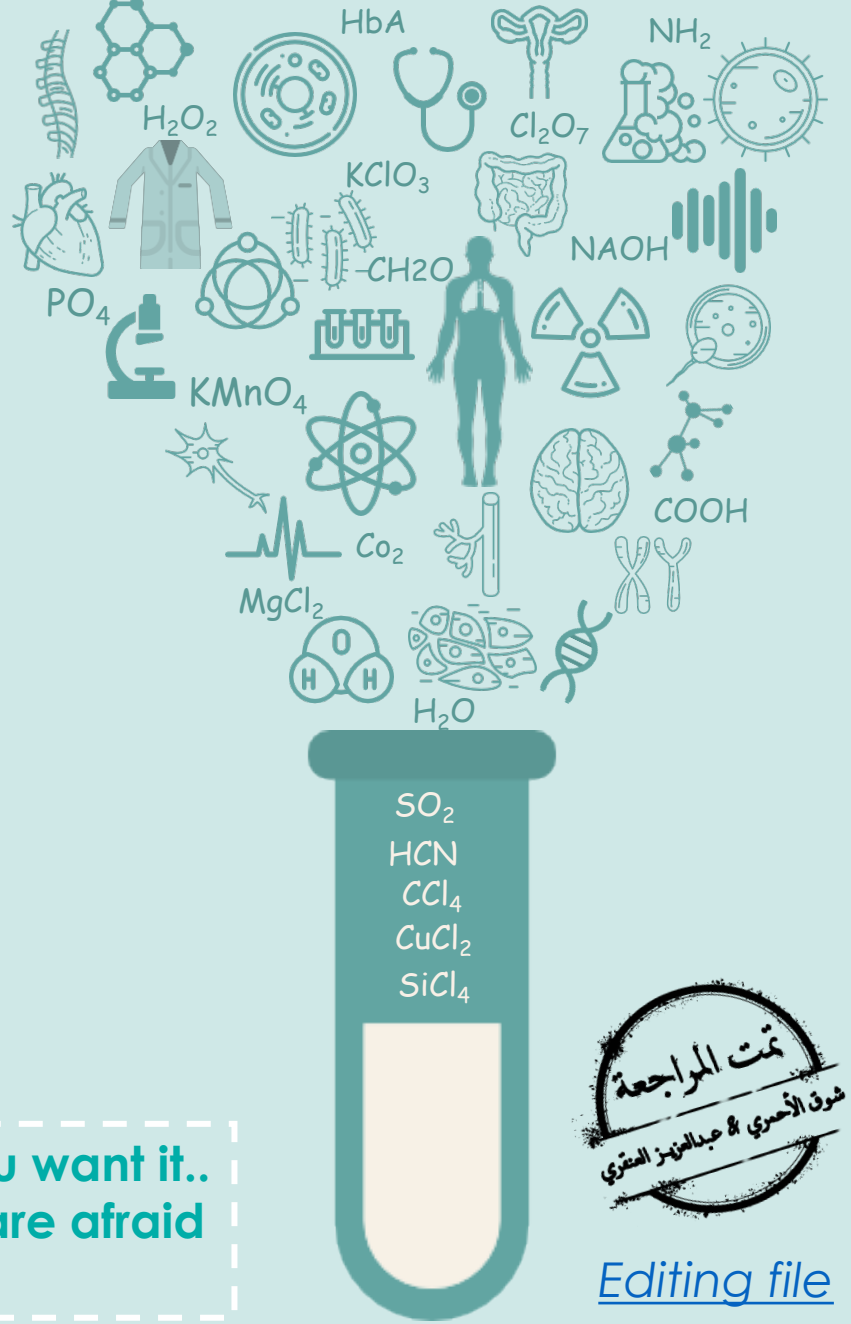
جامعة الملك سعود
King Saud University
1957

436
MEDICINE
KING SAUD UNIVERSITY

BIOCHEMISTRY TEAM 436

Important Doctors slides
Extra Information Doctors notes



Biochemistry

G6PD deficiency and hemolytic anemia

"Decide that you want it.. More than you are afraid of it"

تمت المراجعة
شوق الأحمري & عبدالعزيز العتري

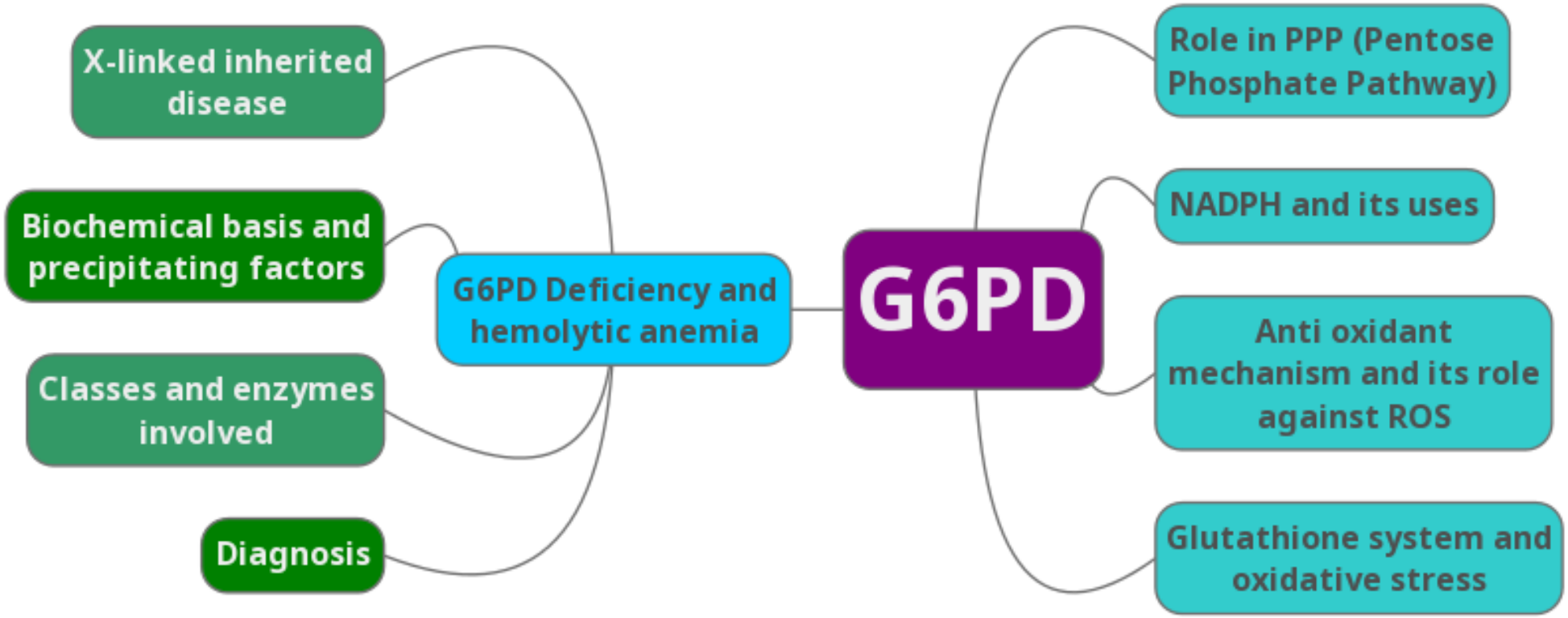
[Editing file]

