Biochemistry Team 435

جامعة الملك سعود
King Saud University 1957

OXIDATIVE STRESS

"I CAN'T CHANGE THE DIRECTION OF THE WIND, BUT I CAN ADJUST MY SAILS TO ALWAYS REACH MY DESTINATION" –JIMMY DEAN

Color index:

- Important
- Extra explanation

*** Please check out this link to know if there are any changes or additions.**

MECHANISMS OF CELL INJURY

In the foundation block pathology, we've talked about the mechanisms of cell injury, one of them was "**the accumulation of oxygen-derived free radicals (oxidative stress)**" which we're going to talk about in this lecture, before we start we have to revise some facts about the free radicals.

- **What are free radicals?**

They're highly reactive and harmful atoms that have single unpaired electron in their outer orbit.

- **how they get produced?**

1- Normal metabolism\respiration: small amounts of harmful reactive oxygen are produced as a "Bi-product" of mitochondrial respiration during normal respiration (through the reduction-oxidation reactions)

2- Ionizing radiation Injury, e.g. UV light which results in the production of free radicals.

3- chemical toxicity.

4- oxygen therapy and reperfusion injury.

5- transition metals such as iron which triggers the production.

- **Common free radicals:** H2O2, NO, OH.

This slide is Extra ..

- **What do they cause?** They cause **damage to** lipids, proteins, and nucleic acids.
- **What are their main damaging effects?**

- **Lipid peroxidation of membranes** → leads to membrane damage.
- **DNA damage** → leads to cell aging and malignant transformation of cells.
- **Oxidative modification of proteins** → leads to protein fragmentation.

- **How does our body fight them?!!!**

- Anti-oxidants: vitamins E,A and C.
- Some enzymes.

NITRIC OXIDE:

This slide is Extra ..

In the foundation block pharmacology, we've talked about the paracrine autocrine mediators and they were classified into so many groups, one of them was "**Nitric oxide**", which we're going to discuss in this lecture also

- **What is Nitric oxide?**

It is a highly diffusible stable gas.

- **Synthesis**

$$\text{L-arginine} + O_2 \xrightarrow{\text{Nitric Oxide Synthase (NOS)}} \text{NO} + \text{Citrulline} + H_2O$$

.

- **NOS Isoforms:**

Type I (N-Nos)	Type III (E-NOS)	Type II (I-NOS)
In the cytosol of neural cells.	Bound to membrane of endothelial cell, platelets,… etc.	Cytosol of macrophages, neutrophils, kupffer cells,… etc.
Constitutive	Constitutive	Inducible.

.

- **Actions:**

1- Vasodilation
2- platelet aggregation
3- Inflammatory cell recruitment.
4- cholesterol deposition.

OBJECTIVES:

- Know the reactive oxygen species (ROS).
- Define the source of ROS.
- Recognize the toxic effects of ROS.
- Identify the productive mechanisms against ROS.
- Describe glucose 6-phosphate dehydrogenase deficiency .

Oxidative stress:

- **What is it?**

- A condition in which cells are subjected to excessive levels of **Reactive Species** (Oxygen or Nutritive species) & they are unable to counterbalance their deleterious effects with Antioxidants.

- **Im**balance between oxidant production and antioxidant mechanisms.

- **It has been implicated in:**

- The aging process.
- Many diseases (e.g., atherosclerosis and coronary heart diseases).

- **It Causes oxidative damage to:**

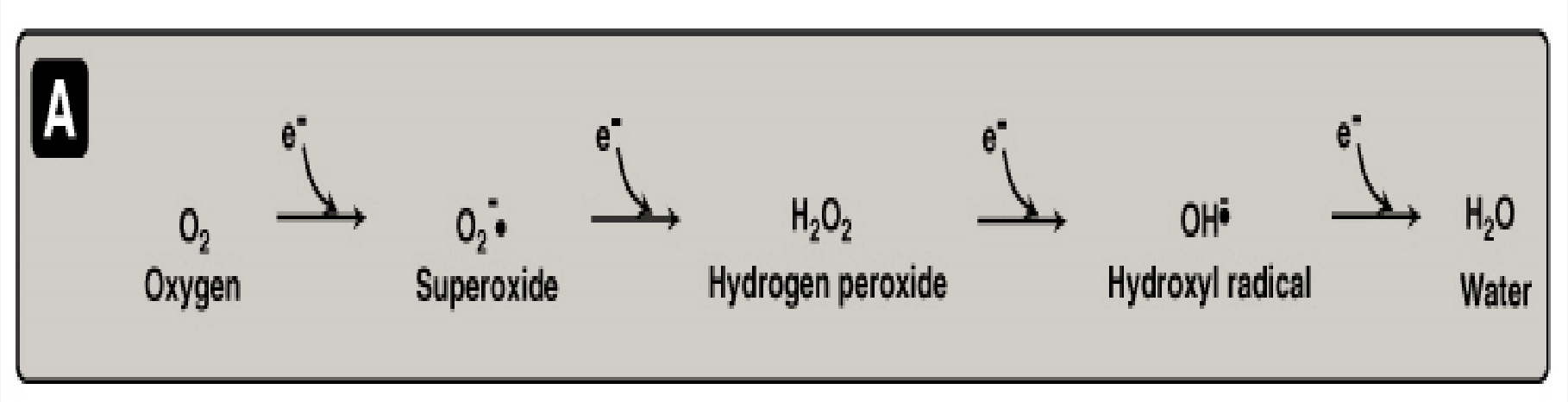
DNA, Proteins and lipids (unsaturated fatty acids)

