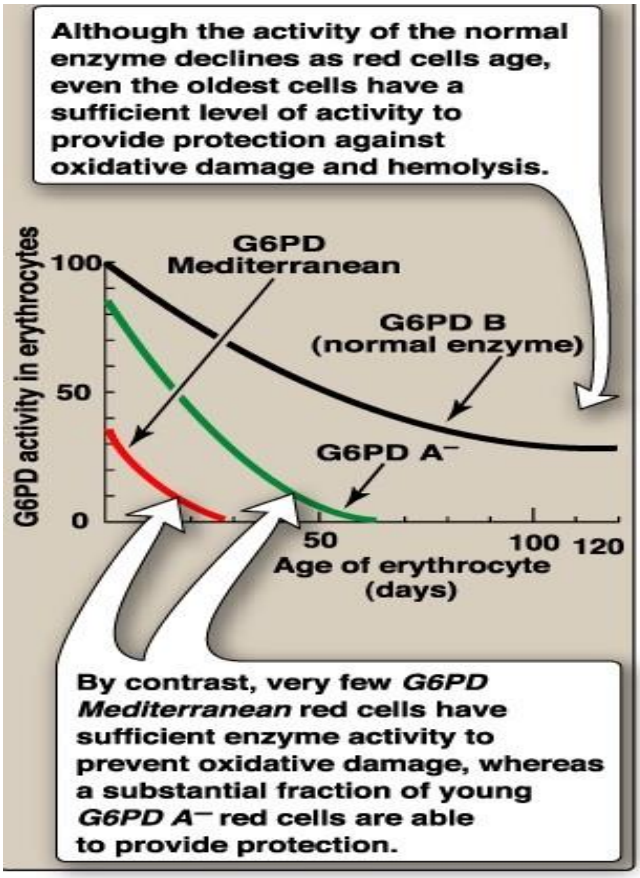
Class I G6PD deficiency is rare class, it is the most severe one, class II G6PD deficiency is moderate, while class III is mild.

No deletion or frameshift mutations طفرات حذف او ازاحة were observed in G6PD gene in the population, because these mutations are not compatible with life, all mutations that were observed in this gene are missense point mutation طفرة موضعية مخطئة التعبير, meaning that one amino acid in the protein would be substituted.



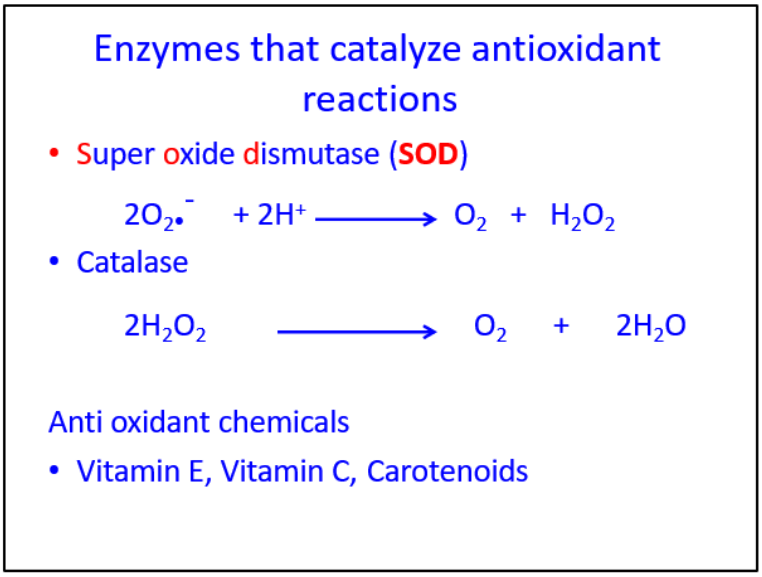
What was mentioned in the lecture:

Again, those are the different classes of G6PD deficiency, class I is very severe (enzymatic activity of G6PD is less than %2), class II (Mediterranean variant) is severe or moderate (enzymatic activity of G6PD is less than %10), class III is moderate or mild (10-50% of the normal enzymatic activity), and class IV has no symptoms(more than 60% of the enzymatic activity).