OBJECTIVES

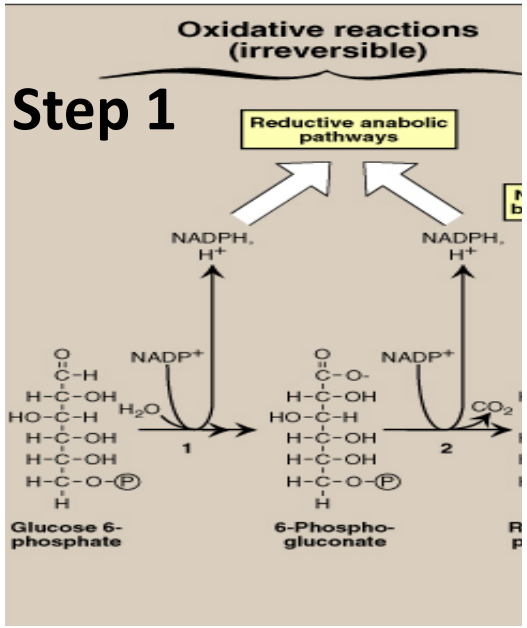
Upon completion of this lecture, the students should be able to :

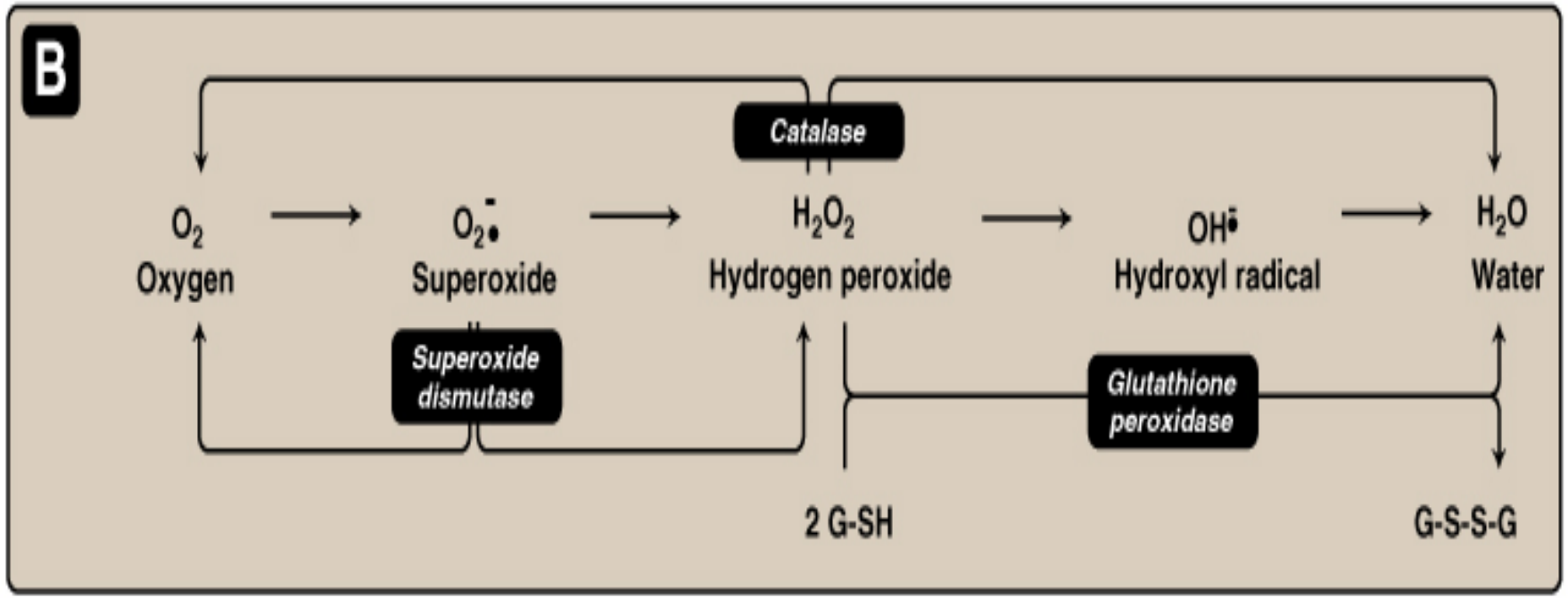
- Explain the biochemical basis of G6PD deficiency anemia
- Recognize the precipitating factors for G6PD deficiency anemia
- Classify various classes of G6PD deficiency anemia (variant enzymes)
- Describe the diagnostic methods for G6PD deficiency anemia

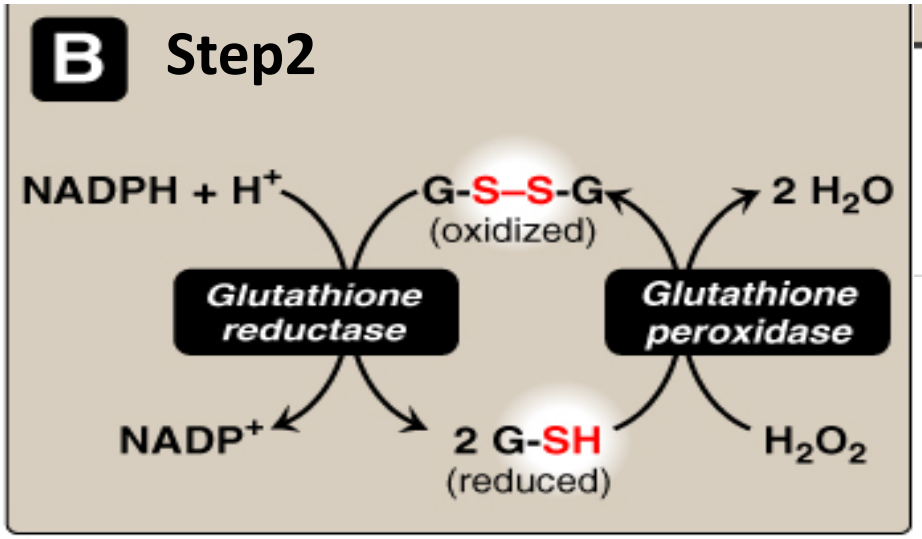
Overview


G6PD
G6PD Deficiency and hemolytic anemia
X-linked inherited disease
Biochemical basis and precipitating factors
Classes and enzymes involved
Diagnosis
Role in PPP (Pentose Phosphate Pathway)
NADPH and its uses
Anti oxidant mechanism and its role against ROS
Glutathione system and oxidative stress

Important to know







عشان ما تتلخبطون وانتوا تدرسون هذه الصور كلها خطوات تؤدي الى نفس الغرض في الاخير اللهم في كل سلايد شارحين جانب يختلف عن الاخر والصورة في السلايد اللي بعدها ملخص لكل السالفة

