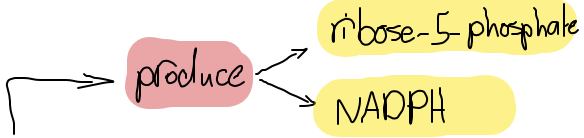
Pentose phosphate pathway and NADPH



The pentose phosphate pathway

1 2

- also called the hexose monophosphate shunt or 6-phosphogluconate pathway
- It occurs in the **cytosol** of the cell. → (NADPH) انتاج
- It consists of two, irreversible oxidative reactions, followed by a series of reversible sugar-phosphate interconversions
- **No ATP** is directly consumed or produced in the cycle.
- Carbon one of glucose 6-phosphate is released as CO_2, and two NADPH are produced for each glucose 6-phosphate molecule entering the oxidative part of the pathway. => One glucose 6-phosphate enter oxidative part of pathway produce :- ① CO_2 ② two NADPH
- The pathway provides a major portion of the body's NADPH, which functions as a biochemical reductant.

هذا المسار يحطي كمية كبيرة من (NADPH) ← يعمل كمركب اختزالي في التفاعلات الحيوية (يعني يصير له اكسدة)

The pentose phosphate pathway

- Ribose 5-phosphate is required for the biosynthesis of nucleotides and provides a mechanism for the metabolic use of five-carbon sugars obtained from the diet or the degradation of structural carbohydrates in the body.

- The oxidative portion of the pentose phosphate pathway occurs in:
  - **Liver** and **lactating mammary glands**, which are active in the biosynthesis of fatty acids
  - **Adrenal cortex**, which is active in the NADPH-dependent synthesis of steroids
  - **Erythrocytes**, which require NADPH to keep glutathione reduced.

محتزل

عشان تتخلص من H_2O_2 و free radical

NADPH + CO_2 + ribulose-5P ← (6-PG dehydrogenase) 6-PG ← (6-PGL hydrolase) 6PGL + NADPH ← (G-6-P dehydrogenase) G-6-P

يعني

Irreversible oxidative reactions

تحويل G-6-P ← 6-PGL عن طريق الإنزيم G-6P dehydrogenase

Dehydrogenation of glucose 6-phosphate (the rate limiting step)

remove hydrogen (H_2)

- **Glucose 6-phosphate dehydrogenase** (G6PD) catalyzes an irreversible oxidation of glucose 6-phosphate to 6-phosphogluconolactone in a reaction that is specific for NADP as its coenzyme which produce one molecule of NADPH
  - maybe glucose one phosphate
- The enzyme is competitively inhibited by NADPH so its regulated by the NADP/NADPH ratio in the cell
  - increase → inhibition enzyme
- Insulin enhances G6PD gene expression (well-fed state)
  - increase → activation enzyme
  - لـ يعني هذا الانزيم شغال وفعال
  - لـ بعد الأكل

Formation of ribulose 5-phosphate

- Phosphogluconolactone is hydrolyzed by **6-phosphogluconolactone hydrolase** (irreversible and not rate-limiting).
- The subsequent oxidative decarboxylation of 6-phosphogluconate is catalyzed by **6-phosphogluconate dehydrogenase** (irreversible) to produce a pentose sugar-phosphate (ribulose 5-phosphate), CO2 (from carbon 1 of glucose), and a second molecule of NADPH