What was mentioned in the lecture:

This curve depicts the G6PD activity in terms of the age of RBCs, the normal life span of RBCs is around 120 days, if the person has G6PD B isoform which is the normal case, the enzymatic activity will not ceases, even after 120 days, but if the person have the G6PD A^- isoform, the enzymatic activity will cease after about 60 days, shortening the life span of RBCs (they'll die young), that will be prominent if the person has the B^- isoform, where the enzymatic activity of G6PD will cease after almost 25 days, even in the first day of the life of RBCs, the enzymatic activity will be only 35%, shortening the life span or RBCs even more. (the enzymatic activity decreases with time because RBCs are not able to synthesize G6PD, they acquire only when they are being born).



What was mentioned in the lecture:

In the context of antioxidant reactions, we must mention also some enzymes and chemicals, superoxide dismutase catalyzes the reaction of conversion of superoxide ($\cdot O_2^-$) radical which is very strong oxidizing agent into hydrogen peroxide which is a less reactive ROS and molecular oxygen.

Catalase also converts H_2O_2 into molecular oxygen and water.

Examples on antioxidant chemicals include vitamin E, vitamin C, and carotenoids which we get from carrots, vitamin C can be oxidized into dehydroascorbic acid, preventing other molecules from being oxidized.

Sources of ROS in the cell

What was mentioned in the slide:

- Oxidases

e- + O_2

Most oxidases produce H_2O_2 (peroxidase)

Oxidases are confined to sites equipped with protective enzymes

- Oxygenases
  - Mono Oxygenases (hydroxylases)
  - Dioxygenases in the synthesis of prostaglandins, Thromboxanes, leukotrienes

Coenzyme Q in Respiratory chain

- Respiratory Burst (during phagocytosis)

O_2 H_2O_2 OH NO HOCl

- Ionizing Radiation
- $OH^{\bullet}$

What was mentioned in the lecture:

From where do these reactive oxygen species come? The first source is oxidases, oxidases are enzymes that catalyze oxidation reactions using molecular oxygen as an electron acceptor, oxidases can mistakenly produce superoxide radical, and most of them actually can produce hydrogen peroxide, hydrogen peroxide is produced by the cell intentionally to be used as an electron acceptor in the reactions catalyzed by peroxidases, but reactions that produce hydrogen peroxide are confined in specific areas in the cell (like the phagolysosome) where protective enzymes (like glutathione peroxidase) that prevent the harmful damage of hydrogen peroxide are ready.

Oxygenases are enzymes that introduce molecular oxygen into the structure of their substrates, they include monooxygenases and dioxygenases, monooxygenases introduce one oxygen atom in the form of hydroxyl groups in the structure of their substrates, that why they are called hydroxylases, while dioxygenases introduce two atoms of oxygen into their substrate, they are involved in synthesis of many inflammatory mediators, including prostaglandins, leukotrienes and thrombaxenes.

Coenzyme Q is a mediator of electron transport chain, it also can produce free oxygen radicals.

Cytochrome P450 Mono oxygenase

What was mentioned in the slide:

- Mixed function oxygenase
- Super family of structurally related enzymes

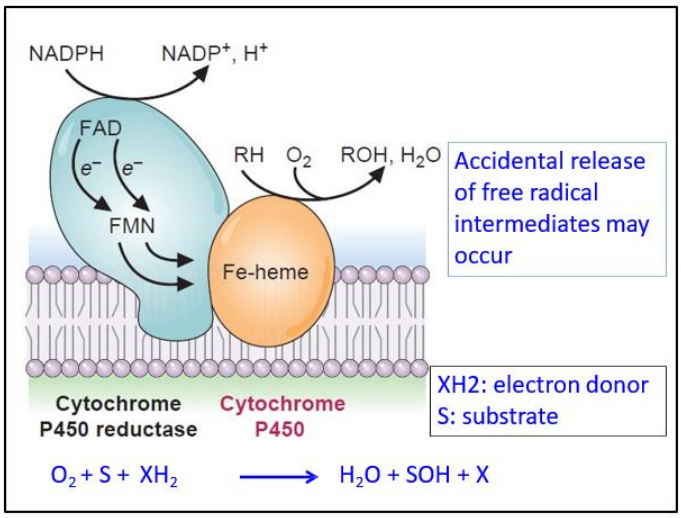
$R\text{-}H + \mathbf{O_2} + NADPH + H^+ \rightarrow R\text{-}\mathbf{O}H + H_2\mathbf{O} + NADP^+$

Mitochondrial system
Hydroxylation of steroids, bile acids, active form of Vit. D
Microsomal system
Detoxification of foreign compounds
activation or inactivation of Drugs
solubilization

What was mentioned in the lecture:

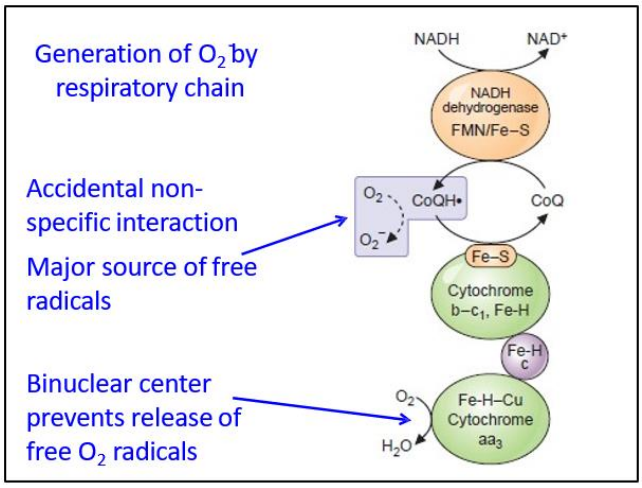
Since we are talking about NADPH, we must talk about cytochrome P450 monooxygenase, it is a mixed-function oxygenase, actually cytochrome P450 is a family of enzymes, those enzymes use molecular oxygen to hydroxylase their substrates, they use one oxygen atom from the O_2 molecule, and the othet oxygen would be reduced by NADPH forming H_2O and $NADP^+$.
Also monooxygenases of the mitochondria are used to add hydroxyl groups to steroids (like in synthesis of steroid hormones), bile acids, and vitamin D (vitamin D is actually a steroid also).

A person can be exposed to toxic materials like petroleum products, pesticides, drugs, etc. the first step in getting rid of these compounds in making them more water soluble to make it possible to excrete them with urine or feces, this is function of the microsomal system which have monooxygenases, this system adds hydroxyl groups to lipophilic foreign materials, making them more water soluble and allows for conjugation with glucuronic acids.
Hydroxylation might cause inactivation of drugs, or even activation of inactive prodrugs.



What was mentioned in the lecture:

NADPH transfers its electron through the cytochrome P450 reductase, this reductase in turn transfers the electron to the cytochrome P450, the cytochrome P450 then hydroxylates its substrate, it is like an electron transport chain but its purpose is to hydroxylase substrates rather than production of ATP, when electrons reaches cytochrome P450, it uses them to reduce one oxygen atom of O_2 molecule, then the other oxygen will be activated, allowing for its addition to the substrate, but in this chain ROS can be generated mistakenly.



What was mentioned in the lecture:

The regular electron transport chain (the one that we use for production of ATP) usually do not involve production of reactive oxygen species when the electron pass from NADH to O_2, but occasionally, some coenzyme Q mistakenly reduce O_2 into superoxide radical, if superoxide radical was produced, the cell converts it to hydrogen peroxide which in turn is reduced by peroxidases into water.

