بسم الله الرحمٰن الرحيم

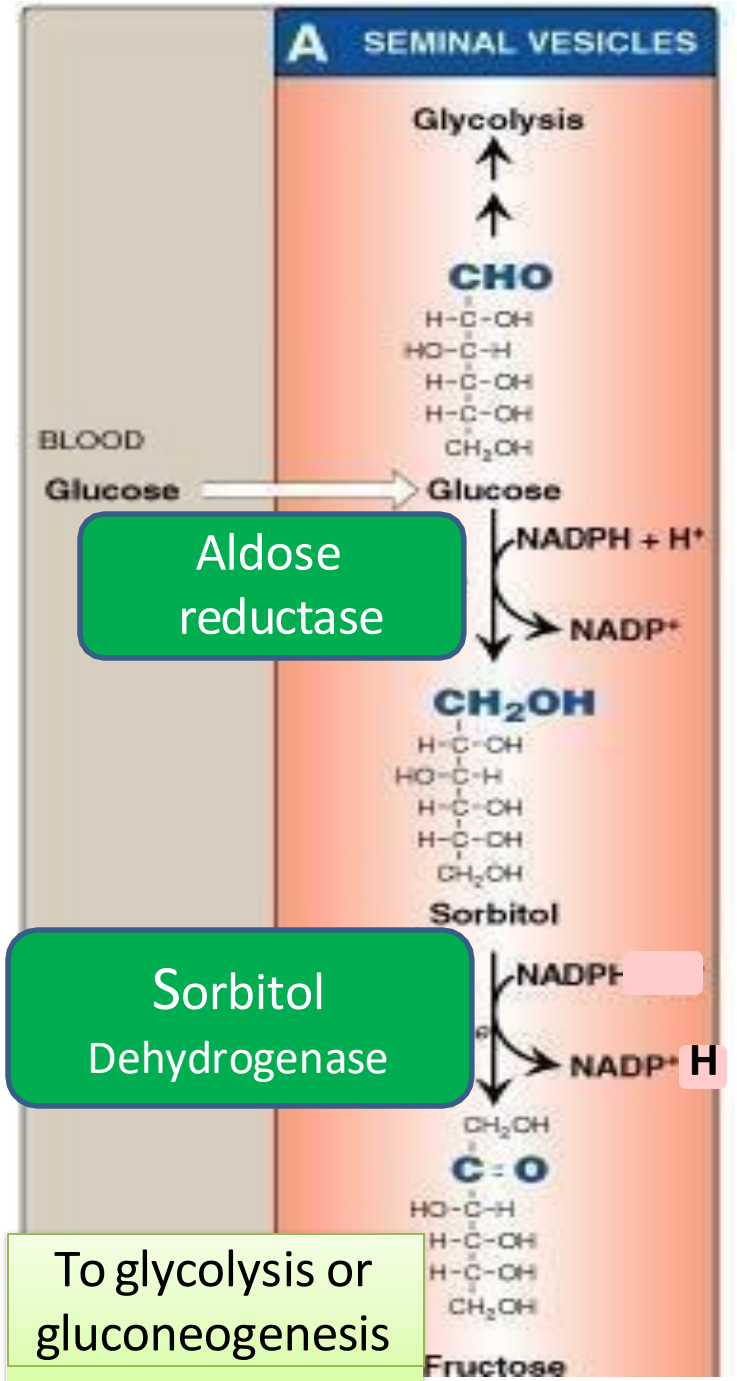
(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | FINAL 3

Metabolism of monosaccharides & disaccharides pt.2 + Phosphate Pentose pathway Pt.1

Written by : DST
NST

Reviewed by : NST
Shahad Alrawi



❑ Conversion of glucose to fructose via sorbitol

➢ An enzyme called **Aldose Reductase:**

Which is found in many tissues; Lens, retina, Schwann cells, liver, kidney, ovaries, and seminal vesicles

- This enzyme reduces aldoses. When glucose enters, it can proceed to glycolysis, pentose phosphate pathway, as well as other pathways. It can also activate aldose reductase in these tissue to reduce glucose to sorbitol, a polyalcohol, oxidizing NADPH to NADP+ in the process.

➢ **Sorbitol Dehydrogenase:**

which is found in Liver, ovaries, and seminal vesicles

- Sorbitol DH is an enzyme that oxidizes sorbitol to fructose (forms a carbonyl at carbon #2). These cells can convert glucose to fructose directly via sorbitol.

Recall: Isomerization mentioned previously was between phosphorylated glucose and phosphorylated fructose.

❖ **Fructose** is the major energy source for sperm cells

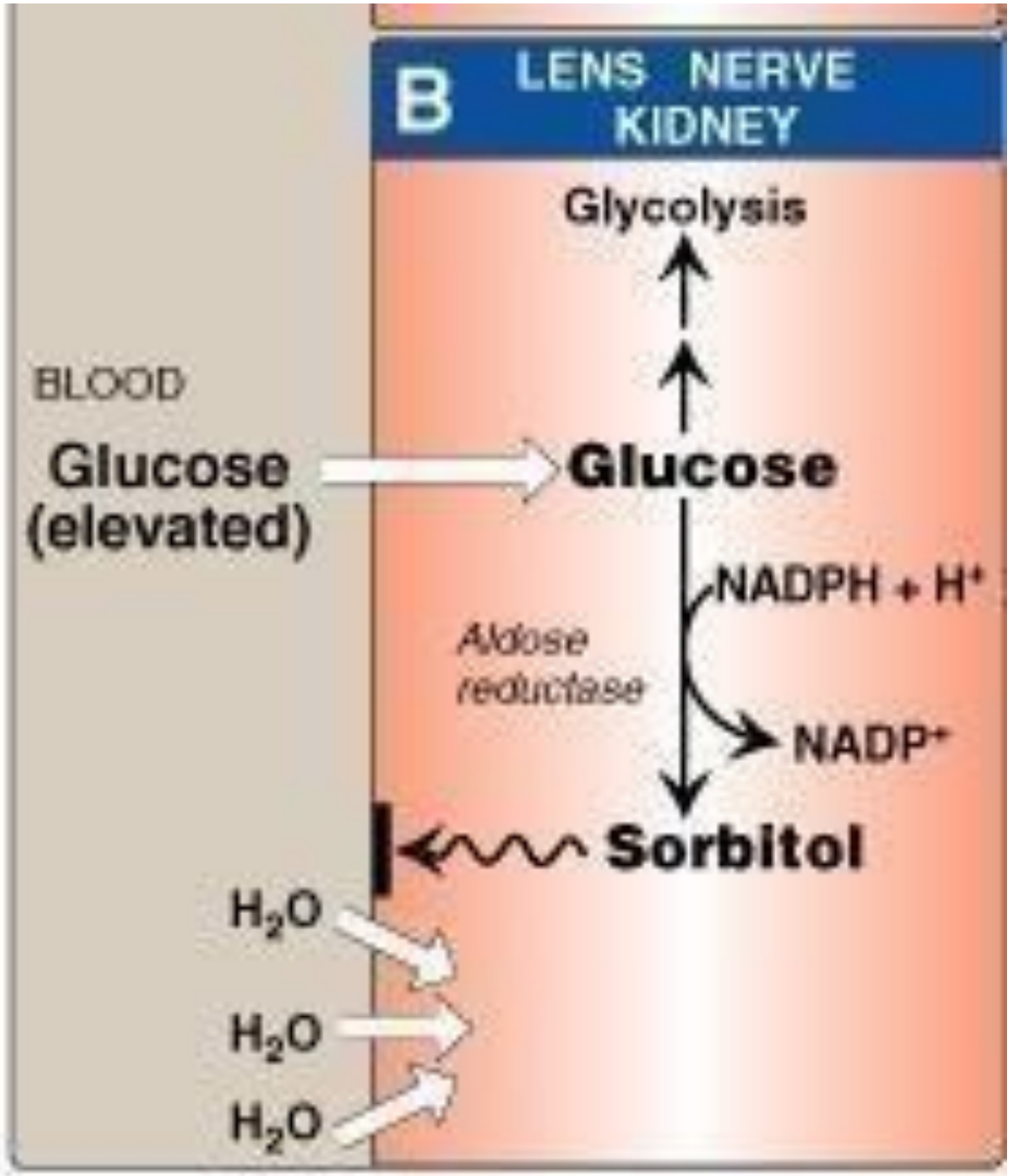
❑ Conversion of glucose to sorbitol & Diabetic Complications

- In uncontrolled diabetic patients (Whether the patient isn't adhering to their treatment, diet isn't working, or even undiagnosed), glucose levels are always high (chronic hyperglycemia). These high levels stimulate the entry of glucose via an insulin independent manner.

Glucose entry is insulin independent in these tissues

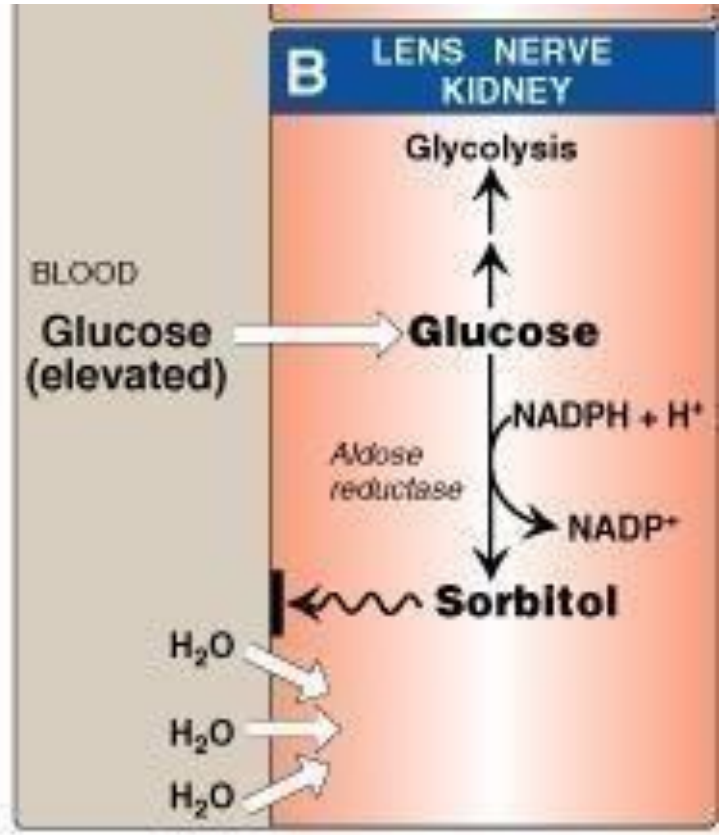
Water retention and cell swelling leading to diabetic complications

Explained in the next slide... :)



Type 1 diabetic patients have an insulin deficiency, and type 2 diabetic patients are resistant to insulin. So, it doesn't make sense that insulin production is increased, nor does the increase of GLUTs, making insulin-independent transport the ideal solution. Once glucose enters the cell, it proceeds to glycolysis and other pathways. However, because it's present in high concentrations, it will activate its reduction to sorbitol. Therefore, sorbitol's concentration will increase, and since polyalcohols don't have transporters, it will accumulate in the cell, increasing the osmotic pressure and causing retention of water and an increase in the cell's size, accordingly.

This is why diabetic patients (especially uncontrolled diabetic patients) go through complications, such as neuropathy, retinopathy, nephropathy, diabetic foot. Because of diabetic foot, injuries in diabetic's feet go unnoticed (due to nerve damage/neuropathy). This ignorance could progress to an infection, and in some cases, gangrene, leaving the patient with no choice but to amputate their limbs (toe, foot, lower leg, etc.)



