Biochemistry Team 435

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G6PD

* Please check out this link to know if there are any changes or additions.

❖ **Note : don't skip the blue notes, they are important!**

Color index: Important | Doctors notes | Further explanation.

OBJECTIVES:

- ✓Explain the biochemical basis of G6PD deficiency anemia.
- ✓Recognize the precipitating factors for G6PD deficiency anemia.
- ✓Classify various classes of G6PD deficiency anemia (variant enzymes).
- ✓Describe the diagnostic methods for G6PD deficiency anemia

BACKGROUND

❖ **What are "Hexose monophosphate pathway (HMP) or Pentose Phosphate Pathway (PPP)"?**

An alternative pathway for glucose.

HMP and PPP products

Produces **ribose-5-phosphate** for nucleotide synthesis

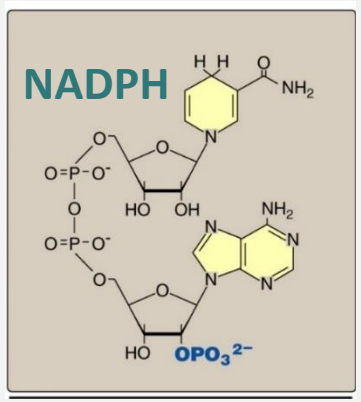
No ATP production

Major pathway for NADPH production

❖ **What is a nucleotide?**
the basic structural unit of nucleus acids such as DNA and RNA

❖ **What is a nucleotide composed of ?**
sugar chain(ribose sugar for RNA and deoxyribose chain for DNA) + nitrogen bases + phosphate group

❖ **What is the major pathway of glucose catabolism?**
Glycolysis followed by electron transport chain (for ATP production)



Uses of NADPH

- Reductive biosynthesis e.g., fatty acid biosynthesis.
- **Antioxidant (part of glutathione system).** So decreased NADPH → increased ROS → hemolytic anemia
- Oxygen-dependent phagocytosis by WBCs. White blood cells during phagocytosis endocytose the organism and act upon it by enzymes, these enzymes require NADPH as a coenzyme.
- Synthesis of nitric oxide (NO).

Because the enzyme nitric oxide synthase requires NADPH as a coenzyme and arginine as a substrate.

PENTOSE PHOSPHATE PATHWAY (PPP)

What you need to know:

1-we start with **glucose 6 phosphate** (we make it by phosphorylation of glucose by hexokinase), if we don't have this enzyme this conversion cannot occur hence glucose will exit the CELL and cannot be used for cellular processes (so in order to use glucose we need hexokinase).