Important to know

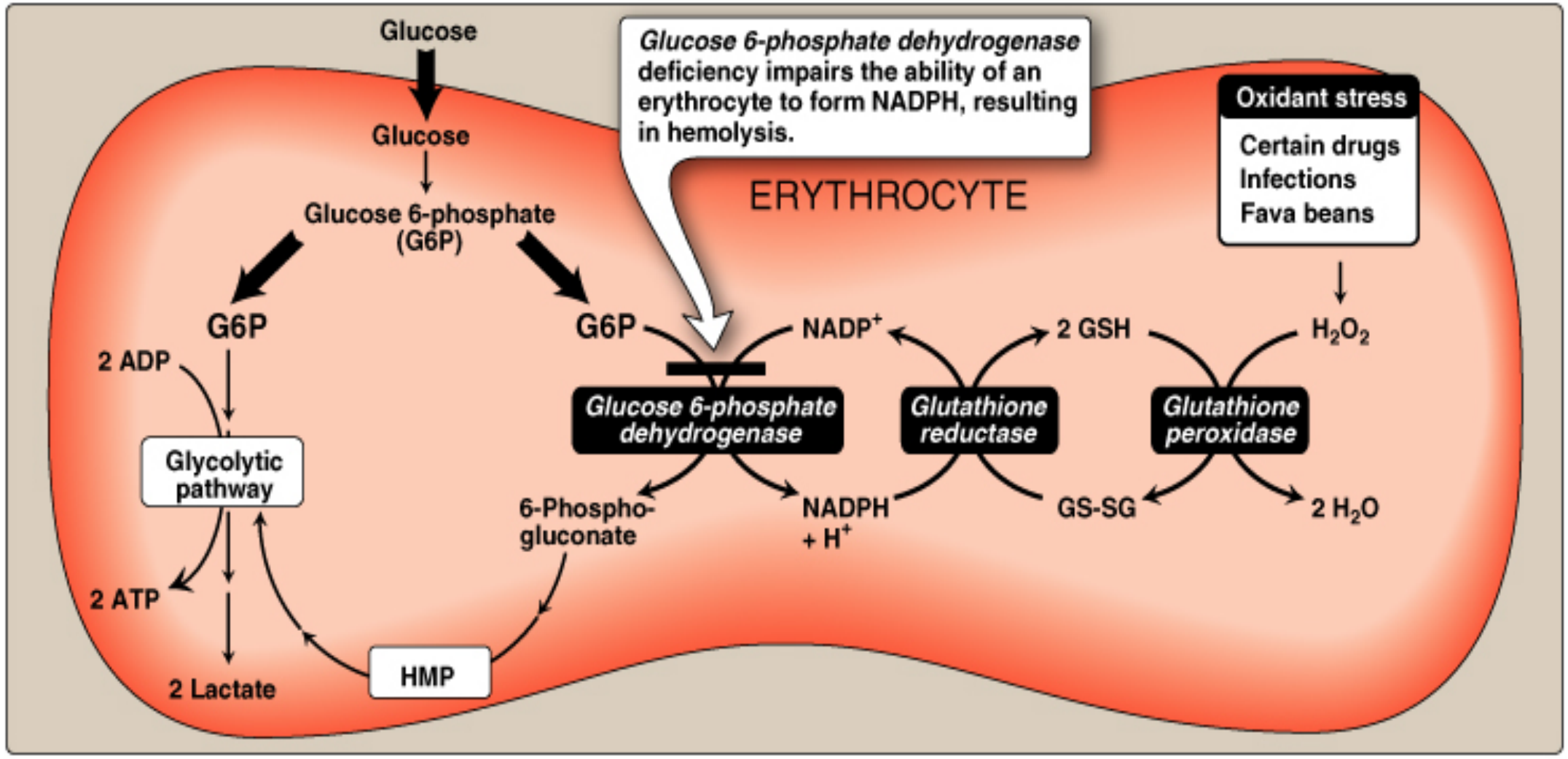


Figure 13.10

Pathways of glucose 6-phosphate metabolism in the erythrocyte.

Background

مهم تفهمون هالحكي عشان تتسهل عليكم باقي المحاضرة

❖ **For explanation :**

- Breakdown of carbohydrates (glucose) takes place in the body by glycolysis followed by tricarboxylic acid cycle (Kreb's cycle) resulting in yield of energy in the form of ATP.
- Glucose can alternatively also undergo a different pathway to produce other products required by the cells.
- One of these alternate pathway is the pentose phosphate pathway or also called as hexose monophosphate pathway in which *oxidation of glucose 6-phosphate takes place to produce pentoses which's required in producing NADPH+ as a coenzyme and ribose 6 phosphate which is required for the nucleotide synthesis*.
- The fate of glucose whether to undergo glycolysis or the hexose monophosphate pathway is decided by the relative concentrations of NADP+ and NADPH.

Background

What's hexose monophosphate or pentose phosphate pathway?