It has been implicated in a number of pathological process

- **Cancers**
- **obesity**
- **Atherosclerosis and coronary artery disease**
- **Inflammatory conditions** e.g. rheumatoid arthritis
- **G6PD deficiency hemolytic anemia**

Oxidative stress

REACTIVE OXYGEN SPECIES (ROS)

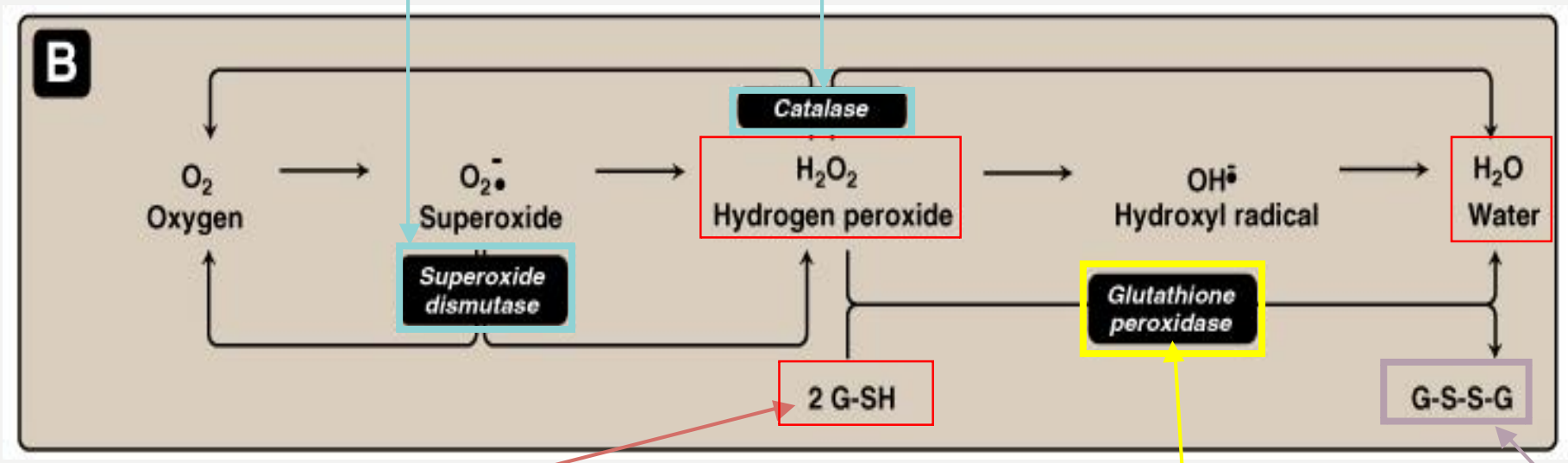


Hydrogen peroxide **(H_2O_2)** is one of a family of reactive oxygen species (ROS) that are formed from the partial reduction of molecular oxygen. These compounds are formed continuously as byproducts of aerobic metabolism, through reactions with drugs and environmental toxins, or when the level of antioxidants is diminished, all creating the condition of "oxidative stress". The highly reactive oxygen intermediates can cause serious chemical damage to DNA, proteins and unsaturated lipids (As mentioned in the previous slides).

ANTIOXIDANTS MECHANISMS

4-additional enzymes such as **superoxide dismutase** and **catalase**, catalyze the conversion of other reactive oxygen intermediates to harmless products. (serve as a defense system to guard against the toxic effects of ROS)

Note:
these process occur Inside the mitochondria . In the absent of these enzymes the free radicals leak out of mitochondria and cause damage.



1-Reduced glutathione (G-SH) which is present in most cells can chemically detoxify H_2O_2 by converting it into water (harmless product).