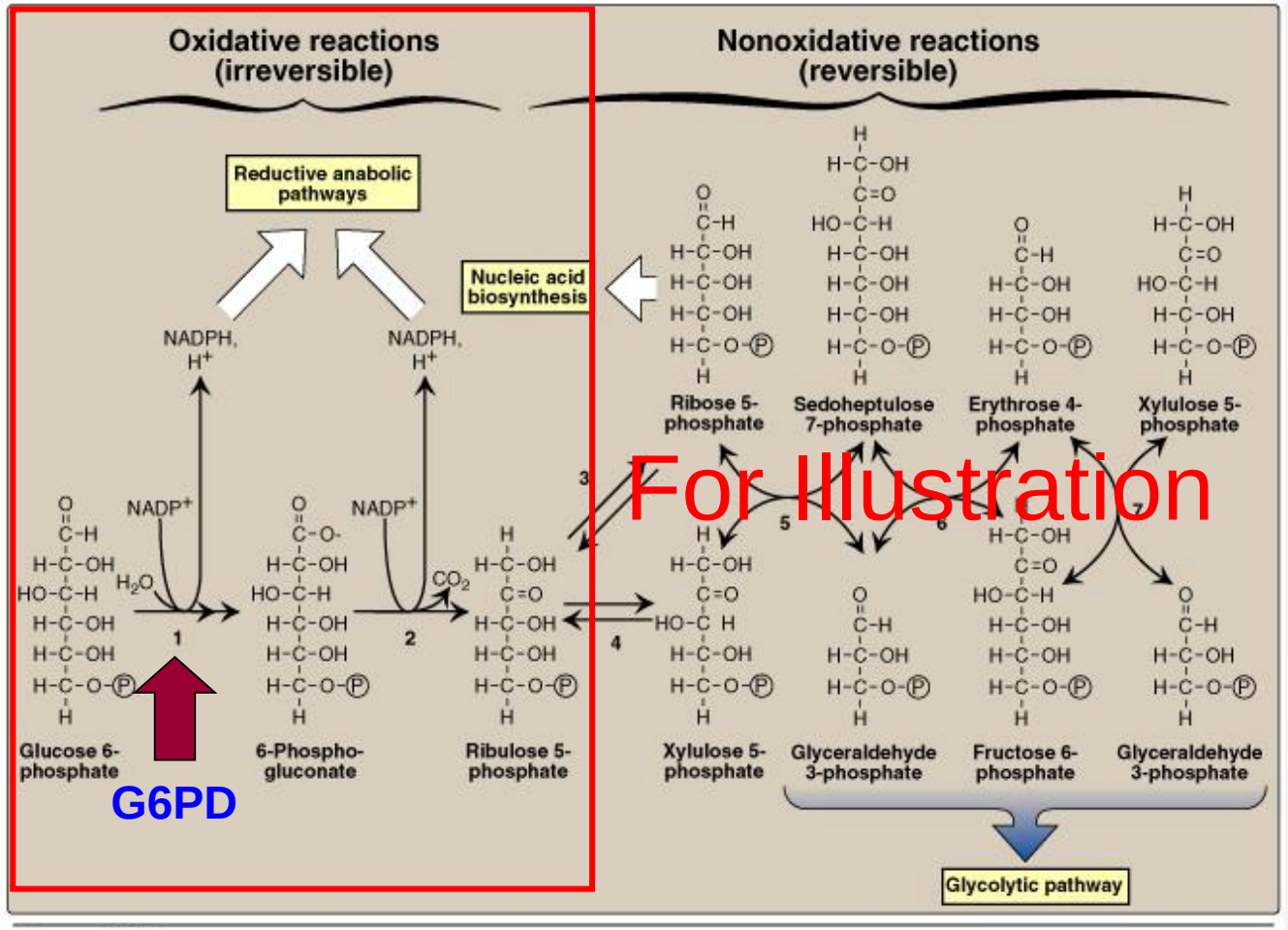
2-**glucose -6- phosphate dehydrogenase** converts glucose 6 phosphate to 6 phosphogluconate.

3- 6 phosphogluconate is converted to ribulose 5 phoaphate (Step 2 and 3 are known as the oxidative steps in HMP pathway, these two steps are the ones that produce NADPH) the rest of the steps are non oxidative.

4- note that ribose 5 phosphate is used in nucleic acid synthesis.

5- glyceraldehyde-3- phosphate and fructose 6 phosphate and glyceraldehyde -3- phosphate (again) are used in the glycolysis pathway.

6-THIS PATHWAY DOES NOT PRODUCE ATP.



What will happen if this pathway doesn't occur?

1-the main thing that will be affected is the NADPH because it cannot be produced by other pathways.

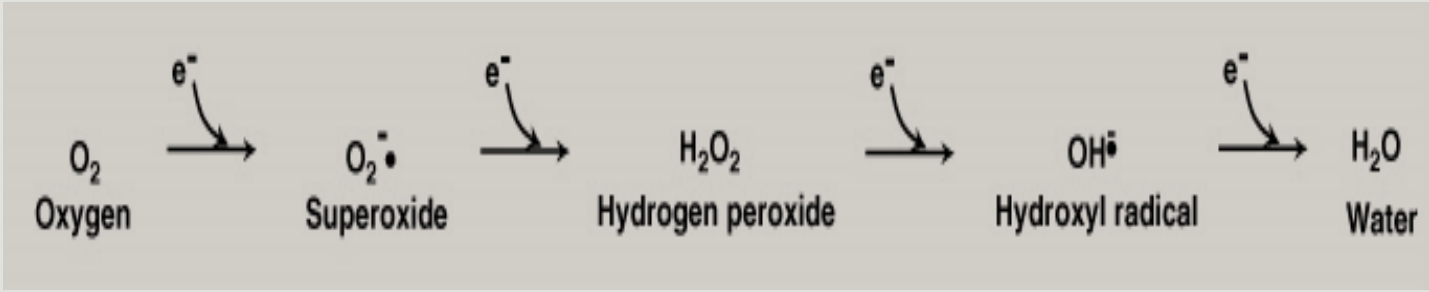
2-the glycolysis intermediate are still being synthesized by the glycolysis pathway itself.

