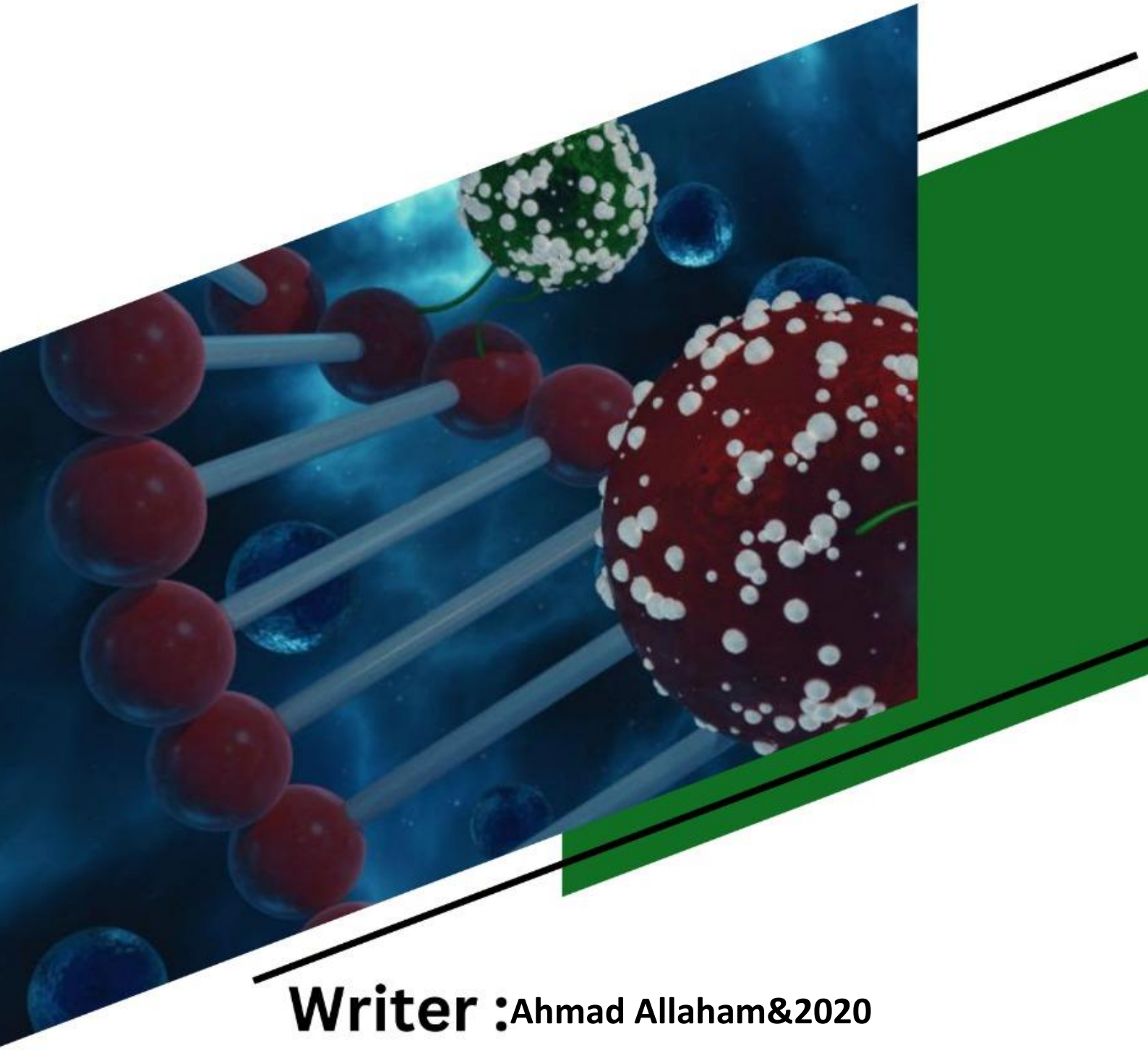
Doctor 021

METABOLISM

Sheet no. 18



Writer :Ahmad Allaham&2020

Corrector :Ahmad Allaham&Rama harb

Doctor : Dr.Diala

Precipitating Factors in G6PD Deficiency

- **Oxidant drugs**
  - **– Antibiotics e.g. Sulfomethxazole**
  - **– Antimalaria Primaquine**
  - **– Antipyretics Acetanalid**
- **Favism due to vicine and covicine in fava beans in some G6PD deficient patients**
- **Infection**
- **Neonatal Jaundice**