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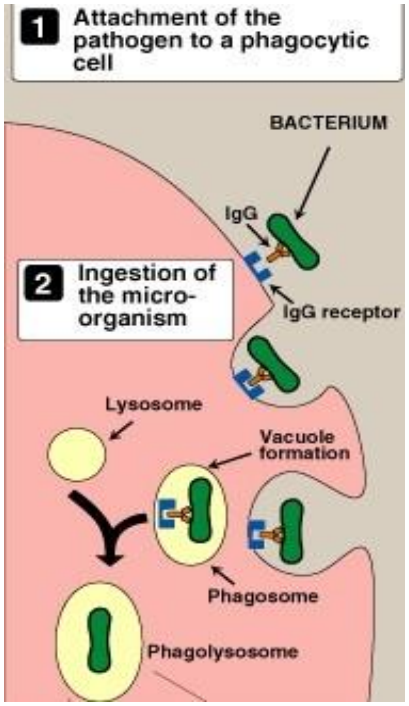
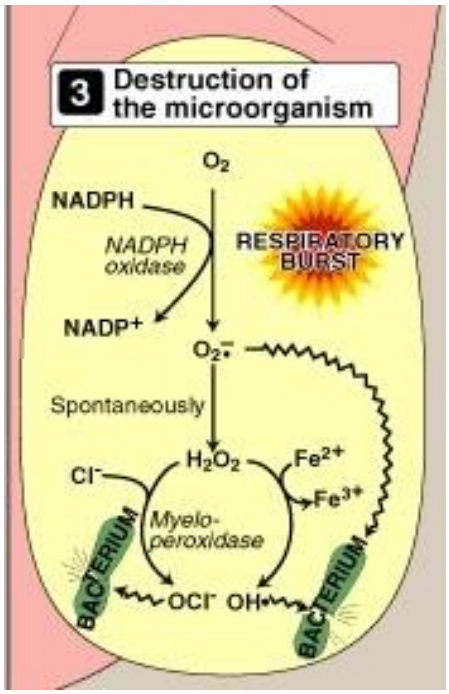
Rapid consumption of O^2 that accompanies superoxide formation

Phagocytosis; the oxygen dependent pathway of microbial killing by White Blood Cells (WBC)