3-the ribose can be synthesized by other pathways

REACTIVE OXYGEN SPECIES (ROS)

ROS	
Oxygen-derived Free radicals	**Non-free radical**
- Superoxide. - hydroxyl radicals.	Hydrogen peroxide (highly reactive).

-Free radicals are formed from different metabolic processes .
-The major source of ATP is aerobic respiration→ glycolysis ,TCA ,ETC, and oxidative phosphorylation .
-What is the final acceptor of electrons in the electron transport chain? Oxygen.
-Oxygen is reduced to water in the end.

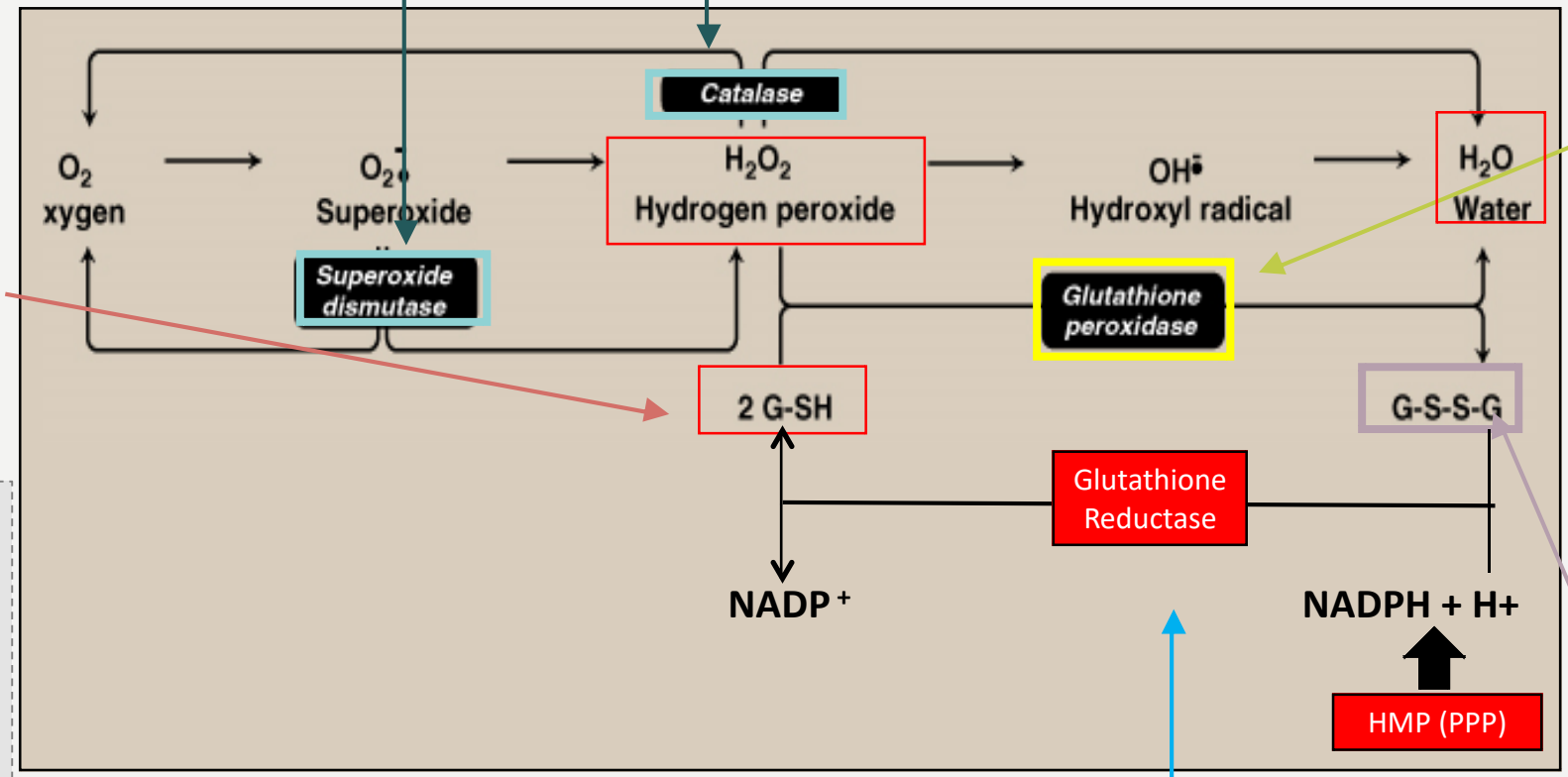


Oxygen is reduced to superoxide by adding an electron to it. Superoxide is reduced to hydrogen peroxide by adding an electron too. Hydrogen peroxide is converted to Hydroxyl radical in the same way. Finally an electron is added to hydroxyl radical to form water .
Note that during the reduction of oxygen to water , free radicals are formed as byproducts of aerobic metabolism

ANTIOXIDANTS MECHANISMS

1- superoxide dismutase is the enzyme that can convert superoxide into oxygen or hydrogen peroxide.
2- catalase can convert hydrogen peroxide into oxygen or water.