-anything that increases oxidative stress exaggerates the deficiency of G6PD, also infections because of the increased production of ROS, neonatal jaundice yellowing of the neonate it's a problem in development especially in those who are born prematurely the enzymes concentration isn't high enough which leads to accumulation of bilirubin.
If the bilirubin concentration is too high we put the infant in an Incubator and make them get exposed to blue fluorescent light which converts bilirubin into a more soluble format but not the exact same to the body's doing however they are similar. Remember that the bilirubin comes from the metabolism of the heme groups.

Clinical Hint: G6PD Deficiency

- **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**

GLUCOSE-6-PHOSPAHTE DEHYDROGENASE DEFICIENCY

In this lecture we will start by discussing a **medical application** for PPP, which is Glucose-6-Phospahte dehydrogenase deficiency also known as التفول , because **fava beans** contain some compounds that increase oxidative stress in cells that induces this condition.

More than 400 mutation can cause G6PD deficiency and every mutation controls the severity of the condition .therefore, this disease is variable among different patients.

Let's take a look on a patient's RBC and why they develop hemolytic anemia :

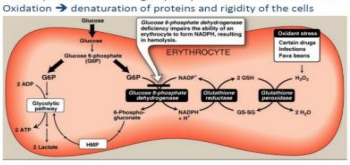
Role of G6PD in red blood cells

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

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

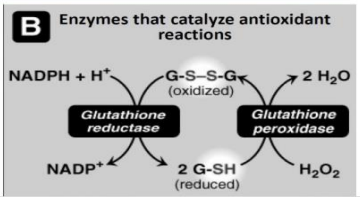
GSH helps maintain the SH groups in proteins in the reduced state

Oxidation → denaturation of proteins and rigidity of the cells



In the case of G6PD deficiency NADPH amounts are decreased so when ROS are made either by infections, fava beans or drugs that will lead to the depletion of NADPH, So the ability for glutathione to fight ROS is going to be reduced **(glutathione won't return to it's reduced state " GSH " that function against ROS)** This will lead to increased oxidative stress in RBCs which will cause their hemolysis.

(this is just a single example on how G6PD deficiency affects the body)



G6PD Deficiency Variants

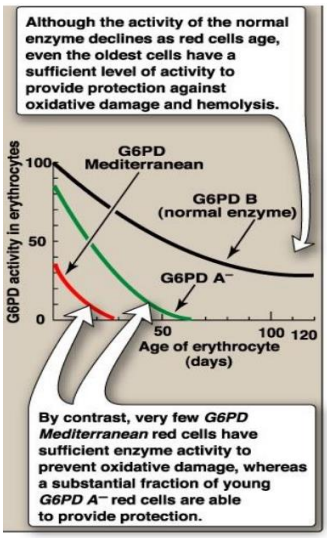
- **Wild type B**
- **Mediterranean Variant B- (Class II) : 563C T**
- **African Variant A- (Class III); two point mutation**
- **African Variant A; Normal activity 80%**
- **Very severe deficiency (Class I)**
- **Majority missense mutation,point mutation**

*large deletion or frame shift; not observed

G6PD DEFICIENCY MUTATIONS :