2-the reaction is catalyzed by **glutathione peroxidase**

3- this reaction will ultimately form an oxidized glutathione (G-S-S-G), which doesn't have the protective properties

REACTIVE OXYGEN SPECIES (ROS): TYPES AND SOURCES

Type:	Examples:	Source:
Oxygen-derived Free radical	Superoxide ($O_2^{\cdot}$)	- Auto-oxidation of hemoglobin and xanthine oxidase. - Partial reduction of molecular oxygen in electron transport chain in mitochondria.
	Hydroxyl radical ($OH^{\cdot}$)	- Fenton reaction (the formation of $OH^{\cdot}$, OH^-, and Fe^{3+} from the non-enzymatic reaction of Fe^{2+} with H_2O_2; a reaction of importance in the oxidative stress in blood cells and various tissues.). - Partial reduction of molecular oxygen in electron transport chain in mitochondria.
	Peroxyl radical ($ROO^{\cdot}$)	-
Non-free radical	Hydrogen peroxide (H_2O_2)	- Partial reduction of molecular oxygen in electron transport chain in mitochondria.

SOURCES

During course of metabolism

- $O_2^{\cdot}$ by auto-oxidation of hemoglobin and xanthine oxidase.
- OH. by Fenton reaction
- O2. , H2O2 , OH. By partial reduction of molecular oxygen in electron transport chain in mitochondria

Ingestion of toxins, chemicals or drugs

Biochemistry Team 435

An **antioxidant** is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells.

Antioxidants

Enzymes

- **Superoxide dismutase**
- **Catalase**
- **Glutathione system** (glutathione, NADPH, reductase, peroxidase & selenium)

Vitamins

- **Vitamin C (ascorbic acid)**
- **Vitamin A and β-carrotenes**
- **Vitamin E**

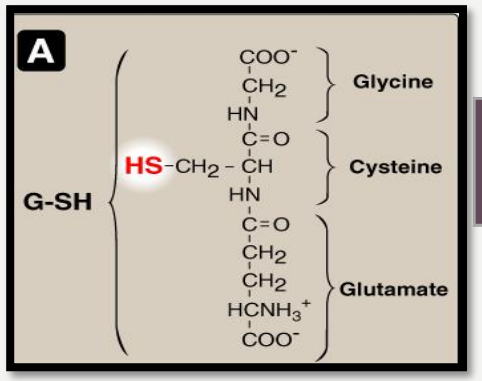
Trace elements

- **Selenium**

Note: Selenium; At molecular level is important in glutathione system. In malnutrition ,there is decrease in selenium level (decrease in antioxidant) , that leads to defect in the glutathione system and increase oxidants level.

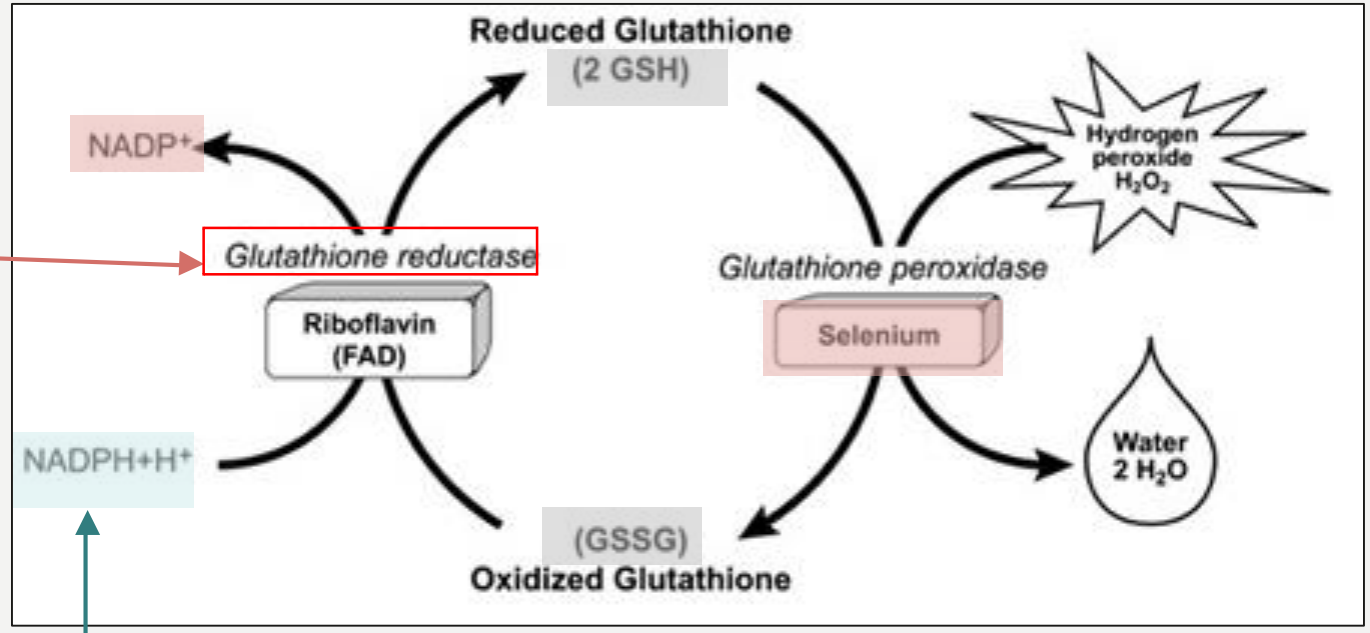
a **trace element** is a dietary **element** that is needed in very minute quantities for the proper growth, development, and physiology of the organism.

