Pentose Phosphate Pathway (PPP) or Hexose Monophosphate Shunt

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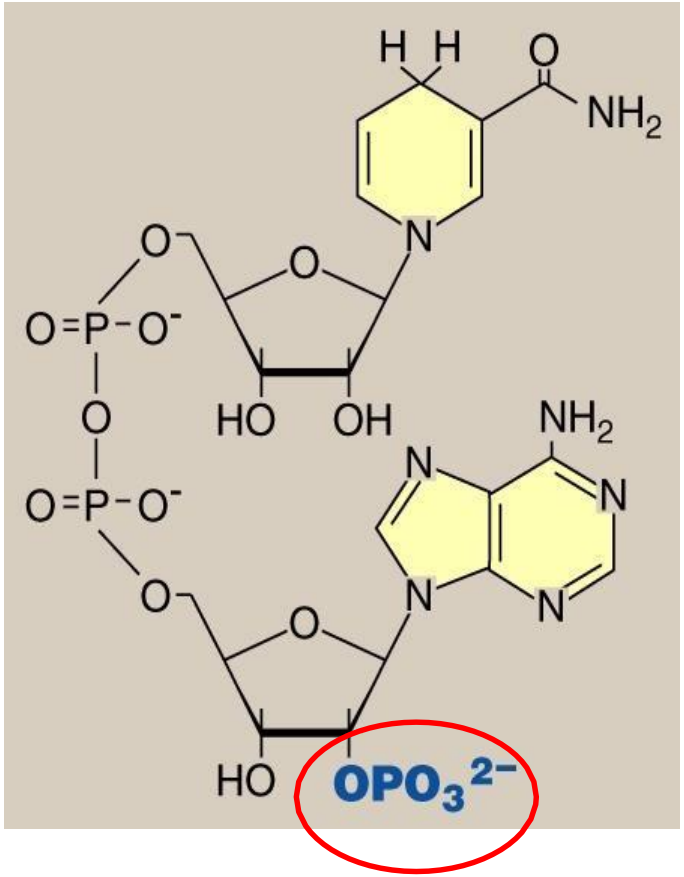
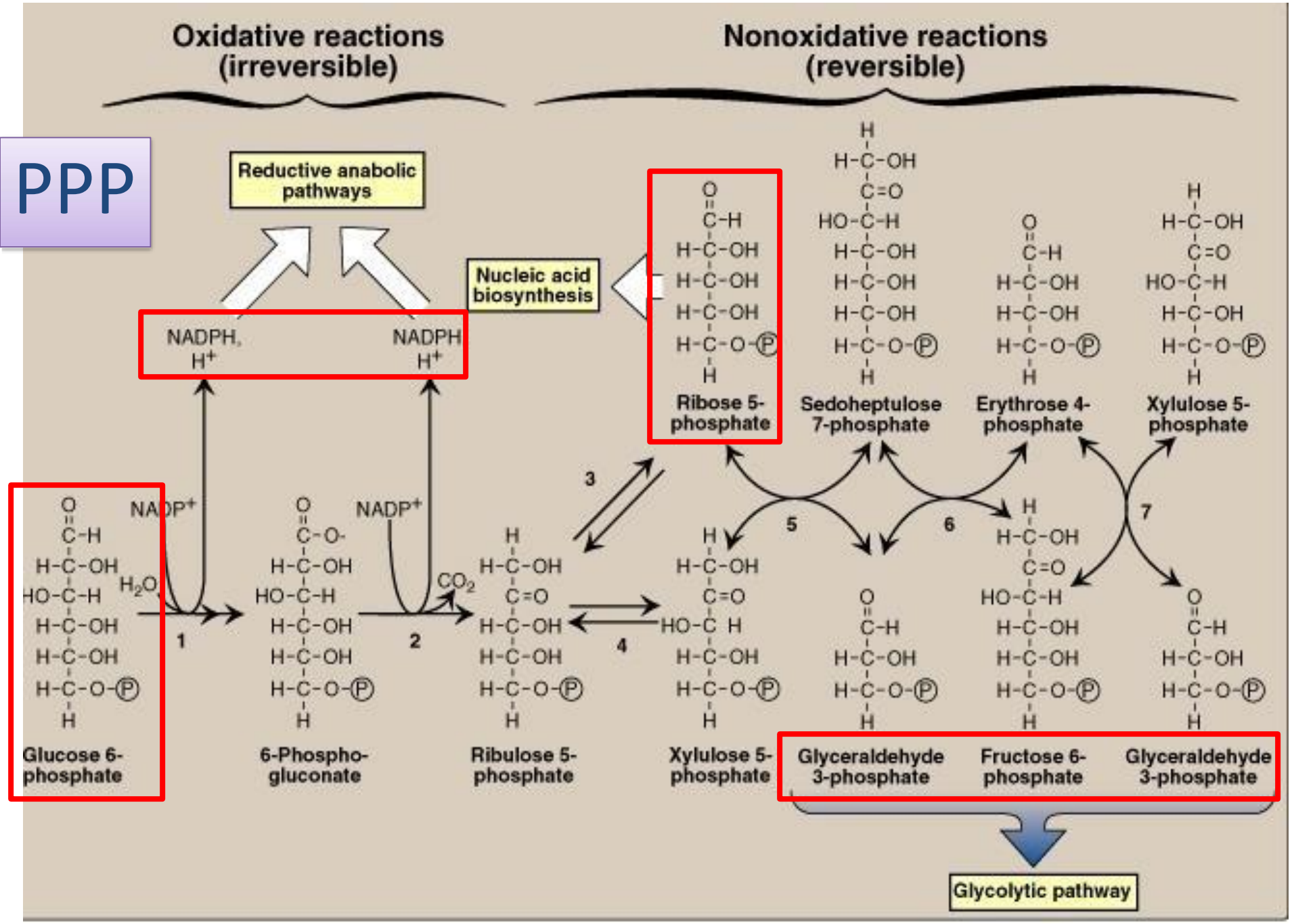
Functions of the PPP

1. Production of NADPH
   - NADPH dependent biosynthesis of fatty acids
     - Liver, lactating mammary glands, adipose tissue
   - NADPH dependent biosynthesis of steroid hormones
     - Testes, ovaries, placenta, and adrenal cortex
   - Maintenance of Glutathione (GSH) in the reduced form in the RBCs



