فريق طوفان الأقصى

METABOLISM

Modified N. 3

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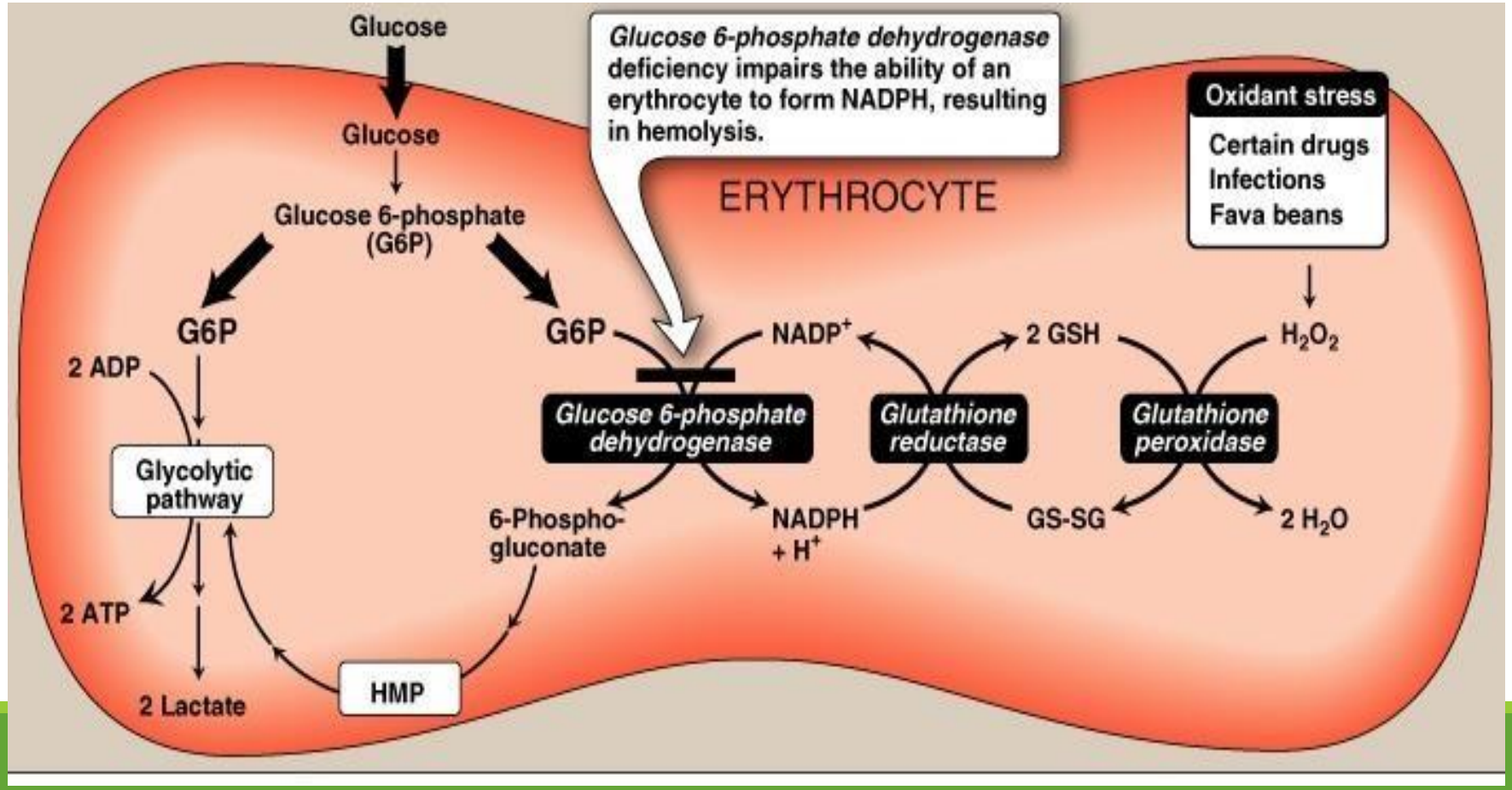
Role of G6PD in red blood cells

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

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

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

Oxidation ➔ denaturation of proteins and rigidity of the cells



- **The complement in this slide:** we talked about G6PD deficiency and this diseases results in reduced in production of NADPH.
- 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.
- Those patients will have hemolysis.
- RBC as u remember is depending on glucose as source of energy.
- When glucose concentration is high it's going to be up taken by ERYTHROCYTE and then phosphorylated to glucose 6-phosphate and continue glycolytic pathway, it may also enter anaerobic respiration producing lactate to provide the low energy demands of this cell.
- In normal conditions this glucose 6-phosphate enters PPP (pentose phosphate pathway) and the reason of PPP in RBC is the NADPH.
- The main purpose is to produce NADPH to fight oxidative stress using Glutathione.

- **In those patients, this pathway is blocked or reduced at an initial stage, which is why a decreased amount of NADPH is produced, leading to the accumulation of more oxidative stress.**
- **These ROS start to react haphazardly with the surrounding proteins(it might be Na+/K+ pump تأثر عليهم بطريقة تخرّب الوظيفة تبعتهم and this will result in more rigidity of the RBC, this will lead to hemolysis(premature death before 120days))**

G6PD Deficiency Variants

NOTE: this disease has many levels of severity:

- 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 deletions or frame shift; Not Observed

The complement in this slide:

- All these 400 mutation most of them are point mutation(nucleotide بدلنا مكانه واحد ثاني)

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(**the wild type of it called B**).