Sorbitol is produced in non-diabetics; however, this pathway is stimulated by very high concentrations of glucose. Remember that glucose is used in many pathway in the cell: glycolysis, pentose phosphate pathway, glucuronate acid synthesis, glycogen synthesis, etc. The production sorbitol is stimulated only when there is a big amount of sugar in the blood (the kind present only in diabetic patients).

- P.S. : Sorbitol is used in sugar-free products because it's absorbed slowly (as we said there are no specific transporter). This limited absorption is why it doesn't cause sharp blood-glucose spikes in diabetic patients.

❑ Galactose Metabolism

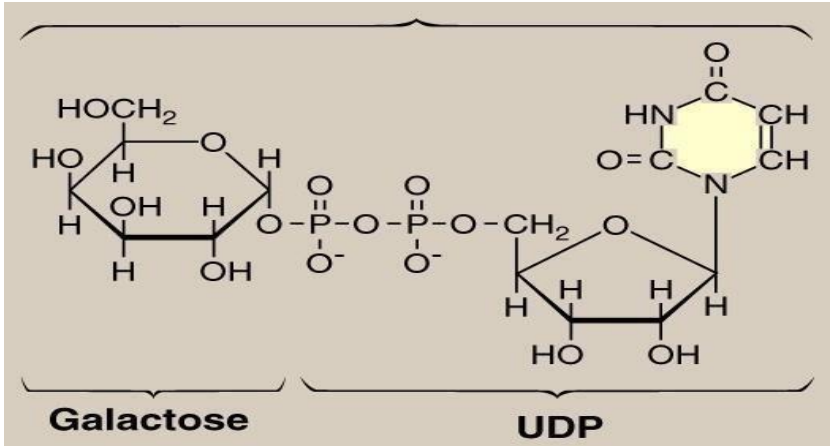
- Galactose is an Epimer and diastereomer of glucose (on Carbon #4)
- Sources: component of lactose, lysosomal degradation glycolipids and glycoproteins
- Entry to cells is insulin independent
- UDP Galactose; an intermediate in galactose metabolism (Just like glucose-UDP in glycogen synthesis): It's a very important intermediate that is used in synthetic pathways, such as glycoproteins, glycolipids, GAGs, etc.

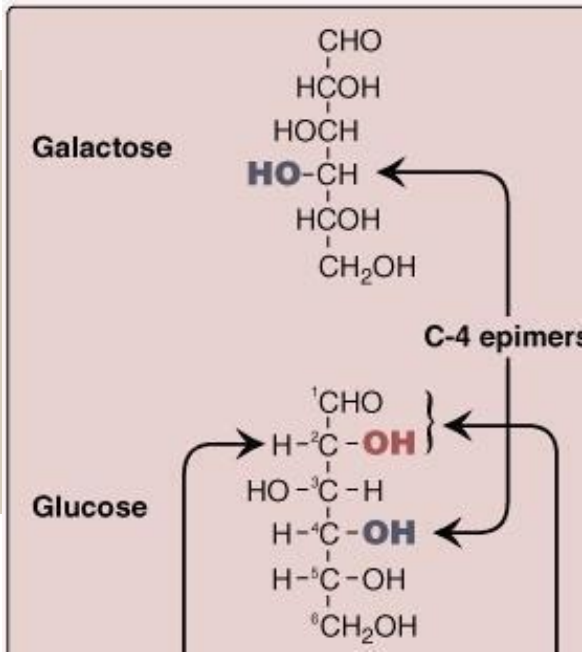
❖ Galactose is rarely found as a galactose monomer. Normally, you'll find it a part of lactose, or another disaccharide. The purpose of galactose metabolism is not energy production (unlike fructose), but rather a precursor for the synthesis of other molecules, such as GAGs. Sugar components of glycoproteins, glycolipids, GAGs are mainly glucose and galactose, as well as modified forms of these sugars. Fructose and galactose don't induce insulin secretions; therefore, they enter the cells in an insulin- independent manner.

Recall: Hexokinase can phosphorylate galactose to produce galactose 6-phosphate. However, galactose 1-phosphate is needed -> galactose must depend on its own specific pathway.

❑ Galactose Metabolism

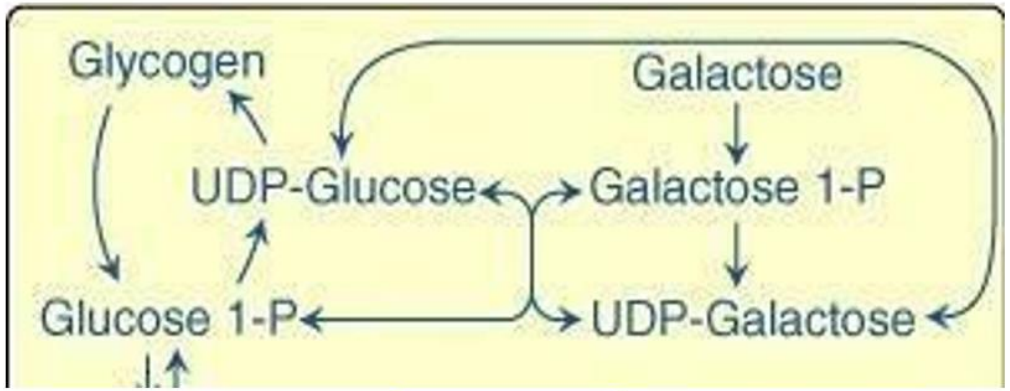
- C4 OH group is on the left in galactose, faces upward in the ring structure
- C4 OH group is on the right in glucose, faces downward in the ring structure





❑ Galactose Metabolism

1. Galactose first gets phosphorylated by galactokinase which produces galactose-1-Phosphate (ATP used) (Irreversible)
2. Then UDP-Glucose (like the one in glycogen synthesis) is used by transferase (GALT), which takes the UDP from glucose & adds it to galactose as well as take the phosphate from galactose & adds it to glucose producing both UDP- galactose and Glucose 1-P (Reversible)
3. UDP-galactose is then used in various ways & also could be epimerized (by epimerase) back to UDP-glucose to be used in glycogen synthesis if there was excess available. (Reversible)



Galactokinase

Galactose + ATP ⟶ Gal. 1-Phosph + ADP

transferase

Gal 1-Phosph + UDP Glc. ⇌ UDP- Galactose + Glc1-Phosph

epimerase

UDP Galactose ⇌ UDP Glucose

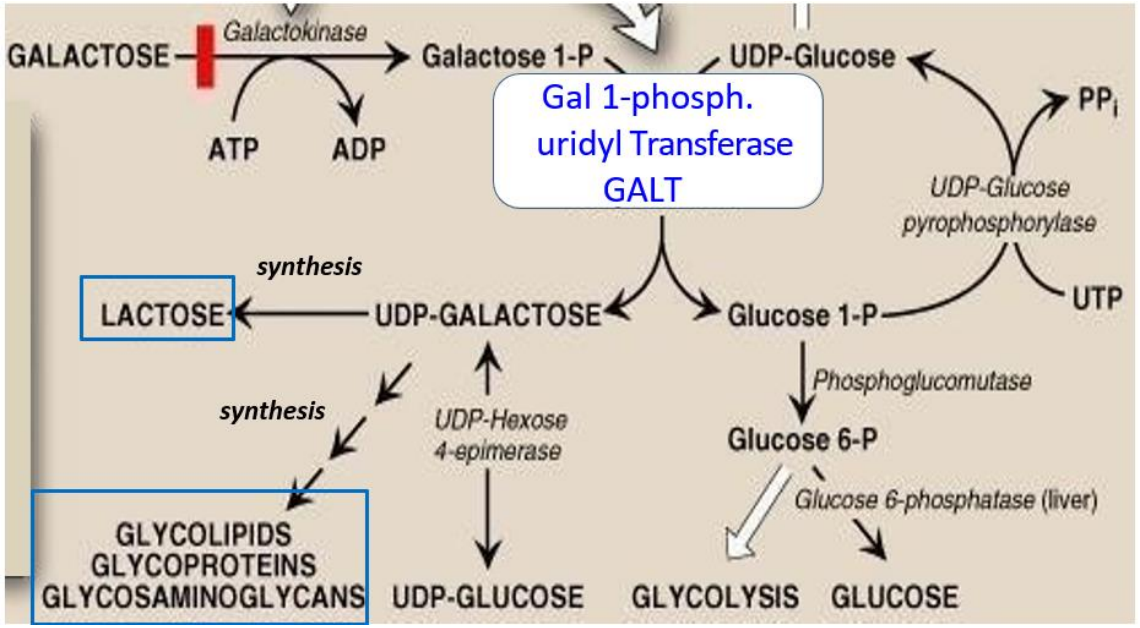
❑ Galactose metabolism and fates

- UDP-galactose can be used in (main uses):

I. lactose synthesis in nursing women (non-nursing women and even men also produce modified lactose with glucose & galactose).
II. Synthesize sugar components (glycolipids, glycoproteins & glycosaminoglycans).
III. If there is excess, it will be epimerized to UDP-glucose used in glycogen synthesis.

- Glucose 1-p (by phosphoglucomutase) becomes glucose 6-p & used in:

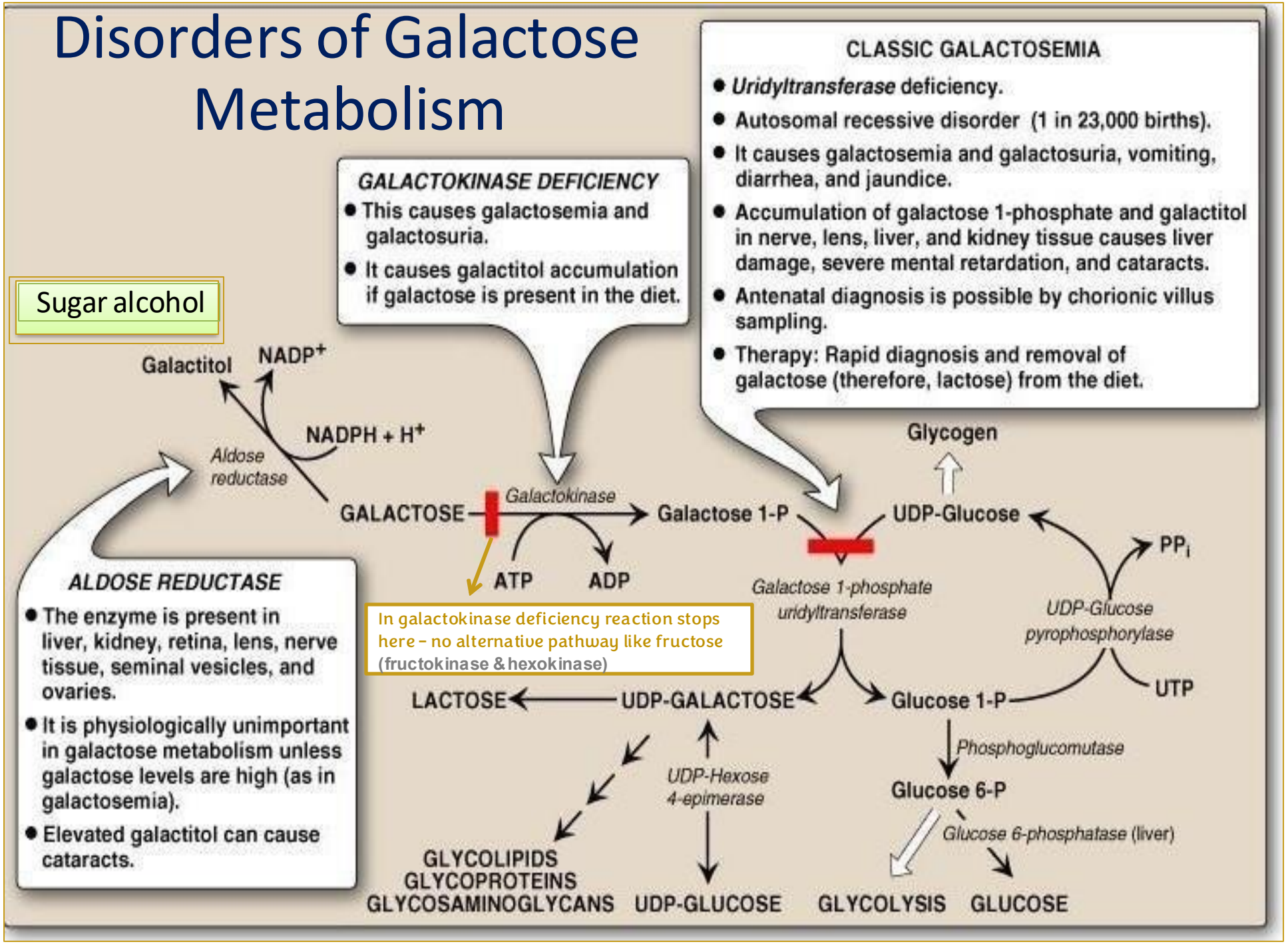
I. In cells like muscle, process stops here and glucose 6-p is used in glycolysis.
II. Other cells like liver, phosphate is removed to produce glucose by phosphatase (so basically, we produced glucose from galactose in the end).
III. Or glucose 1-p can react with UTP to produce UDP-glucose directed to glycogen synthesis or reacts again with a new galactose.