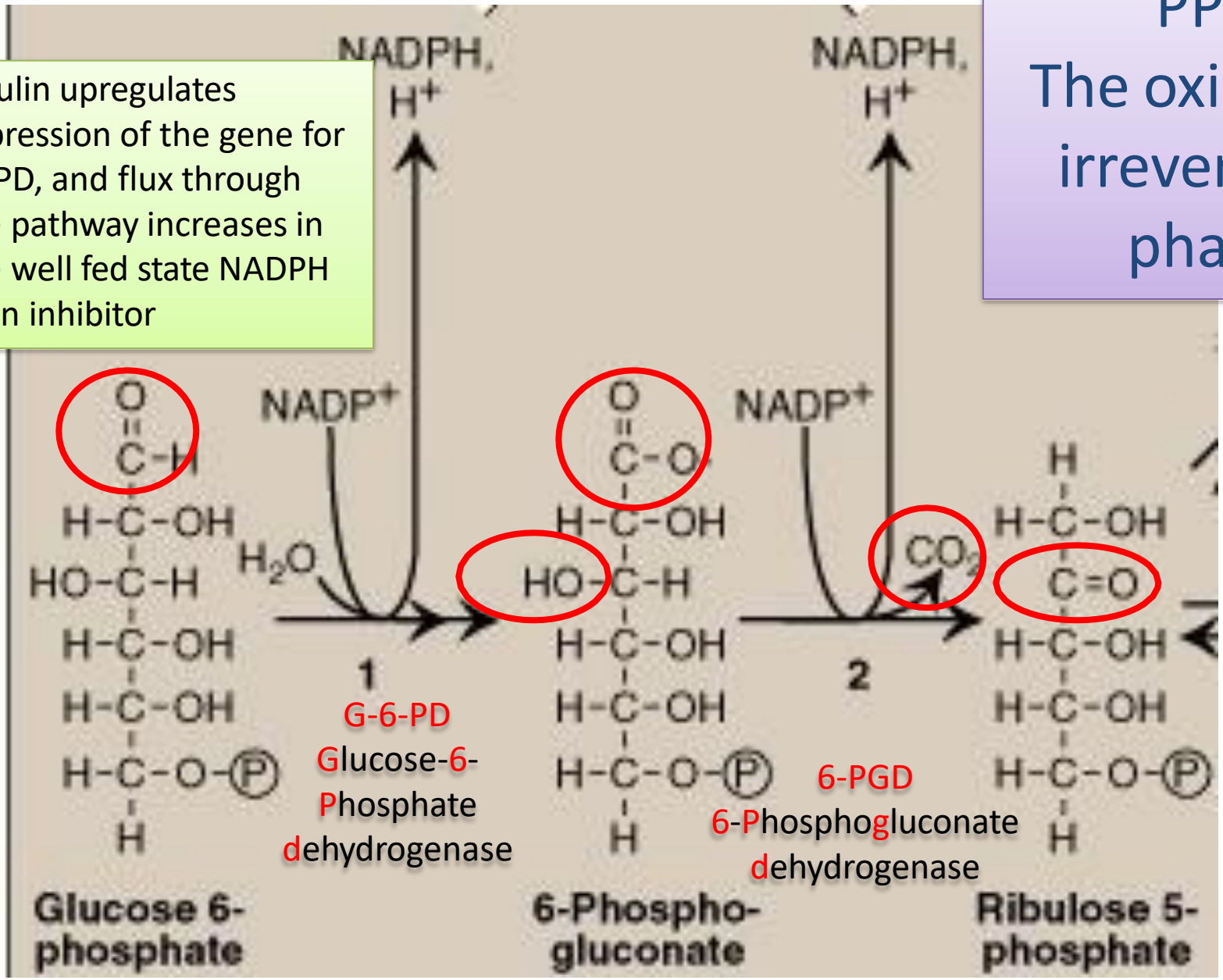
OH in NADH

2. Metabolism of five-carbon sugars (Pentoses)
   - Ribose 5-phosphate (nucleotide biosynthesis)
   - Metabolism of pentoses


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

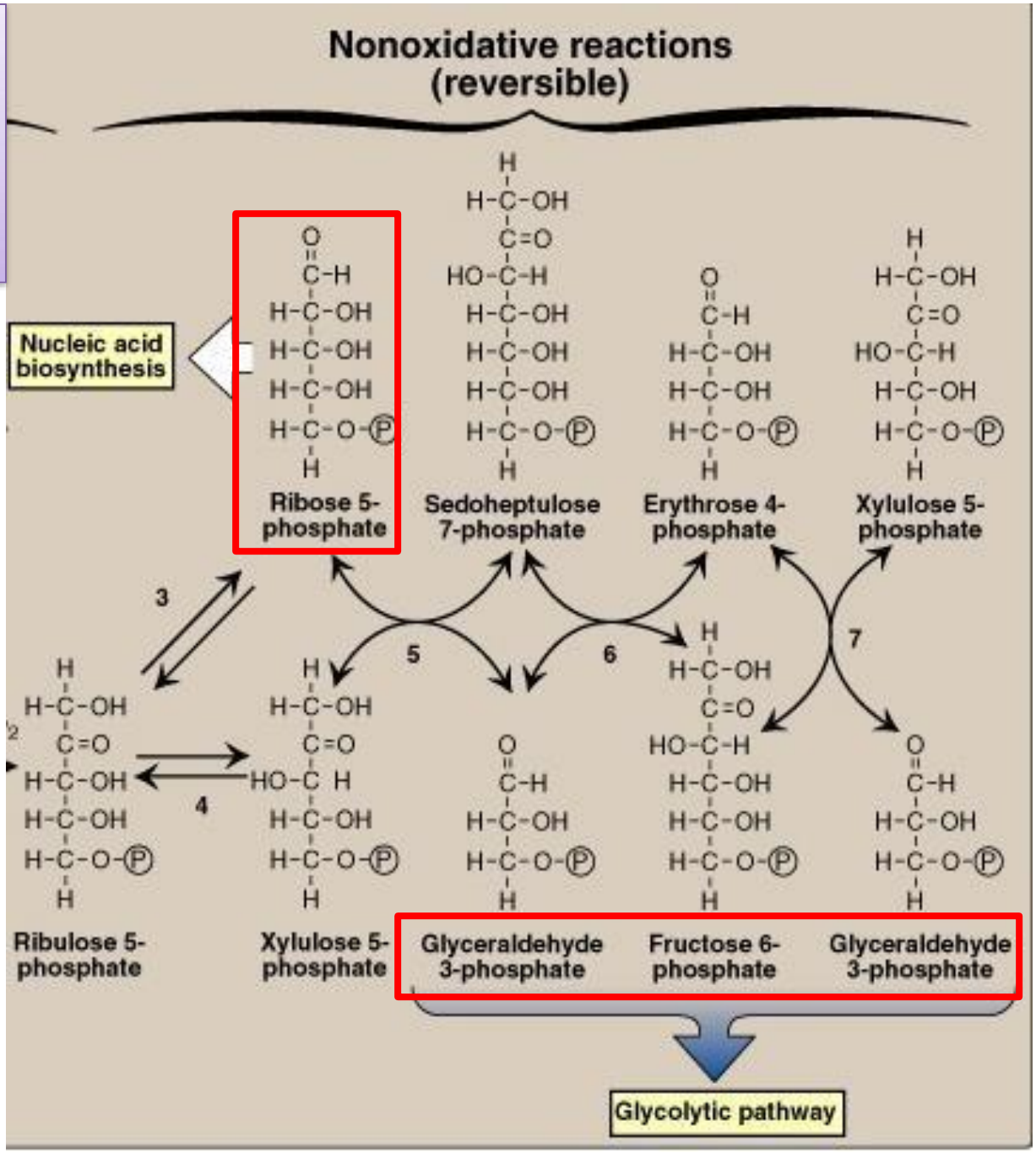
PPP
The oxidative irreversible phase

Insulin upregulates expression of the gene for G6PD, and flux through the pathway increases in the well fed state NADPH is an inhibitor