An alternative pathway for glucose

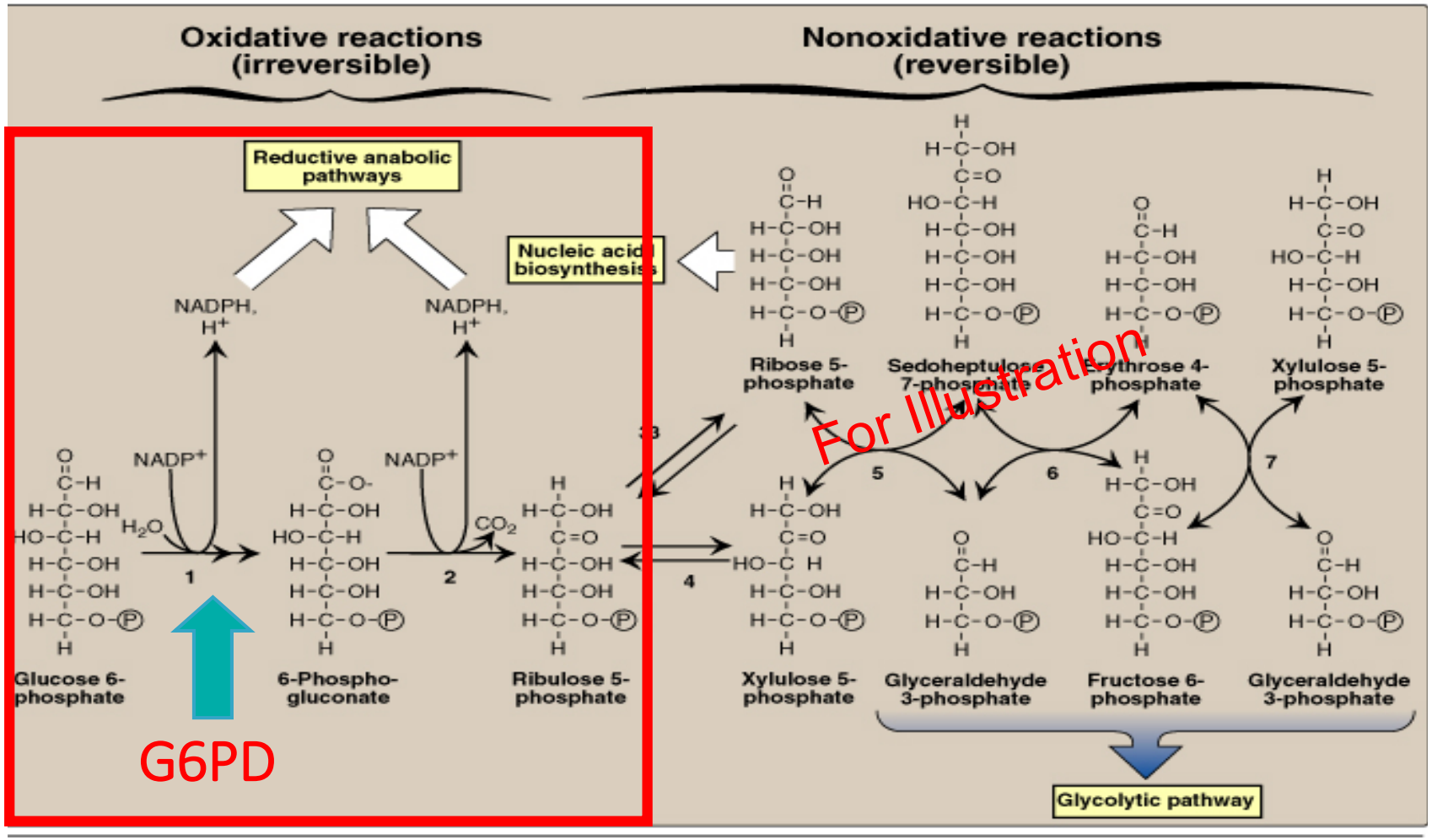
HMP or PPP products

- Produces **ribose-5-phosphate** for nucleotide synthesis
- **No ATP production**
- **Major pathway for NADPH production**

NADH transvers electrons to oxygen and simultaneously it produces ATP) (NADPH required when there is reductive biosynthesis ex: fatty acid synthesis)

Pentose Phosphate Pathway (PPP)

Also known as Hexose monophosphate pathway (HMP)



G-6-P will get converted to 6PG by the enzyme (Glucose 6 phosphate dehydrogenase) which leads to the reduction of NADP+ producing NADPH.

FOCUS on this (This reaction is catalyzed be G6PD enzyme)

You don't have to memorize the entire pathway (only the circled one)

NADPH

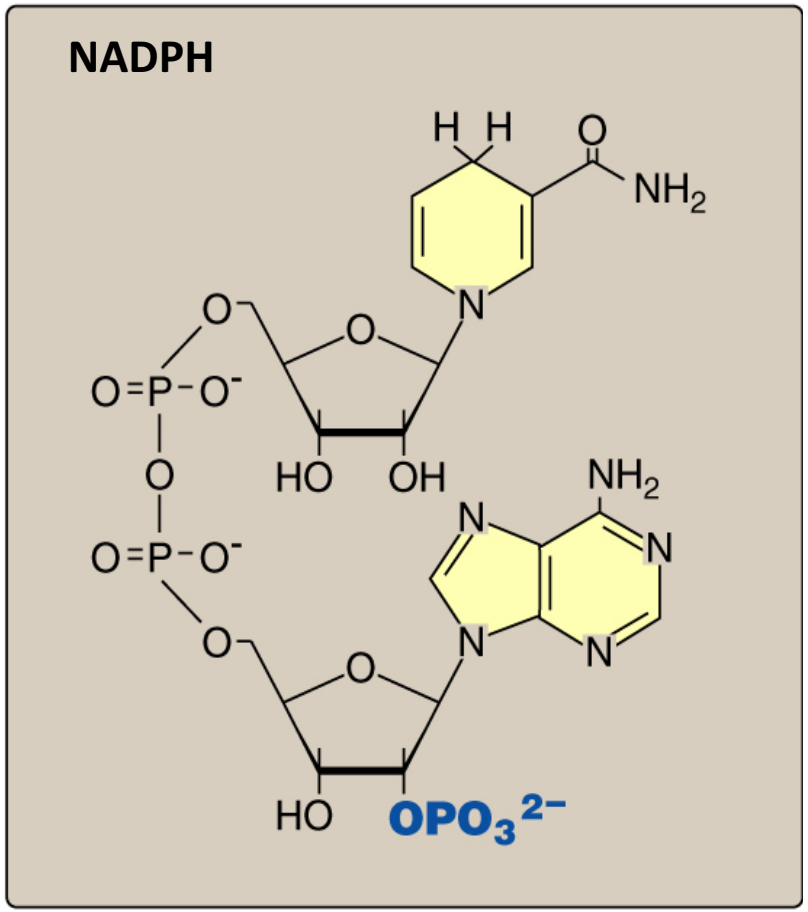
Uses of NADPH

1. **Reductive biosynthesis** e.g., fatty acid biosynthesis.

2. **Antioxidant (part of glutathione system)**.
When glutathione gets oxidized it needs to be reduced by the action of NADPH

3. **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.

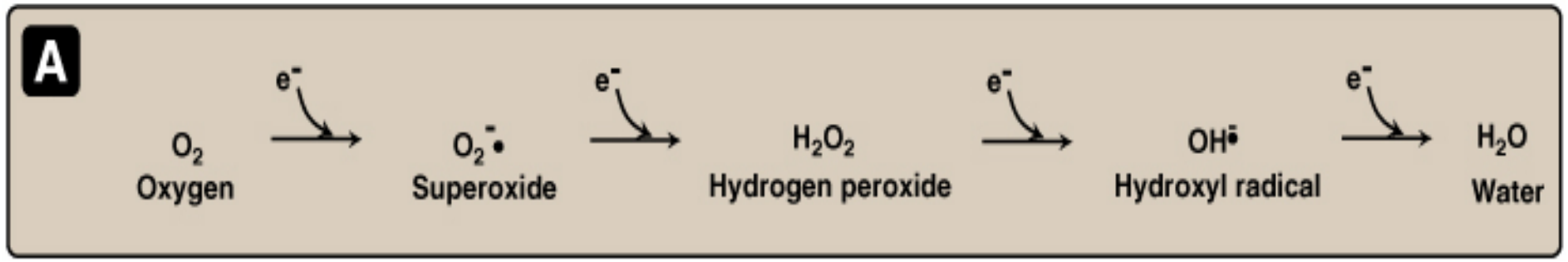
4. **Synthesis of nitric oxide (NO).** Because the enzyme nitric oxide synthase requires NADPH as a coenzyme and arginine as a substrate.