3-Reduced glutathione (G-SH) which is present in most cells can also detoxify H_2O_2 by converting it into water (harmless product).



3-the reaction is catalyzed by **glutathione peroxidase** which is a selenium containing enzyme **and it is part of** the glutathione system (glutathione + glutathione peroxidase)

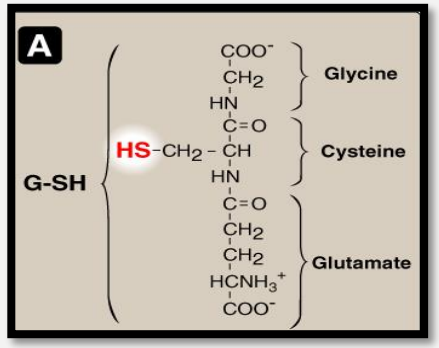
Important: G-S-S-G : oxidized form of glutathione (2S).
G-SH : reduced form -by glutathione reductase - (reduced to 1S.).

4- this reaction will ultimately form an oxidized glutathione (G-S-S-G), which doesn't have the protective properties

5- In order to have a protective function it must be converted to its reduced form which is done by the **glutathione reductase** enzyme, this enzyme **requires the NADPH** from **HMP pathway** in order to do so.
Then 2G-SH (reduced glutathione) again will give hydrogen to hydrogen peroxide to form water.
-so it is important to know that the reduction of hydrogen peroxide was by the electrons or the reducing equivalent was provided from NADPH.