❑ Disorders of Galactose Metabolism

- Deficiency of GALT (Hereditary disease) ➔ Classic Galactosemia
- Accumulation of Galactose 1-Phosphate and galactose
  (Completes first step & gets halted at second step)
- Similar consequences to those in fructose intolerance
- Galactose ➔ Galactitol production
  Uses ATP for nothing → drop in ATP → increase in AMP → Uric acid or hyperuricemia, accumulation of both galactose 1-p and galactose, conduction of galactitol & burst of the cell → causes cataracts, mental retardation due to neurons getting damaged & dying
- Deficiency of Galactokinase
  → Activates aldose reductase to produce galactitol (a polyalcohol)
  → Worse than fructokinase deficiency (has no solution)
- Accumulation of Galactose ➔ Galactitol
  Galactitol (like sorbitol) gets trapped in the cell which drags H2O molecules, causes water retention & swelling & burst.

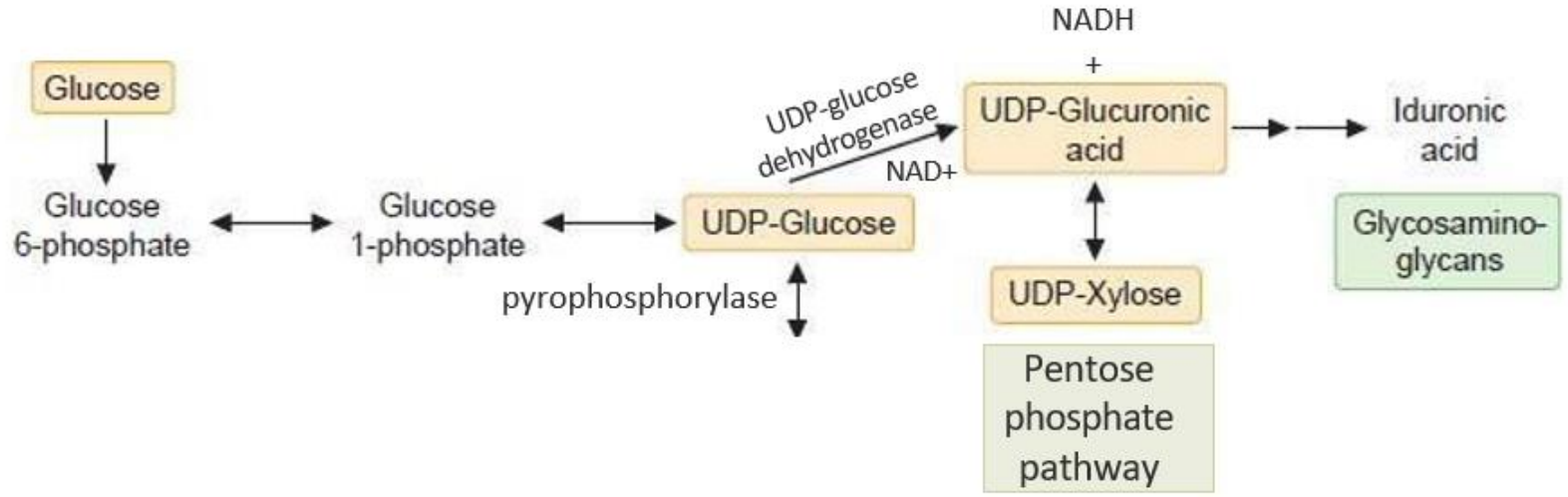
Disorders of Galactose Metabolism



❑ Metabolism of Glucuronic acid Oxidized Glucose

- Is a quantitatively minor route of glucose metabolism (uses Glucose to produce modified sugars)
- It provides biosynthetic precursors and interconverts some less common sugars to ones that can be metabolized.

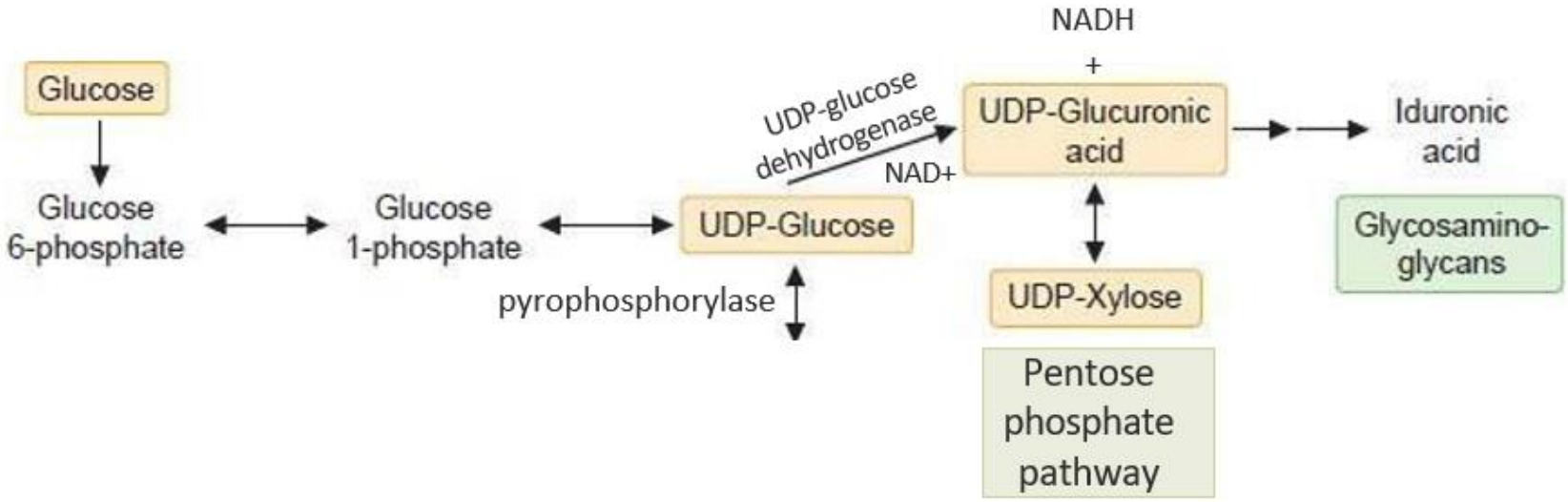
- Used mainly in glycosaminoglycans synthesis
- Sugars in glycosaminoglycans are either: Oxidized / Sulfated / Aminated
- GAGs synthesis is an anabolic pathway that needs a huge amount of energy to produce such polymer, needs well- fed state (exception to this is gluconeogenesis).



Metabolism of Glucuronic acid (Diagram Explanation)

1. High glucose levels stimulate phosphorylation of glucose to glucose-6-phosphate (same first step as glycolysis).
2. Glucose-6-phosphate is isomerized to glucose-1-phosphate.
3. Glucose-1-phosphate reacts with UTP to form UDP-glucose.
4. UDP-glucose dehydrogenase oxidizes UDP-glucose to UDP-glucuronic acid, reducing NAD^+ to NADH.
5. UDP-glucuronic acid is then used for:

- Synthesis of glycosaminoglycans
- Formation of other derivatives, including supplying intermediates connected to the pentose phosphate pathway (PPP)

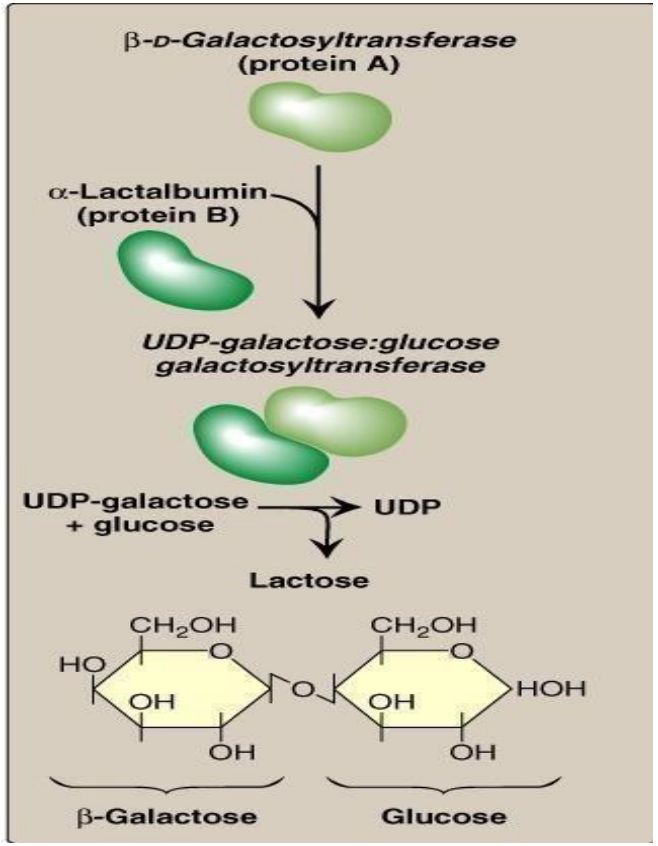


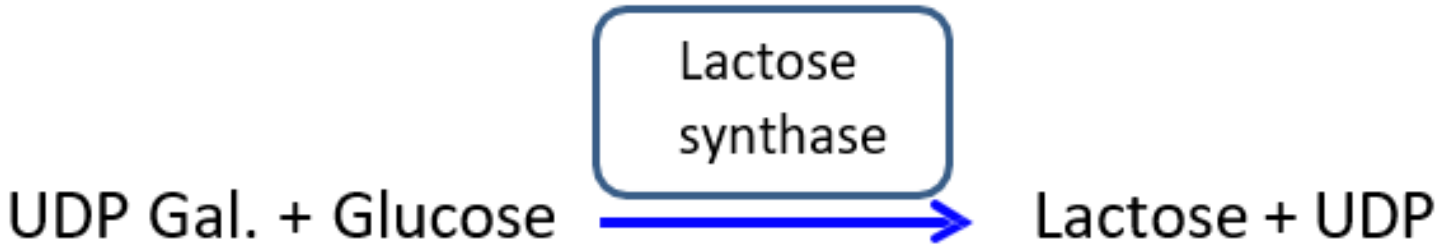
❑ Lactose Synthesis

(Galactosyl β (1→4) glucose)