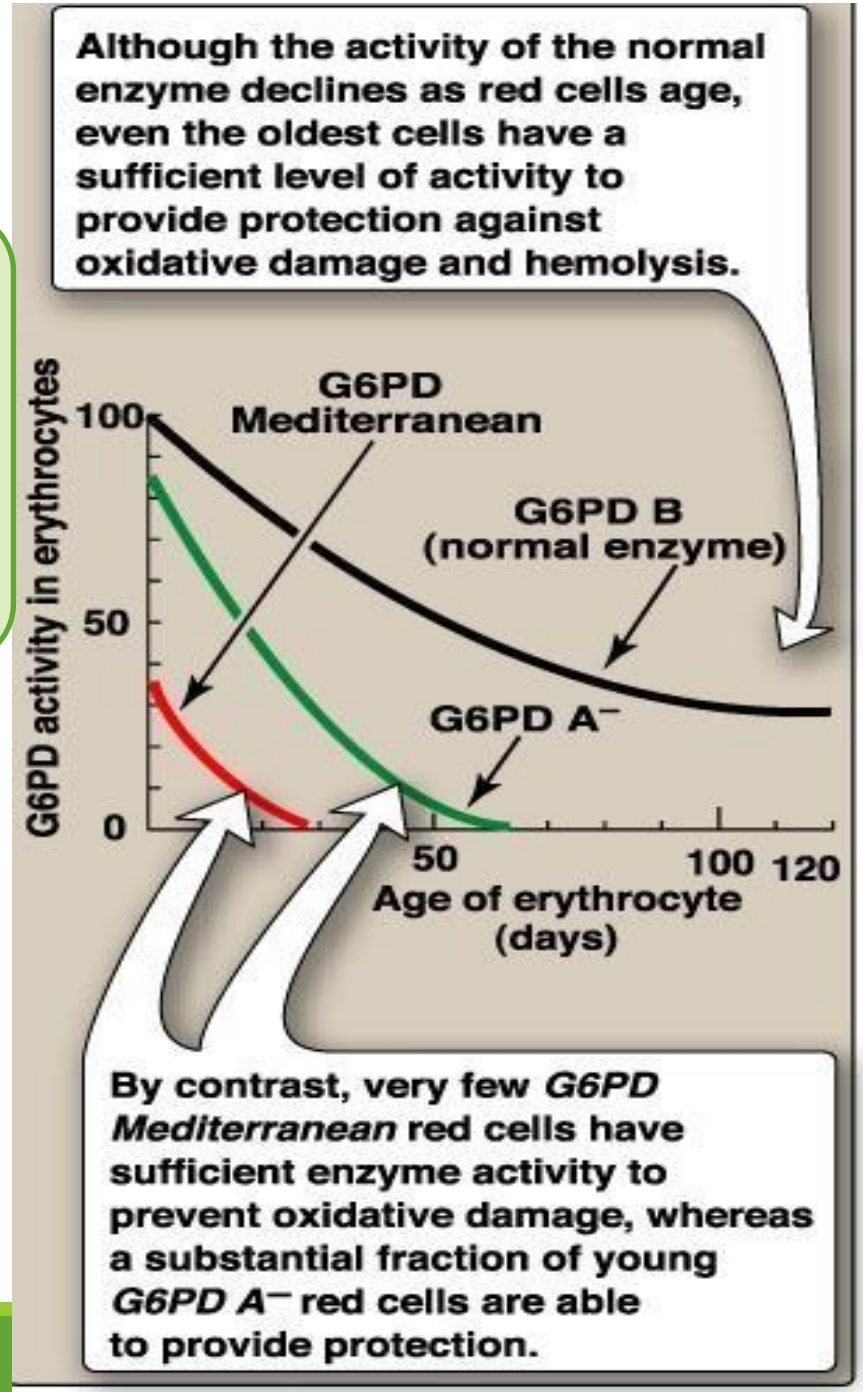
3- Class III (**A- Variant**): more predominant in **African population** , their enzyme activity is close to normal, made up from **2-point mutation**, however the amount of deficiency is less(**when it is affected, we call it A- but when it is normal, we call it A**).

4- Class IV: is the least severe, mostly normal, expression of the enzyme is high.

Look at the picture in the next slide.

Classification of G6PD Deficiency Variants

NOTE: focuse on the day of death and the activity



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

NOTE: i need more than %60 of the enzyme for full function of pathway

Enzymes that catalyze antioxidant reactions

NOTE: Oxidative stress can be handled by NADPH by different molecules like **Glutathione,** another ways:

2. Super oxide dismutase (**SOD**)

$$2O_2\bullet^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

NOTE: Super Oxide Dismutase (SOD), acts on super oxide ion and coverts it into Hydrogen peroxide

2) Hydrogen peroxide (H_2O_2) can be degraded by catalase enzyme into water and oxygen

3. Catalase (in the peroxisome)

$$2H_2O_2 \longrightarrow O_2 + 2H_2O$$

Anti oxidant chemicals

- Vitamin E, Vitamin C, Carotenoids

NOTE: Mechanisms from outside the body like diet (Vitamins): Carotenoids (Vitamin A): Beta carotene get cleavage and it releases Vitamin A.

- **NOTE:** we said that ROS always will be side product of your metabolism, is that mean that every reaction and every enzyme can produce ROS? **No**
- In specific enzymes whose functions involve oxygen, they are the ones that will produce reactive oxygen species (ROS) as a side product of that reaction.

Sources of ROS in the cell

- Oxidases

  e- + O_2

Most oxidases produce H_2O_2 (peroxidase)

Oxidases are confined to sites equipped with protective enzymes

- Oxygenases
  - Mono oxygenases (hydroxylases)
  - Dioxygenases in the synthesis of prostaglandins, thromboxanes, leukotrienes

- **The complement in this slide:** the enzymes that are consider as Oxidases (Peroxidase as a sub group) peroxidase phosphate can produce H2O2 (substrate react with O2)
- Oxidases also classify as **Oxidoreductases.**
- Oxygenases also classify as **Oxidoreductases**
- Also by using O2 and they can be subdivided into:

Monooxygenase (like hydroxylases)

Dioxygenase like: COX 1 AND 2

Sources of ROS in the cell

- Coenzyme Q in Respiratory chain
- Respiratory Burst (during phagocytosis)

  O_2^- H_2O_2 $OH^\bullet$ NO HOCl
- Ionizing Radiation

  $OH^\bullet$

NOTE: like UV light, X ray, nuclear Radiation

- **The complement in this slide:** another place that as Coenzyme Q(**ubiquinone**) in the respiratory chains.
- Carries Electrons from Complex 1(NADH dehydrogenase(NADH)) and 2(Succinate dehydrogenase(FADH2)).
- The last electron acceptor in ETC is **O2**, so it is an **Oxygen rich environment.**
- When electrons are moving, I might lose some of these electrons and get accepted by the Oxygen to produce **Superoxide ion** O_2^-.
- Respiratory burst occur during **phagocytosis of microorganisms** (viruses and bacteria) and their phagocytosis into phagosome that fuses with lysosome (Lysosomes have acidic environment and have same enzymes that can produce a huge amount of ROS/RNOS to lyse and damage the invading microorganisms).