NADPH

Reactive oxidative species (ROS)

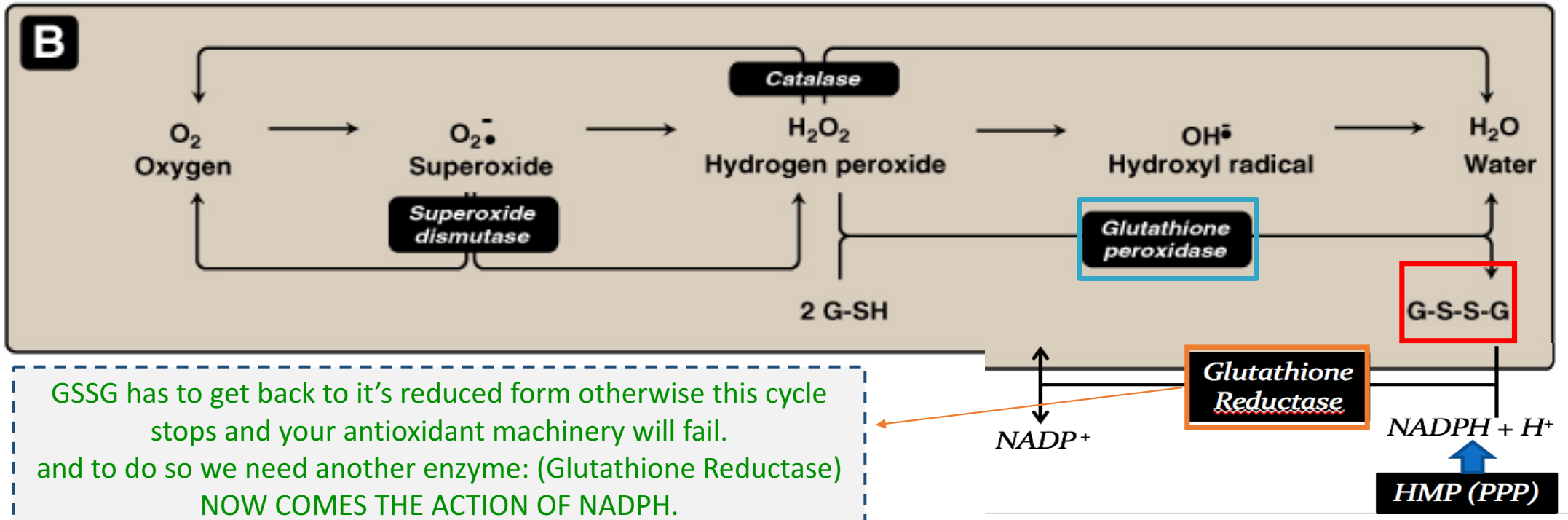
Reactive oxidative species	
Oxygen-derived free radicals	Non-free radicals
e.g. Superoxide and hydroxyl radicals because they have long chain of electrons.	e.g. Hydrogen peroxide (highly reactive)



This picture shows the process of reduction by adding an electron in each step.
Note that during the reduction of oxygen to water , free radicals are produced and the body has to decrease the amount of them normally

Antioxidant mechanism

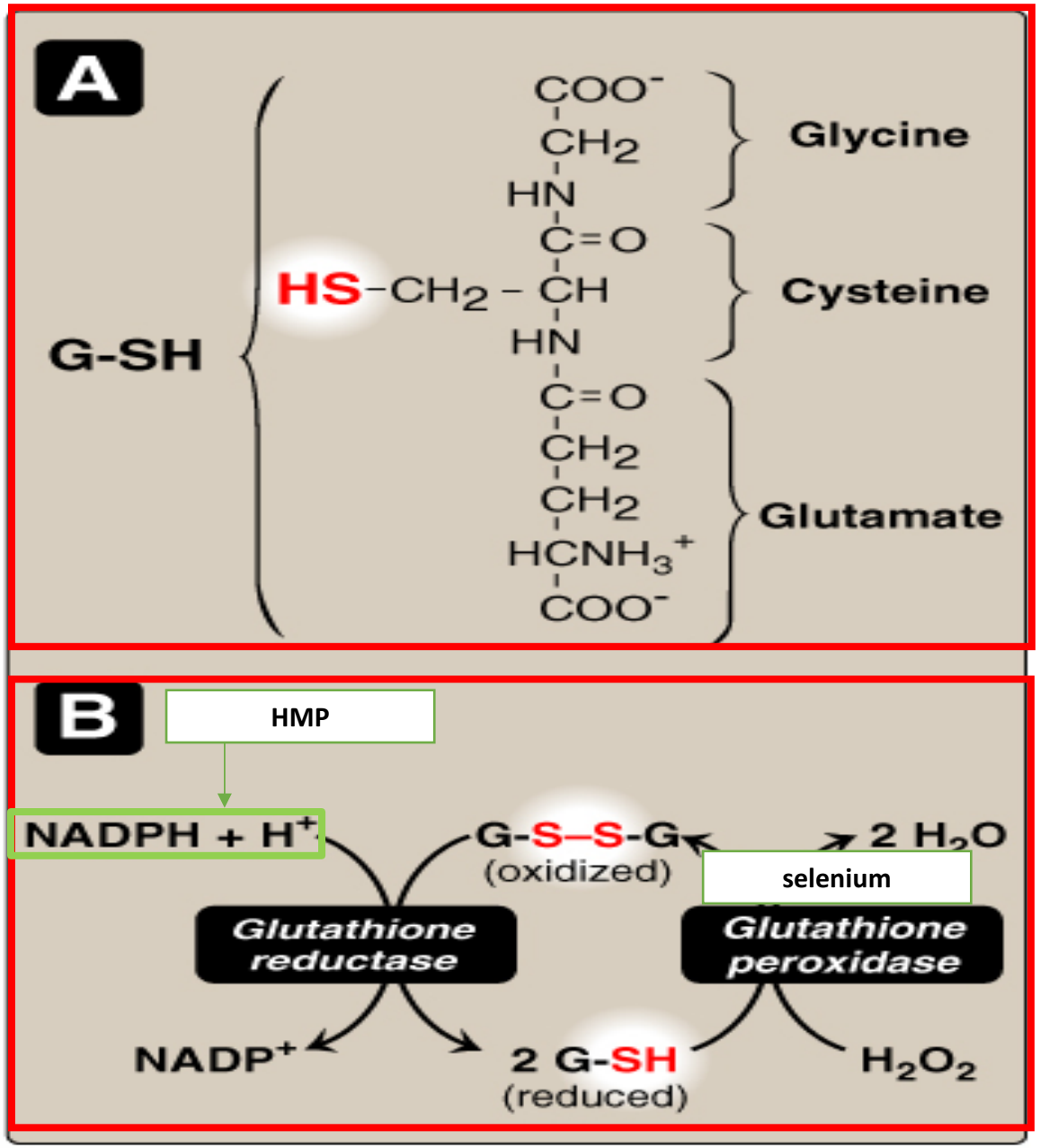
Here we will focus on Glutathione system in which NADPH is working.



GSSG has to get back to it's reduced form otherwise this cycle stops and your antioxidant machinery will fail.
and to do so we need another enzyme: (Glutathione Reductase)
NOW COMES THE ACTION OF NADPH.
From where this NADPH came from?
from PPP which is catalyzed by G-6-PD.
(conversion of glucose 6 phosphate into 6 phospho-gluconate)
كل شيء مرتبط بالآخر.
So if this enzyme is inactive or deficient PPP will not work and NADPH is not going to be produced
Which will lead to increasing in the ROS in the system which will lead to oxidative damage to the cell.