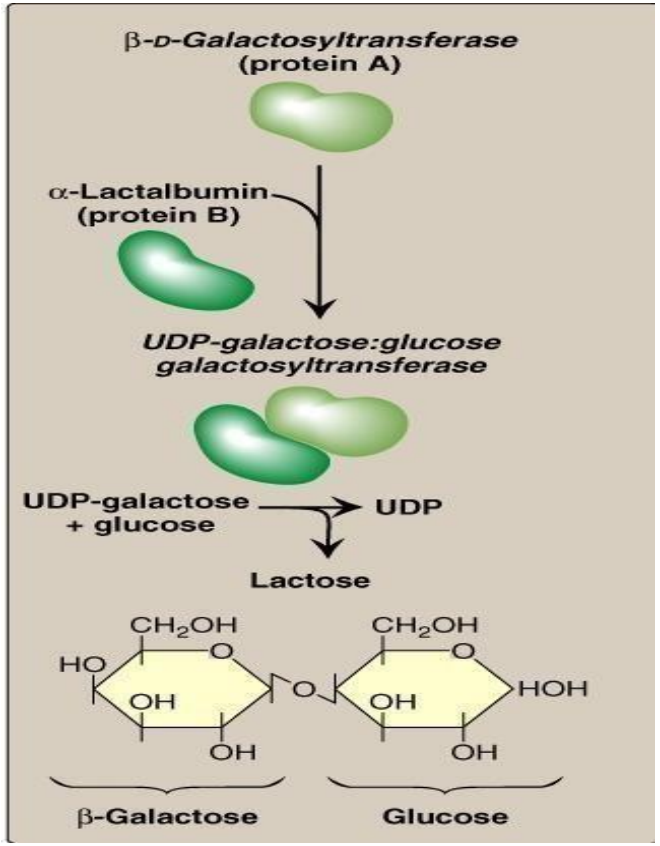
- ➢ Produced by mammary glands (for milk)
- ➢ Galactosyl β (1→4) glucose is found in glycolipids and glycoproteins

- ✓ Lactase synthase forms a reaction between UDP-galactose (has to be this form) & a glucose residue and connects them via beta(1-4) linkage and releases UDP, producing lactose
- ✓ The process happens in mammary glands (with A & B protein complex)





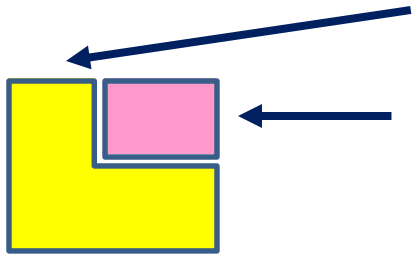
❑ Lactose Synthesis (Lactose Synthase structure)



- Lactose Synthase: complex of 2 proteins:

I. Galactosyl transferase (Protein A)
II. α-lactalbumin (Protein B)

- Only in mammary glands, its synthesis is stimulated by prolactin



- Galactosyl transferase (yellow part): An enzyme (Protein A) that connects both UDP-galactose & glucose
- α-lactalbumin (pink part): It is a protein, not enzyme (protein B), Its role is ensuring enzyme part of lactose synthase (part A) takes only glucose in mammary glands to guarantee formation of lactose (selective)

- In glycolipids and N-linked glycoprotein synthesis + Can be used for GAGs too, etc.

- Other cells have no protein B, only protein A (like in men or non pregnant women). So modified glucose like N-acetyl glucosamine can enter, this allows modified lactose to form.

$$\text{UDP-Gal} + \text{N-acetyl glucosamine} \xrightarrow{\textbf{Protein A}} \text{N-acetyllactosamine}$$

Pentose Phosphate Pathway (PPP) or Hexose Monophosphate Shunt

Dr. Diala Abu-Hassan

❑ Pentose Phosphate Pathway (PPP) or Hexose Monophosphate Shunt

- The **pentose phosphate pathway** is named as such because it produces pentose (five-carbon) sugars and NADPH molecules (which include phosphate).
- This pathway is also referred to as the "hexose monophosphate shunt" because it begins with a six-carbon sugar (mainly glucose) that has a single phosphate group (monophosphate). It acts as an alternative route/detour (shunt) , where glucose-6-phosphate does not immediately enter glycolysis but instead goes through a different pathway before eventually rejoining glycolysis at a later stage.
- This pathway is active in the well-fed state

❑ Phases of the PPP

➢ The pentose phosphate pathway (PPP) is composed of two phases :
✓ Oxidative Phase : This phase is **irreversible** and involves oxidation-reduction reactions.
✓ Non-Oxidative Phase : This phase is **reversible** and does not involve oxidation. No loss of carbons.

❖ Phase One : Oxidative Phase

Begins with glucose-6-phosphate, produced when glucose is phosphorylated by either glucokinase or hexokinase. This traps glucose in the cell , diverting it into the PPP.

I. Step 1 :

- Oxidation Reaction: Glucose-6-phosphate is oxidized at C1 by glucose-6-phosphate dehydrogenase, forming 6-phosphogluconate and NADPH.
- The enzyme glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes this step , reducing NADP+ to NADPH the first goal pf this pathway. NADPH is a co-enzyme involved in oxidation reduction reactions differ from the NADH it is mainly needed as NAD+ get reduced during reactions, this means that the main substrate get oxidized, NAD+ is mainly active in the catabolic reactions. NADPH is mainly present in its reduced form so its gets oxidized during reactions while the main substrate get reduced its important in anabolic reactions.
- G-6-PDH is essential in the PPP and is associated with several common diseases(deficiency).
- Target gene of insulin.

II. Step 2 :

- Oxidative Decarboxylation : The removable of the carboxyl group from 6-phosphogluconate as CO_2 , and oxidation of the hydroxyl group on carbon 3 (of 6-phosphogluconate) to form a ketose (carbonyl on C2)
- This reaction produces ribulose-5-phosphate (a pentose)(ketose), catalyzed by 6-phosphogluconate dehydrogenase (6-PGDH).
- Another NADP+ is reduced producing NADPH molecule.

❖ Phase Two : Non-Oxidative Phase

III. Step 3 :

- Ribulose-5-phosphate is converted to ribose-5-phosphate through an isomerization reaction (ketose to aldose), catalyzed by an isomerase enzyme in a reversible reaction.
- Ribose-5-phosphate can exit the pathway for nucleotide synthesis , especially during the S phase of cell division when energy and nucleotide demand is high (during well-fed state) - or may continue the pathway-
- However , to continue the pathway , a new molecule of glucose-6-phosphate enters Phase One , producing more ribulose-5-phosphate.
- Any loss in carbons happens in phase one not phase two

IV. Step 4 :

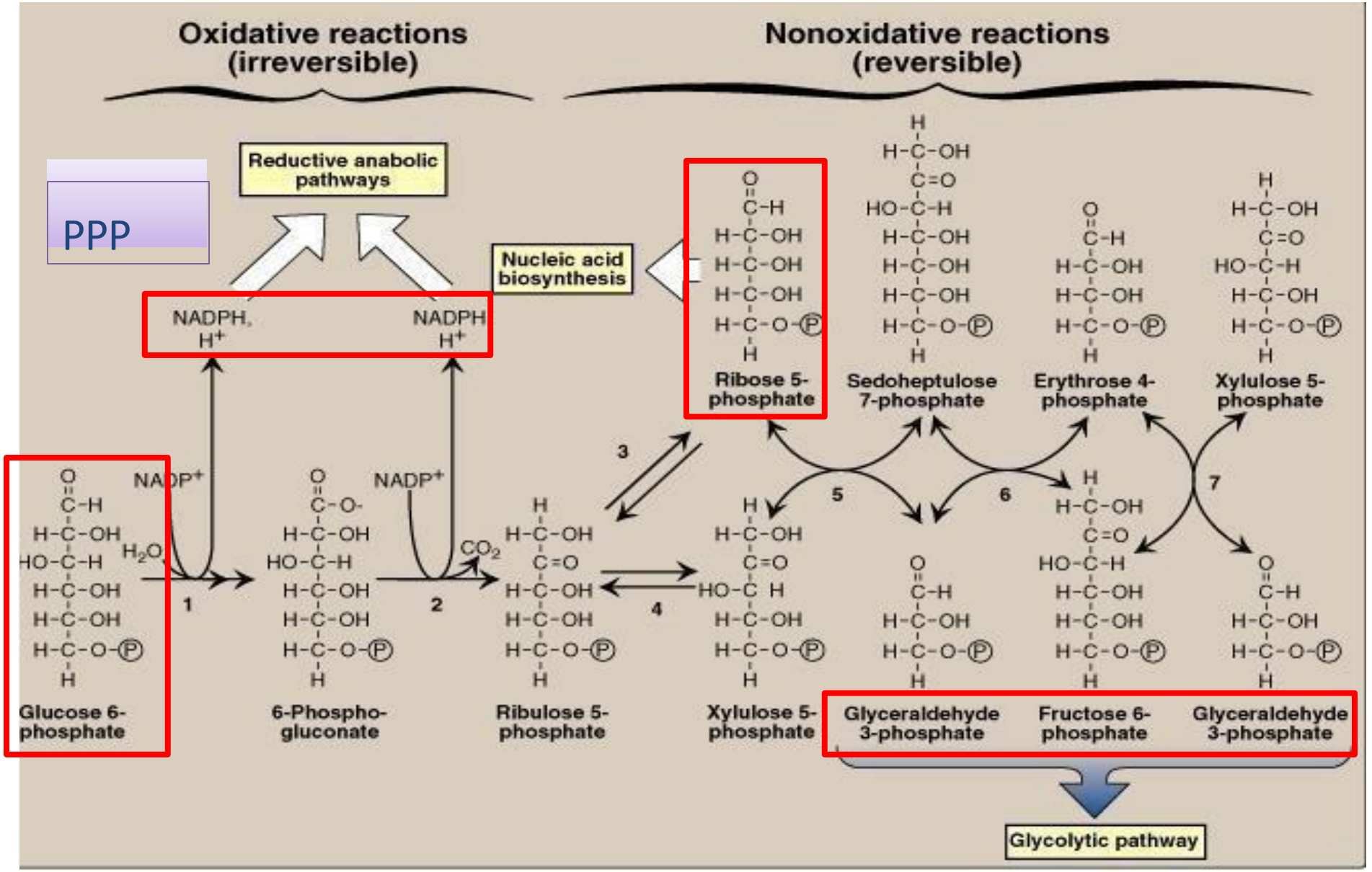
- Ribulose-5-phosphate is converted into xylulose-5-phosphate (a ketose) by an epimerization reaction catalyzed by epimerase. This reaction is separate from the isomerization to ribose-5-phosphate (Step 3)
- At this stage, we have two 5-carbon molecules : ribose-5-phosphate and xylulose-5- phosphate
- Xylulose-5-phosphate transfers two carbons to ribose-5-phosphate by transketolase , forming sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate.
- Sedoheptulose-7-phosphate then transfers three carbons to glyceraldehyde-3-phosphate by transaldolase, producing fructose-6-phosphate and erythrose-4-phosphate.
- This process is repeated with a new glucose-6-phosphate entering Phase One, converting to ribulose-5-phosphate and then to xylulose-5-phosphate , which transfers two carbons to erythrose-4-phosphate by transketolase
- This results in another fructose-6-phosphate molecule and glyceraldehyde-3-phosphate from xylulose(intermediate of glycolysis).
- Transketolase was used twice and transaldolase was used once.