Cytochrome P450 Mono oxygenase

- Mixed function oxygenase
- Super family of structurally related enzymes

Mitochondrial system

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

Microsomal system

Detoxification of foreign compounds

Activation or inactivation of Drugs

Solublization to facilitate excretion in urine or feces

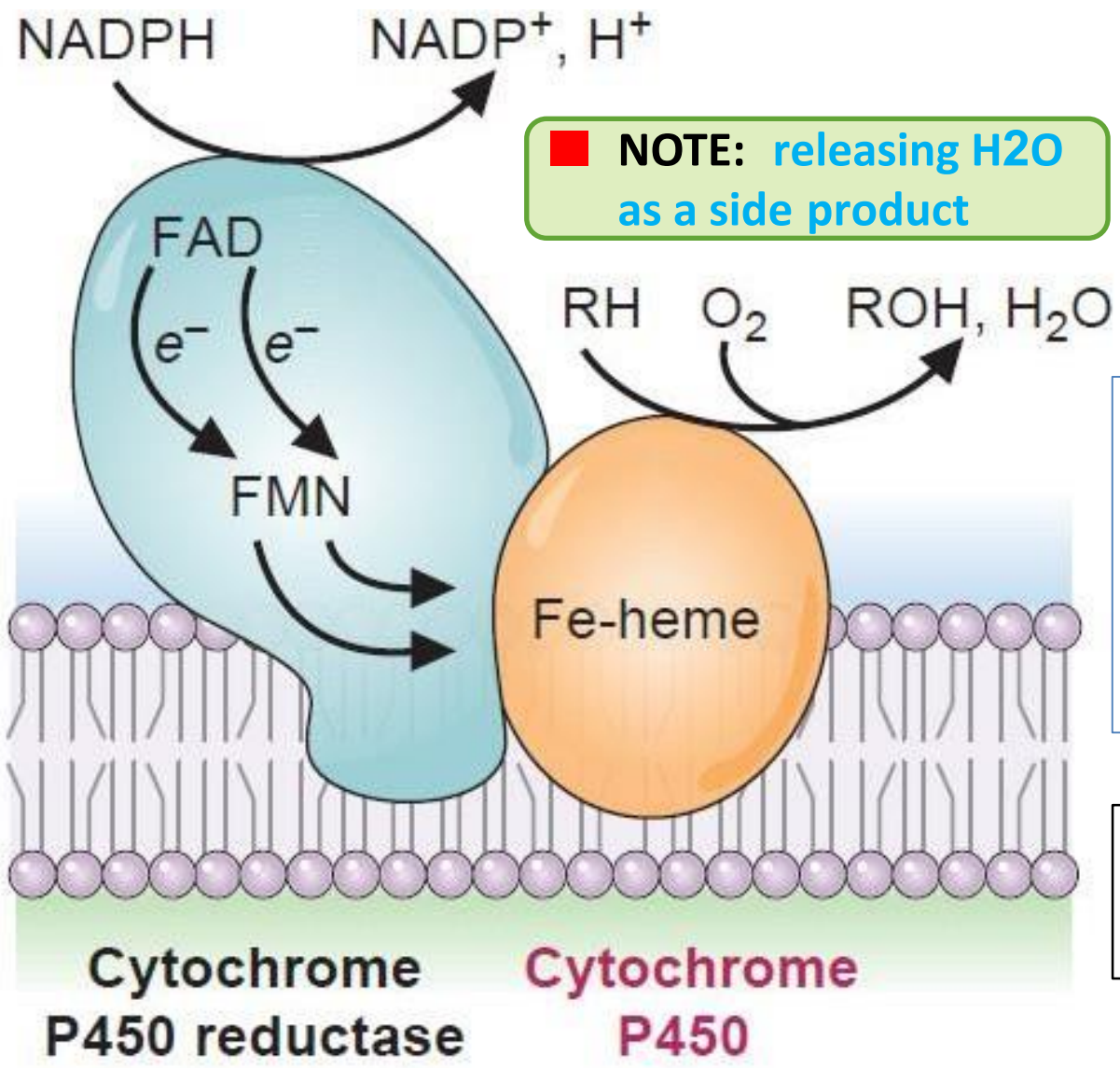
The complement in this slide:

- 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.

- P450 system 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.

- Microsomal system which is present in the ER , its main function is detoxification of drugs and foreign compounds, 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) by solubilization.

Cytochrome P450 Mono oxygenase



NOTE: releasing H2O as a side product

Accidental release of free radical intermedia tes may occur

XH2: electron donor
S: substrate

NOTE: Reaction in the picture is the main mechanism.