GLUTATHIONE SYSTEM



Structure of reduced glutathione (G-SH), note that cysteine is linked to glutamate through a gamma-carboxyl rather than an alpha-carboxyl.

The cell regenerates G-SH in a reaction catalyze by "**glutathione reductase**", using NADPH as a source of reducing equivalents,



بعد ماحولنا الـ H_2O_2 ، إلى نتائج أقل ضررا على الخلية، نحتاج نرجع (G-SH) زي ماكان علشان نستخدمه مره ثانيه ، كيف؟
عن طريق (**glutathione reductase**) اللي راح يأخذ هيدروجينز من (NADH) ويحوله من الأوكسدايزد فورم إلى الرديوسد فورم (اللي ينفعنا ونحتاجه)

From where do we get the NADPH?

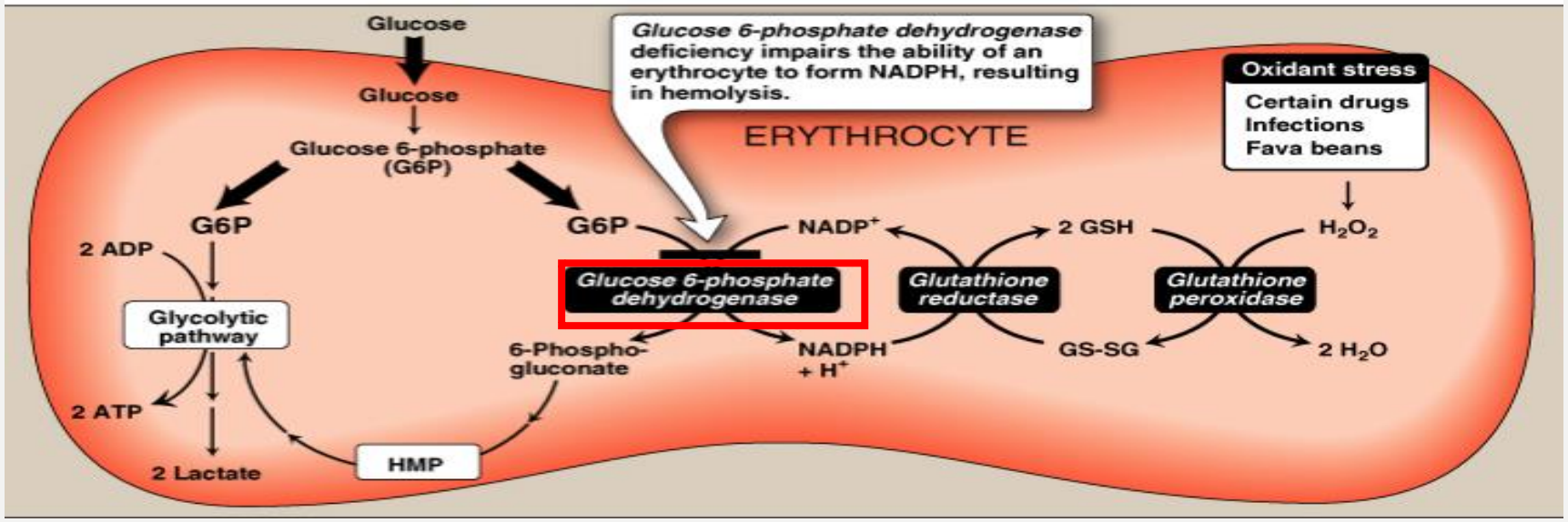
Glucose-6-phosphate dehydrogenase (**G-6-PD**) is **the main source** for NADPH generation and is, therefore, essential for proper function of glutathione system.
(without it we cannot have the NADPH, therefore we can't convert the oxidized glutathione into the reduced form "the beneficial form")

- **Biochemical Basis of G6PD Deficiency Hemolytic Anemia:**
- **For better understanding :**

- It happens within the RBCs.