Lets talk about mutation once again, the majority of these Mutations are Point Substitution for a single nucleotide that causes a missense translation, and as we said there are different types and classes for this mutation : 1- Class I : the most severe mutation which causes the greatest deficiency of the enzyme. 2- Class II (B- variant): cytosine is replaced with thymine on position 563 on the gene and this type is the most common type in Mediterranean countries , this contains severe deficiency of G6PD. 3- Class III (A- Variant): more predominant in African population , their enzyme activity is really close to normal, made up from 2 point mutations. Notice how affected erythrocytes have much lower life span than that of normal ones (check the graph -->): 30 days for class 2 RBCs 60 days for class 3 RBCs.this deficiency is less severe

Class4- is the least severe and it's almost normal



Classification of G6PD Deficiency Variants

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

-note with aging the enzyme activity gets less with time however it's still functional in normal erythrocyte,observe the graph very well, decreased enzyme activity leads to hemolytic anemia because of the increased oxidative stress and because the RBCs are dying before 120 days.

2. Enzymes that catalyze antioxidant reactions

Super oxide dismutase (**SOD**)

Super Oxide Dismutase (SOD), acts on super oxide ion and converts it into Hydrogen peroxide (H2O2) , Hydrogen peroxide can be degraded by catalase enzyme into water and oxygen.

3. Catalase

Side note: the first one is GSH peroxidase. From previous lecture

-Other anti-oxidant chemicals like Vitamin E,C and carotenoids (mainly Vitamin A).

-carotenoids are the compounds that give the carrots their orange color in our bodies they get cleaved from the middle releasing Vitamin A which is also important for the vision.

Sources of ROS in the cell:

- **Oxidases e- + O2**

Most oxidases produce H2O2 (peroxidase). Oxidases are confined to sites equipped with protective enzymes.

- **Oxygenases – Mono oxygenases (hydroxylases)**

– Dioxygenases in the synthesis of prostaglandins, thromboxanes, leukotrienes

-as an example of dioxygenases COX1/COX2.

-repeated inflammations can increase the probability of cancer because of the increased production of ROS it's a strong theory.(not proofed yet).

- **Coenzyme Q in Respiratory chain.(can be a source for super oxide ion " O2 - ")**
- **Respiratory Burst (during phagocytosis) O2 -,H2O2,OH●,NO,HOCl**
- **Ionizing Radiation**

OH●

-Respiratory Burst: it's the condition where super oxide ion is produced in high concentrations in the phagolysosome during the destruction of an invading microorganism.
-Note : It starts with super oxide ,then other ROS may be produced such as NO, H2O2, HOCL and OH. . also Nitrogen Oxygen RS , and in this case they are produced on purpose to fight microorganisms.

- Ionizing Radiation for ex. xray (over exposure produce OH.) : like lab technicians and Radiologists, those people will be continuously tested for the amount of radiation they have. because overtime, ROS can develop cancer.

CYTOCHROME P450 MONOOXYGENASE SYSTEM :

- **Mixed function oxygenase.**
- **Super family of structurally related enzymes.**

RELATION TO FORMATION AND DESTRUCTION OF ROS:

A) It takes an O2 molecule and use one atom to form an OH group , and another one will interact with H + to form water, the source of H+ will be NADPH oxidation.it produces ROS increasing the oxidative stress.

$$R\text{-}H + \mathbf{O_2} + NADPH + H^+ \longrightarrow R\text{-}\mathbf{O}H + H_2\mathbf{O} + NADP^+$$

Mitochondrial system

Synthesis by hydroxylation of steroids, bile acids, active form of Vit. D

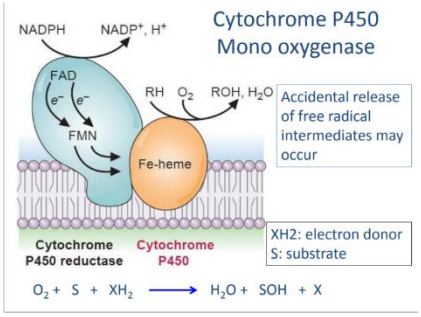
B) P450 system is mostly in hepatocytes, and it is present in multiple places within the cell , like the mitochondria (that is mainly concerned with hydroxylation Reactions which happens during steroids, bile acid and vitamin D synthesis. Note: The active form of Vitamin D contains multiple hydroxyl groups, and remember that vitamin D was originally a cholesterol molecule.