irreversible oxidative reaction produce 2NADPH CO_2 ribulose-5P

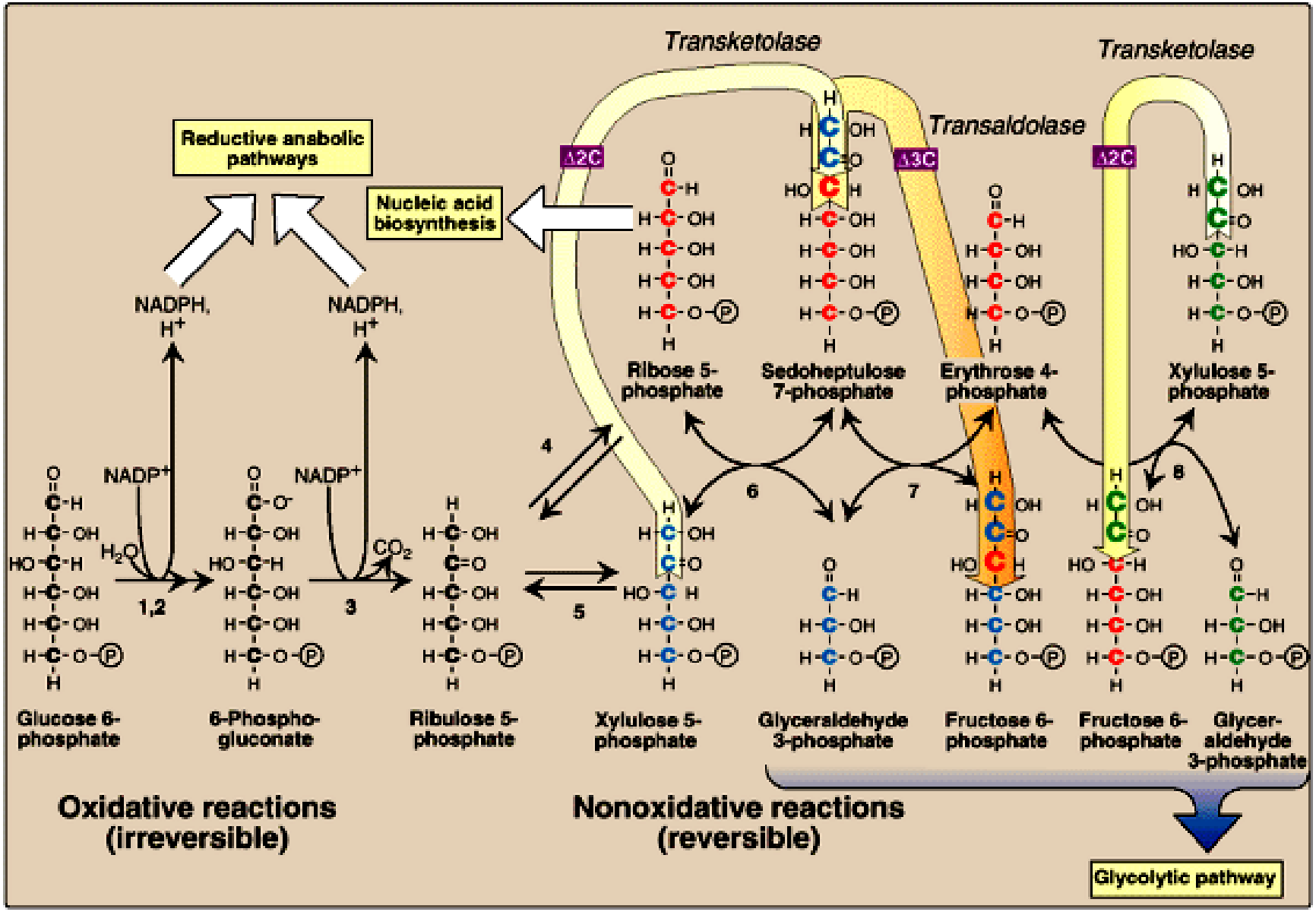
Reversible nonoxidative reactions

- The nonoxidative reactions of the pentose phosphate pathway occur in all cell types synthesizing nucleotides and nucleic acids. These reactions catalyze the interconversion of three-, four-, five-, six-, and seven- carbon sugars.

- These reversible reactions permit ribulose 5-phosphate to be converted either to[1] ribose 5-phosphate or to[2] intermediates of glycolysis-fructose 6- phosphate and glyceraldehyde 3-phosphate.