Glutathione peroxidase reduces H2O2 into water at the same time converts Glutathione into it's oxidized form GSSG.
هذا الإنزايم يحتاج سلينيم عشان يشتغل.

Glutathione system



When we have G6PD deficiency all of the cells will get affected (RBCs in specific) because other cells have their main machinery to make NADPH, Malic dehydrogenase enzyme can make NADPH when it converts it to pyruvate.
RBC the only source of NADPH is PPP.

This system is also known as **gamma glutamyl cystinyl glycine**.

Pic. B : Glutathione reduced detoxifies H2O2 to water and itself gets oxidized and gets reduced again by glutathione reductase which requires NADPH and it's supplied by PPP

- ❖ HMP provides NADPH which provides the reducing equivalent to the oxidized form of glutathione.
- Then , in the presence of glutathione reductase, 2 molecules of reduced GSH are formed.
- 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

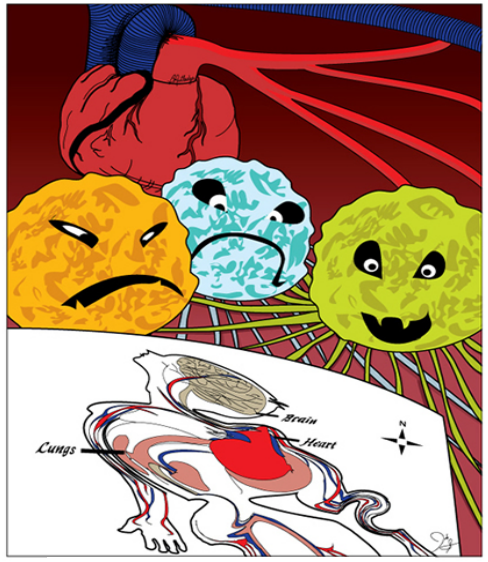
What is it ?

Imbalance between oxidant production and antioxidant mechanism

Causes Oxidative damage to

1. DNA
2. Proteins
3. Lipids *(unsaturated fatty acids)*

ROS



Oxidative stress and diseases

- **Inflammatory conditions** e.g., Rheumatoid arthritis
- Atherosclerosis and coronary heart diseases
- Obesity
- Cancers
- G6PD deficiency hemolytic anemia

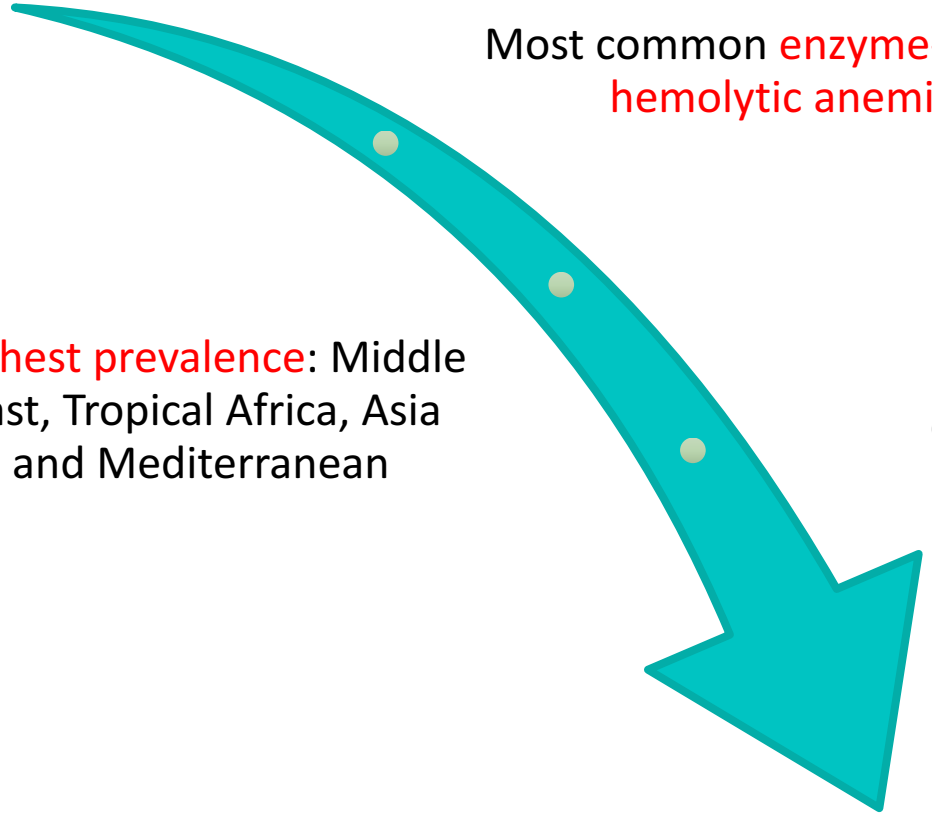
G6PD deficiency hemolytic anemia

Inherited X-linked recessive disease

Most common enzyme-related hemolytic anemia

Highest prevalence: Middle East, Tropical Africa, Asia and Mediterranean

~ 400 different mutations (Point mutations) affect G6PD gene, but only some can cause clinical hemolytic anemia



G6PD deficient patients have increased resistance to infestation by falciparum malaria

Because F.Malaria needs RBCs to complete it's life cycle. The RBCs in G6PD deficient patient are dying (no RBCs for the malaria to live inside)

Precipitating factors of G6PD deficiency hemolytic anemia

1. Intake of oxidant drugs (AAA)

1. **Antibiotic** e.g: sulfa preparation
2. **Antimalarial** e.g: primaquine
3. **Antipyretic** (Fever drugs)

- Because these drugs increase production of reactive nitrogen species.

2. Ingestion of fava beans

1. Favism
2. Mediterranean variant

- They increase production of H2O2 BUT NOT IN EVERY ONE.

3. Exposure to infection

❖ **Chronic non-spherocytic anemia:**

✓ Hemolytic attack in absence of precipitating factors.

✓ Severe form due to **class I mutation**

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

✓ When that happens the membrane become more rigid. Which leads to increase break down of RBCs.