- In normal red blood cells , G6PD (Glucose 6-phosphate dehydrogenase) is responsible of the conversion of NADP into NADPH, then NADPH will help Glutathione reductase to convert Glutathione from the oxidized form (GS-SG) to the reduced form (2 GSH), and finally 2 GSH will activate Glutathione peroxidase which will transform H2O2 into water.

so when there is an impairment or a deficiency of G6PD, the oxidized Glutathione will accumulate in the RBCs , which will lead to the accumulation of H2O2 , and ultimately will cause hemolysis of the RBCs.



Effects of ROS

1. Molecular Effects.

- **Lipid peroxidation** (oxidative degradation of lipids, it's the process in which free radicals steal electrons from lipids cell membrane, resulting in cell damage).

(polyunsaturated fatty acids is most often affected in lipid peroxidation)

- **Protein denaturation.**
- **Inactivation of enzymes.**
- **DNA damage.**
- **Cell signaling effects.**

(e.g., release of Ca2+ from intracellular stores)

- **Cytoskeletal damage.**
- **Chemotaxis** .

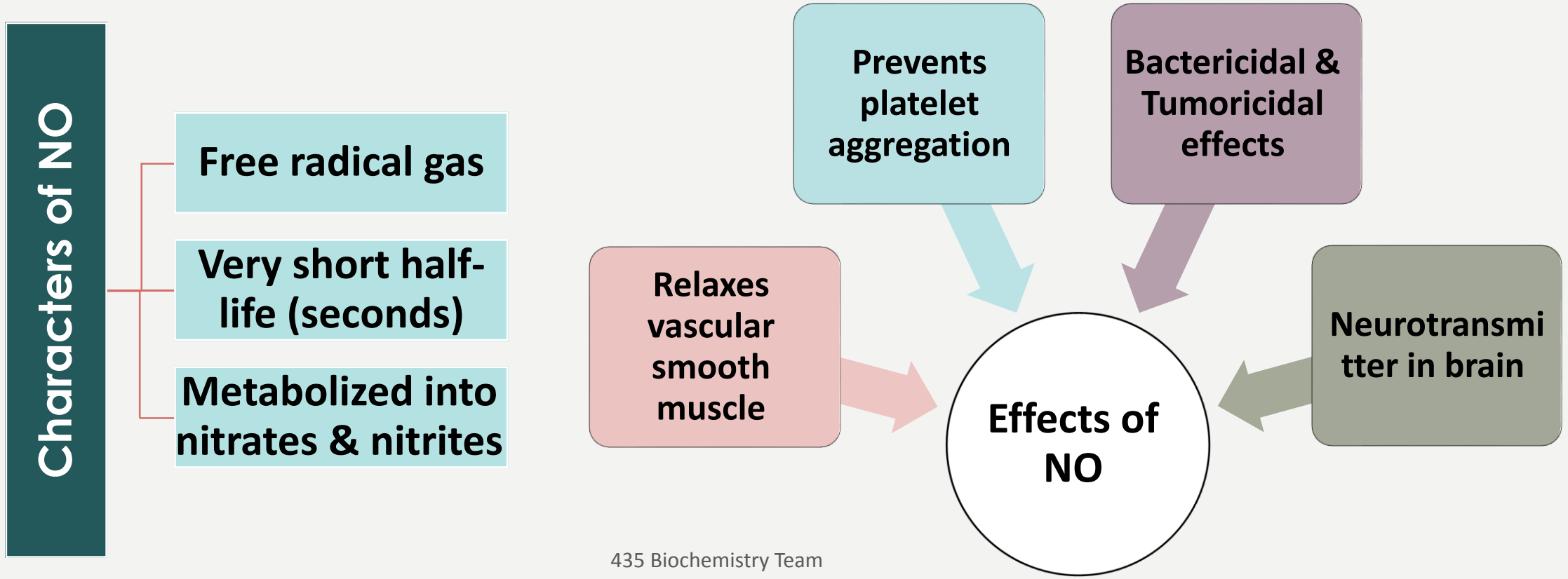
2. .Vascular Effects.

- **Altered vascular tone .**
- **Increased endothelial cell permeability.** (that's why there is usually an inflammation and edema with oxidative stress).