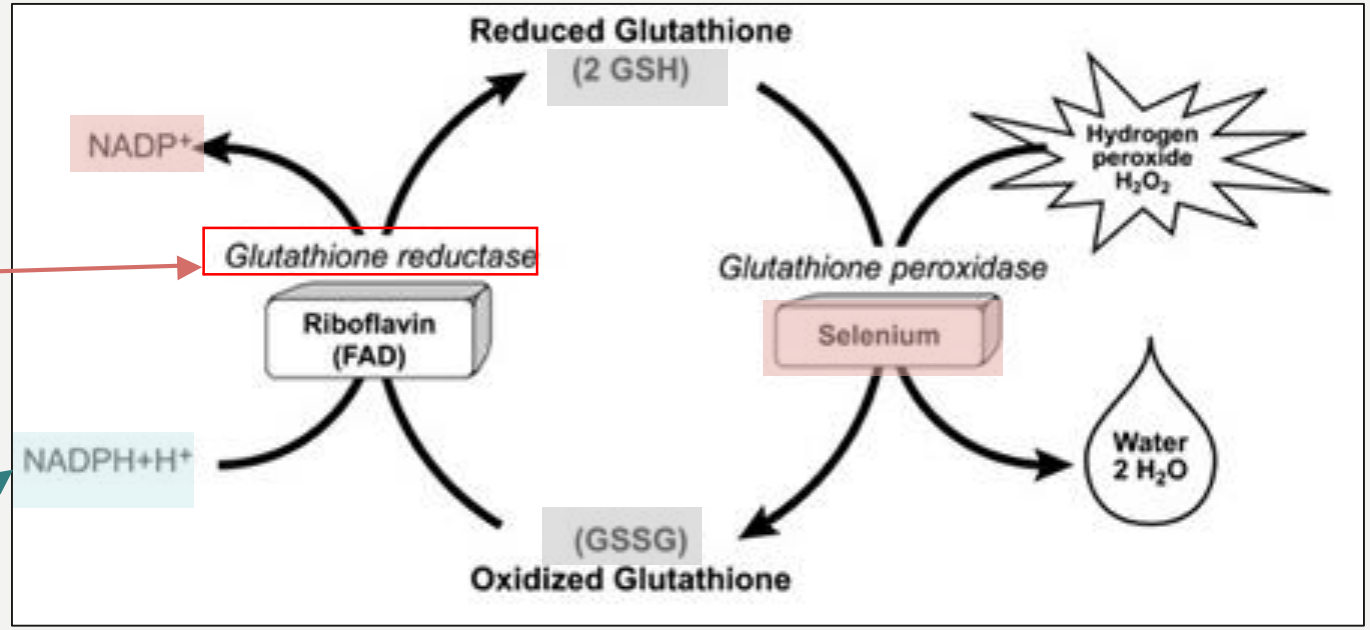
GLUTATHIONE SYSTEM

Another name is gamma glutamyl cystinyl glycine



Structure of reduced glutathione (G-SH), note that cysteine is linked to glutamate through a gamma-carboxyl rather than an alpha-carboxyl.

The cell regenerates G-SH in a reaction catalyze by "**glutathione reductase**", using NADPH as a source of reducing equivalents,



From where do we get the NADPH?
From HMP (PPP)

1- HMP provides NADPH which provides the reducing equivalent to the oxidized form of glutathione
2-then , in the presence of glutathione reductase, 2 molecules of reduced GSH are formed
3- these two molecules of reduced glutathione will be used by glutathione peroxidase (which contains selenium) to convert hydrogen peroxide into 2 molecules of water

Oxidative stress

❖ What is it?

- A condition in which cells are subjected to excessive levels of **Reactive Species** (Oxygen or Nutritive species) & they are unable to counterbalance their deleterious effects with Antioxidants.
- **Im**balance between oxidant production and antioxidant mechanisms. Due to inflammation or drugs that act as oxidants or infection.. anything that increases the amount of free radicals being produced by the body Oxidative stress can also be due to a defect in the antioxidant machinery in the body.

❖ It Causes oxidative damage to:

- DNA.
- Proteins
- Lipids (unsaturated fatty acids)

It has been implicated in a number of pathological process

- **Cancers**
- **obesity**
- **Atherosclerosis and coronary artery disease**
- **Inflammatory conditions** e.g. rheumatoid arthritis
- **G6PD deficiency hemolytic anemia**

Note that G6PD deficiency leads to → decreased NADPH, which leads to → defective glutathione system, which will lead to → increased oxidative stressm which will lead to → RBC damage.

-why do only RBCs get damaged and not all cells?? because the other cells have other ways to make NADPH ,but mature RBCs do not.

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G6PD Deficiency Hemolytic Anemia

- Inherited **X-linked** recessive disease
  - So males are effected if the mother was a carrier, but females require both parents to have the gene in order to have this disease.
- **Most common** enzyme-related hemolytic anemia
- ~400 different mutations affect G6PD gene, but **ONLY SOME** can cause clinical hemolytic anemia.
  - Note that the mutations that cause G6PD hemolytic anemia are point mutations (single nucleotide alteration in the gene).
- G6PD deficient patients have **increased resistance** to infestation by **falciparum malaria**
  - These diseases actually protect from G6PD deficiency because the life span of the RBCS is reduced If the case of malaria, the causative organism is plasmodium falciparum, which multiplies in the liver & RBCS.
- **Highest prevalence:** Middle East, Tropical Africa, Asia and Mediterranean.
  - Middle East, Tropical Africa, Asia and Mediterranean: These areas have more thalassemia , malaria, and sickle cell disease.

Precipitating Factors for G6PD Deficiency Hemolytic Anemia

Intake of oxidant drugs (AAA):

- **Antibiotics** e.g., sulfa preparation
- **Antimalarial:** e.g., Primaquine
- **Antipyretics**

Exposure to infection:

How does infection lead to attack of hemolysis?
During infection we have increased inflammation which will increase free radicals and increased macrophages response.
So Why do the free radicals in the macrophages enter and damage the blood cells?
because the build up of free radicals in the macrophages will lead to their diffusion into the RBC.

Ingestion of fava beans

- **Favism (انيميا الفول):**

  A condition characterized by hemolytic anemia (breakup of red blood cells) after eating fava beans. the mechanism is unknown but it is suggested that the fava beans have oxidants that cause damage.

- **Mediterranean variant.**

❖ Chronic nonspherocytic anemia:

Hemolytic attack in **absence** of precipitating factors. Severe form due to class I mutation.