C) Microsomal system which is present in the ER , it's main function is detoxification of drugs and xenobiotics, sometimes this system activates the drug. The main purpose of this degradation is to produce excretable (mainly in Urine) form of the drug (convert it from lipid soluble to water soluble).
Note :Monooxygenase is present in both Mitochondria and ER but in each site it has a different function.

Microsomal system

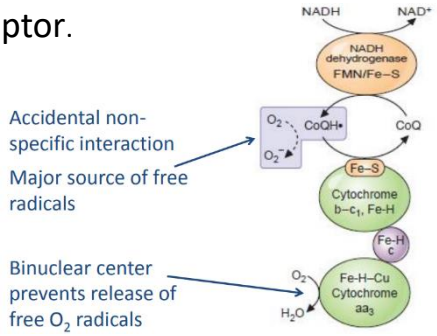
- Detoxification of foreign compounds
- Activation or inactivation of Drugs
- Solublization to facilitate excretion in urine or feces

D) Also FAD and FMN can aid in getting rid of ROS by reacting with the heme group which will make the Fe in the heme group to sway between ferrous and ferric states, but sometimes accidental release of free radicals may occur. Notice that p450 can fight and also produce ROS.

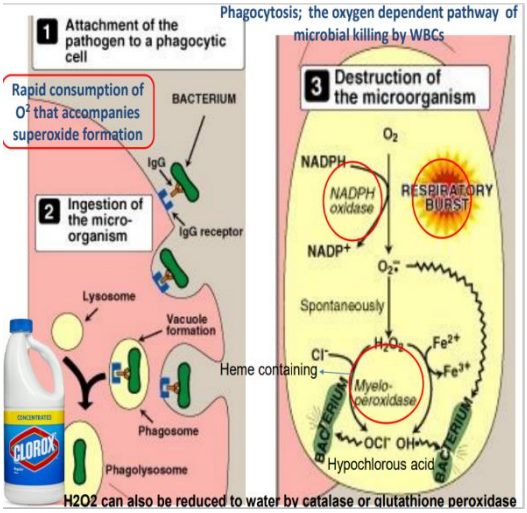


GENERATION OF O2- BY the respiratory chain :

Co- enzyme Q receives electrons from complex 1 and 2, these electrons can be added to oxygen to form O2 - as a byproduct.
Note : ROS amount produced by this method is Low due to the fact that the main function of O2 in ETC is to be the last electron acceptor.

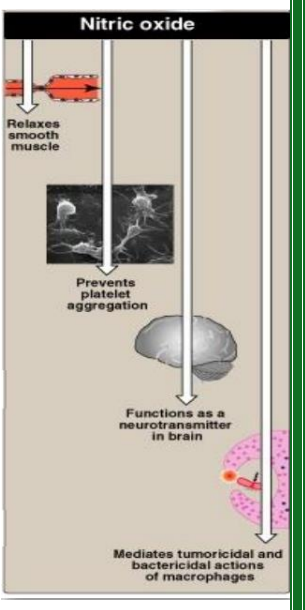


RESPIRATORY BURST :



Associated with immune responses and phagocytosis of microorganism.
When IgG and IgG receptors interact the production of ROS will be activated.
Inside the phagolysosome, oxygen is going to be converted to O2 - , this reduction reaction is catalyzed by NADPH oxidase , O2 - can act directly on bacteria or it can be converted to H2O2 through SOD, H2O2 can be converted to OH^- (Hydroxyl radical). Or OCl- with the involvement of Cl- and Fe2+ .
Note : OCl- is used in disinfectants like Clorox because it's a very strong microorganism killer (more potent that alcohol) .