Summary of the non-oxidative reactions

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

- Rearrangment of sugars
- 3 pentose phosph.. $\rightleftharpoons$ { 2 hexose phosph + 1 triose phosph. }

PPP



Note: the phosphate group is always on the last carbon of the molecule

❑ Functions of the PPP

1. **Production of NADPH** (Most Important)
   - ✓ 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

2. **Metabolism of five-carbon sugars (Pentoses)**
   - ✓ Ribose 5-phosphate (nucleotide biosynthesis)
   - ✓ Metabolism of pentoses

SEE NEXT SLIDES FOR FURTHER EXPLANATION

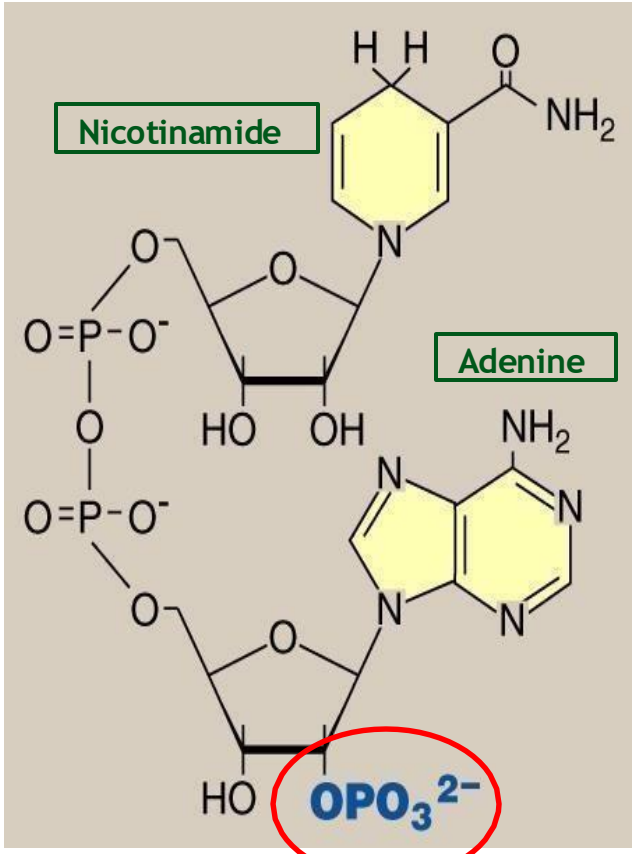
Functions of the PPP /1

1 Production of NADPH

- In general , degradative (catabolic) pathways involve the oxidation of the main substrate and the reduction of a coenzyme. In contrast , synthetic (anabolic) pathways involve the reduction of the main substrate and oxidation of a coenzyme. Oxidation of NADPH to NADP+
- This setup ensures that the cell is equipped (هلا ناحبس)with two pools of coenzymes : one predominantly in an oxidized form and the other in a reduced form. This arrangement allows the cell to carry out the needed reactions , whether it requires oxidizing or reducing the main substrate.
- For instance , in synthetic pathways such as fatty acid synthesis, steroid hormone synthesis (e.g. sex hormones) , aldosterone or cortisol , etc , the main substrate is reduced, and the coenzyme , primarily NADPH, is oxidized.
- Additionally , NADPH plays a key role in regenerating glutathione , a tripeptide that acts as an antioxidant. This regeneration is essential to maintain adequate levels of glutathione(recycle), enabling it to counteract reactive oxygen species (ROS) and protect cells from oxidative damage.

Structure of NADPH

- NADPH stands for nicotinamide adenine dinucleotide phosphate
- As shown , the connection between the two sugar molecules in NADPH is through phosphate groups
- The additional phosphate -marked in red- (which differentiates NADPH from NADH) is attached to the sugar bound to adenine.
- This phosphate group does not affect the site of oxidation-reduction reactions (it occurs at the nicotinamide) , as these occur at the same location in both NADH and NADPH. However, enzymes are able to distinguish between NADH and NADPH , allowing each to play distinct roles in cellular processes.



OH in NADH

Functions of the PPP /2

2- Metabolism of five-carbon sugars (Pentoses)

- The second , less critical function of the pentose phosphate pathway is the production of pentose sugars , which are essential for synthesizing various nucleotides.

- This pathway is active when glucose levels are high (bcz it uses glucose-6-phosphate) , such as in a well-fed state. Additionally , other related anabolic pathways are generally active during this well-fed state (NADPH level is also high) .

End products of the PPP

- **End of Phase One** : produces two NADPH molecules per glucose-6-phosphate molecule.

- **End of Phase Two** : produces two fructose-6-phosphate molecules and glyceraldehyde-3-phosphate , both are intermediates of glycolysis.

- Key Outcome of the Pentose Phosphate Pathway :

This pathway , an alternative to glycolysis , generates NADPH along with glycolytic intermediates.

- **NADPH** is critical for antioxidant function , specifically in regenerating glutathione to protect cells from reactive oxygen species (ROS)

Phase	# of Step	Reaction Type	Enzyme	Reactants (Or intermediates)	Products	Coenzyme
Oxidative phase	Step 1	Oxidation - Reduction	Glucose-6-phosphate dehydrogenase	Glucose-6-phosphate	6-phosphogluconate	NADP+ → NADPH
	Step 2	Oxidative decarboxylation	6-phosphogluconate dehydrogenase	6-phosphogluconate	Ribulose-5-phosphate	NADP+ → NADPH
Non-oxidative phase	Step 3	Isomerization	Isomerase	Ribulose-5-phosphate	Ribose-5-phosphate (may exit the pathway or may continue)	
	Step 4 (Bypass step 3 for second molecule)	Epimerization	Epimerase	Ribulose-5-phosphate	Xylulose-5-phosphate	
		2-carbon transfer	Transketolase	Xylulose-5-phosphate + Ribose-5-phosphate	Sedoheptulose-7-phosphate + Glyceraldehyde-3-phosphate	
		3-carbon transfer	Transaldolase	Sedoheptulose-7-phosphate + Glyceraldehyde-3-phosphate	Fructose-6-phosphate + Erythrose-4-phosphate	
		(Cycle repeated with new molecule) 2-carbon transfer	Transketolase	Xylulose-5-phosphate + Erythrose-4-phosphate	Fructose-6-phosphate + Glyceraldehyde-3-phosphate	

Before starting (;

In the first 10 slides, We will swiftly and thoroughly go through what have been said in the previous lecture or modified that was just before the first exam.
just to refresh your brains, since We have been through a lot in the MID exams!!.
You can skip them safely if you have studied them before.(;

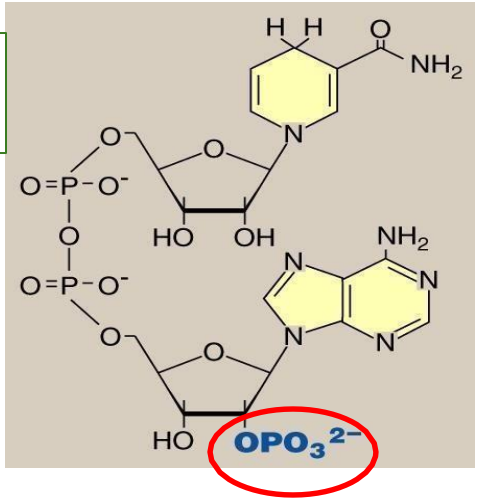
Functions of the PPP

1. Production of NADPH **"the more important."**
   - 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
2. Metabolism of five-carbon sugars (Pentoses) **"the less important."**
   - Ribose 5-phosphate (nucleotide biosynthesis)
   - Metabolism of pentoses

Sex hormones and adrenocortical hormones; for instance: aldosterone and cortisone ….etc.

Generally speaking:

- **Degradative pathway: Oxidation of the main substrate and reduction of the coenzyme**
- **Synthetic pathway: reduction of the main substrate and oxidation of the coenzyme**



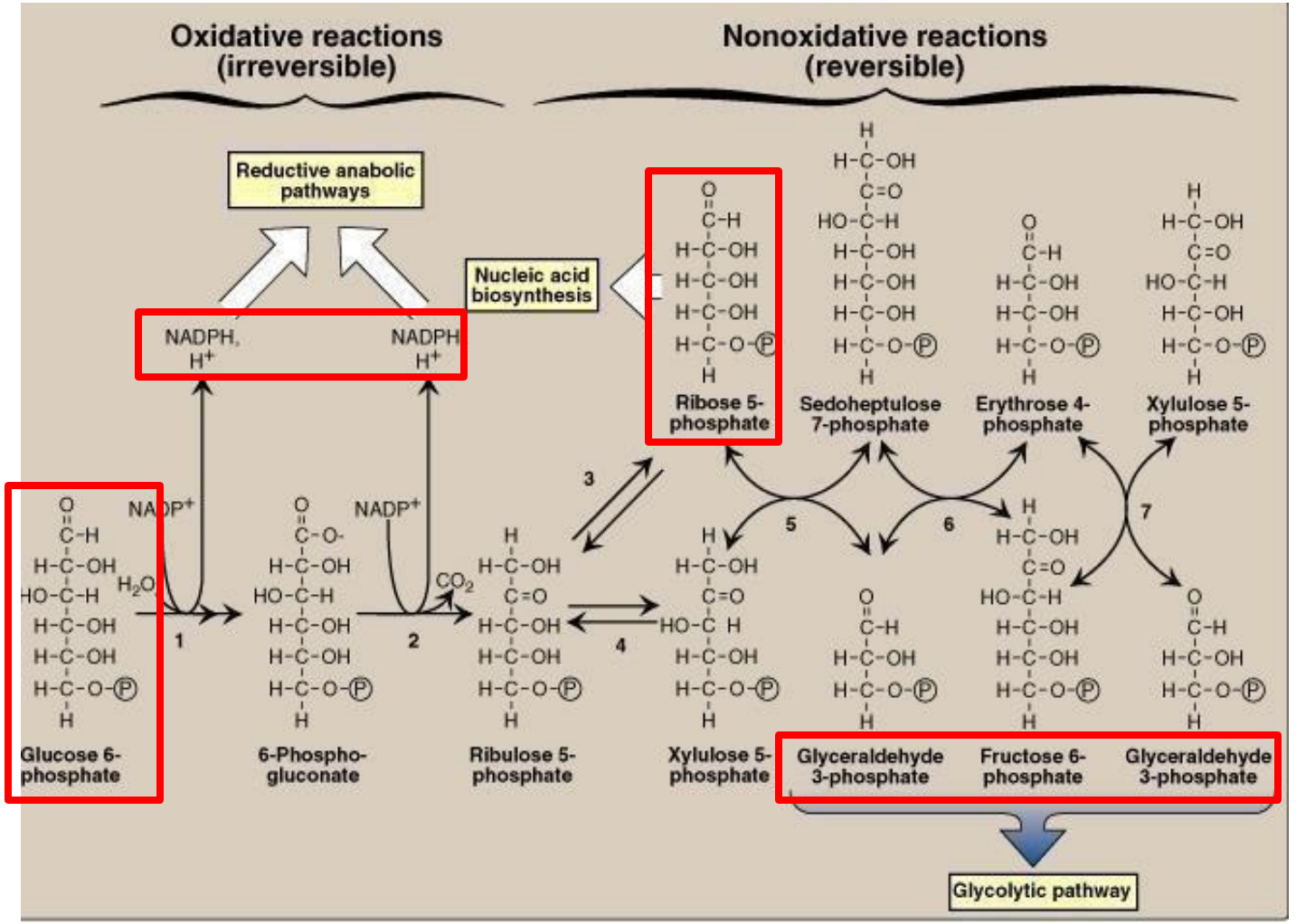
OH in NADH

A simple review of the PPP 0r Hexose Monophosphate Shunt.

