"اللَّهُمَّ لا سَهْلَ إلاَّ ما جَعَلْتَهُ سَهْلاً، وأنْتَ تَجْعَلُ الحَزْنَ إذَا شِئْتَ سَهْلاً "

# G6PD

Biochemistry Team 437

**Color index:**
Doctors slides
Doctor's notes
Extra information
Highlights

GNT block

# Objectives:

- By the end of this lecture, the student should be able to:

  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

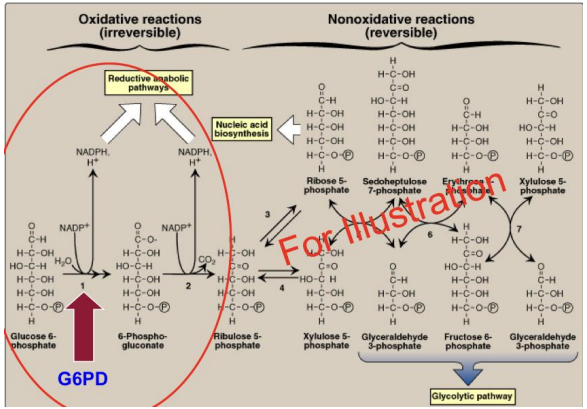
# Background

Hexose monophosphate pathway (HMP) or Pentose Phosphate Pathway (PPP):

- An alternative oxidative pathway for glucose "happens in RBCs mostly"
- No ATP production
- Major pathway for NADPH production, "which we need for glutathione system as we are going to see in the lecture"
- Produces ribose-5-phosphate for nucleotide synthesis

-The body will enter this shunt when the conc of NADPH is low.
-We have another way for production of the NADPH(MINOR) ,but not in RBC why?
Because it doesn't have any mitochondria to enter the kryps cycle.

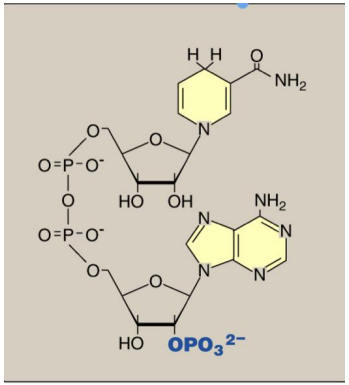
# Pentose Phosphate Pathway (PPP)




2 reactions :
**1-** conversion of glucose 6-phosphate to ribulose 5 - phosphate
Results in Release of co2 **and NADPH**
**"reduced NADP"**
**2-** ribulose 5-phosphate either continue to glycolytic pathway to give co2 and energy OR helps in nucleic acid biosynthesis , according to the needs of the body, this reaction is reversible

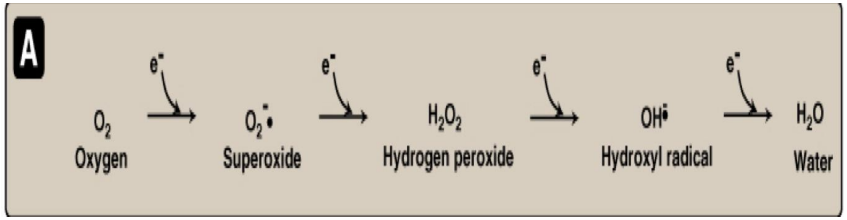
# Uses of NADPH

1. Reductive biosynthesis in the liver
   ex: fatty acid biosynthesis
2. Antioxidant (part of glutathione system) prevents cancer
3. Oxygen dependent phagocytosis by WBCs*
4. Synthesis of nitric oxide (NO)




*The enzymes that is required for phagocytosis need NADPH as coenzyme to work.

# Reactive Oxygen Species




- Oxygen-derived free radicals ex: superoxide and hydroxyl radicals.
- Non-free radical: Hydrogen peroxide

- To keep the body healthy, we need to have a balance between oxidants and antioxidants
- Imbalance between them causes oxidative stress.
- Everything we have around us like foods, toxins, radiatio all lead to generation of oxidants
- Reactive O2 species are normally produced in our body as a byproduct of metabolism or through some reactions with drugs and toxins, to counteract their effect, our bodies have special antioxidant machinery.
- Some ROS have a free electron called a free radical which damages the cells.