NO and Reactive Nitrogen Oxygen Species (RNOS):

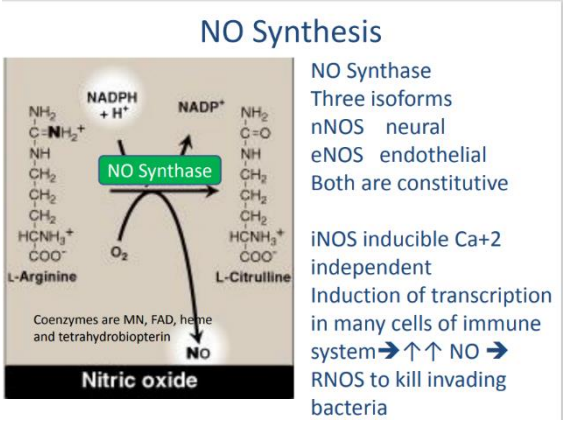


- **Diffuses readily.**
- **Essential for life and toxic.**
- **Neurotransmitter , vasodilator.**
- **↓Platelet aggregation.**
- **At high concentration combines with O_2^- (Peroxide) or O2 to form RNOS.**
- **RNOS are involved in neurodegenerative diseases and inflammatory diseases.**

-NO; is a signaling molecule that is produced in the cell and is also considered as a ROS. It's a small gaseous molecule so it's diffused very easily. Therefore, it's effect is really wide and it may cause toxicity in higher amounts.

The effects of NO:

1- Vasodilation
2- Neurotransmitter in the Brain
3- Reduce platelet aggregation
4- Can react with ROS producing RNOS



How NO is produced ?
It's produced from arginine amino acid (because it contains a plenty of Nitrogen atoms) and that's through NO synthase with the interference of NADPH as indicated in the slide.
NO synthase has different isoforms :

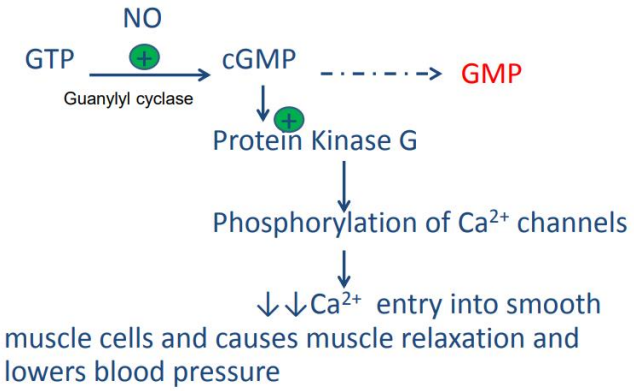
1- nNOS "neural" .
2- eNOS "endothelial".
3- iNOS (inducible Ca2+ independent) which is activated under certain conditions, one of these conditions is the process of fighting an infection (to kill a microorganism) which will induce the production of RNOS to kill invading bacteria.
4- Note : nNOS & eNOS have constitutive actions , which means that they don't need a certain stimulus to induce their action.

Action of NO on vascular endothelium:

Synthesis by endothelia cells → smooth muscle

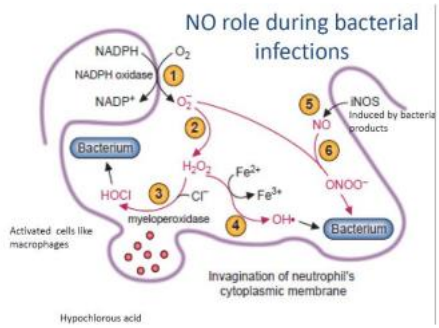
how NO act as a vasodilator ?

1- it's going to activate Guanylyl cyclase that converts GTP to cGMP.
2- which leads to the activation of protein kinase G.
3- phosphorylate Ca2+ channels.
4- causes entry of Ca2+ into SER and cause muscle relaxation.
5- which decreases the blood pressure.