NOTE: the accident when O2 interacts with electrons --> free radicals

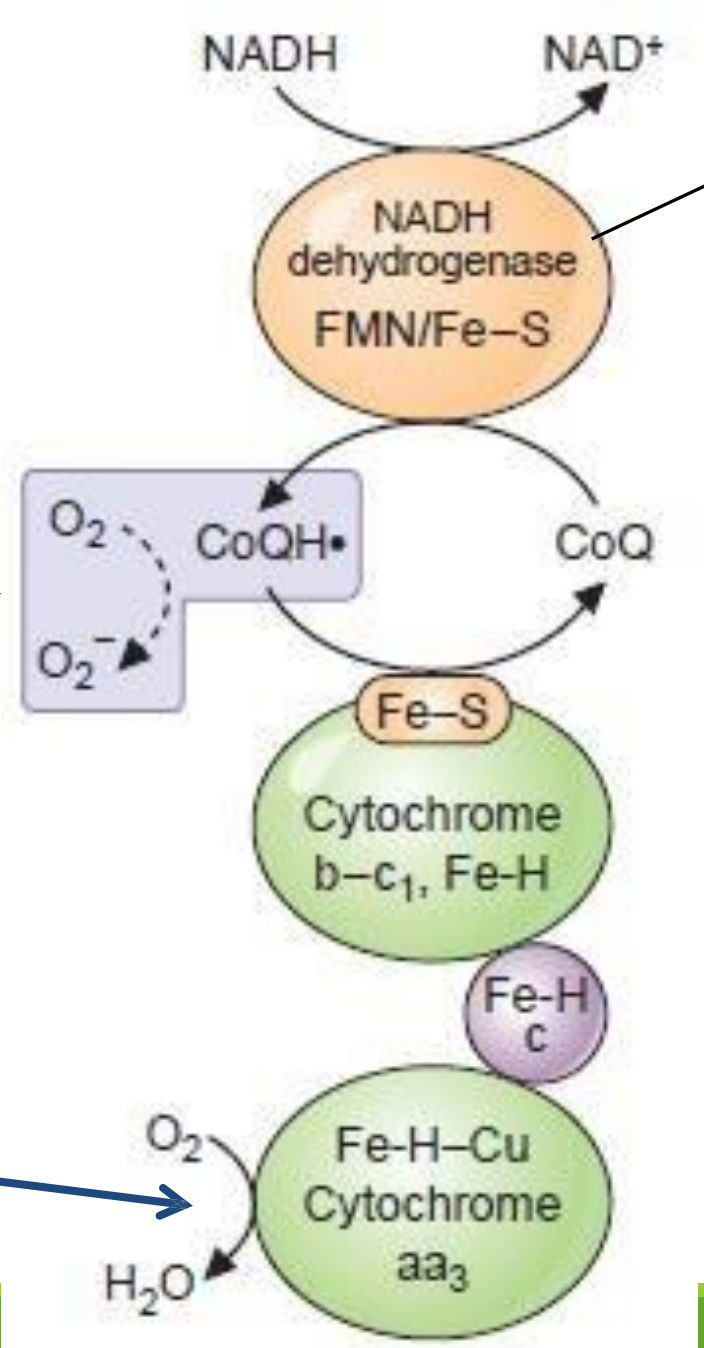
$$O_2 + S + XH_2 \longrightarrow H_2O + SOH + X$$

Generation of O_2^- by the respiratory chain

Accidental non-specific interaction

Major source of free radicals

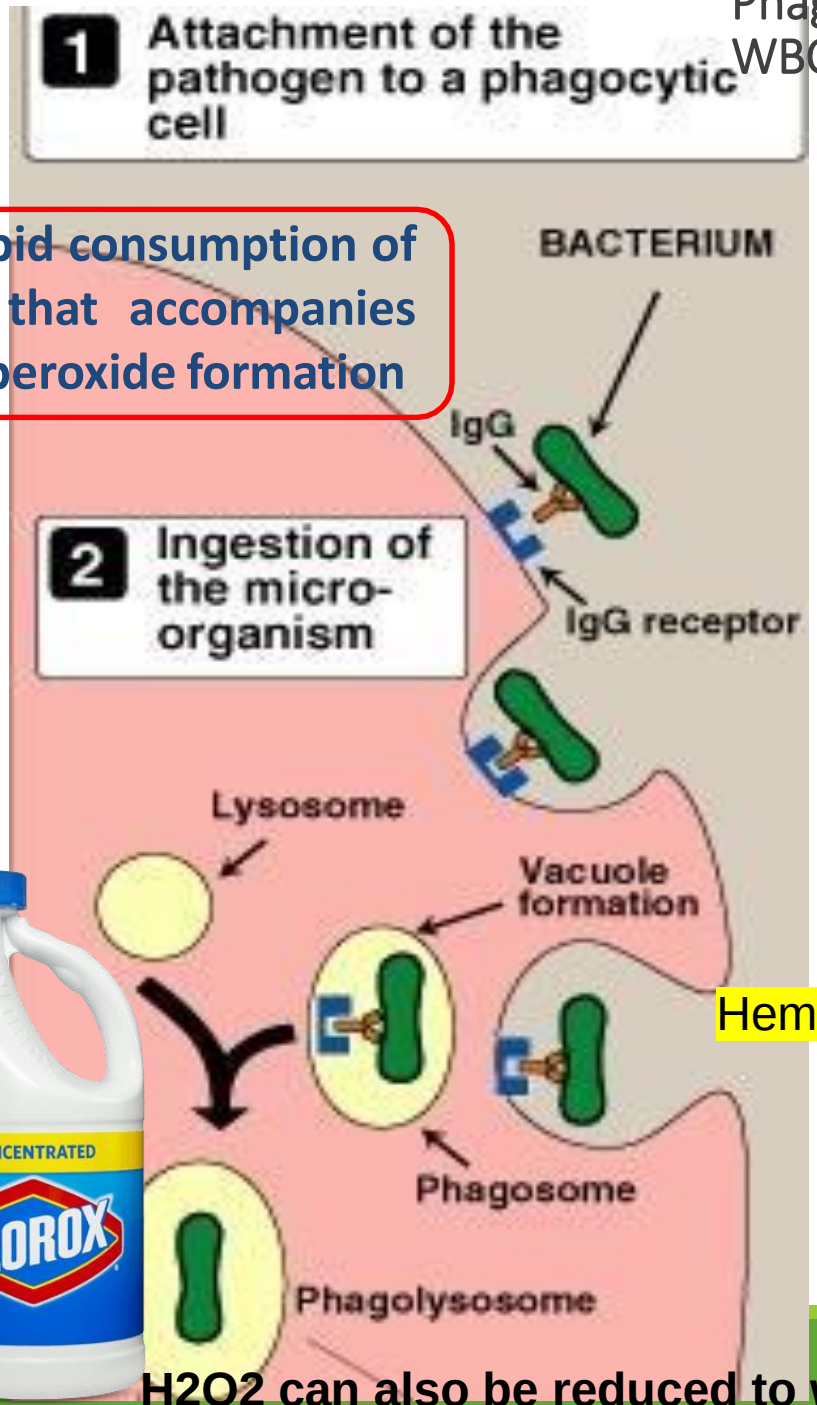
Binuclear center prevents release of free O_2 radicals