- In reductive biosynthetic reactions, there is a great need for NADPH, so transketolase (which transfers two-carbon units) and transaldolase (which transfers three-carbon units) convert the ribulose 5-phosphate to glyceraldehyde 3-P and fructose 6-P, which are intermediates of glycolysis

- At increased demands for ribose to synthesize nucleic acids, the non-oxidative reactions can provide the biosynthesis of ribose 5-P from G-3-P and F-6-P in the absence of the oxidative steps


Transketolase
Transketolase
Transaldolase
Reductive anabolic pathways
Nucleic acid biosynthesis
Δ2C
Δ3C
Δ2C
NADPH, H+
NADPH, H+
NADP+
NADP+
H2O
CO2
1,2
3
4
5
6
7
8
Ribose 5-phosphate
Sedoheptulose 7-phosphate
Erythrose 4-phosphate
Xylulose 5-phosphate
Glucose 6-phosphate
6-Phospho-gluconate
Ribulose 5-phosphate
Xylulose 5-phosphate
Glyceraldehyde 3-phosphate
Fructose 6-phosphate
Fructose 6-phosphate
Glycer-aldehyde 3-phosphate
Oxidative reactions (irreversible)
Nonoxidative reactions (reversible)
Glycolytic pathway

حسب تركيز NADPH, NADH يتم استخدام أحدهم في عمليات ال metabolism

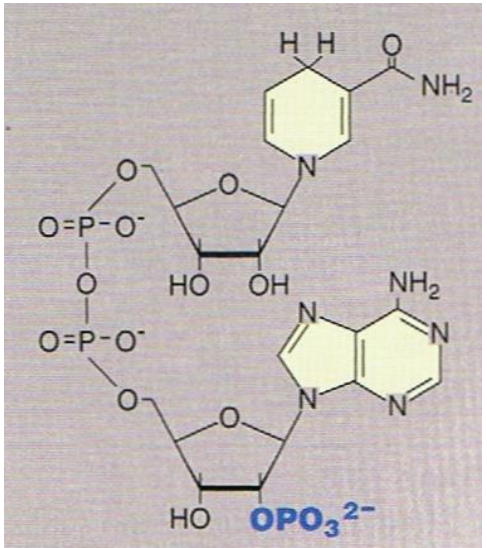
NADPH

reduction process :- oxidation NADPH to $NADP^+$

oxidation process :- reduction NAD^+ to NADH

لـ نسبة أكبر من NADH

- The coenzyme NADP differs from NAD only by the presence of a phosphate group (PO_4^-) on one of the ribose units
- The steady-state ratio of NADP/NADPH in the cytosol of hepatocytes is approximately 0.1, which favors the use of NADPH in reductive biosynthetic reactions
- This contrasts with the high ratio of NAD/NADH approximately 1000 in the cytosol of hepatocytes, which favors an oxidative role for NAD



Uses of NADPH

A. Reduction of hydrogen peroxide

- Hydrogen peroxide is formed from the partial reduction of molecular oxygen

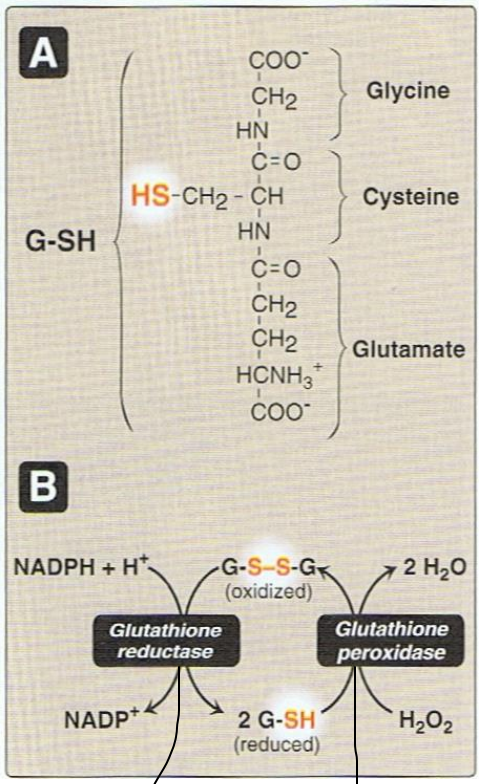
- It is formed continuously as by-products of aerobic metabolism, through reactions with drugs and environmental toxins, or when the level of antioxidants is diminished, all creating the condition of oxidative stress. (causes this condition) → three point above

- These highly reactive oxygen intermediates can cause serious chemical damage to DNA, proteins, and unsaturated lipids, and can lead to cell death.