- Glucose 6-phosphate, instead of entering glycolysis, can enter this alternative pathway, eventually producing glycolytic intermediate, Hence the name, ‘shunt’, just like traffic shunt!!.
- The PPP (Pentose Phosphate Pathway) is a pathway that occurs in the well-fed state, and it consists of two phases:
- 1: The Oxidative irreversible phase: 2NADPH are produced per Glucose 6-phosphate.
- 2: Non-Oxidative phase: two fructose 6-phosphate and glyceraldeyhde-3-phosphate.

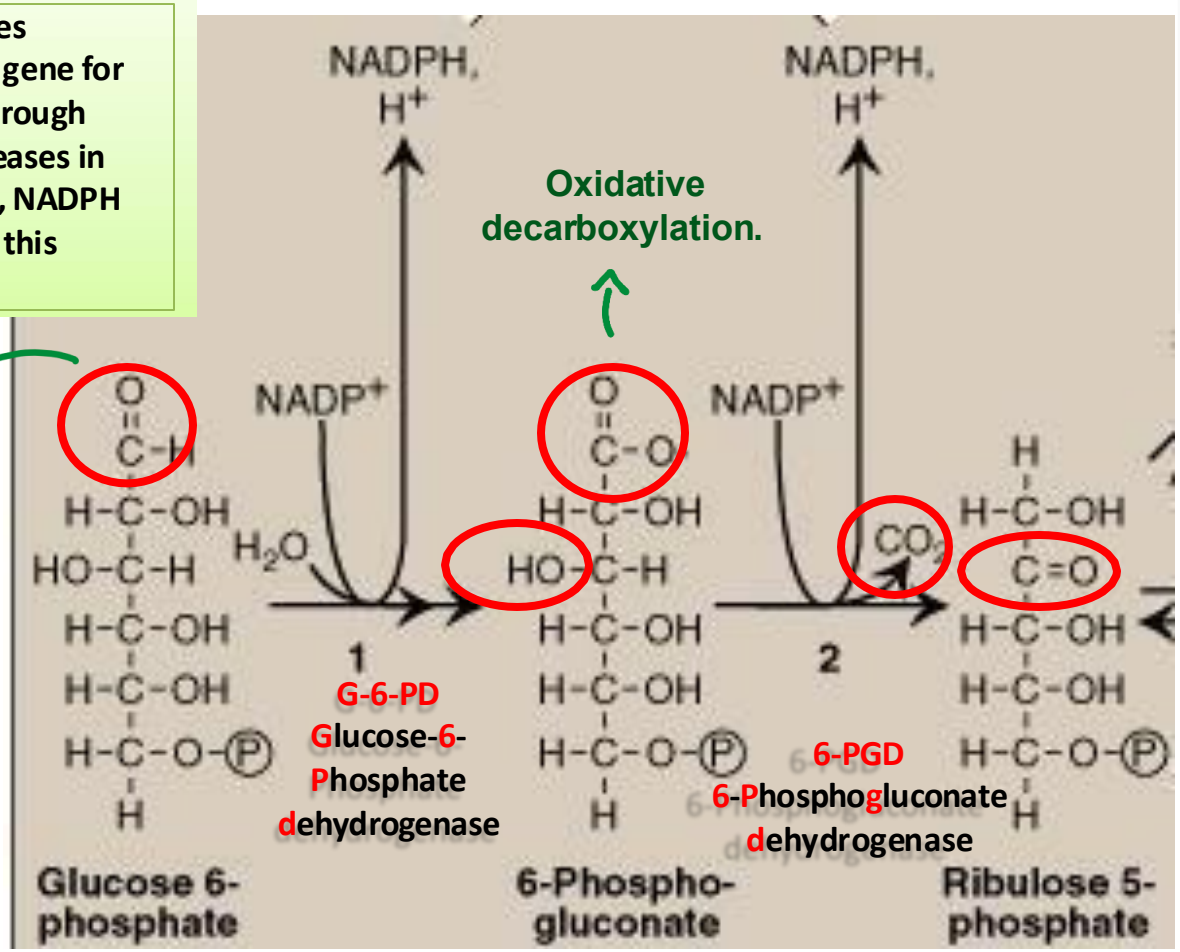
Please See the next few slides for more clarification.

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

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

PPP
The oxidative irreversible phase



-Oxidation of the first carbon "the aldehyde carbon" yielding Gluconate.

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note that glucuronate results from oxidation of the last carbon, just for you to know.

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

The oxidative irreversible phase.

Reaction 1:

G-6-PD
Glucose-6- Phosphate dehydrogenase

Glucose 6-Phosphate + $NADP^+$ ⟶ 6-phospho-gluconate + NADPH

Reaction 2:

6-PGD
6-Phosphogluconate dehydrogenase

6-phospho-gluconate + $NADP^+$ ⟶ Ribulose 5-Phosphate + NADPH + CO_2

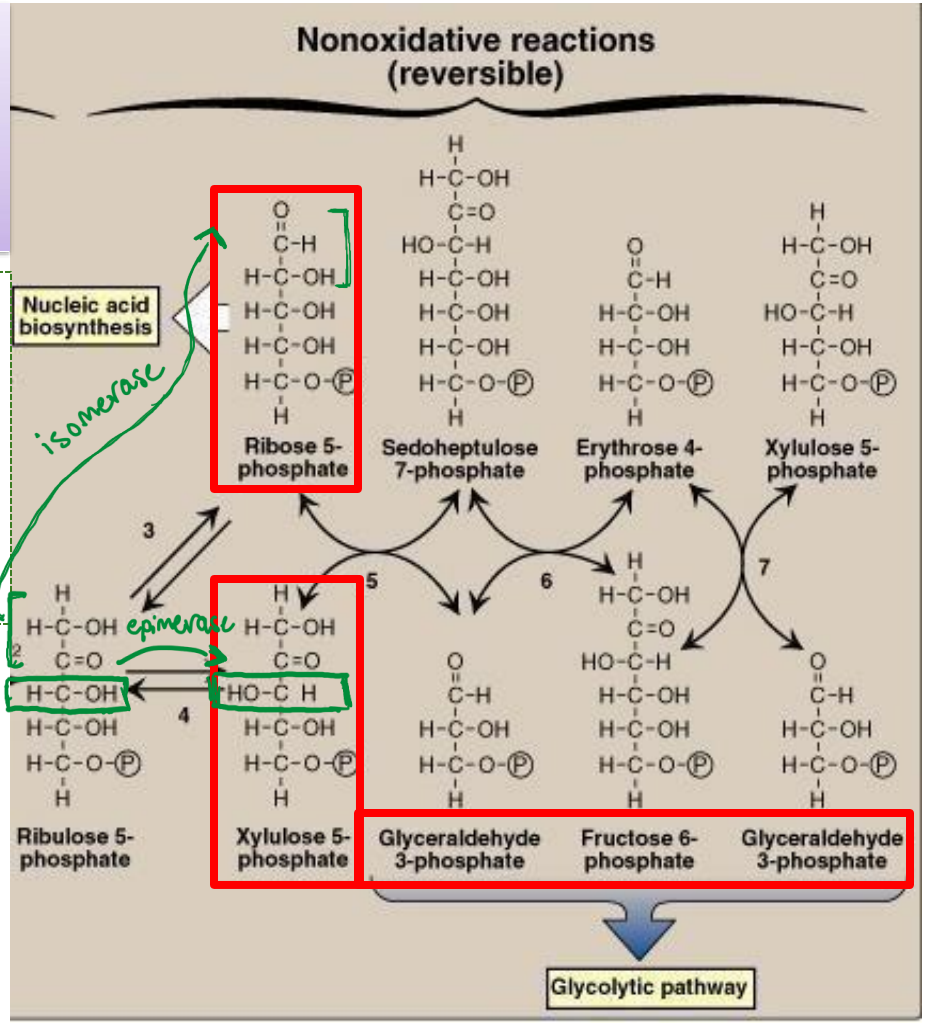
Total:

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

PPP
The non-oxidative reversible phase

Once Ribulose 5-Phosphate (ketose) is produced, it can be ***isomerized*** into Ribose 5-phosphate (aldose) by ***isomerase*** enzyme, which continues into Nucleic acid biosynthesis pathways that is mostly activated at the S phase of the cell cycle, where there is high demand of energy. This partially explains why PPP typically occurs in the well-fed state during which anabolic pathways are strongly favored.

Now second molecule of Glucose 6-phosphate must repeat the same steps until Ribulose 5-Phosphate is produced, so that it can be ***epimerized*** into Xylulose 5-phosphate carried out by ***epimerase*** enzyme, only altering the configuration around carbon number 3.