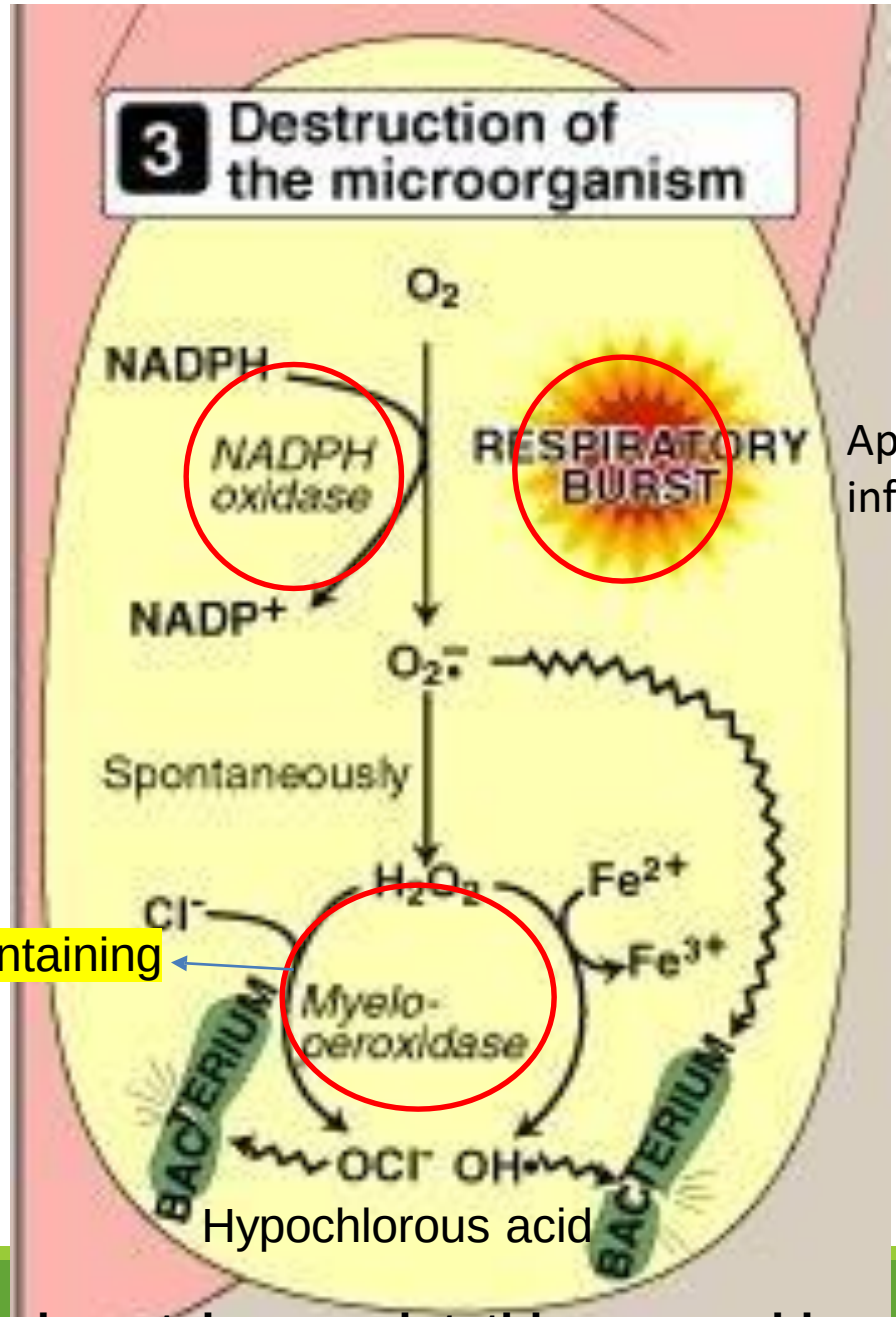
NOTE: complex 1

- **The complement in this slide: :** Co-enzyme Q receives electrons from complex 1 and 2, these electrons can be added to oxygen because they are in oxygen rich environment to form superoxide O2- as a byproduct.

Phagocytosis; the oxygen dependent pathway of microbial killing by WBCs



Rapid consumption of O^2 that accompanies superoxide formation



Heme containing

Appears during infections

H2O2 can also be reduced to water by catalase or glutathione peroxidase

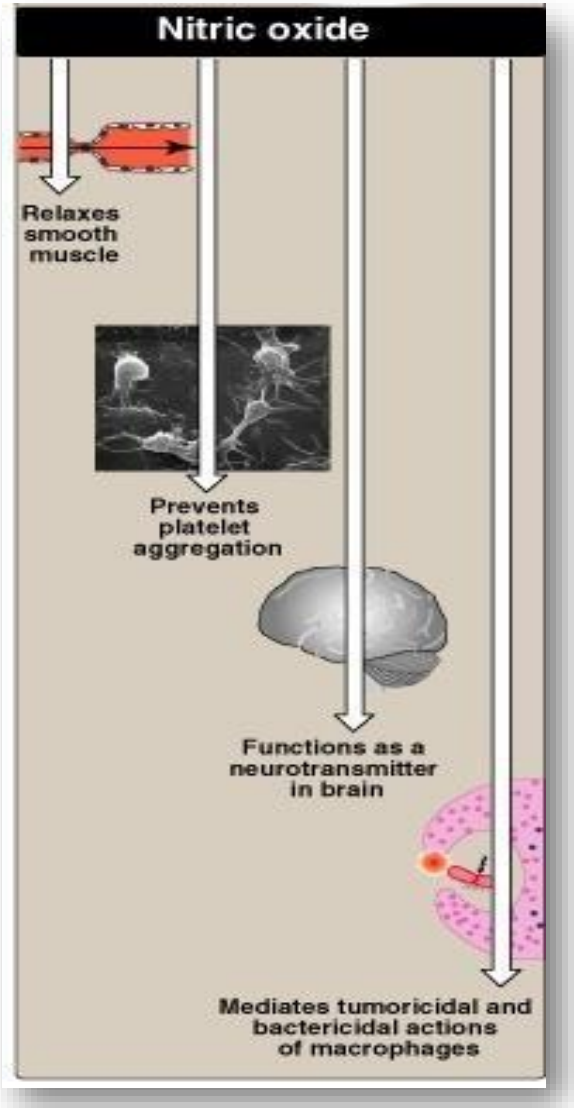
- **The complement in this slide:** An invading bacterium is recognized by the immune system(IGg) and attacked by antibodies that bind it to a receptor on a phagocytic cell. Inducing the ingestion of the bacterium in cell forming phagosome. Then it will fuse with lysosome forming phagolysosome, This will induce the respiratory burst (kind of reaction makes ROS and NROS), then NADPH oxidase is activated which leads to:

  O_2 ----> $O_2^{\bullet-}$ (superoxide) ----------> H_2O_2 (spontaneous)

Then H_2O_2 can bind with CL- in the presence of MPO (Myeloperoxidase) → OCL^- Hypochlorous acid

OR H_2O_2 can accept an electron from Fe^{+2} ,to form OH^-

NOTE: all of them ROS and NROS (H_2O_2 , OCL^-, OH^-, $O_2^{\bullet-}$) can destruct the bacteria.
ALSO, H_2O_2 can also be reduced to water by catalase or glutathione peroxidase.



NO and Reactive Nitrogen Oxygen Species (RNOS)

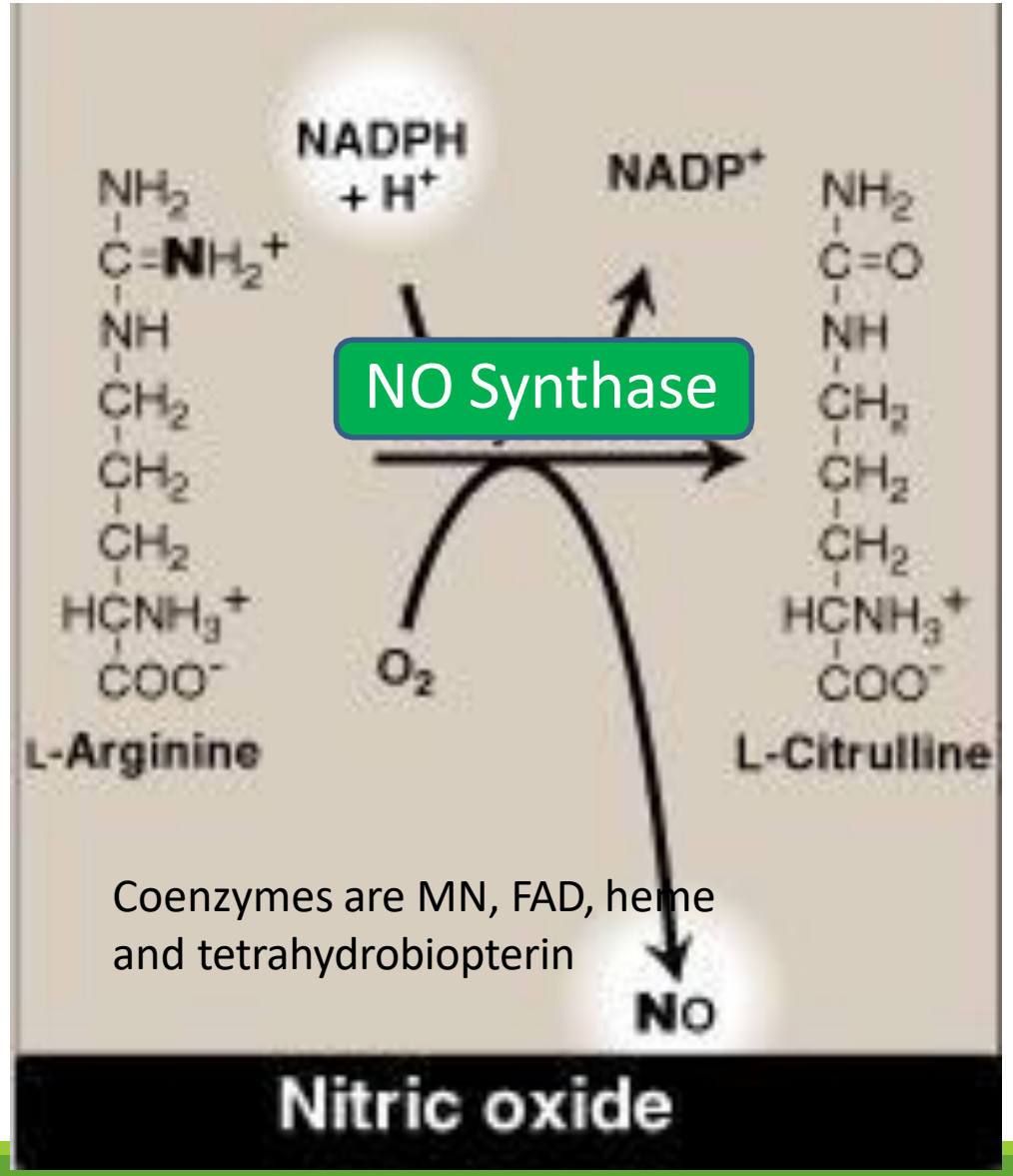
- Diffuses readily
- Essential for life and toxic
- Neurotransmitter , vasodilator (by relaxation of smooth muscles)
- ↓Platelet aggregation
- At high concentration combines with $O_2\bullet^-$ or O_2 to form **RNOS**
- **RNOS (if there concentration increase)** are involved in neurodegenerative diseases and inflammatory diseases

Read the picture important!!!!!

- **The complement in this slide:** 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 wide, and it may cause toxicity in higher amounts, because it is source of RNOS.

- How is NO 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". (they are always active and expressed)
- 3- iNOS (inducible -need stimulus- Ca^{+2} 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.