Glc. 6 Phosph. +2 $NADP^+$ ⟶ Ribulose 5-Phosph. + CO_2 + 2 NADPH

PPP
The non-oxidative reversible phase



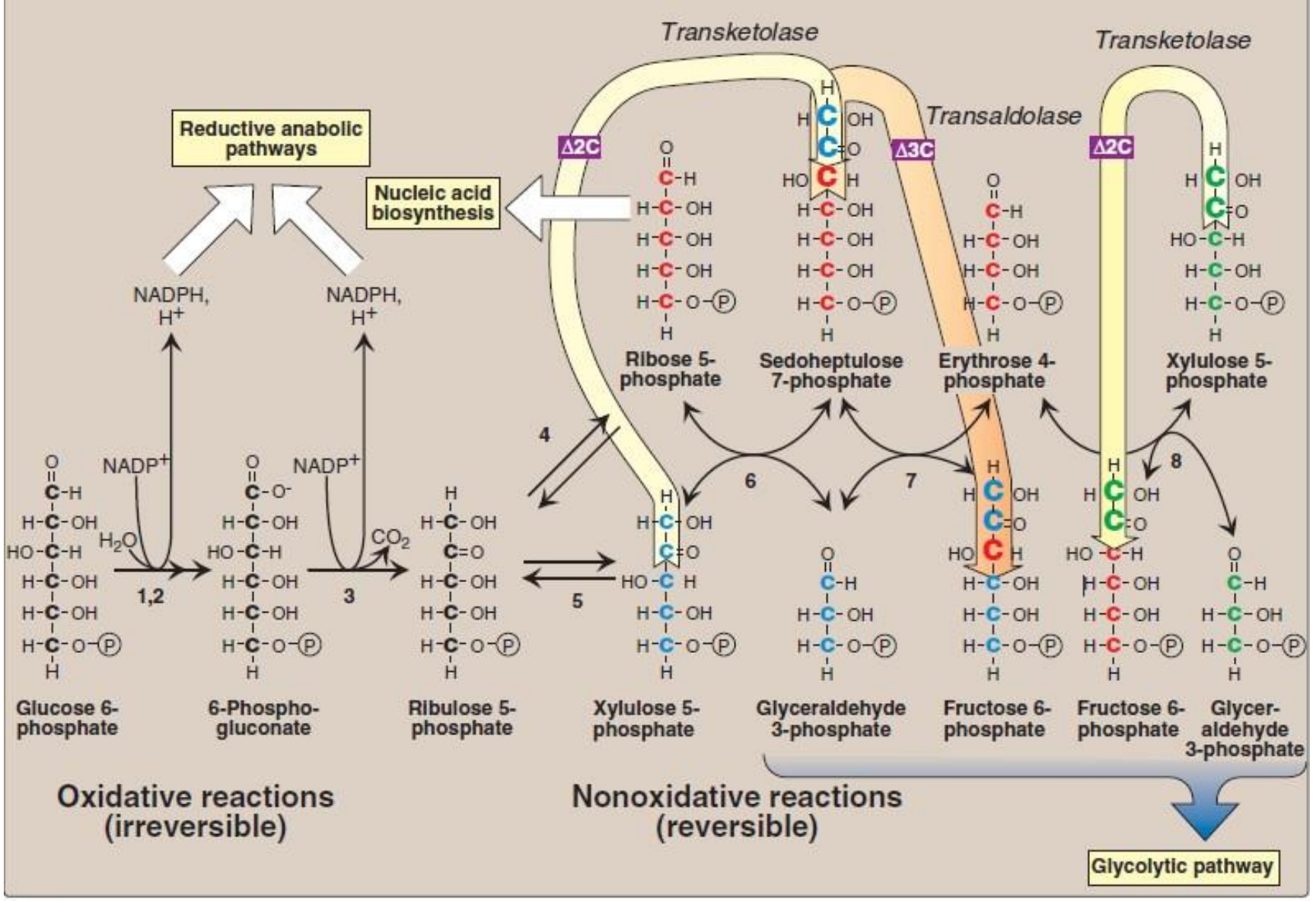


Figure 13.2

Reactions of the hexose monophosphate pathway. Enzymes numbered above are: 1,2) *glucose 6-phosphate dehydrogenase* and *6-phosphogluconolactone hydrolase*, 3) *6-phosphogluconate dehydrogenase*, 4) *ribose 5-phosphate isomerase*, 5) *phosphopentose epimerase*, 6) and 8) *transketolase* (coenzyme: thiamine pyrophosphate), and 7) *transaldolase*. Δ2C = two carbons are transferred in *transketolase* reactions; Δ3C = three carbons are transferred in the *transaldolase* reaction.

Carbon movements in non-oxidative reactions

Ketose + Aldose ⇌ Aldose + Ketose

C5 + C5 ⇌ C3 + C7

C7 + C3 ⇌ C4 + C6

C5 + C4 ⇌ C3 + C6

Summary of the non-oxidative reactions

- Reversible reactions
- Transfer of 2 or 3 carbon fragment
- Transketolase (2C), Transaldolase (3C)
- Ketose + aldose ⇌ ketose + aldose
- From ketose to aldose

- Rearrangment of sugars
- 3 pentose phosph.. ⇌ { 2 hexose phosph + 1 triose phosph. }

The net non-oxidative reaction

- 3 Ribulose 5-phosph. ⇌ { 2 Fructose 6-phosph. + Glyceraldehyde 3-phosph. }
- Multiply by 2
- 6 Ribulose 5-phosph. ⇌ { 4 Fructose 6-phosph. + 2 Glyceraldehyde 3-phosph. }

↓

↓

↓

5 Fructose. 6-Phosph.

Net Products of the 2 Phases

3 Glc. 6-P + 6 $NADP^+$ → 2 Glc. 6-P + GA3P + 3 CO_2 + 6 NADPH

6 Glc. 6-P + 12 $NADP^+$ → 4 Glc. 6-P + 2 GA3P + 6 CO_2 + 12 NADPH

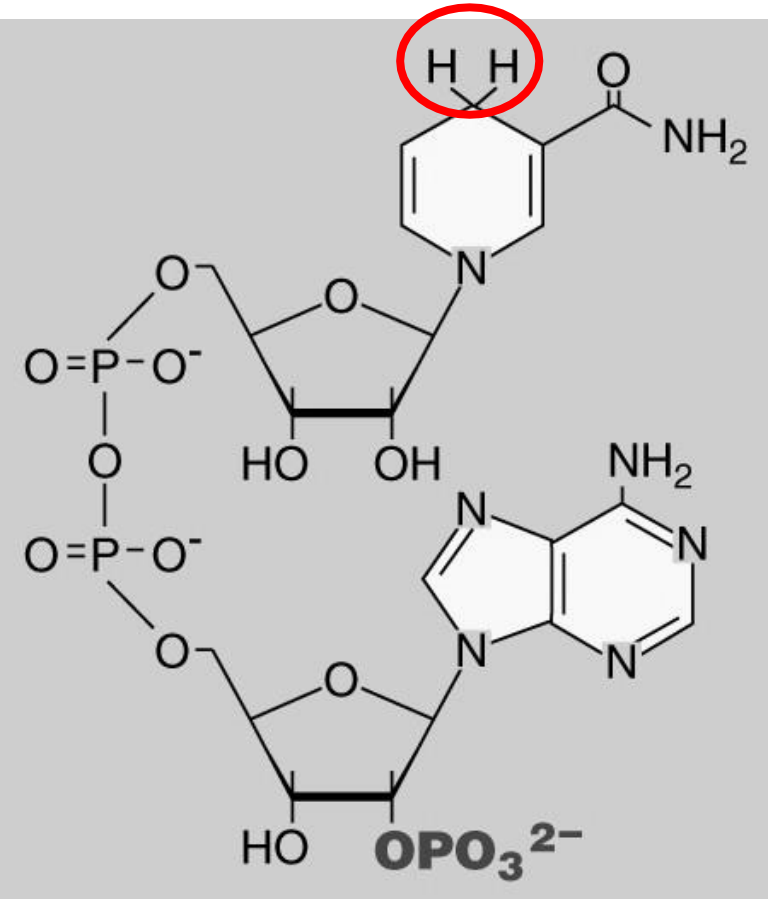
↓

Glc. 6-P

6 Glc. 6-P + 12 $NADP^+$ → 5 Glc. 6-P + 6 CO_2 + 12 NADPH

NADPH vs NADH

- Enzymes can specifically use one NOT the other
- NADPH and NADH have different roles
- NADPH exists mainly in the reduced form (NADPH)
- NADH exists mainly in the oxidized form (NAD^+)
- In the cytosol of hepatocyte
  - $NADP^+/NADPH \approx 1/10$
  - $NAD^+/NADH \approx 1000/1$



What are the uses of NADPH?

1. Reductive Biosynthesis

- Some biosynthetic reactions require high energy electron donor to produce reduced product
- Examples: Fatty acids, Steroids …

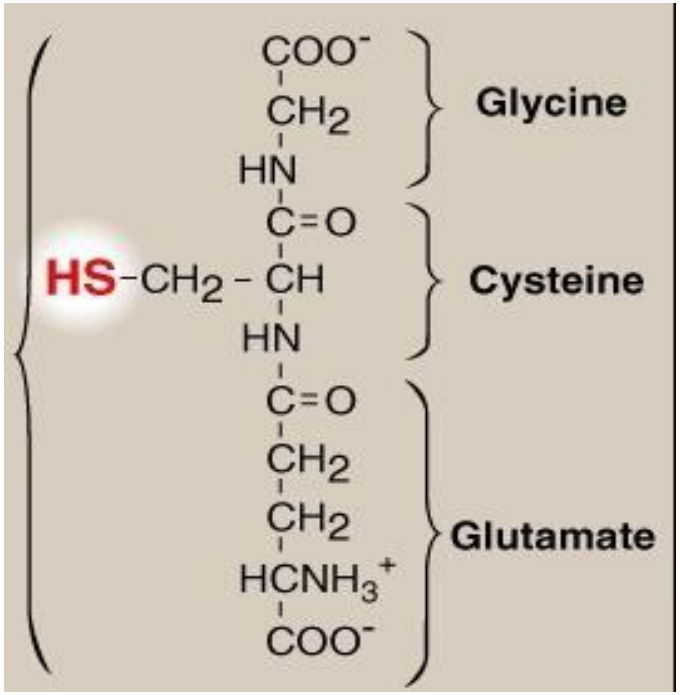
2. Reduction of Hydrogen Peroxide

- H_2O_2 one of a family of compounds known as Reactive Oxygen Species (**ROS**)
- Other: Super oxide, hydroxyl radical,
- Formed continuously
  - As by products of aerobic metabolism
  - Interaction with drugs and environmental toxins
- Can cause chemical damage to proteins, lipids and DNA ➔cancer, inflammatory disease, cell death