Nitric Oxide (NO)

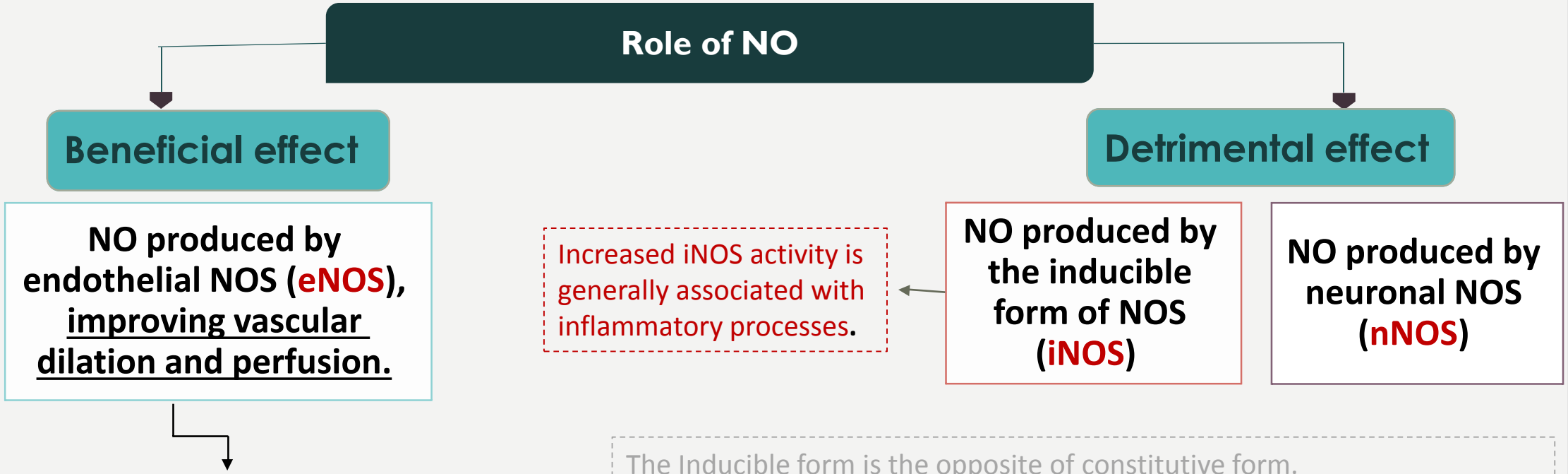
Synthesis of Nitric Oxide (NO):

- The enzyme involved in synthesis of Nitric Oxide: NO Synthase (NOS).
- Precursor: L-Arginine (Not R-Arginine).
- The Arginine will turn to Citrulline in presence of NOS, and that will lead to formation of nitric oxide.



Oxidative Stress: Role of Nitric Oxide (NO)

The role of NO in oxidative stress may be both beneficial and detrimental (pathologic), depending upon when and where NO is released.



Vasodilators such as nitroglycerin is metabolized into NO and causes vasodilatation.

people with angina takes usually a sublingual tablets that releases nitric oxide which relief the angina by vasodilation.

The Inducible form is the opposite of constitutive form.

Constitutive form has a basal level of activity, so it doesn't need a stimulus to get expressed, while the inducible form needs a stimulus for expression.

In simple terms : constitutive expression is like the muscle tone, no stimulus is needed. And inducible expression is like muscle contraction, which needs stimulus to happen.

Pathogenesis of Atherosclerosis

Modified (oxidized) LDL (Oxidative stress)

(imbalance between oxidants and antioxidants)

→ **Endothelial injury of arterial wall**

↓

Adherence of monocytes to endothelial cells and their movement into intima where it becomes macrophages

←

Uptake of oxLDL by macrophage scavenger receptor class A (**SR-A**)

↓

Foam cell transformation: Accumulation of excess lipids inside the cells (unregulated receptor)