NO Synthesis



NO Synthase

Three isoforms

nNOS neural

eNOS endothelial

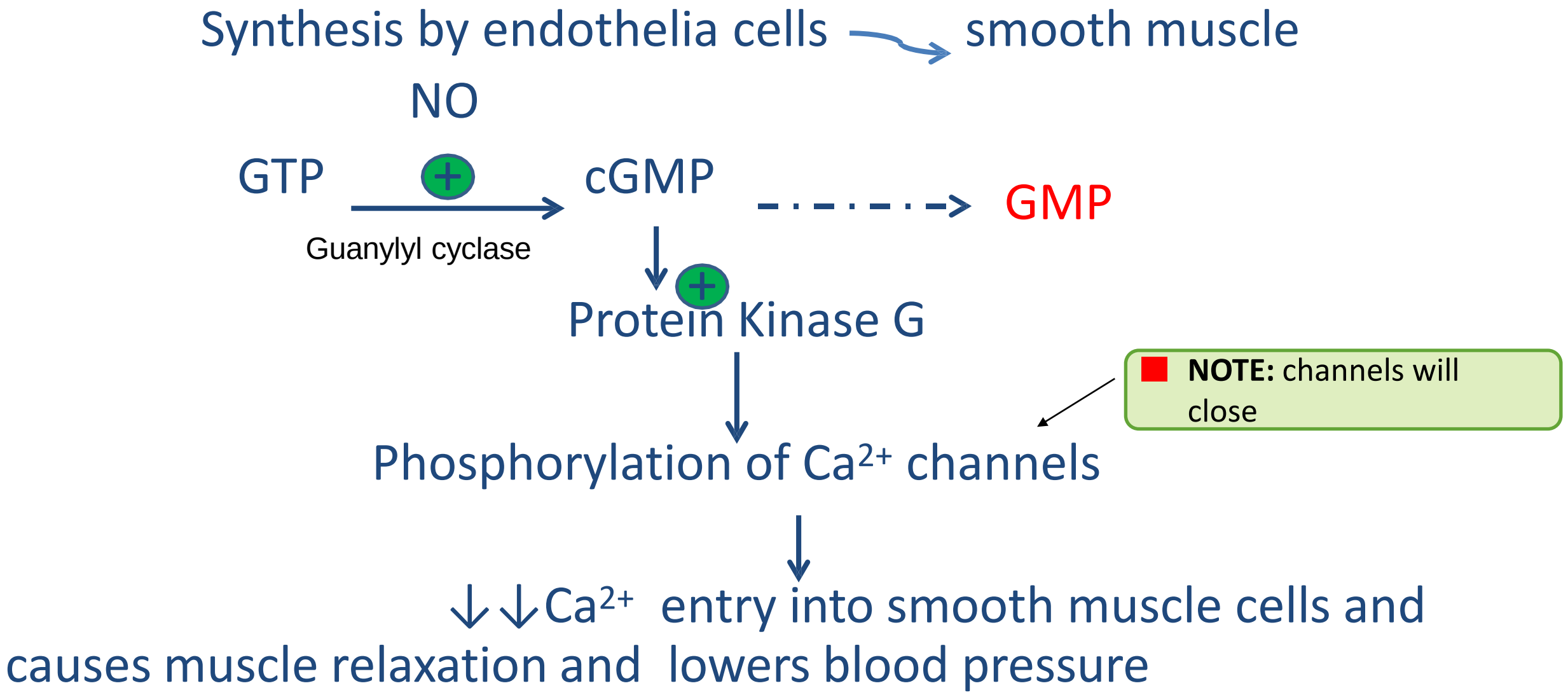
Both are constitutive

iNOS inducible Ca+2 independent

Induction of transcription in many cells of immune system➔↑↑ NO ➔ RNOS to kill invading bacteria