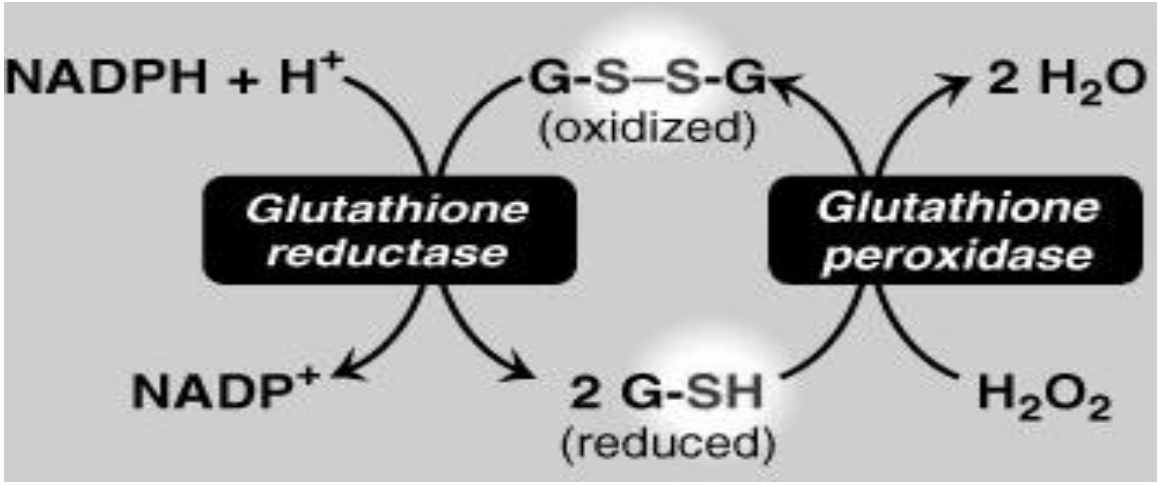
Enzymes that catalyze antioxidant reactions

1. Glutathione peroxidase

- Glutathione is a reducing agent
- Tripeptide
- GSH is the reduced form
- Oxidation ➔two molecules joined by disulfide (GSSG)
- 2 GSH ⟶ GSSG



Glutathione peroxidase is Selenium requiring Enzyme RBCs are totally dependent on PPP for NADPH production