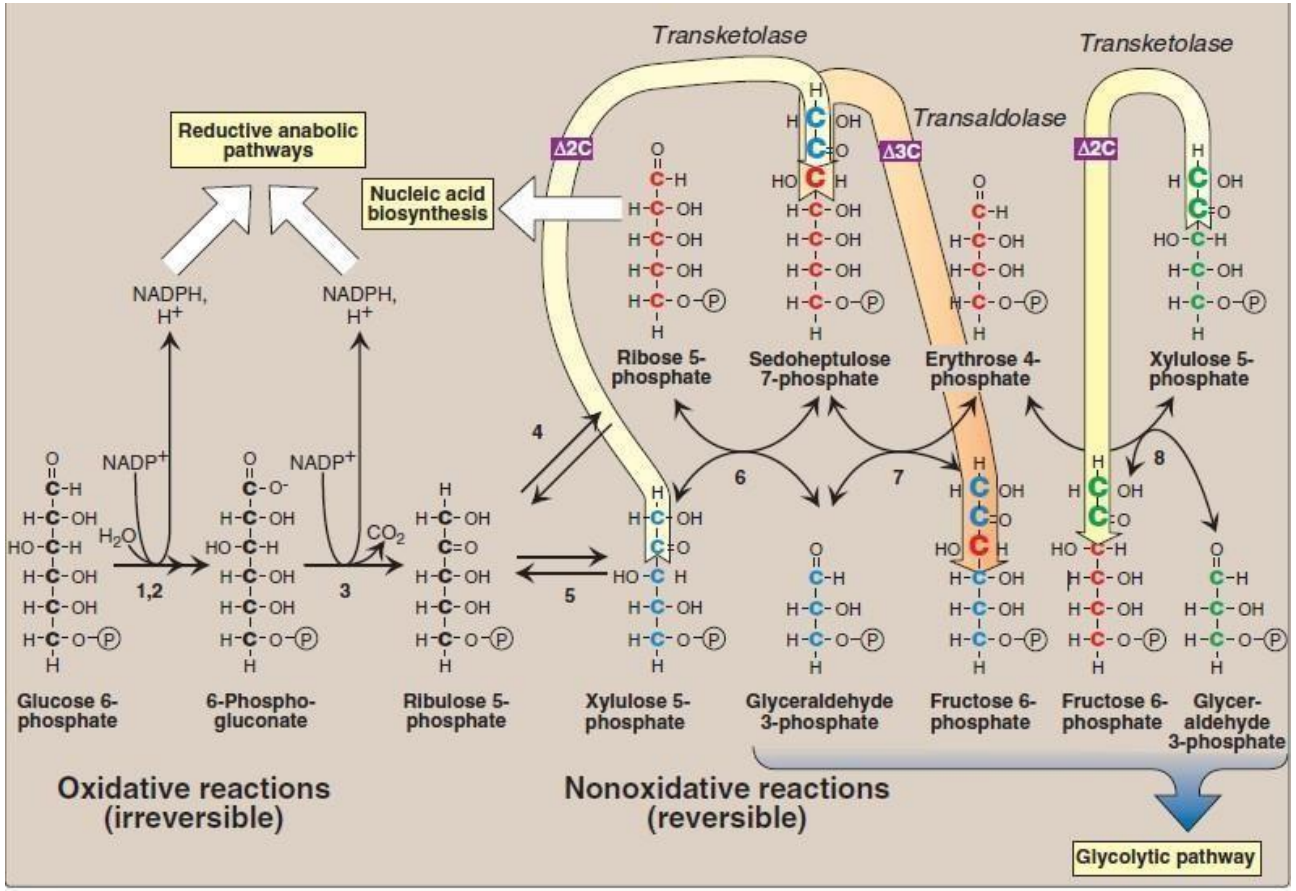


Figure 13.2
Reactions of the hexose monophosphate pathway. Enzymes numbered above are: 1,2) *glucose 6-phosphate dehydrogenase* and *6-phosphogluconolactone hydrolase*, 3) *6-phosphogluconate dehydrogenase*, 4) *ribose 5-phosphate isomerase*, 5) *phosphopentose epimerase*, 6) and 8) *transketolase* (coenzyme: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

In the second phase ketose is converted to aldose and aldose to ketose

The non-oxidative reversible phase.

Do not memorize just understand the concept.

Reaction 3:

Ribulose 5-Phosphate —(isomerase)→ Ribose 5-phosphate

Reaction 4:

Ribulose 5-phosphate —(epimerase)→ Xylulose 5-phosphate

Reaction 5:

Xylulose 5-phosphate + Ribose 5-phosphate —(transketolase)→ Sedoheptulose 7-phosphate + Glyceraldehyde 3-phosphate

Reaction 6:

Sedoheptulose7-phosphate + Glyceraldehyde3-phosphate —(transaldolase)→ Fructose 6-phosphate + Erythrose 4-phosphate

Reaction 7:

A third molecule of G6P must enter to give this Xylulose.

Erythrose 4-phosphate + Xylulose 5-phosphate —(transketolase)→ Fructose6-phosphate +Glyceraldehyde3-phosphate

Carbon movements in non-oxidative reactions

Do not memorize just understand the concept.

Ketose + Aldose ⇌ Aldose + Ketose

The whole concept is that each aldose becomes a ketose, and each ketose becomes an aldose by the transfer of 3C or 2C, catalyzed by transaldolase or transketolase enzymes respectively.

C5 + C5 ⇌ C3 + C7

C7 + C3 ⇌ C4 + C6

C5 + C4 ⇌ C3 + C6

Summary of the non-oxidative reactions

NO NET CARBON LOSS

- 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.. ⇌ **Fructose 6-P** 2 hexose phosph + 1 triose phosph. **Glyceraldeyhde 3-P**

Ribulose 5-P

The net non-oxidative reaction

Do not memorize just understand the concept.

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

↓

↓

↓

5 Fructose. 6-Phosph.

The two G3P turn into one F6P as if they were in the glycolysis.

6 molecules of Ribulose (5 carbons), a total of 30 carbons, The final yield of this pathway is 5 fructose molecules (6 carbons) giving a total of 30 carbons.. NO NET CARBON LOSS

Net Products of the 2 Phases

Do not memorize just understand the concept.

Now indeed here the actual yield is Fructose 6-phoshphate not Glucose 6-phosphate, however we desire to unify the compounds for a purpose.

$3\ Glc.\ 6\text{-}P + 6\ NADP^+ \rightarrow 2\ Glc.\ 6\text{-}P + GA3P + 3\ CO_2 + 6\ NADPH$

MULTIPLY BY 2

$6\ Glc.\ 6\text{-}P + 12\ NADP^+ \rightarrow 4\ Glc.\ 6\text{-}P + 2\ GA3P + 6\ CO_2 + 12\ NADPH$

$2\ GA3P \rightarrow Glc.\ 6\text{-}P$

$6\ Glc.\ 6\text{-}P + 12\ NADP^+ \rightarrow 5\ Glc.\ 6\text{-}P + 6\ CO_2 + 12\ NADPH$

- **The whole concept is that there will be loss of one Glucose 6-phosphate in the form of six CO2 molecules for each six G6P molecules that enter this pathway, instead of all the six Glucose 6-phosphate typically entering the glycolytic pathway.**
- **The importance of this pathway in NADPH production and Nucleic acid biosynthesis comes at the cost of this loss of carbons.**

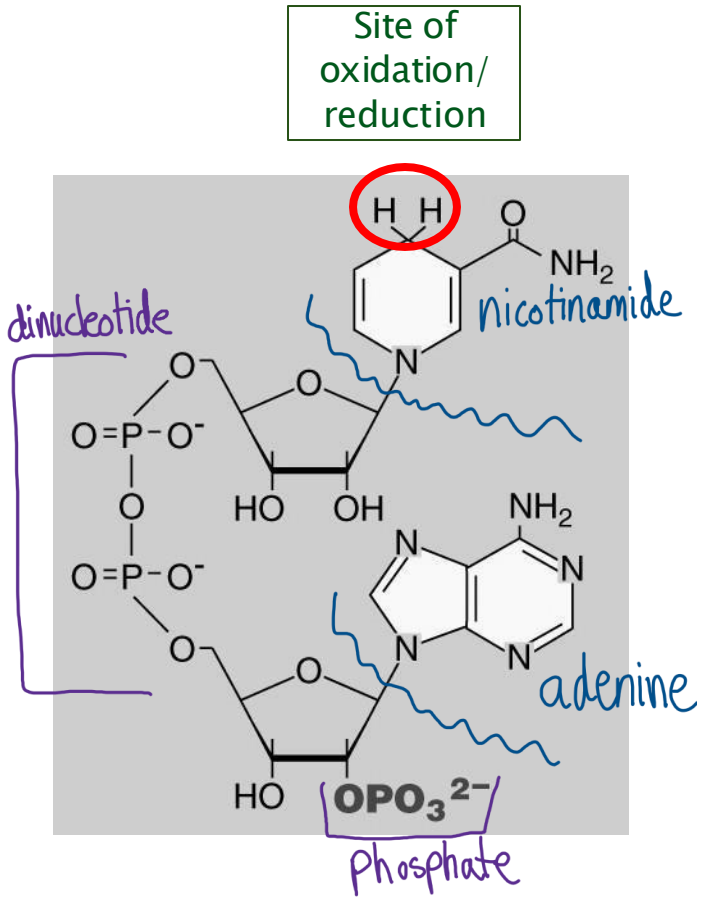
NADPH vs NADH

Since NADPH is primarily oxidized in the biosynthetic pathways reducing the main substrate.

On the other hand, NAD+ is primarily reduced in the degradative pathways oxidizing the main substrate.

Therefore, there are two pools, one is more oxidized while the other is more reduced, this facilitates REDOX reaction inside the cell, whether oxidation or reduction reaction is carried out.

- 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
  - $NADP^+/NADPH \approx 1/10$
  - $NAD^+/NADH \approx 1000/1$



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

+ cholesterol (precursor of steroids).

- ...

Reduction of H2O2 indirectly by reduction of Glutathione directly for the recycling. to be discussed.

2. Reduction of Hydrogen Peroxide

- H_2O_2 one of a family of compounds known as Reactive Oxygen Species (**ROS**)

Highly reactive molecules with excess electrons form covalent bonds when reacting

- Other: Super oxide, hydroxyl radical,
- Formed continuously
  - As by products of aerobic metabolism
  - Interaction with drugs and environmental toxins

ROS are normally produced as byproduct of the metabolism.

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

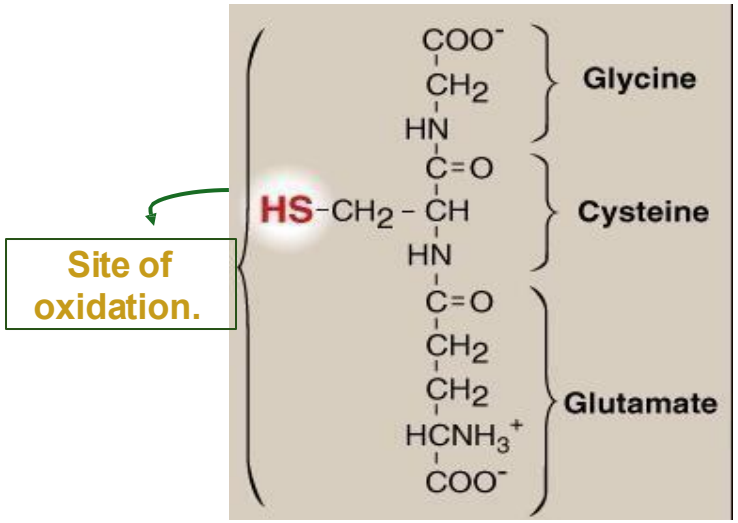
They are hazardous if present in large amounts

Enzymes that catalyze antioxidant reactions

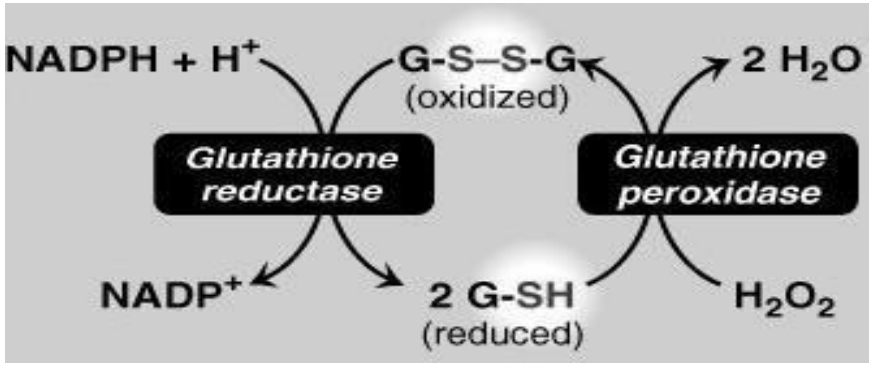
Please See next slide for more clarification.

1. Glutathione peroxidase

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



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



Glutathione peroxidase mechanism.

- H_2O_2 reacts with two reduced Glutathione molecules (free sulfhydryl groups "GSH"), and by the activity of *Glutathione peroxidase*, H_20_2 will be reduced to water alongside the oxidation of two GSH yielding disulfide bridge (GS-SG).

- Now the oxidized Glutathione (GS-SG) must be recycled back to two (GSH) by *glutathione reductase* which oxidizes NADPH to NADP+, breaking up the disulfide bond and ensuring that Glutathione is mostly present in the reduced form so that it can function in the clearance of ROS again.