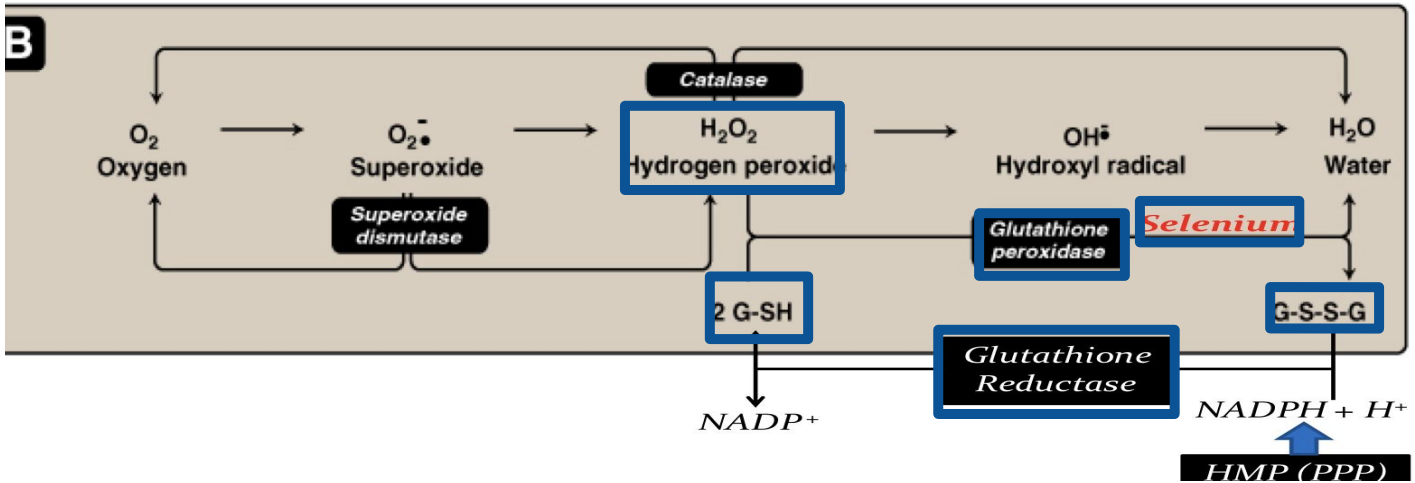
Some Antioxidants in our body:
-Glutathione system
-vitamins:
Vit A , Vit C, Vit E
-minerals: zinc, cellenium

# Oxidative stress

Oxidative stress is an imbalance between oxidant production and antioxidant mechanisms.

| Oxidative **damage** to | Oxidative stress **diseases** |
| --- | --- |
| 1. DNA<br>2. Proteins<br>3. Lipids (unsaturated fatty acids) | 1. Inflammatory conditions e.g., Rheumatoid arthritis<br>2. Atherosclerosis and coronary heart diseases<br>3. Cancers<br>4. Obesity<br>5. G6PD deficiency hemolytic anemia |

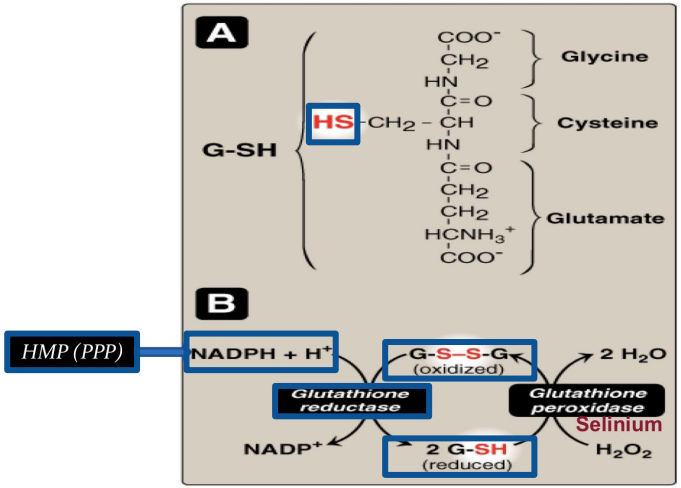
# Antioxidant Mechanisms




Let's say that hydrogen peroxide [H2O2] is accumulated, how will the body get rid of itt?