Enzymes that catalyze antioxidant reactions

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

3. Catalase

Anti oxidant chemicals

- Vitamin E, Vitamin C, Carotenoids

Clinical Hint: G6PD Deficiency

- A 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

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

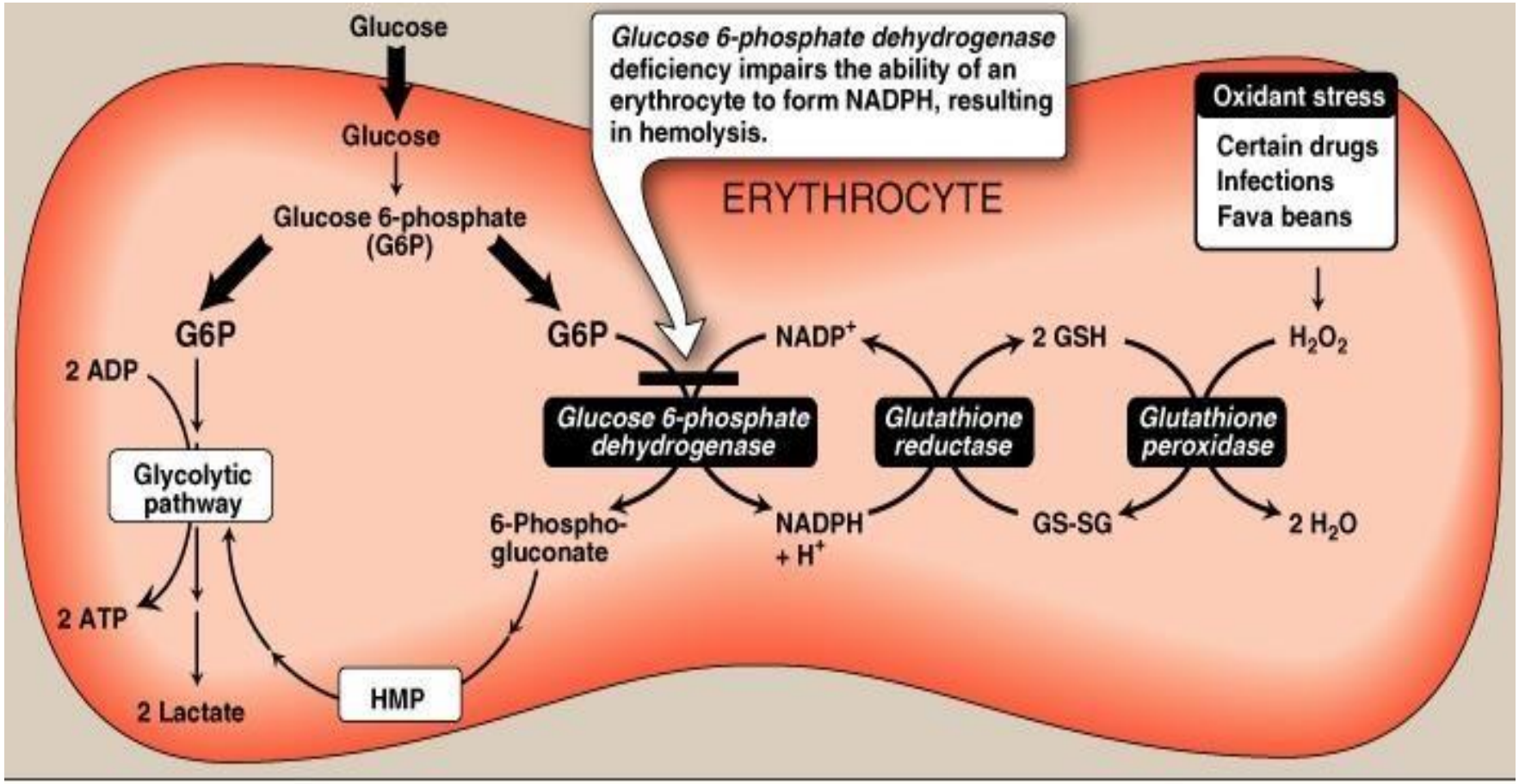
Role of G6PD in red blood cells

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

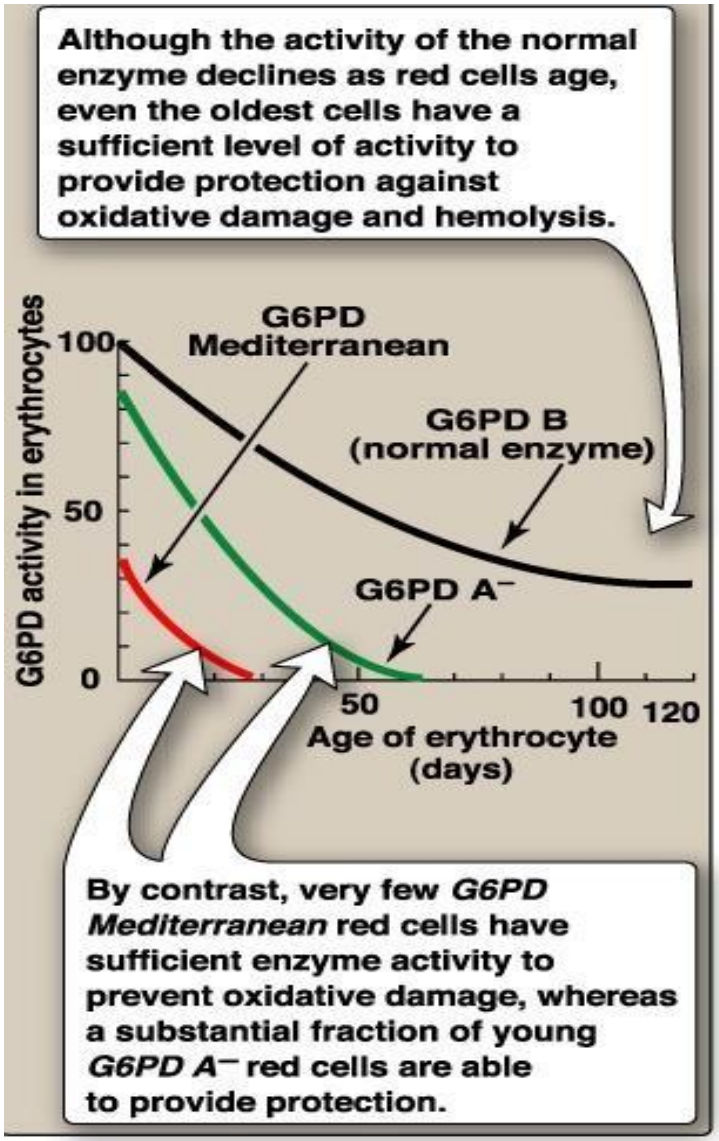
$G\text{-}S\text{-}S\text{-}G + NADPH \longrightarrow 2GSH + NADP+$

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

Oxidation ➔ denaturation of proteins and rigidity of the cells



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%

- Wild type B
- Mediterranean Variant B^- (Class II) : 563C→T
- African Variant A^- (Class III); two point mutation
- Majority missense mutation, point mutation
- Large deletions or frame shift; Not Observed

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
- Coenzyme Q in Respiratory chain
- Respiratory Burst (during phagocytosis) O_2^-, $OH^\bullet$, NO, HOCl, H_2O_2
- Ionizing Radiation $OH^\bullet$

Cytochrome P450 Mono oxygenase

- Mixed function oxygenase
- Super family of structurally related enzymes

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

✓ Mitochondrial system

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

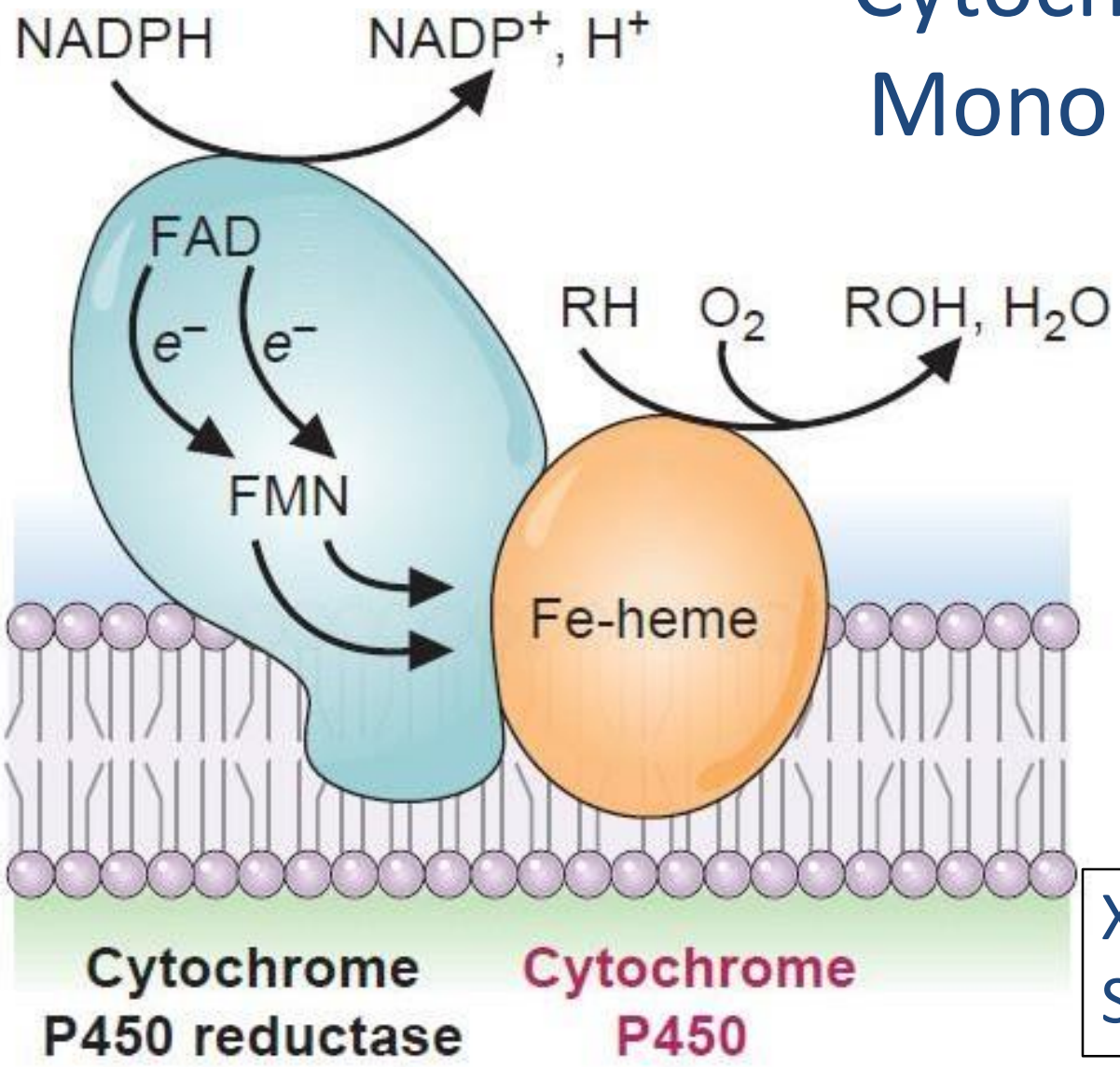
✓ Microsomal system

Detoxification of foreign compounds

Activation or inactivation of Drugs

Solublization to facilitate excretion in urine or feces

Cytochrome P450
Mono oxygenase



Accidental release of free radical intermediates may occur

XH2: electron donor
S: substrate

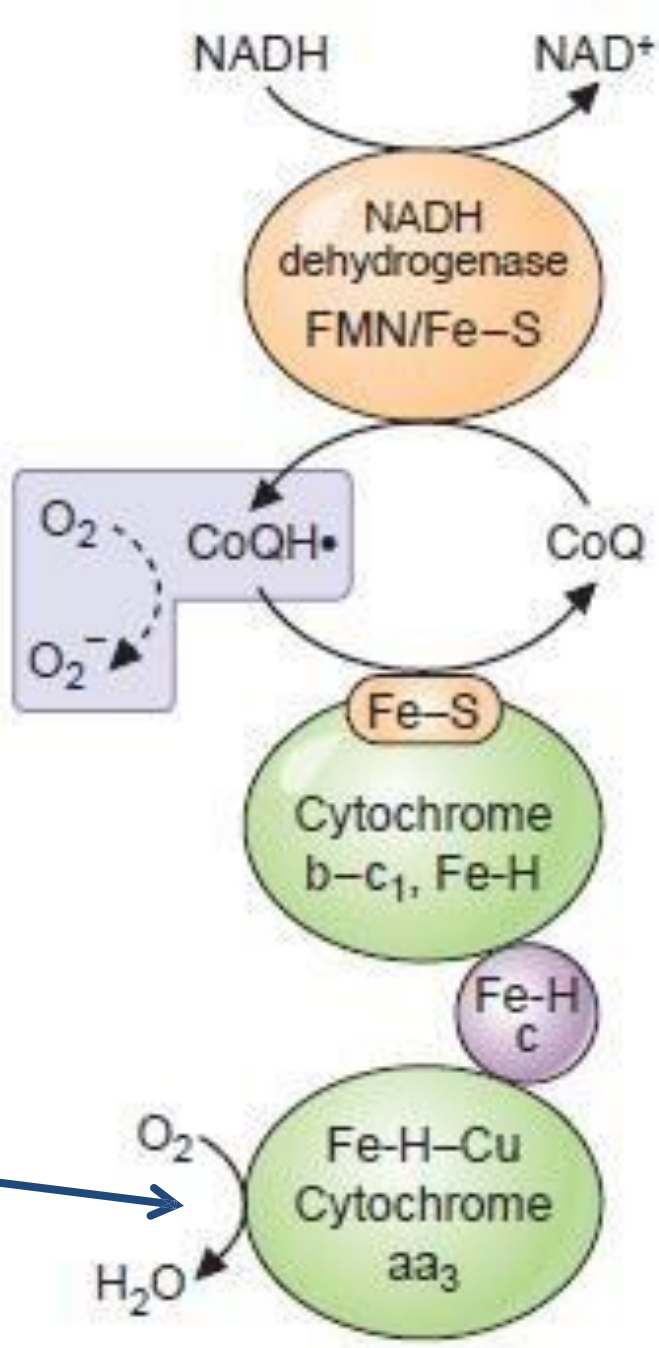
$$O_2 + S + XH_2 \longrightarrow H_2O + SOH + X$$