- The cell has several protective mechanisms that minimize the toxic potential of these compounds.

Uses of NADPH
A. Reduction of hydrogen peroxide

- Enzymes that catalyze antioxidant reactions:
  - Reduced glutathione, a tripeptide-thiol present in most cells, can chemically detoxify hydrogen peroxide that is catalyzed by the selenium-requiring glutathione peroxidase, forms oxidized glutathione, which no longer has protective properties
  - The cell regenerates reduced glutathione in a reaction catalyzed by glutathione reductase, using NADPH as a source of reducing electrons. NADPH indirectly provides electrons for the reduction of hydrogen peroxide
  - Erythrocytes are totally dependent on this pathway for their supply of NADPH so any defect, hydrogen peroxide will accumulate, threatening membrane stability and causing red cell lysis

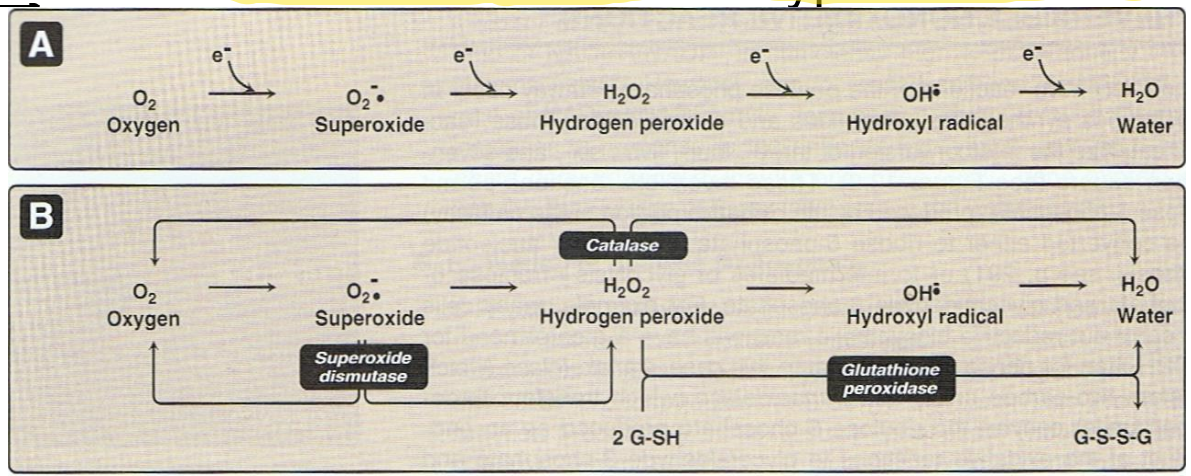


Uses of NADPH
A. Reduction of hydrogen peroxide

- Superoxide dismutase and catalase, catalyze the conversion of other toxic oxygen intermediates to harmless products so guard the cell against the toxic effects of reactive oxygen species.

- Antioxidant chemicals: A number of intracellular reducing agents such as ascorbate, vitamin E, and β-carotene, are able to reduce and detoxify oxygen intermediates in the laboratory.

- Consumption of foods rich in these antioxidant compounds has been correlated with a reduced risk for certain types of cancers



Uses of NADPH

B.Cytochrome P450 monooxygenase system

- Monooxygenases incorporate one atom from molecular oxygen into a substrate (creating a hydroxyl group), with the other atom being reduced to water. ← تكوين
- In the cytochrome P450 monooxygenase system, NADPH provides the reducing equivalents required by this series of reactions
  (لـ عامل اختزال (مصدر الإلكترونات
- The overall reaction catalyzed by a cytochrome P450 enzyme is:

$$R\text{-}H + O_2 + NADPH + H^+ \xrightarrow{\text{CYP بوجود}} R\text{-}OH + H_2O + NADP^+$$

- where R may be a steroid, drug, or other chemical ← increase polarity → increase excretion
- Mitochondrial system: involved in the hydroxylation of steroids that makes them more water soluble.
  - in the steroid hormone-producing tissues, such as the placenta, ovaries, testes, and adrenal cortex, it is used to hydroxylate intermediates in the conversion of cholesterol to steroid hormones
  - The liver uses this system in bile acid synthesis
  - the kidney uses it to hydroxylate vitamin 25-hydroxycholecalciferol (vitamin D) to its biologically active 1 ,25-hydroxylated form.