Role of NO in hydrolyzing microorganism:

iNOS is induced by bacterial products, NO combines with O2 - producing ONOO- which hydrolyze bacteria.



Summary from lippincott for pentosephosphate pathway:

The pentose phosphate pathway includes an irreversible oxidative phase followed by a series of reversible sugar–phosphate interconversions (Fig. 13.14). No ATP is directly consumed or produced in the pathway. The reduced nicotinamide adenine dinucleotide phosphate (NADPH)-producing oxidative portion of the pathway is important in providing reducing equivalents for reductive biosynthesis and detoxification reactions. In this part of the pathway, glucose 6-phosphate is irreversibly converted to ribulose 5-phosphate, and two NADPH are produced. The regulated step is catalyzed by ***glucose 6-phosphate dehydrogenase*** (***G6PD***), which is strongly inhibited by a rise in the NADPH/$NADP^+$ ratio. Reversible nonoxidative reactions interconvert sugars. This part of the pathway converts ribulose 5-phosphate to ribose 5-phosphate, required for nucleotide and nucleic acid synthesis, or to fructose 6-phosphate and glyceraldehyde 3-phosphate (glycolytic intermediates). NADPH is a source of reducing equivalents in reductive biosynthesis, such as the production of fatty acids in liver, adipose tissue, and the mammary gland; cholesterol in the liver; and steroid hormones in the placenta, ovaries, testes, and adrenal cortex. It is also required by red blood cells (RBC) for hydrogen peroxide reduction. Reduced glutathione (G-SH) is used by ***glutathione peroxidase*** to reduce the peroxide to water. The oxidized glutathione (G-S-S-G) produced is reduced by ***glutathione reductase***, using NADPH as the source of electrons. NADPH provides reducing equivalents for the mitochondrial ***cytochrome P450 monooxygenase*** system, which is used in steroid hormone synthesis in steroidogenic tissue, bile acid synthesis in the liver, and vitamin D activation in the liver and kidneys. The microsomal system uses NADPH to detoxify foreign compounds (xenobiotics), such as drugs and a variety of pollutants. NADPH provides the reducing equivalents for phagocytes in the process of eliminating invading microorganisms. ***NADPH oxidase*** uses molecular oxygen (O_2) and electrons from NADPH to produce superoxide radicals, which, in turn, can be converted to peroxide by ***superoxide dismutase***. ***Myeloperoxidase*** catalyzes the formation of bactericidal hypochlorous acid from peroxide and chloride ions. Rare genetic defects in ***NADPH oxidase*** cause chronic granulomatous disease characterized by severe, persistent, infections and granuloma formation. NADPH is required for the synthesis of nitric oxide (NO), an important free radical gas that causes vasodilation by relaxing vascular smooth muscle, acts as a neurotransmitter, prevents platelet aggregation, and helps mediate macrophage bactericidal activity. NO is made from arginine and O_2 by three different NADPH-dependent ***NO synthases*** (***NOS***). The endothelial (***eNOS***) and neuronal (***nNOS***) isozymes constantly produce very low levels of NO for vasodilation and neurotransmission, respectively. The inducible isozyme (***iNOS***) produces large amounts of NO for defense against pathogens. ***G6PD*** deficiency impairs the ability of the cell to form the NADPH that is essential for the maintenance of the G-SH pool. The cells most affected are RBC because they do not have additional sources of NADPH. ***G6PD*** deficiency is an X-linked disease characterized by hemolytic anemia caused by the production of free radicals and peroxides following administration of oxidant drugs, ingestion of fava beans, or severe infections. The extent of the anemia depends on the amount of residual enzyme. Class I variants, the most severe (and least common), are associated with chronic nonspherocytic hemolytic anemia. Babies with ***G6PD*** deficiency may experience neonatal jaundice.

بالتوفيق للجميع لا تنسونا من صالح دعائكم