Generation of O_2^- by the respiratory chain

Accidental non-specific interaction

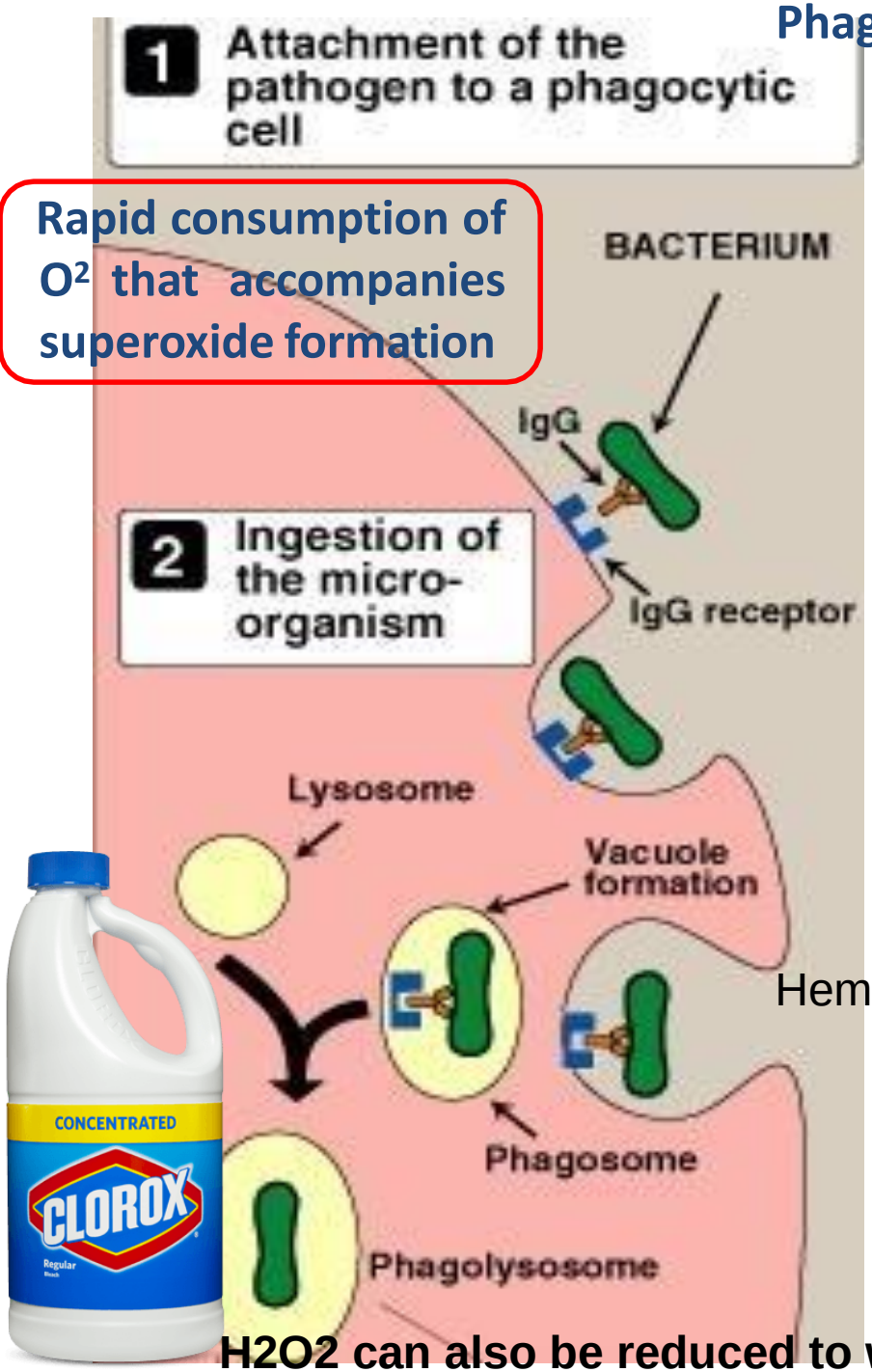
Major source of free radicals

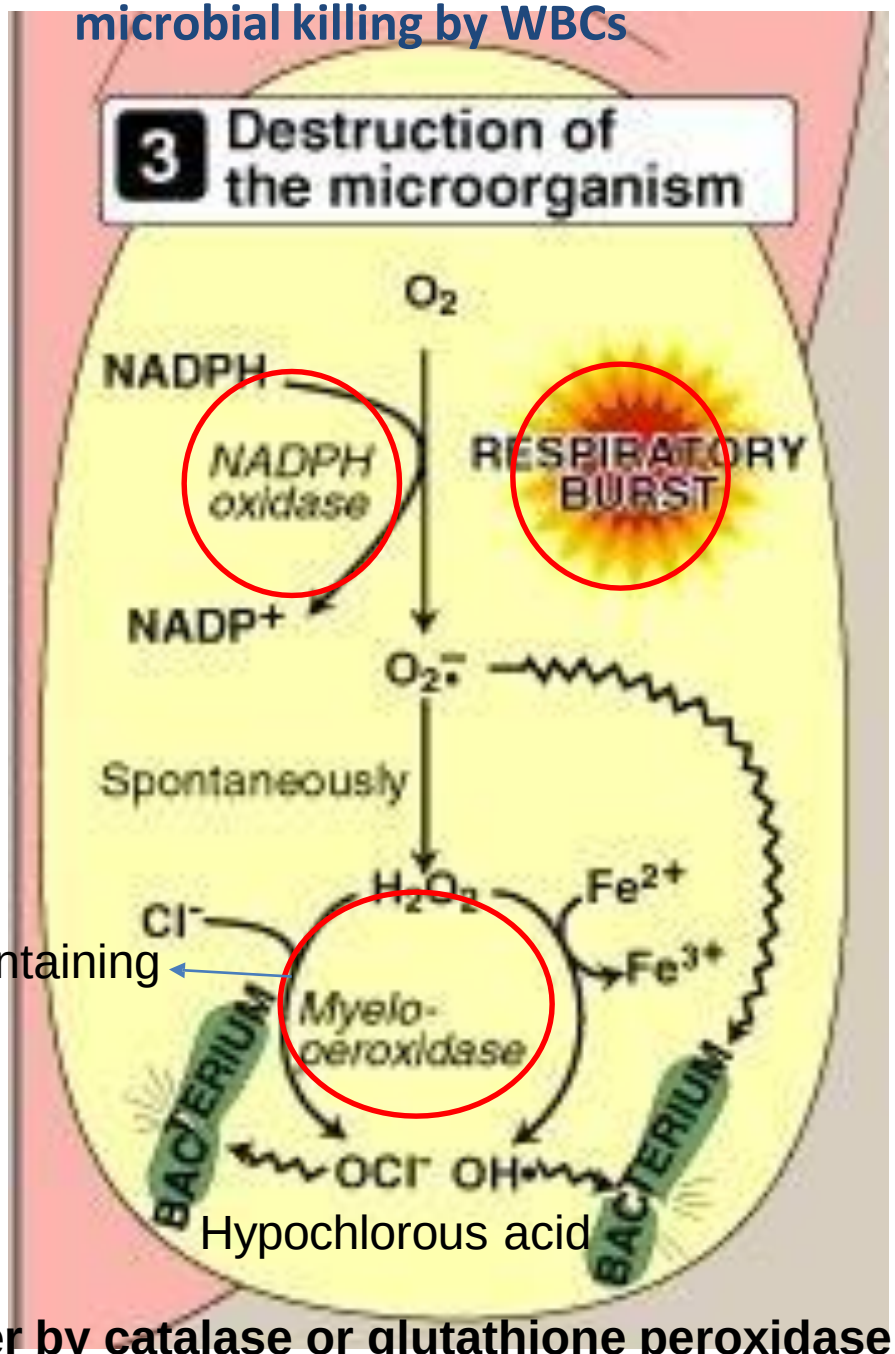
Binuclear center prevents release of free O_2 radicals