NOTE: **This reaction consumes NADPH, so it is bad when it happens alot**

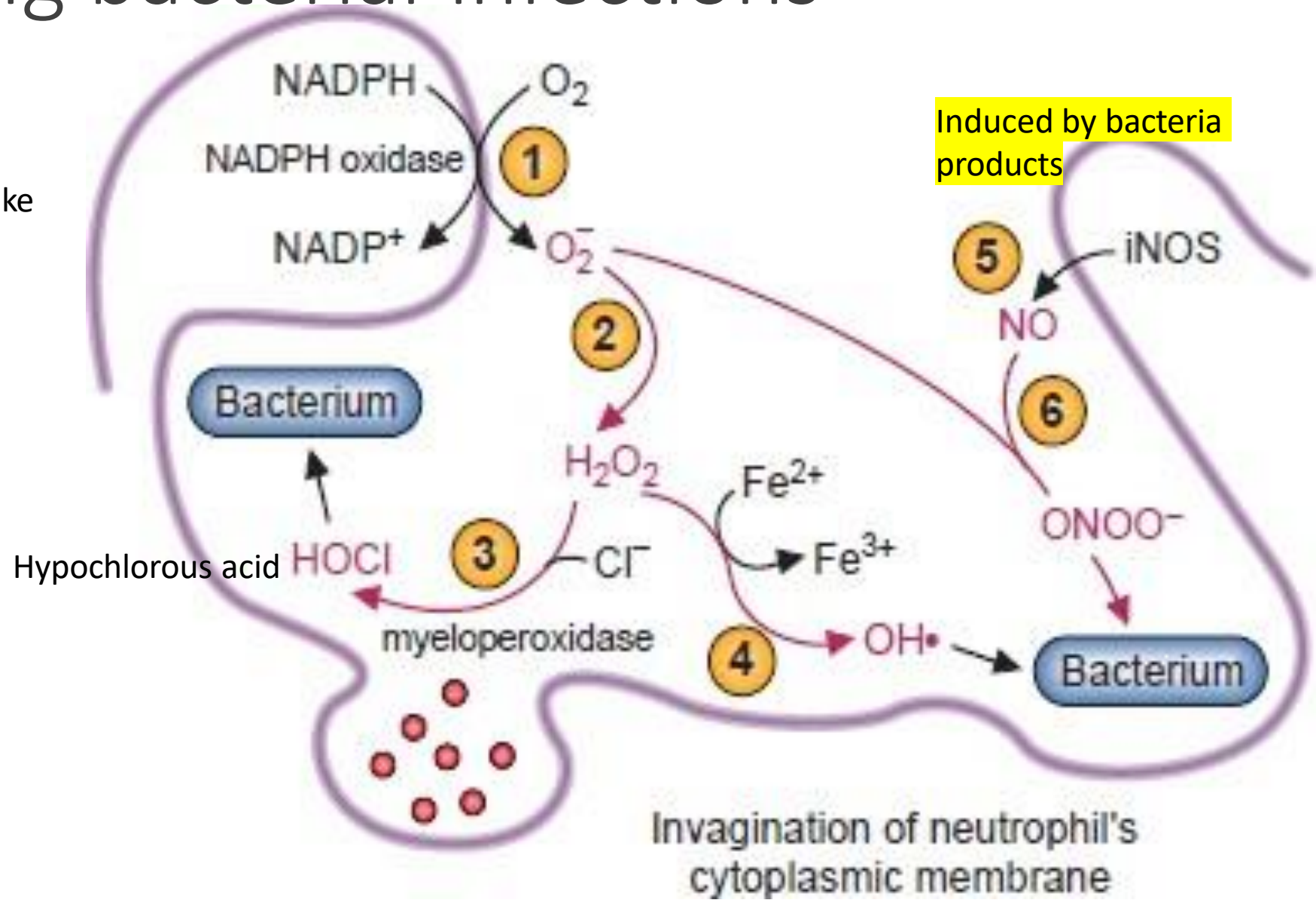
Action of NO on vascular endothelium



NO role during bacterial infections

Activated cells like macrophages

- **NOTE:** from 1 – 4 we took previously, last 2 points 5, 6 briefly:
- iNOS is induced by bacteria → NO synthesis → $ONOO^-$ → destruct bacterium



Insulin Resistance (IR)

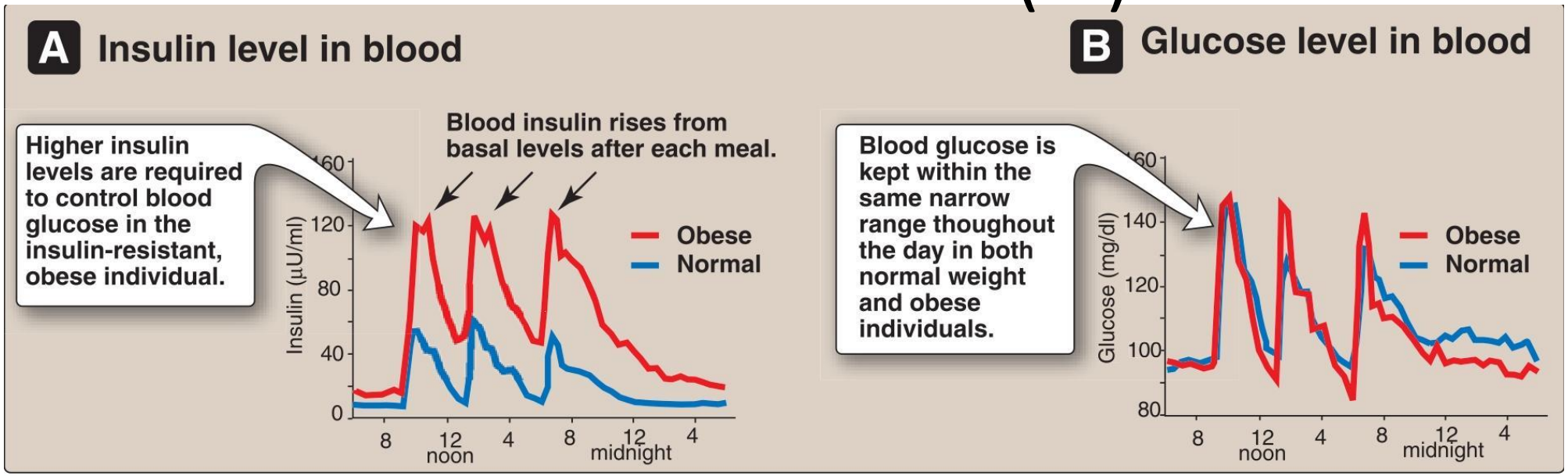


Figure 25.7
Blood insulin and glucose levels in normal weight and obese subjects.

- ✓ **IR is the decreased ability of target tissues, such as liver, adipose, and muscle, to respond properly to normal (or elevated) circulating concentrations of insulin.**
- ✓ **IR is characterized by uncontrolled hepatic glucose production, and decreased glucose uptake by muscle and adipose tissue.**
- ✓ **Obesity is the most common cause of IR**
- ✓ **IR alone will not lead to type 2 diabetes.**
- ✓ **Type 2 diabetes develops in insulin-resistant individuals who also show impaired B-cell function**

- **The complement in this slide:** IR happens in patients with diabetes type 2 (in adults)
- Type 1: in children, there might be disfunction of Pancreatic cells because of autoimmune disease, so the pancreas will not produce insulin or makes very little amount(you can take insulin as a therapeutic, but it is more serious than type 2).
- **Type 2** usually happen to **obese people.**
- When u eat a large meal (even if you are not obese) the pancreas will release **large amount of insulin** Proportional to glucose concentration.
- The more sugar in the blood, there will be increase in insulin secretion , this will lead to draining of the pancreas(asking him to work a lot), in the end whatever the person is normal or obese he will reach fasting blood sugar after 2 hours.
- The same amount of insulin **doesn't produce** the same effect, like **Tolerance**
- And this will exhaust the pancreas.
- NOTE: When insulin levels are elevated (obese people) --> bodily cells develop resistance to insulin.

لما يضل الجسم يفرز كميات كبيرة من الأنسولين وبصورة متكررة و(خصوصا الشخص السمين) فيؤدي الجسم الى افراز كميات أكبر من الأنسولين لإدخال نفس كمية السكر,و هذا يسمى ب مقاومة الأنسولين

V2 slide 19

OR H_2O_2 can accept an electron from Fe^{+2} ,to form OH^-

ALSO, H_2O_2 can also be reduced to water by catalase or glutathione peroxidase.