→ **Atherosclerotic plaque formation**

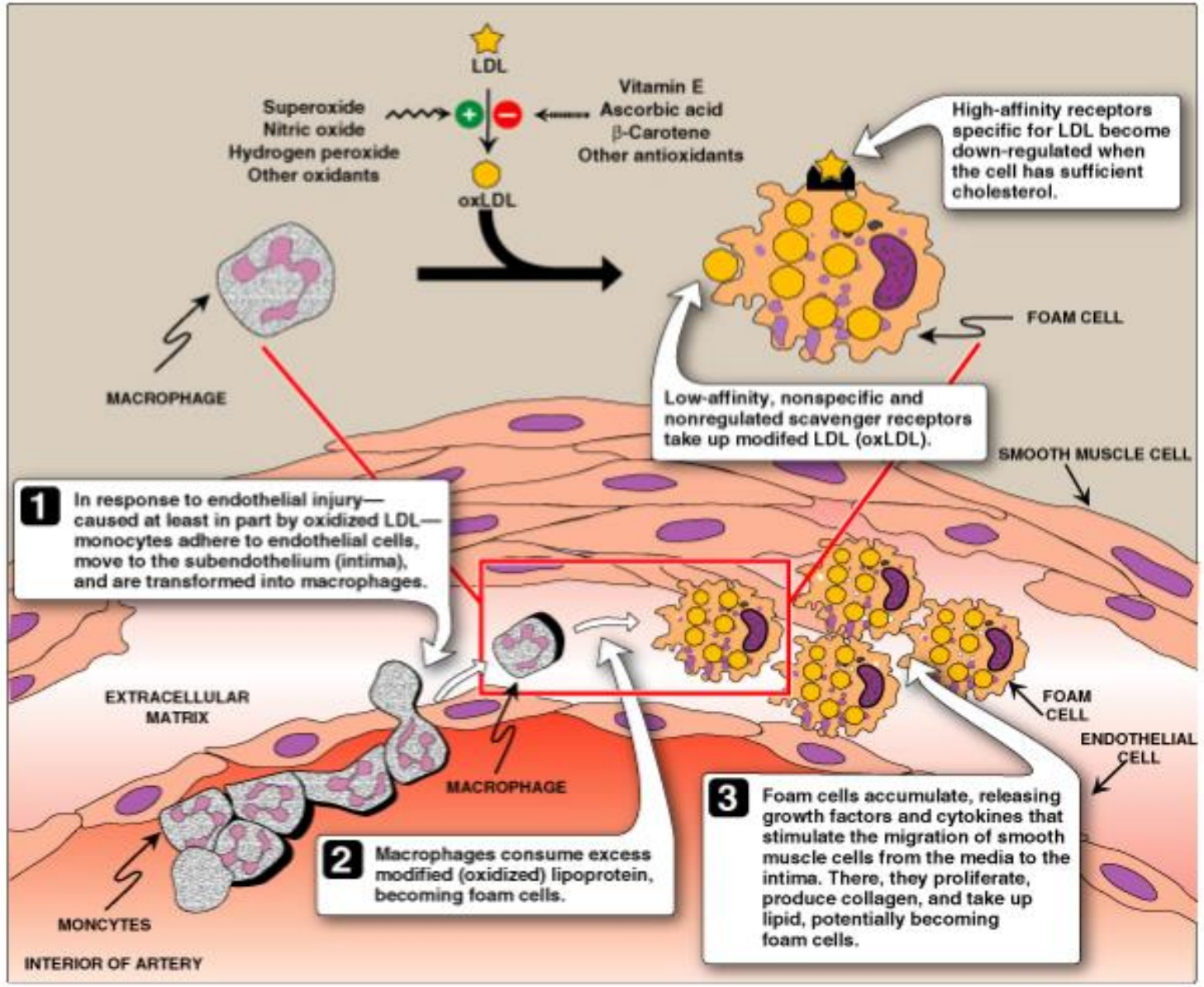
REMEMBER:

regular LDL receptor is tightly regulated can regulate the amount of cholesterol inside the cell ,but this SR-A receptor is un-regulated and allows more cholesterol inside the cell, which will turn into foam cells and secrete growth factors and inflammatory cytokines

Scavenger receptor class A (SR-A):

Low-affinity, non-specific, Un-regulated receptor.

Atherosclerotic plaque Formation



Atherosclerosis plaque formation

Extra explanation for memory refreshing:

- If the superoxide, NO, hydrogen peroxide, or other oxidants are increased, LDL will turn to oxLDL.
- Then, it will bind to scavenger receptors type A.
- After that, the cell will be filled of cholesterol , so the macrophage will turn to foam cell.
- Foam cells secrete growth factors and cytokines.

Summary

Oxidative stress

What is it ?	A condition in which cells are subjected to excessive levels of Reactive Species (Oxygen /Nitrative species) & they are unable to counterbalance their deleterious effects with antioxidants
Implicated in	Ageing process & many diseases (e.g.: Atherosclerosis and coronary heart diseases)
Diseases	Inflammatory conditions (Rheumatoid arthritis) Atherosclerosis and coronary artery diseases Obesity Cancers G6PD deficiency hemolytic anemia

Reactive Oxygen Species (ROS)

$$O_2 \rightarrow O_2^{\bar{}}\cdot \rightarrow H_2O_2 \rightarrow OH^{\bar{}}\cdot \rightarrow H_2O$$

Superoxide ($O_2^{\bar{}}\cdot$)	Free radicals	Sources: • Ingestion of toxins, chemicals, or drugs. • During course of metabolism:	• auto-oxidation of hemoglobin • xanthine oxidase • partial reduction of molecular oxygen in ETC (to make water)
Hydroxyl radical ($OH^{\bar{}}\cdot$)			• Fenton reaction (ferric\ferrous) • partial reduction of molecular oxygen in ETC (to make water)
Peroxyl radical ($ROO^{\bar{}}\cdot$)			-
Hydrogen peroxide (H_2O_2)	Non free radical		• partial reduction of molecular oxygen in ETC(to make water)