- Hydrogen peroxide will react with 2 Molecules of reduced glutathione [G-SH] by the enzyme **glutathione peroxidase.** This enzyme **requires selenium** as a cofactor
- Due to this reaction, the hydrogen peroxide will be reduced to Water "becomes safe with no oxidative activity" and glutathione will become oxidized [G-S-S-G]. This reaction is considered oxidation reduction reaction.
- To keep this reaction going, I need to reduce glutathione again, or else the cycle will stop and the antioxidant machinery will fail
- To reduce glutathione, we need the enzyme **glutathione reductase**, and **NADPH** "reduced NADP"
- In this reaction, glutathione will be reduced by glutathione reductase and NADPH will be oxidized to NADP
- Now we have another problem, we need to restore NADPH for this reaction to continue.
- Through the HMP/PPP pathway "revise the first slide" glucose-6-phosphate "GSP" is converted to 6-phosphogluconate by the enzyme **G6PD "glucose-6-phosphate dehydrogenase"** and NADP will be reduced to NADPH, which we can use again in the glutathione system.

# Glutathione System




- Dr.Rana said that the composition of G-Sh is not important just know that there is a sulfhydryl group of cysteine(SH)

In Summary:

- HMP provides NADPH which is required for the reduction of G-S-S-G to 2 molecules of 2G-SH( reduced) ;these two molecules of reduced glutathione will be used by glutathione peroxidase + selenium (coenzyme) to convert hydrogen peroxide into 2 molecules of water

- Remember, PPP/HMP pathway is the main glycolysis pathway in the RBSc, If G6PD was deficient , PPP will not work and NADPH is not going to be produced → the glutathione system will fail → increased oxidative stress in the RBCs → hemolytic anemia

# G6PD Deficiency Hemolytic Anemia

- **Inherited** X-linked recessive disease
- Most common enzyme-related hemolytic anemia*
- Highest prevalence: Middle East, Tropical Africa Asia and Mediterranean [1]
- ~400 different mutations affect G6PD gene, but only some can cause clinical hemolytic anemia
- G6PD deficient patients have increased resistance to infestation by falciparum malaria [2]

1- Most severe
2- Because the short life of the G6PD deficient RBCs will not allow the malaria to grow and become fully mature.
*more common than pyruvate kinase deficiency.

# Precipitating Factors for G6PD Deficiency Hemolytic Anemia

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Important!

G6PD deficient patients will develop hemolytic attack upon:

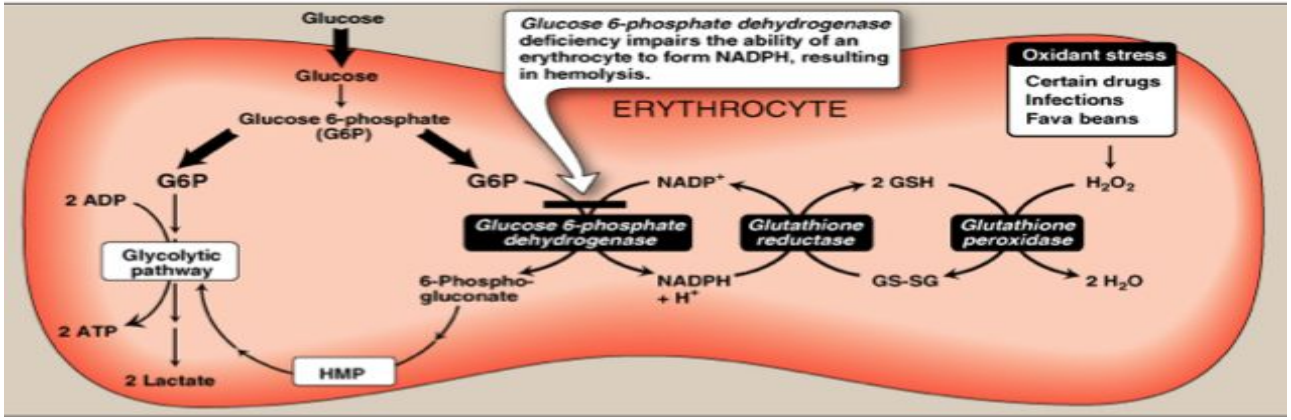
**1.Intake of oxidant drugs (AAA):**
Antibiotics. e.g.,sulfa preparation Antimalarial. e.g.,Primaquine
Antipyretics.