G6PD deficiency

G6PD Deficiency (USMLE)

Biochemical Basis of G6PD Deficiency Hemolytic Anemia

Oxidation of sulfhydryl (SH) groups of proteins inside RBCs, Causes: → 1-Protein denaturation → 2- Formation of **insoluble masses (Heinz bodies)** that attach to RBCs membranes.

❖ **Although G6PD deficiency affects all cells, it is most severe in RBCs …… Why?**

Other cells have **OTHER** sources for NADPH production: e.g., **Malic enzyme (**Called malate dehydrogenase) that converts malate into pyruvate.

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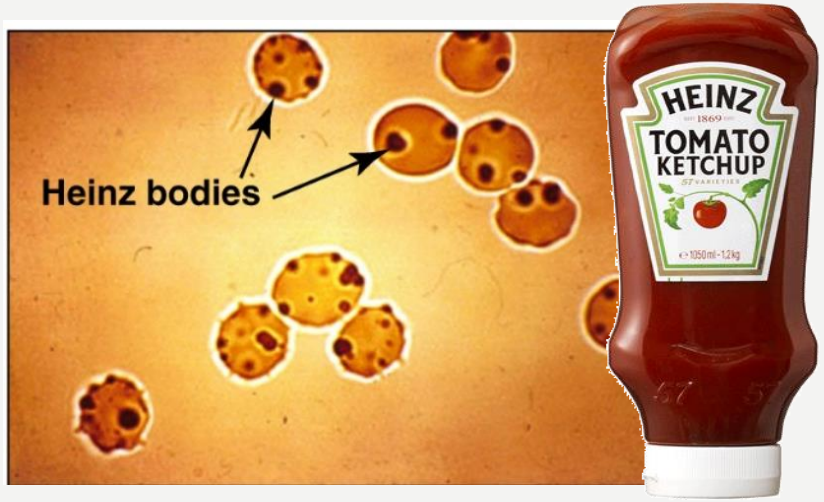
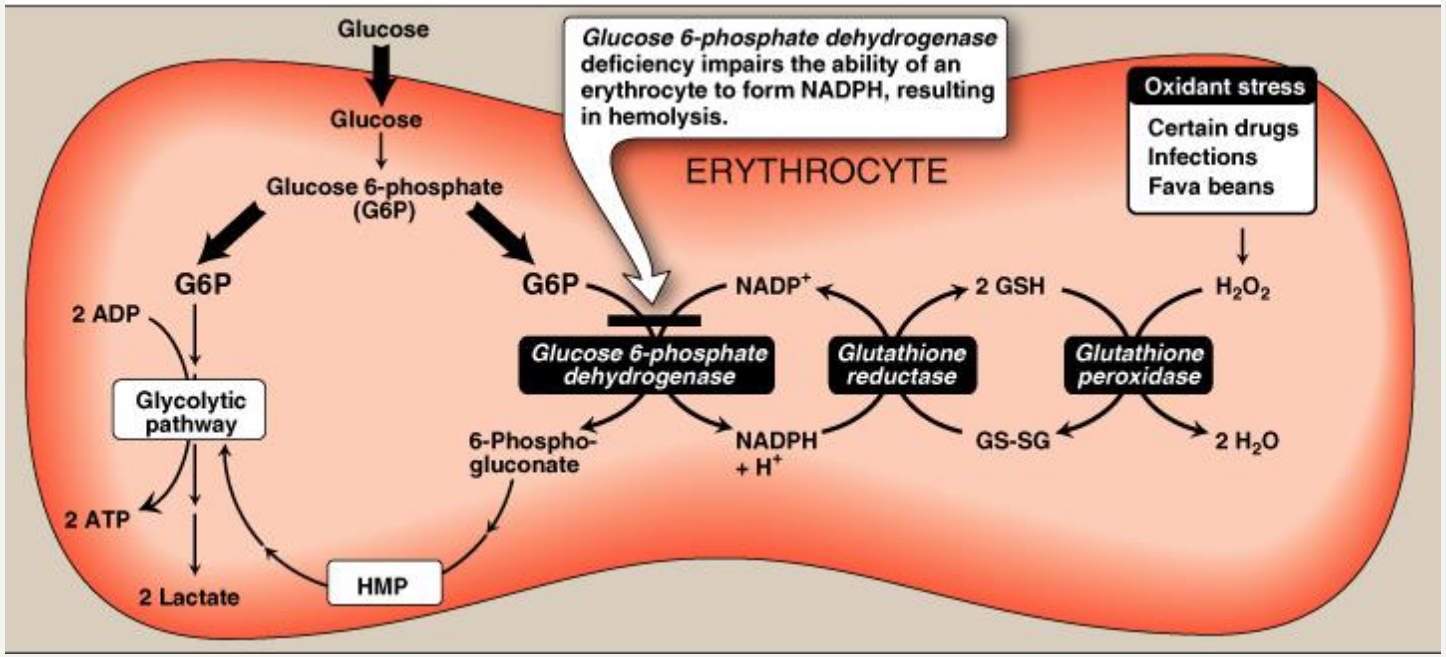