Summary

Antioxidants

enzymes	Superoxide dismutase	Converts superoxide into oxygen or hydrogen peroxide
	Catalase	Converts hydrogen peroxide into oxygen or water
	Glutathione system	Converts hydrogen peroxide into oxygen or water
vitamins	Vitamin C (ascorbic acid) / Vitamin A and β-carotenes / Vitamin E	
Trace elements	Selenium	

Glutathione system

Glutathione ?	glycine + cysteine (with SH) + glutamate
What happens ?	• Reduced glutathione(2G-SH) donates its hydrogen and gets oxidized into oxidized glutathione (G-S-SG) by glutathione peroxidase (why?) to reduce H_2O_2 into 2 H_2O • Now the oxidized glutathione should be reduced back.. So it takes H from NADPH by glutathione reductase
The NADPH	It is formed in HMP(hexose monophosphate) pathway by glucose-6-phosphate dehydrogenase "so as we said there is an oxidative stress in G-6-PD deficiency hemolytic anemia "

Effects of ROS

Molecular effects	• Lipid peroxidation (especially polyunsaturated fatty acids) • Protein denaturation (inactivation of enzymes - cytoskeletal damage) • DNA damage leading to mutations • Cell signaling effects (e.g.: release of Ca2+ from intracellular stores) • Chemotaxis
Vascular effects	• Altered vascular tone • Increased endothelial cell permeability

Summary

Nitric Oxide (NO)

What is it ?	• Free radical gas with very short half-life "it metabolized into nitrates & nitrites in seconds"	
synthesis	Enzyme: NO synthase (NOS)	Precursor: L-Arginin
effects	• Relaxes vascular smooth muscle • Prevents platelet aggregation • Bactricidal & Tumoricidal effects of macrophages • Neurotransmitter in brain	

The role of NO can be both beneficial and detrimental, depending upon when and where it is released

eNOS	beneficial	• Endothelial NO synthase • improving vascular dilation and perfusion.
nNOS	detrimental	• Neural NO synthase
iNOS		• Induced NO synthase • increased iNOS activity is generally associated with inflammatory processes

Vasodilators (nitroglycerin) is metabolized into NO and causes vasodilatation

Pathogenesis of atherosclerosis

1	Modified (oxidized) LDL "oxidative stress"	
2	Endothelial injury of arterial wall	
3	Adherence of monocytes to endothelial cells they move into intima and become macrophages	
4	Uptake of oxLDL by macrophage scavenger receptor	Scavenger receptor class A (SR-A) Low-affinity, non-specific & un-regulated receptor
5	Foam cell transformation ➔accumulation of excess lipids inside the cells ➔Atherosclerotic plaque formation	

MCQs

1-What enzyme, or combination of enzymes, protects cells against superoxide generated in oxidation reactions?

A. G6PD
B. Catalase
C. Superoxide dismutase and catalase

2-Which of the following is unable to protect the cell against free radical damage?

A. Vitamin c
B. Xanthine oxidase
C. Superoxide dismutase.
D. Glutathione peroxidase.

3-which of the following lipoproteins is involved in the pathogenesis of atherosclerosis ?

A. Low density of lipoproteins
B. very low density lipoproteins
C. High density lipoproteins
D. Chylomicrons

4-the free radical nitric oxide is generated in cells by reaction catalyzed by:

A. catalase
B. NOS
C. 6 phospho gluconate

5-Tangier disease is a disease of cholesterol transport .Due to mutation in transport protein , cholesterol cannot properly exit the cell. Which of the following is an important risk factor for such patient due to decreased level of HDL?

A. Diabetes mellitus
B. Fatty liver
C. Atherosclerosis

6-Which of the following doesn't generate reactive oxygen species?

A. Reduction of oxygen to water by cytochrome oxidase.
B. Spontaneous oxidation of hemoglobin to methemoglobin.
C. Phagocyte defense mechanisms.

7- an atom that has unpaired electron in an outer orbit is:

A. Free radical
B. Reactive atom
C. Isotope

1-c
2-b
3-a
4-b
5-c
6-a
7-a

Team Members:

- نوره الرميح.
- منيره العمري.
- رهف بن عباد.
- دلال الحزيمي.
- بدور جليدان.
- أثير النشوان.
- علا النهير.
- أفنان المالكي.
- خوله العريني.
- غاده القصيمي.
- نوف الرشيد.

- خالد النعيم .
- ثاني معافا .
- فارس المطيري.
- زياد العنزي .
- محمد الصهيل .
- إبراهيم الشايع .
- عبدالله الشنيفي .
- أحمد الرويلي .
- فراس المؤمن .

Team Leaders:

- شهد العنزي.
- عبدالله الغزي.

* نستقبل اقتراحاتكم وملاحظاتكم على:

@435biochemteam

435biochemistryteam@gmail.com

@biochemteam435