Phagocytosis; the oxygen dependent pathway of microbial killing by WBCs

Rapid consumption of O^2 that accompanies superoxide formation

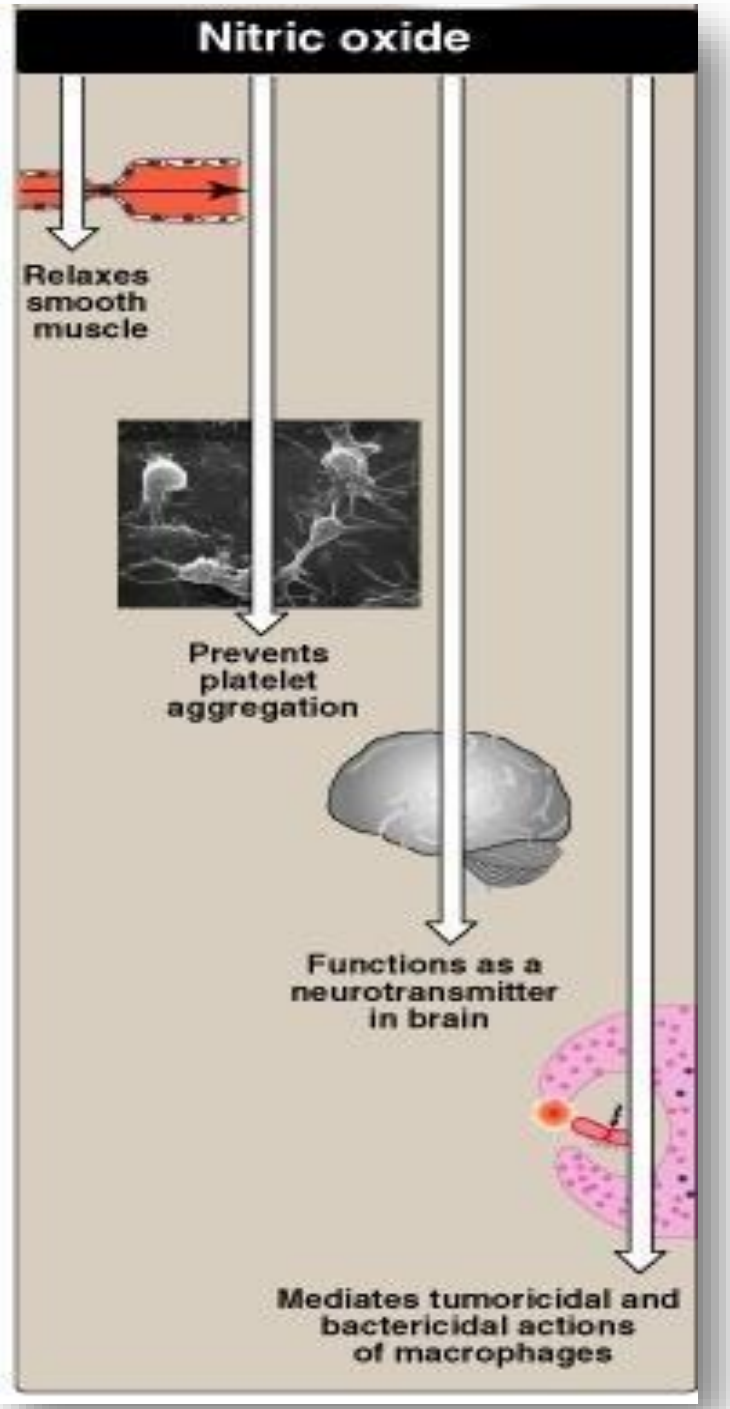




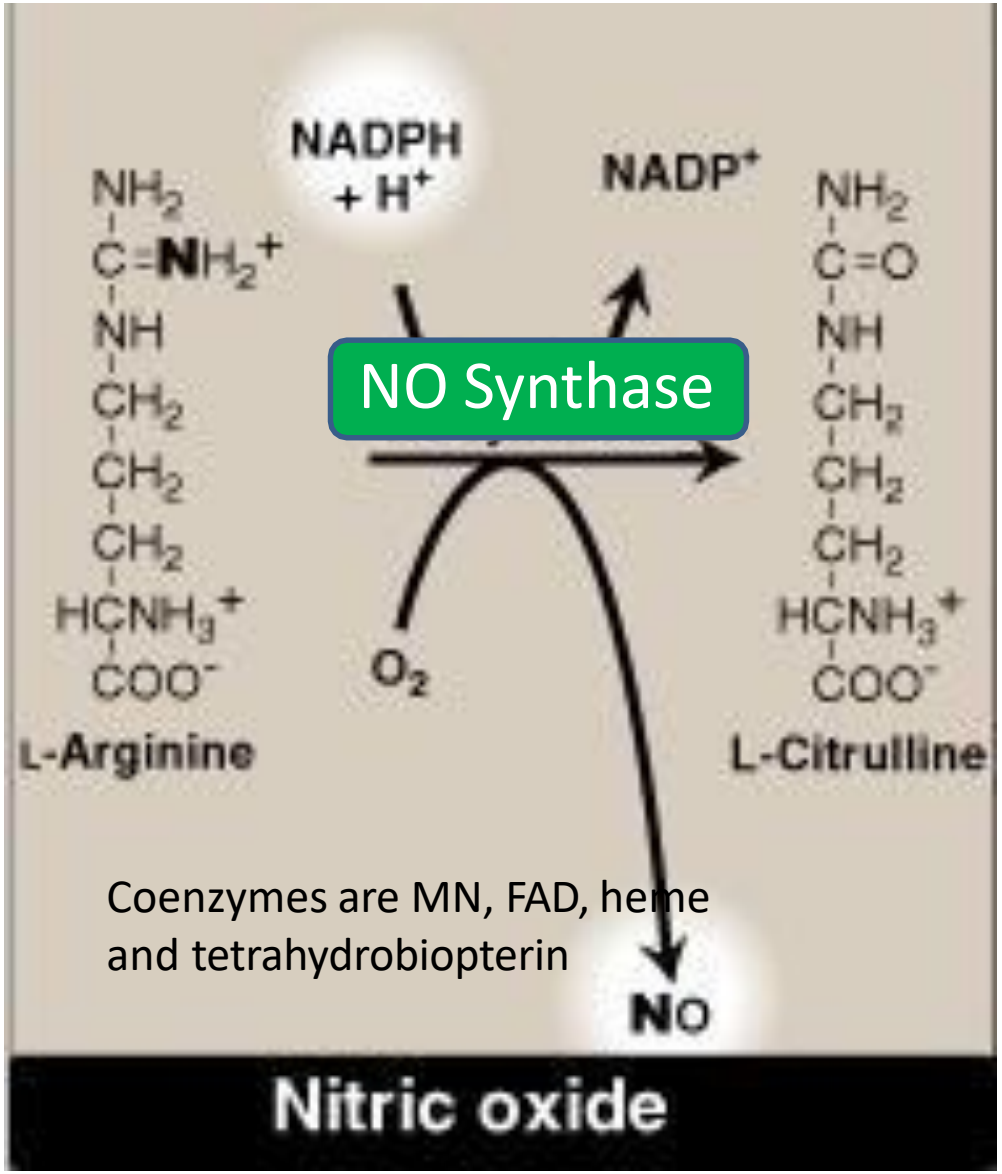
H2O2 can also be reduced to water by catalase or glutathione peroxidase

NO and Reactive Nitrogen Oxygen Species (RNOS)