**2.Ingestion of fava beans:**
(favism, Mediterranean variant)

**3.Exposure to infection**
Because they lead to increase in the production of ROS which Increase hemolysis

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

# Biochemical Basis of G6PD Deficiency Hemolytic Anemia




-G6PD convert G6P to 6-phosphogluconate and make NADPH  So if I don't have this enzyme “G6PD” I will not have NADPH and I will not have reduced glutathione thus I can not convert hydrogen peroxide“H2O2” to H2O

-Accumulation of H2O2 will cause oxidative stress that will damage the proteins  and this include the cell membrane of the RBCs which is protein leading to hemolysis

This reaction happen everywhere in the body but if its happened in RBCs it will lead to hemolytic anemia why? Because the RBCs don't have any other resources of NADPH like glycolysis so it's only depend on this pathway and if we have G6PD deficiency this will lead to hemolytic anemia

simply no G6PD => no NADPH => no reduced glutathione => no converting of H2O2 to H2O => hemolysis

G6PD deficiency impairs the ability of the erythrocyte to form NADPH resulting in hemolysis

# Biochemical Basis of G6PD Deficiency Hemolytic Anemia

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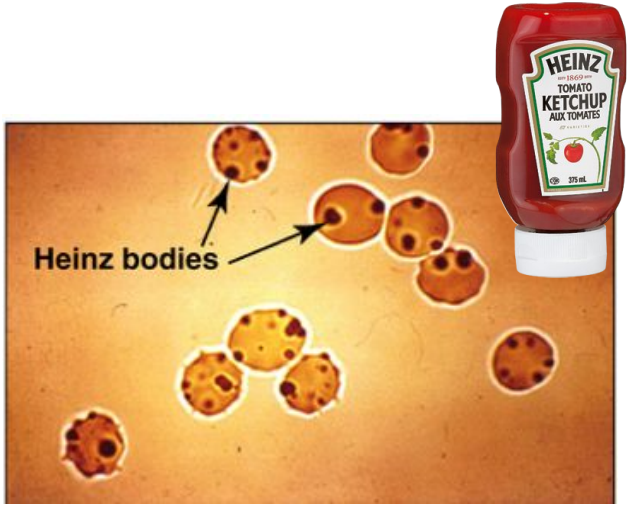
Oxidation of sulfhydryl (SH) groups of proteins inside RBCs causes protein denaturation and formation of insoluble masses (Heinz bodies)* that attach to RBCs membranes




Normally the RBCs are flexible but when we have denaturation protein attached to the cell membrane of RBCs "Heinz body" it will be rigid so when it pass throw the blood vessels it will breakdown / rupture and that leads to hemolysis
**"So simply the present of Heinz bodies will lead to hemolysis"**

*Help in detection of hemolytic anemia due to G6PD deficiency

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 that converts malate into pyruvate

# Different Classes of G6PD Deficiency Hemolytic Anemia

- This classification is based on the residual enzyme activity [1] (Least in class I, and Highest in class IV)

- There are 4 different classes:

| |
|---|
| I (Very severe) |
| II (Severe, e.g. Mediterranean) |
| III: (Moderate: G6PD A-) |
| IV: (Normal) [2] |

1- How much of the enzyme is still active

2- symptoms appear with precipitating factors, Especially infection and some drugs