Uses of NADPH
B.Cytochrome P450 monooxygenase system

- Microsomal system: found associated with the membranes of the smooth endoplasmic reticulum (particularly in the liver) is the detoxification of foreign compounds (xenobiotics). These include numerous drugs and such varied pollutants as petroleum products, carcinogens, and pesticides

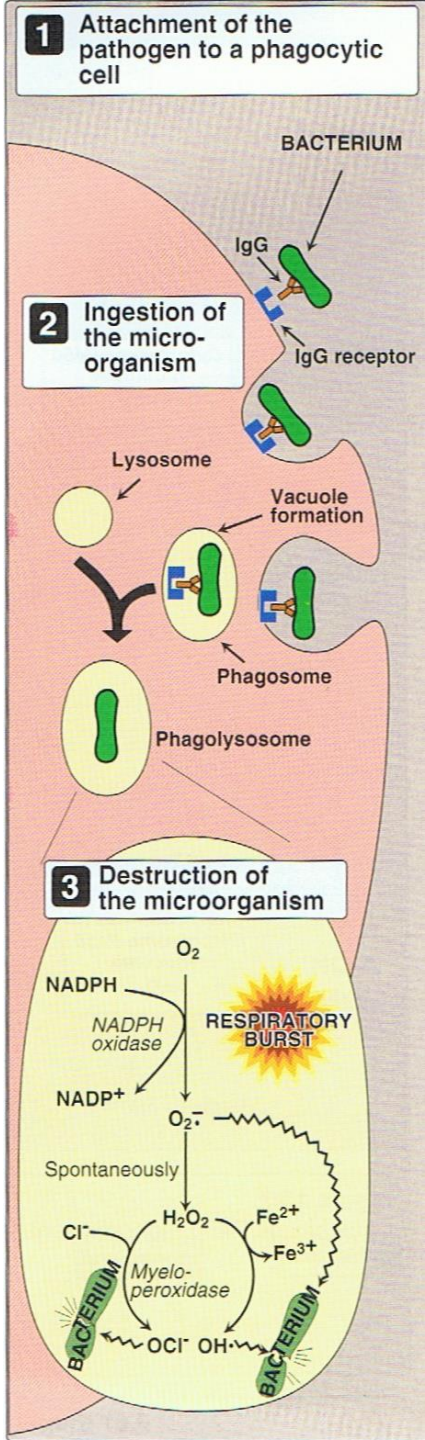
لـ سرطانات لـ مبيدات حشرية لـ ملوثات متنوعة مثل مشتقات البترول

- It can be used to hydroxylate these toxins, using NADPH as the source of reducing equivalents in order to:
  - activate or inactivate a drug
  - make a toxic compound more soluble, thus facilitating its excretion in the urine or feces
  - Frequently the new hydroxyl group will serve as a site for conjugation with a polar compound, such as glucuronic acid, which will significantly increase the compound's solubility.

Uses of NADPH

C.Phagocytosis by white blood cells

- NADPH provides the reducing equivalents for phagocytes in the process of eliminating invading microorganisms
  الغازية
- **NADPH oxidase** uses molecular oxygen and NADPH electrons to produce superoxide radicals, which can be converted to peroxide, hypochlorous acid, and hydroxyl radicals using Myeloperoxidase enzyme.
  ← تحطيم الميكروبات حيث أن هذه المركبات تهاجم المكونات الحيوية للبكتيريا (DNA)
- A genetic defect in NADPH oxidase causes chronic granulomatosis, a disease characterized by severe, persistent, chronic infections.
  ← مستمرة ← تستمر ← الجزيء الذي يصير فيه تدمير البكتيريا
- Any superoxide that escapes the phagolysosome is converted to hydrogen peroxide by superoxide dismutase (SOD).
  ← يتحول إلى ماء
- Excess peroxide is either neutralized by catalase or by glutathione peroxidase



Uses of NADPH

D. Synthesis of nitric oxide

(vasodilation)

- Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems.

function of nitric oxide ?!