- Another thing that glutathione does is to protect the sulfhydryl groups in proteins in cells including RBCS, so if glutathione is not present the S-H (sulfahydryl group) will be oxidized, so these proteins become denatured and hence become insoluble masses which we call **Heinz bodies.**
- These Heinz bodies move from the periphery of the cell of the RBC and attach themselves to the cell membrane which make the RBC a rigid structure, this is problematic, **why?** Because they won't be able to squeeze into the small capillaries

Biochemical Basis of G6PD Deficiency Hemolytic Anemia



- Glucose enters the cell and gets converted to glucose -6- phosphate by hexokinase.
- Some of the Glucose-6-phosphate goes to the **glycolytic pathway** and some go to the **HMP pathway**.
- **In HMP pathway:**
  - First glucose -6- phosphate is acted upon by G6PD and is converted to 6-Phosphogluconate which enters into the HMP pathway and as a by product **NADPH IS PRODUCED BY THIS REACTION!!**
  - NADPH **reduces** glutathione and 2-GSH is produced by the action of glutathione reductase.
  - The reduced glutathione reduces hydrogen peroxide into 2 molecules of water by the action of the selenium containing enzyme.

❖ **What will happen in G6PD deficiency ?**

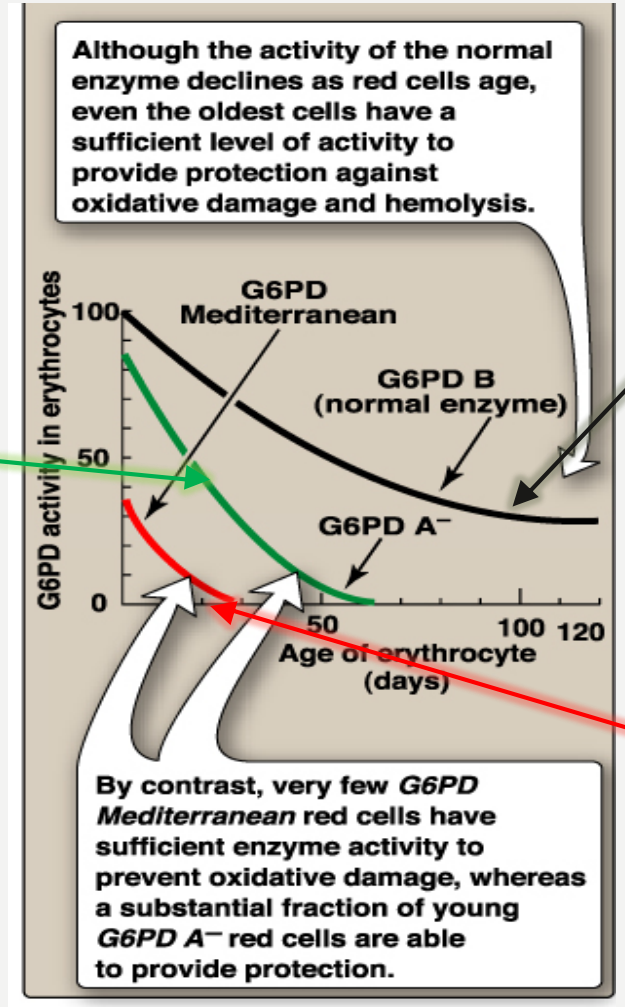
No enzyme is present to form NADPH → **no** reduction of glutathione → oxidized form of glutathione **cannot** provide the hydrogen needed for the conversion of hydrogen peroxide into water → hydrogen peroxide **accumulates** and **damages RBC** → **hemolytic anemia** ☹☹☹☹☹

Different Classes of G6PD Deficiency Hemolytic Anemia

❖ Based on the **residual enzyme activity**, G6PD deficiency hemolytic anemia is classified into 4 classes:

Class I	Mediterranean (Class II)	G6PD A- (Class III)	Class IV
Very severe	Severe	Moderate	Normal
LOWEST ☹	Residual activity		HIGHEST
is most severe there is a hemolytic attack without any precipitating factors ,fatal, non spherocytic hemolytic anemia The enzyme activity is less than 10%	- Affect **all** RBCs (both young and old).	- **young** RBCs.	-Normal means that symptoms are not present but there is a decrease in enzyme activity especially when the RBC reaches 120 days. But you must know that even with the decrease, the enzyme is still sufficient. -here the patient has a mutation, but the symptoms don't appear unless one of precipitating factors is present (e.g. favism).
	- Enzyme with **decreased stability** Resulting in **decreased activity** (severe).	- contain enzymatic activity. **Unstable enzyme**, but kinetically normal.	
	Severe form and also less than 10% activity. It is also called Favism, and it is episodic. (why ? Because it occurs only due to the ingestion of fava beans.)	Moderate form and is not active unless there is a precipitating factor People usually live asymptomatic And even if the hemolytic anemia occurred , it resolves on its own within 10 days. Enzyme activity is 10-16 %. NOT Lethal.	

The green curve:
The younger RBCS has a good amount of activity But as they age they start losing their activity and finally die Because when they lose their activity the accumulation of free radicals and Heinz bodies will occur finally leading to the death of said RBCS.



The black curve is class four :
When the RBC is young = normal activity (almost 100%) But as the RBC ages , the enzyme activity decreases, and by the time it reaches 120 it has lost a considerable amount of its activity (40% of its activity is left) which is sufficient for protection.

The Red curve:
It dies within the first 15-20 days.

- The cause of G6PD deficiency is mainly point mutation. This point mutation may either affect:
  - **the binding site of the enzyme** thus decreasing activity Or **another site** this will affect the enzyme stability.
- **General concept:** So if the enzyme stability (not the activity) is affected but the RBC is young, the enzyme will have enough activity But as they grow old the activity of the enzyme will go down.
  - The severe cases include an affect on both activity and stability of the enzyme.

Diagnosis of G6PD Deficiency Hemolytic Anemia

Diagnosis of hemolytic anemia

- Complete Blood Count (CBC)
- Reticulocytic count

-Decreased RBC count but increased number of reticulocyte on blood smear : Heinz bodies.
-Reticulocytes number normally is lower than CBC number or equal to it at most, so if it was more than CBC it may indicate this type of anemia.

Screening:

- Qualitative assessment of G6PD enzymatic activity (UV-based test)

Tells you that enzyme activity is low if it becomes positive, do confirmatory test

Confirmatory test:

- Quantitative measurement of G6PD enzymatic activity

Tells you exactly the amount of active enzyme

Molecular test:

- Detection of G6PD gene mutation

Tells you what kind of mutation it is and the type of deficiency And it will assist in the classification of the deficiency

Check your understanding!

Q1: Which one of the following describes biochemical consequences of G6PD deficiency that lead to hemolytic anemia?

A. Low ATP
B. Low NAD
C. Low NADPH
D. Low Hb

Q2: Which one of the following is a characteristic feature of class IV G6PD?

A. Low residual activity
B. High residual activity
C. Severe RBCs hemolysis
D. None of the above

Q3: One of the histological findings that aids in the diagnosis of an active G6PD deficiency is the inclusion of insoluble masses in the RBCs.

A. True
B. False

Q4: G6PD deficiency affects RBC's severely because…..

A. RBCs are not nucleated
B. RBCs lack alternate NADPH production pathways
C. Oxidation of sulfhydryl (SH) groups of proteins inside RBCs causes protein denaturation
D. B + C

Q5: Which of the following is NOT a precipitating factor for G6PD-deficiency hemolytic anemia?

A. Fava Beans
B. Anti-malarial drugs
C. Fiber-rich diet
D. Exposure to infection

Q6: The products of G6PD's action on Glucose 6-phosphate are:

A. 6-Phosphogluconate and NADPH
B. 2-GSH and NADPH
C. 6-Phosphogluconate and NADP+
D. 2-GSH and NAD+

1.C 2.B 3.A 4.D 5.C 6.A

Done by:

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"Every struggle in your life has shaped you into the person you are today. Be thankful for the hard times; they can only make you stronger."

Resources:

- 435's slides and notes.
- Lippincott's illustrated reviews: Biochemistry – sixth edition.

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