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

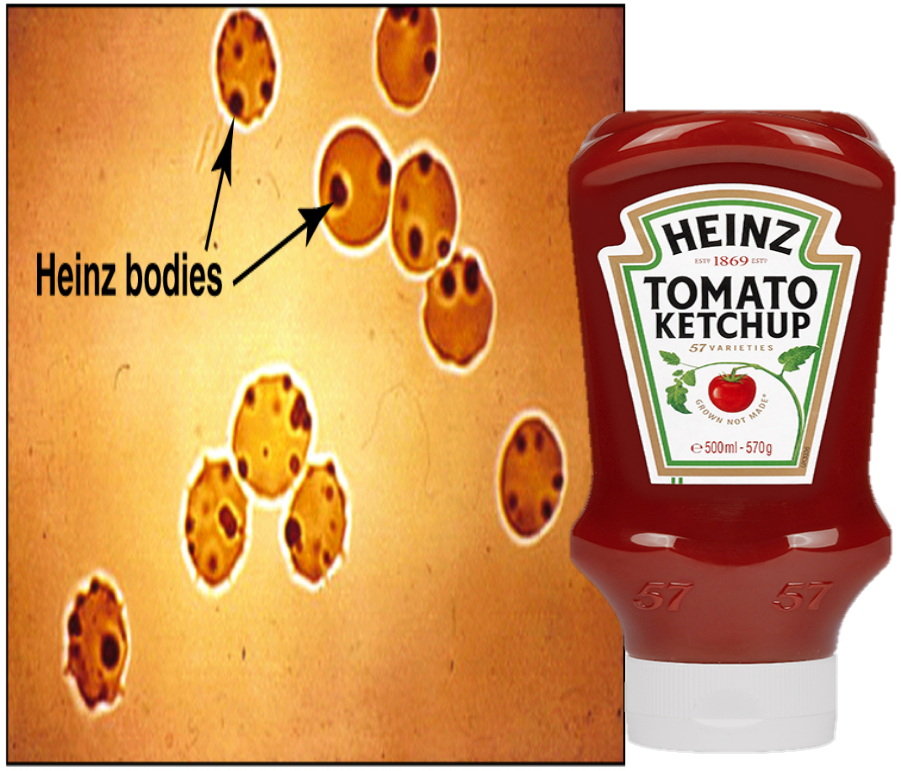


Figure 13.11

Heinz bodies in erythrocytes of patient with *G6PD* deficiency.

Biochemical basis of G6PD deficiency hemolytic anemia

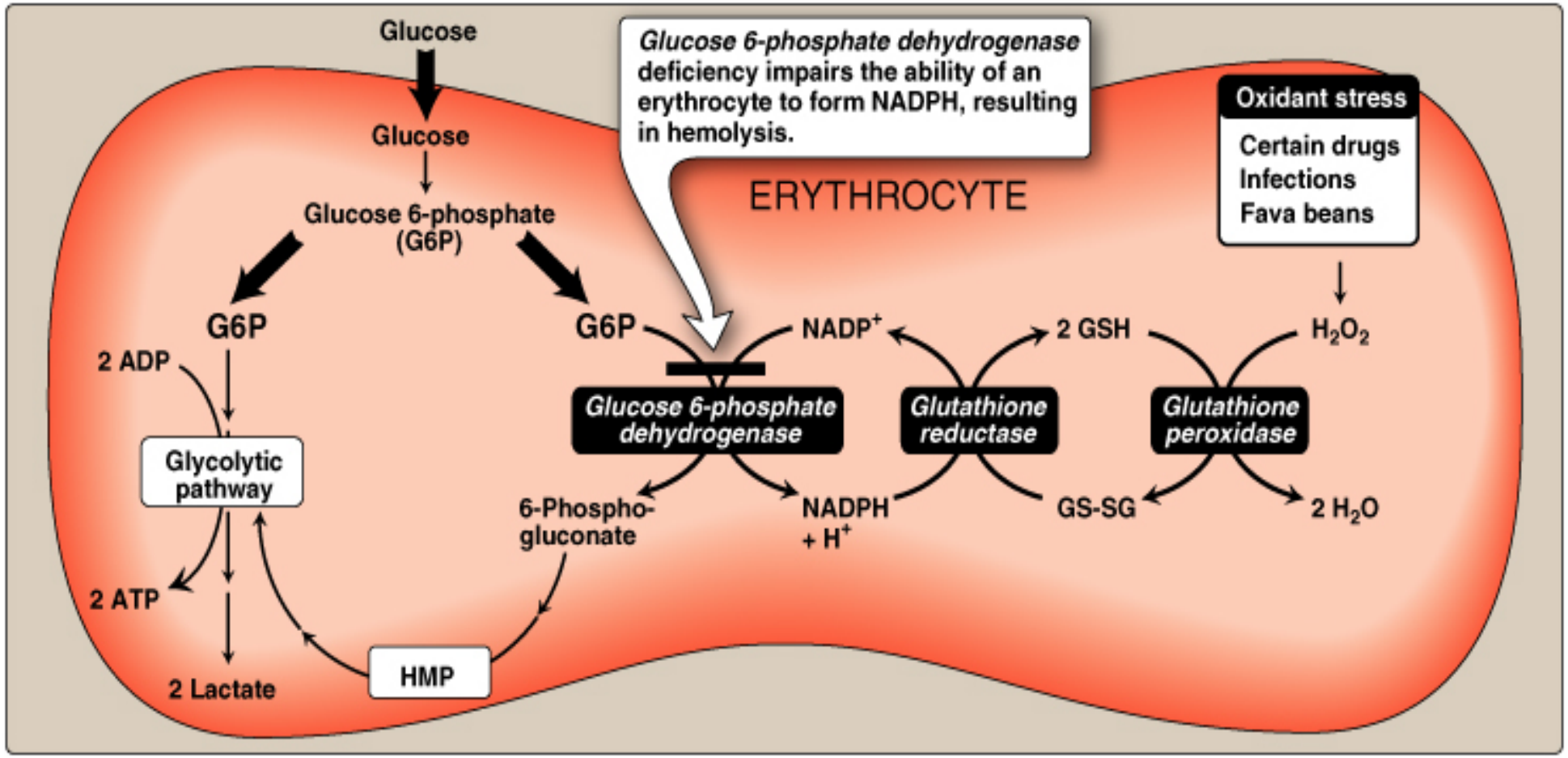


Figure 13.10

Pathways of glucose 6-phosphate metabolism in the erythrocyte.

Anything increases the ROS will increase the severity of hemolysis For example when the hemolytic anemia patient has an infection the macrophages will start producing ROS which will be excessive in the addition of the ROS in the body and will diffuse to the RBCs and kill them

Classes of G6PD deficiency hemolytic anemia

❖ **This classification is based on the residual enzyme activity** *(Least enzyme activity in class I, and Highest enzyme activity in class IV)*

There are four different classes

- **I (Very severe)**
- **II episodic (Severe, e.g. Mediterranean)**
- **III: (Moderate: G6PD A-)**
- **IV: (Normal)**