- This reaction is particularly essential in the erythrocytes for several reasons, their internal environment is highly saturated with O_2, the raw material from which ROS are primarily generated, besides the absence of mitochondria in RBC, which partially aid in the removal of these hazardous products in other cells other than RBC.

$$H_2O_2 + 2GSH \longrightarrow GS\text{-}SG + 2H_2O$$

$$GS\text{-}SG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

Enzymes that catalyze antioxidant reactions

2. Superoxide dismutase (**SOD**)

Can be removed by either Glutathione peroxidase or catalase enzymes.

3. Catalase

Heme protein present in peroxisomes.

Antioxidant chemicals

- Vitamin E, Vitamin C, Carotenoids (source of Vitamin A)

Many of these chemicals are present in the cosmetics for their antioxidant characteristics.

Clinical Hint: G6PD Deficiency التفول

- A 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.

It is X-linked recessive ,so Males are more susceptible than females, as they only need one copy of the abnormal allele.

Mutations are usually point mutations (missense mutation), however frame shift, addition or deletion mutations are not observed.

Precipitating Factors in G6PD Deficiency

- Oxidant drugs

Please See next slide for more clarification.

  - Antibiotics e.g. Sulfomethxazole
  - Antimalaria Primaquine
  - Antipyretics Acetanalid

وفا

- Favism due to vicine and covicine in fava beans in some G6PD deficient patients
- Infection

During infection or inflammation there will be excessive ROS formation. Specially in phagocytes (precipitating factors)

- Neonatal Jaundice

This is actually another clinical manifestation of G6PD Deficiency, in addition to hemolytic anemia, besides being a Precipitating factor. During the degradation of heme groups, typically as a part of the renewal process of erythrocytes, a toxic byproduct, Bilirubin, is normally produced. in the case of G6PD deficiency, there is premature death of RBC, that is, unusual rapid turn over of these cells occurs, producing excessive amounts of Bilirubin that exceed the functional capacity of our body to detoxify and eliminate these highly toxic compounds, giving the clinical outcome of patient yellowing, mostly affecting neonates and damaging their central nervous system.

Precipitating Factors in G6PD Deficiency and how RBC are affected

- A deficiency in this enzyme hinders the PPP, resulting in decrease in the concentration of NADPH reducing the cellular ability to function against ROS, And Eventually accumulation of these harmful compounds and excessive oxidative stress that can not be handled any longer, leading to senescence or premature death of the affected cells.

- Erythrocytes are particularly most affected since they are more vulnerable and sensitive to the oxidative stress, **for several reasons mentioned in slide 18**, in addition, RBC do not have any other pathway to produce NADPH unlike other tissues. Therefore, this results in rapid turn over of RBC and early death before 120 days. they as well have no nucleus nor ribosomes so they can not replenish their supply of this enzyme and, moreover, other tissues have alternative pathways like *$NADP^+$-dependent malate dehydrogenase* to produce NADPH. This additionally accounts for the vulnerability of erythrocytes to this condition.

- Precipitating factors are the factors that aggravate the problem by contributing to the oxidative stress or at least making patients more susceptible to it. avoidance of which, besides antioxidants supplementation are essential for the patient to prevent crisis, due to the acute hemolytic anemia that would otherwise result from the exposure to these factors.

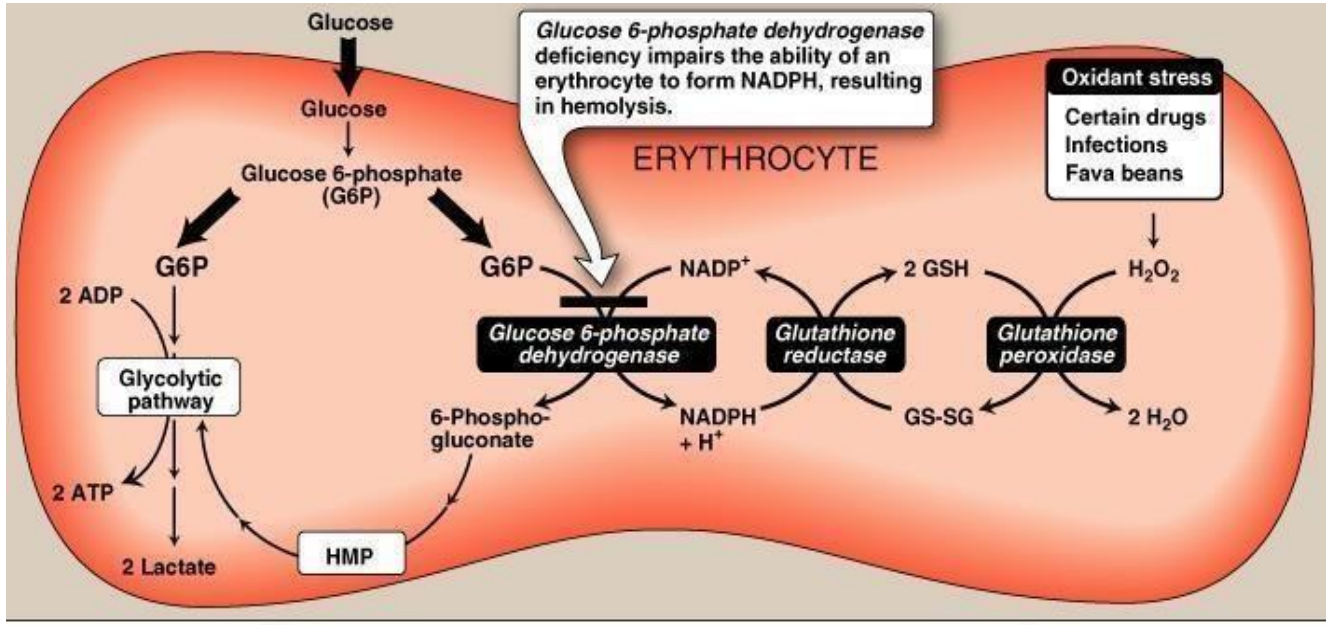
Role of G6PD in red blood cells

$H_2O_2 + GSH \longrightarrow G\text{-}S\text{-}S\text{-}G + 2H_2O$

$G\text{-}S\text{-}S\text{-}G + NADPH \longrightarrow 2GSH + NADP+$

GSH helps maintain the SH groups in proteins in the reduced state

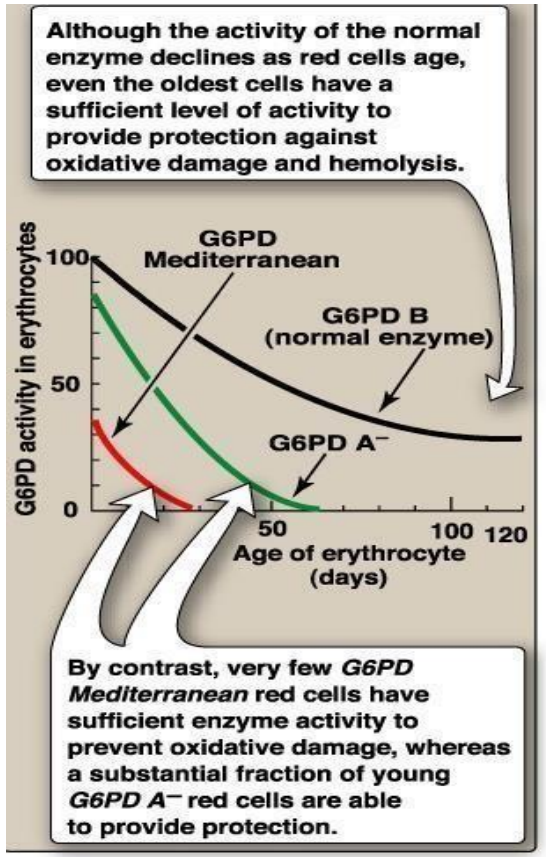
Oxidation ➔ denaturation of proteins and rigidity of the cells



Decreasing the NADPH/NADP+ pool in erythrocytes would ultimately reduce GSH/GS-SG pool which in turn increases the ROS. those highly reactive molecules can oxidize sulfhydryl groups of the cellular proteins including hemoglobin, leading to the formation of denatured proteins. Oxidation of the membrane proteins by ROS results in rigid membrane.

Classification of G6PD Deficiency Variants

Please Take a closer look at the figure

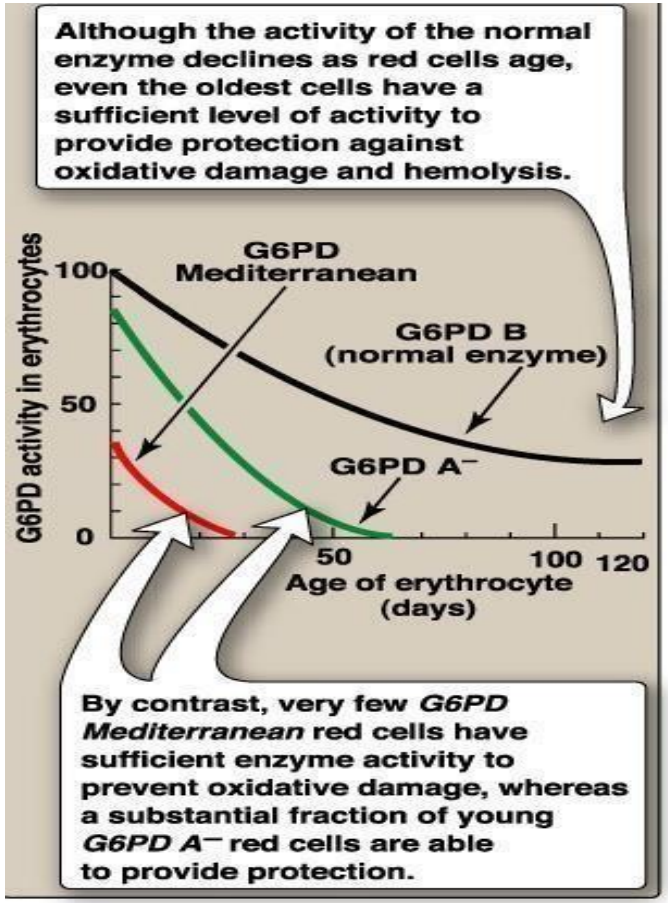


Class	Clinical symptoms	Residual enzyme activity
I	Very severe	<2% Almost deficient
II	Severe	<10%
III	Moderate	10–50%
IV	None	> 60%

Different mutations dictates different severities and classes.

- Wild type B: Normal type
- Mediterranean Variant B- (Class II) : 563C → 563T
- African Variant A- (Class III); two point mutation
- Majority missense mutation, point mutation
- Large deletions or frame shift; Not Observed

elucidation of the diagram



- This graph represents the decrease of G6PD activity during the life span of the erythrocyte with the wild type B, Mediterranean variant B^- and African variant A^-.
- The Black curve: represents the normal erythrocyte (wild type B) with 120 days life span, G6PD activity declines with time but never approaches zero, this means that the cell dies, and the enzyme still has some activity.
- The Green curve: represents African variant A^-, erythrocyte lives only for 60 days, the enzyme has no activity at the time of death, and interestingly notice how the enzymatic activity of variant A^- after 30 days resembles that of normal cell (Wild-type) at death!.
- The Red curve: represents Mediterranean variant B^- (class), erythrocyte lives only for 30 days, the enzyme has no activity at the time of death, also notice how the enzymatic activity of variant B^- early in its life span resembles that of normal cell (wild-type) at death!.

رسالة من الفريق العلمي:

أيْنَ وصَلَ خَيْط مُصْحَفك؟
أتُرَاهُ يَنتَقِل أمْ أصَابَهُ
الْهجْرَان .

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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			