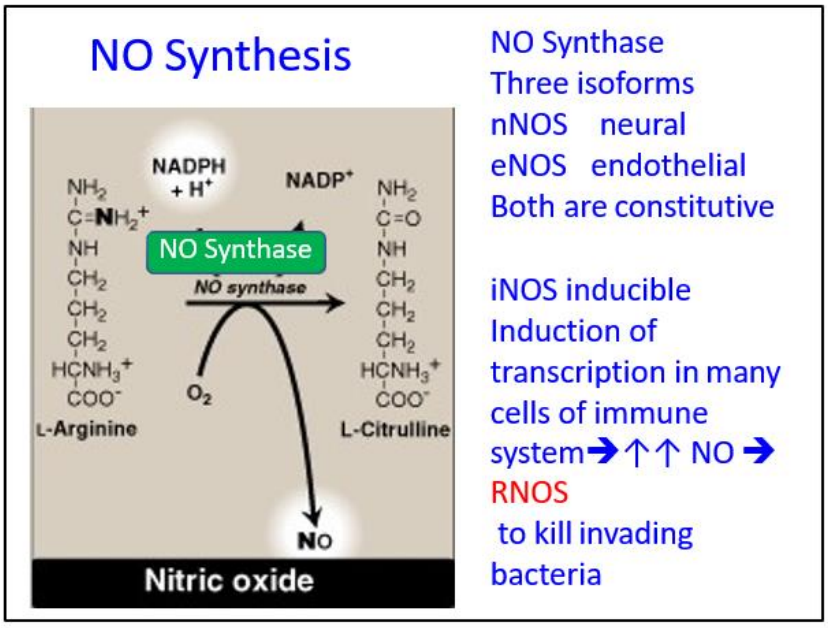


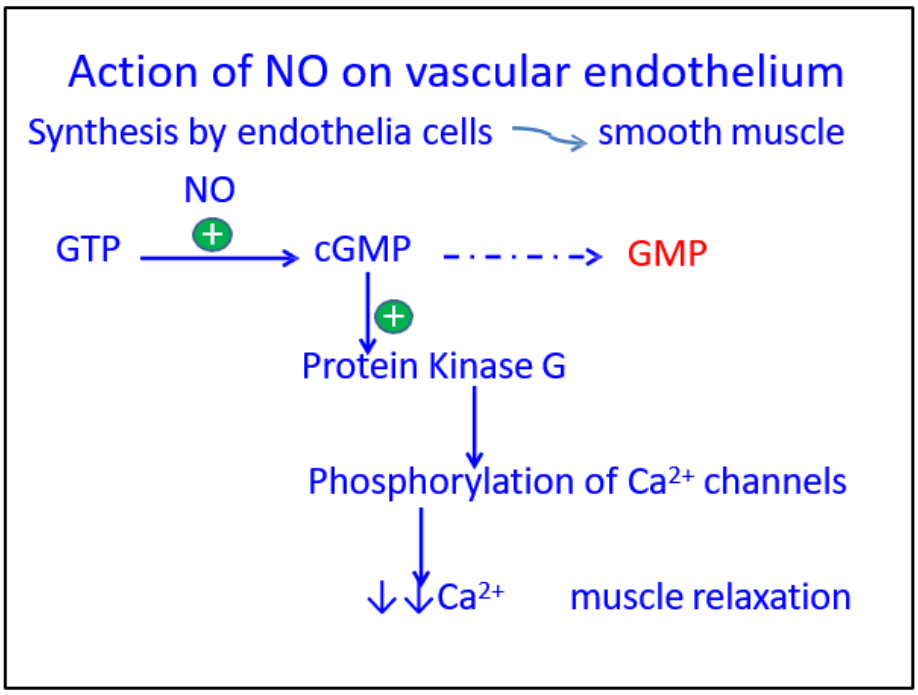
What was mentioned in the lecture:

The cell can make benefit from reactive oxygen species in killing of microbes, they use them as a weapon, when a cell phagocytes microbes after the microbes was recognized by immunoglobulins, the phagosome fuses with lysosome forming a phagolysosome, after that, NADPH is used to produce the superoxide which is harmful for microbial cells, there is an enzyme called NADPH oxidase, this enzyme will reduce molecular oxygen using NADPH by adding only of one electron, forming the superoxide radical, superoxide radical can be also converted to hydrogen peroxide which is also harmful for bacterial cells, also hydrogen peroxide can react with chloride ion forming by the aid of myeloperoxidase hypochlorite (hypochlorous acid) (OCl^-) which is also harmful, so ROS are also can be produced as a defense mechanism.
They utilized this idea in creating bleaching agents that are disinfectants, those agents contain hypochlorous acid.
Phagocytosis of microbes leads to increasing of oxygen demands in the cell, because we'll need oxygen to produce ROS, this is called respiratory burst, it is a reflex that happens after cells phagocytose microbes.

NO and Reactive Nitrogen Oxygen Species (RNOS)

- Free radical diffuses readily
- Essential for life and toxic
- Neurotransmitter, vasodilator
- ↓Platelet aggregation
- At high concentration combines with O_2● or O_2 to form **RNOS**
- **RNOS** are involved in neurodegenerative diseases and inflammatory diseases




Action of NO on vascular endothelium
Synthesis by endothelia cells
smooth muscle
NO
GTP
cGMP
GMP
Protein Kinase G
Phosphorylation of Ca2+ channels
Ca2+
muscle relaxation