- NO is the endothelium-derived relaxing factor, which causes (1) **vasodilation** by relaxing vascular smooth muscle. NO also acts as a (2) **neurotransmitter**, (3) prevents **platelet aggregation**, and (4) plays an essential role in **macrophage function**

- NO is a free radical gas that has a very short half-life in tissues (three to ten seconds) because it reacts with oxygen and superoxide, and then is converted into nitrates and nitrites.

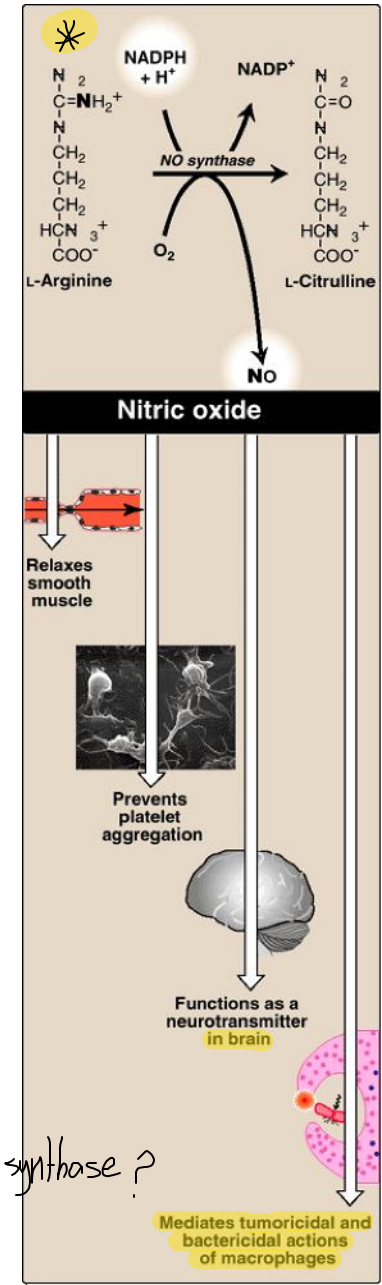
why ?!

- Synthesis of NO:

L-arginine + O_2 —(NO synthase; NADPH → NADP+)→ L-Citrulline + NO

  - It is synthesized by the cytosolic NO synthase.
  - (1) Flavin mononucleotide (FMN), (2) flavin adenine dinucleotide (FAD), (3) heme, and (4) tetrahydrobiopterin are coenzymes for the enzyme → NO synthase ?

four



Glucose 6-Phosphate dehydrogenase deficiency

- This deficiency is a genetic disease characterized by hemolytic anemia. G6PD deficiency impairs the ability of the cell to form the NADPH that is essential for the maintenance of the reduced glutathione pool.
- The cells most affected are the red blood cells because they do not have additional sources of NADPH. Free radicals and peroxides formed within the cells cannot be neutralized, causing denaturation of protein (as hemoglobin) and membrane proteins.
- The cells become rigid, and they are removed by the reticuloendothelial system of the spleen and liver.
- Hemolytic anemia can be caused by the production of free radicals and peroxides following the taking of oxidant drugs, ingestion of fava beans or severe infections.

Glucose 6-Phosphate dehydrogenase deficiency

- Babies with G6PD deficiency may experience neonatal jaundice appearing one to four days after birth.
- The degree of severity of the anemia depends on the location of the mutation in the G6PD gene
- Class I mutations are the most severe (for example, G6PD Mediterranean). They are often associated with chronic nonsphercytic anemia.
- Class III mutations (for example, G6PD A-) cause a more moderate form of the disease