# Variant Enzymes of G6PD Deficiency Hemolytic Anemia

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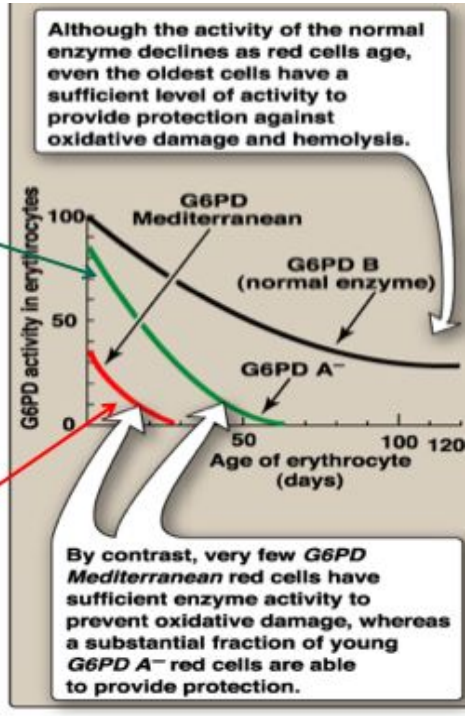
G6PD A- (class III):
Moderate, **young RBCs** contain enzymatic activity.
Unstable enzyme, but kinetically normal

G6PD Mediterranean (II)
Enzyme with decreased stability Resulting in decreased activity (severe).
Affect all RBCs
**(both young and old)**