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



NO Synthesis

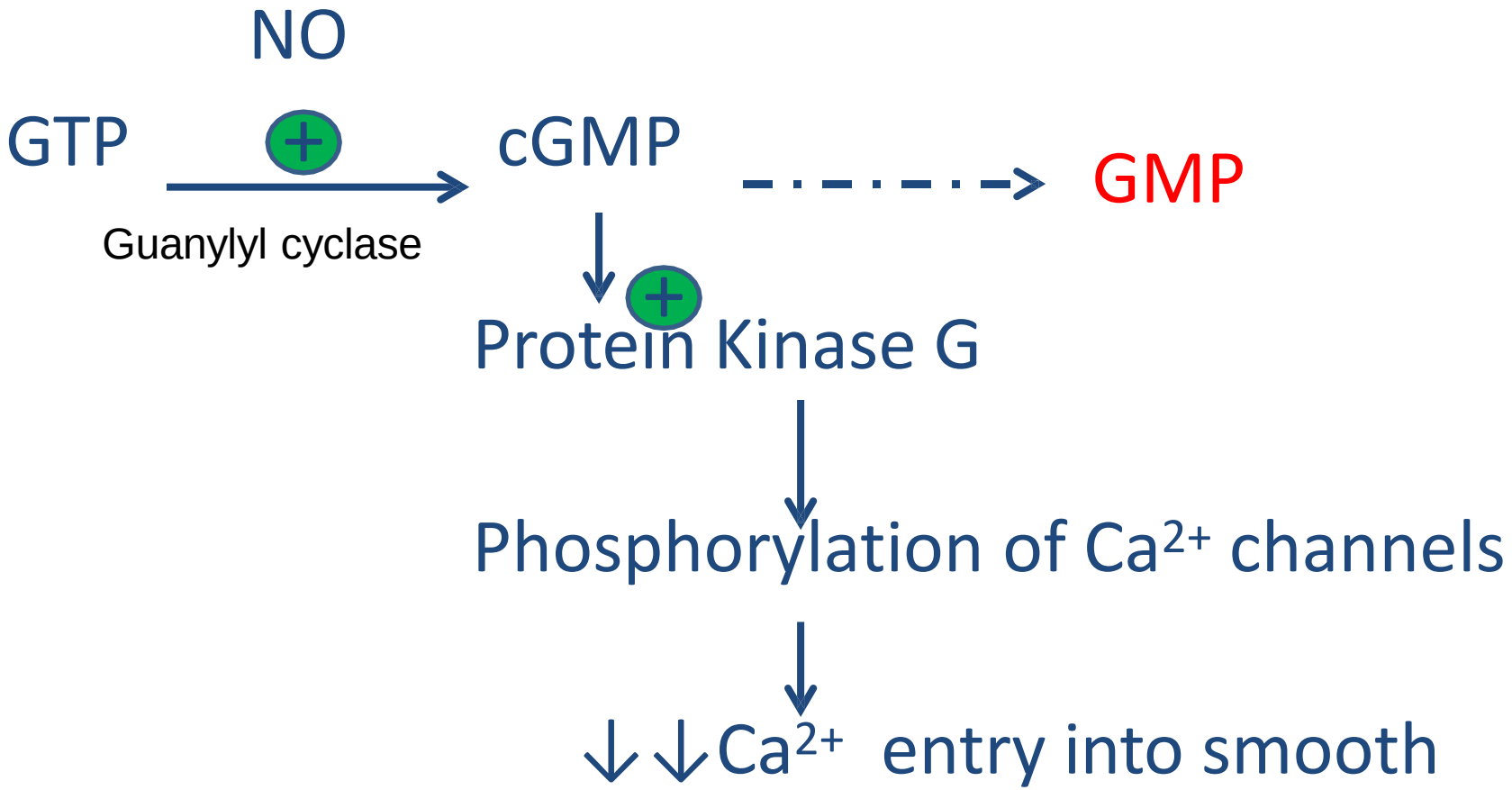


NO Synthase
Three isoforms
nNOS neural
eNOS endothelial
Both are constitutive

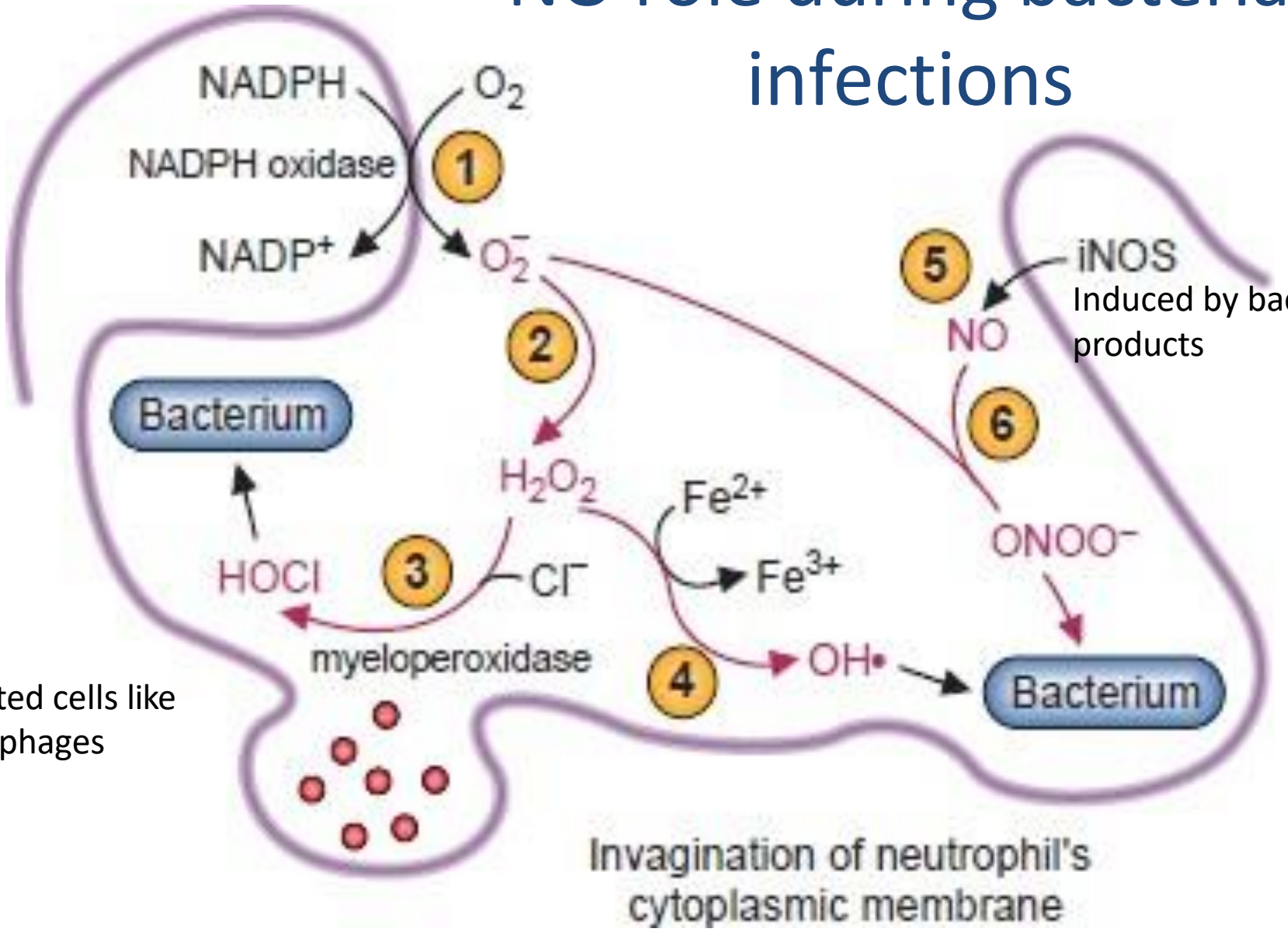
iNOS inducible Ca+2 independent
Induction of transcription in many cells of immune system➔↑↑ NO ➔ RNOS to kill invading bacteria

Action of NO on vascular endothelium

Synthesis by endothelia cells → smooth muscle



NO role during bacterial infections



Activated cells like macrophages

Hypochlorous acid