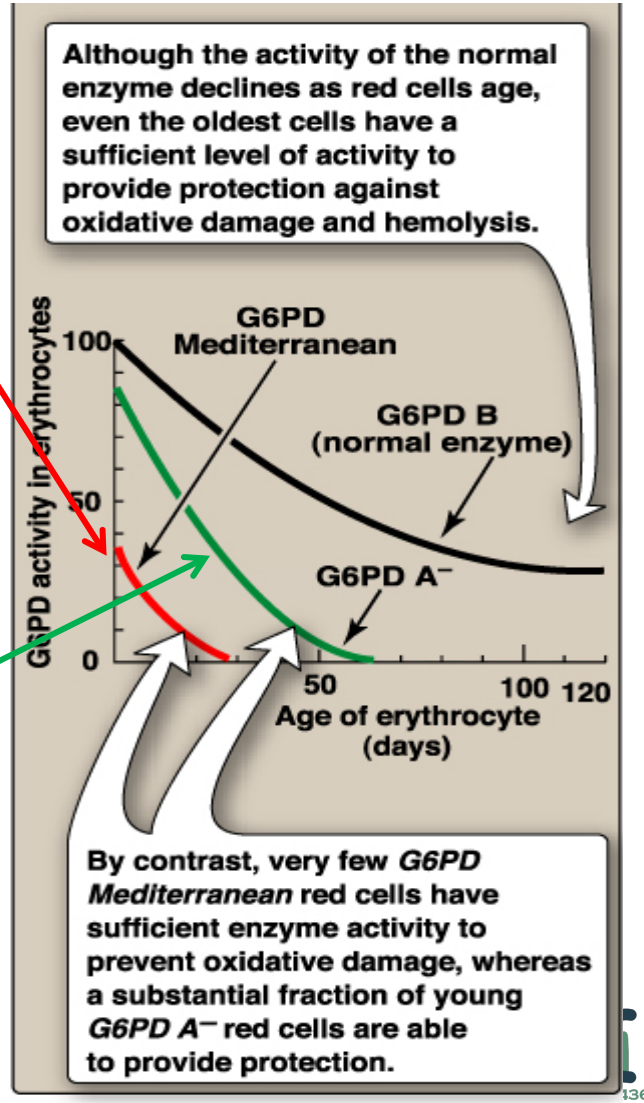
Variant enzymes of G6PD deficiency hemolytic anemia

G6PD Mediterranean (II)

- Enzyme with decreased stability and activity (severe).
- Affect all RBCs (both young and old)

G6PD A- (class III):

- Moderate, young RBCs
- contain enzymatic activity.
- Unstable enzyme, but kinetically normal



Diagnosis of G6PD deficiency hemolytic anemia

Investigation	Results
Diagnosis of hemolytic anemia	1. Complete Blood Count (CBC) 2. Reticulocytic count immature RBCs
Screening	Qualitative assessment of G6PD enzymatic activity (UV-based test)
Confirmatory test	Quantitative measurement of G6PD enzymatic activity
Molecular test **to detect what kind of mutation**	Detection of G6PD gene mutation

Explanation

- 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 severe infections.
- G6PD deficiency is classified according to the residual activity of the G6PD
- Class I variant (the most severe) class is associated with chronic non-spherocytic hemolytic anemia.

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 severe infections.
- G6PD deficiency is classified according to theresidual activity of the G6PD
- Class I variant (the most severe) class isassociated with chronic nonspherocytic hemolyticanemia.

Summary

Uses of NADPH	• Reductive biosynthesis. • Antioxidant (part of glutathione system). • Oxygen-dependent phagocytosis by WBCs. • Synthesis of nitric oxide (NO)
Oxidative Stress: Imbalance between oxidant production and antioxidant mechanisms.	Oxidative damage to: DNA ,Proteins and Lipids (unsaturated fatty acids) diseases: Inflammatory conditions - Atherosclerosis and coronary heart diseases – Obesity - Cancers - G6PD deficiency hemolytic anemia
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) • most severe in RBCs because **Other cells have other sources for NADPH production** • **Precipitating Factors**: **1-**Intake of oxidant drugs. **2-**Exposure to infection **3-**Ingestion of fava beans • **Different Classes: I (Very severe)/ II (Severe) / III: (Moderate) / IV: (Normal)** • **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.

QUIZ

Q1 : Which one of these can produce NADPH ?

A. Malic enzyme
B. Transferrin
C. Superoxide dismutase
D. All of them

Q2 : Which of the following best describes biochemical consequences of G6PD deficiency that leads to hemolytic anemia ?

A. Low RBC Hemoglobin
B. Low NADPH
C. Low Glucose 6-Phosphate
D. Deficient Aspartate aminotransferase

Q3 : Which of the following is a Precipitating Factor for G6PD Deficiency Hemolytic Anemia ?

A. Captopril
B. Mediterranean Tomato Salad
C. Anti-Epileptic Drugs
D. bacillary dysentery “Shigella infections”

Q4 : Which one of these is NOT a diagnostic method for G6PD Deficiency Hemolytic Anemia ?

A. Detection of G6PD gene mutation
B. Complete Blood Count (CBC)
C. Radiological methods
D. Qualitative assessment of G6PD enzymatic activity

Q5 : Which one of the following enzymes converts H2O2 to H2O?

A. Glutathione Peroxidase
B. Glutathione Reductase
C. Glutathione Synthetase
D. None of them

Q6 : Oxidation of sulfhydryl (SH) groups of proteins inside RBCs lead to ?

A. Protein denaturation
B. Formation of insoluble masses (Heinz bodies)
C. Production ATP
D. A & B

QUIZ

Q7 : In male patients who are homozygous for glucose 6-phosphate dehydrogenase (G6PD) deficiency, pathophysiologic consequences are more apparent in erythrocytes (RBC) than in other cells, such as, in the liver. Which one of the following provides the most reasonable explanation for this different response by these individual tissue types ?

A. Excess glucose-6-phosphate in the liver, but not in RBCs, can be channeled to glycogen, thus averting cellular damage.
B. Liver cells, in contrast to RBCs, have alternative mechanisms for supplying the NADPH required for keeping metabolic and cellular integrity.
C. Glucose 6-phosphatase activity in RBCs removes the excess glucose 6-phosphate, thus resulting in cell damage. This does not happen in the hepatocyte.
D. Because RBCs do not have mitochondria, production of ATP required to keep cell integrity depends exclusively on the routing of glucose 6-phosphate to the pentose phosphate pathway.
E. The catalytic properties of the liver enzyme are significantly different than those of the RBC enzyme.

Q8 : What is the name of Major pathway for NADPH production?

Pentose Phosphate Pathway (PPP).

Q9 : What is the difference between G6PD Mediterranean and G6PD A ?

G6PD Mediterranean (class II) :Enzyme with normal stability but low activity (severe) . Affect all RBCs (both young and old)
G6PD A- (class III): unstable enzyme, but kinetically normal (moderate). Young RBCs

Q10 : G6PD Deficiency is classified into 4 classes according to what ?

Residual enzyme activity

Suggestions and recommendations

1) A 2) B 3) D 4) C 5) A 6) D 7) B

Biochemistry Team 436

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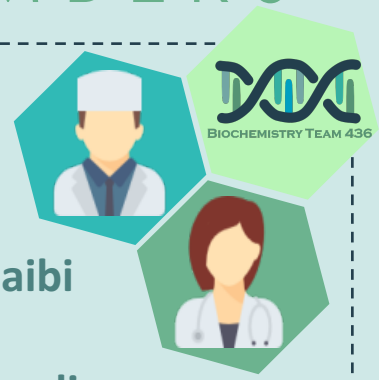

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THANK YOU

FOR CHECKING OUR WORK

PLEASE CONTACT US IF YOU HAVE ANY ISSUE

- Lippincott's Illusrated Reviews Biochemistry 6th E

Review the notes

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