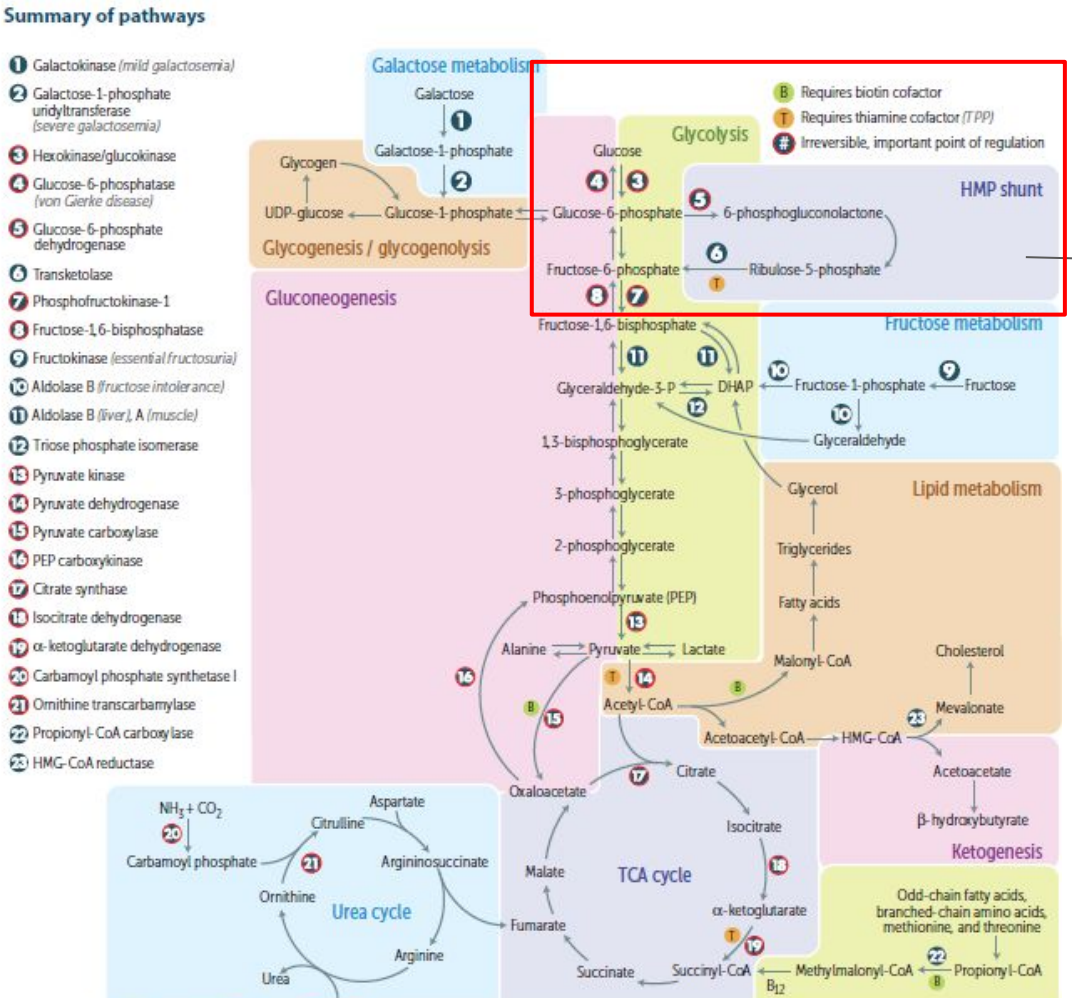


# Diagnosis of G6PD Deficiency Hemolytic Anemia

| | |
|---|---|
| **Diagnosis of hemolytic anemia:** | Complete Blood Count (CBC) & reticulocyte count<br>"we will find hemolysis + Heinz bodies " |
| **Screening:** | Qualitative assessment of G6PD enzymatic activity (UV-based test)<br>"Old technique, by fluorescence in a dark room" |
| **Confirmatory test:** | Quantitative measurement of G6PD enzymatic activity |
| **Molecular test:** | Detection of G6PD gene mutation |

# Summary

| | |
|---|---|
| Uses of NADPH | • Reductive biosynthesis.<br>• Antioxidant (part of glutathione system).<br>• Oxygen-dependent phagocytosis by WBCs.<br>• Synthesis of nitric oxide (NO) |
| Oxidative Stress:<br>Imbalance between oxidant production and antioxidants mechanisms. | Oxidative damage to: DNA ,Proteins and Lipids (unsaturated fatty acids)<br>diseases: Inflammatory conditions - Atherosclerosis and coronary heart diseases – Obesity - Cancers - G6PD deficiency hemolytic anemia<br>Oxidant : Hydrogen peroxide , Superoxide , hydroxyl radical.<br>Antioxidants: glutathione system |
| G6PD Deficiency Hemolytic Anemia | • Biochemical Basis: Oxidation of sulfhydryl (SH) groups of proteins inside RBCs causes : 1- protein denaturation. 2- formation of insoluble masses (Heinz bodies)<br>• most severe in RBCs because Other cells have other sources for NADPH production<br>• Precipitating Factors: 1-Intake of oxidant drugs. 2-Exposure to infection 3-Ingestion of fava beans<br>• Different Classes: I (Very severe)/ II (Severe) / III: (Moderate) / IV: (Normal)<br>• Diagnosis: 1-Complete Blood Count (CBC) & reticulocytic count. 2-Qualitative assessment of G6PD enzymatic activity (UV-based test). 3- Quantitative measurement of G6PD enzymatic activity 4-Detection of G6PD gene mutation.<br>Inheritance: Inherited X-linked recessive disease<br>Benefit: increase resistance to infection by falciparum malaria |




This picture summarizes all the pathways of glucose metabolism

HMP shunt aim to generate NADPH which we will discuss thoroughly in this lecture

# Take Home Messages

- G6PD deficiency impairs the ability of cells to form NADPH.
- RBCs are particularly affected because they do not have other sources of NADPH.
- □ NADPH is essential for the anti-oxidant activity of Glutathione peroxidase/reductase system.
- G6PD deficiency is an X-linked disease characterized by hemolytic anemia.
- □ The precipitating factors of hemolysis includes administration of oxidant drugs, ingestion of fava beans or sever infections. □
- G6PD deficiency is classified according to the residual activity of the G6PD.
- Class I variant (the most severe) class is associated with chronic nonspherocytic hemolytic anemia.

# MCQs:

1 Which one of the following is not a use of NADPH?
A carbohydrates biosynthesis
B fatty acid biosynthesis
C Antioxidant
D Synthesis of nitric oxide

2 Which one of the following cause Heinz bodies?
A Reduction of disulfide
B Oxidation of disulfide
C Reduction of sulfhydryl
D Oxidation of sulfhydryl

3 Mediterranean considered as
A Class I of G6PD Deficiency Hemolytic Anemia
B Class II G6PD Deficiency Hemolytic Anemia
C Class III G6PD Deficiency Hemolytic Anemia
D Class IV G6PD Deficiency Hemolytic Anemia

4 G6PD deficient patients will develop hemolytic attack If he take
A diuretics
B antihypertensive
C NSAID
D Antibiotics

5 Which one of the following enzymes converts H2O2 to H2O? (this Q from 436 team)
A Glutathione Peroxidase
B Glutathione Reductase
C Glutathione Synthetase
D None of them

1- A
2- D
3- B
4- D
5- A

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## Girls team

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## Boys team

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