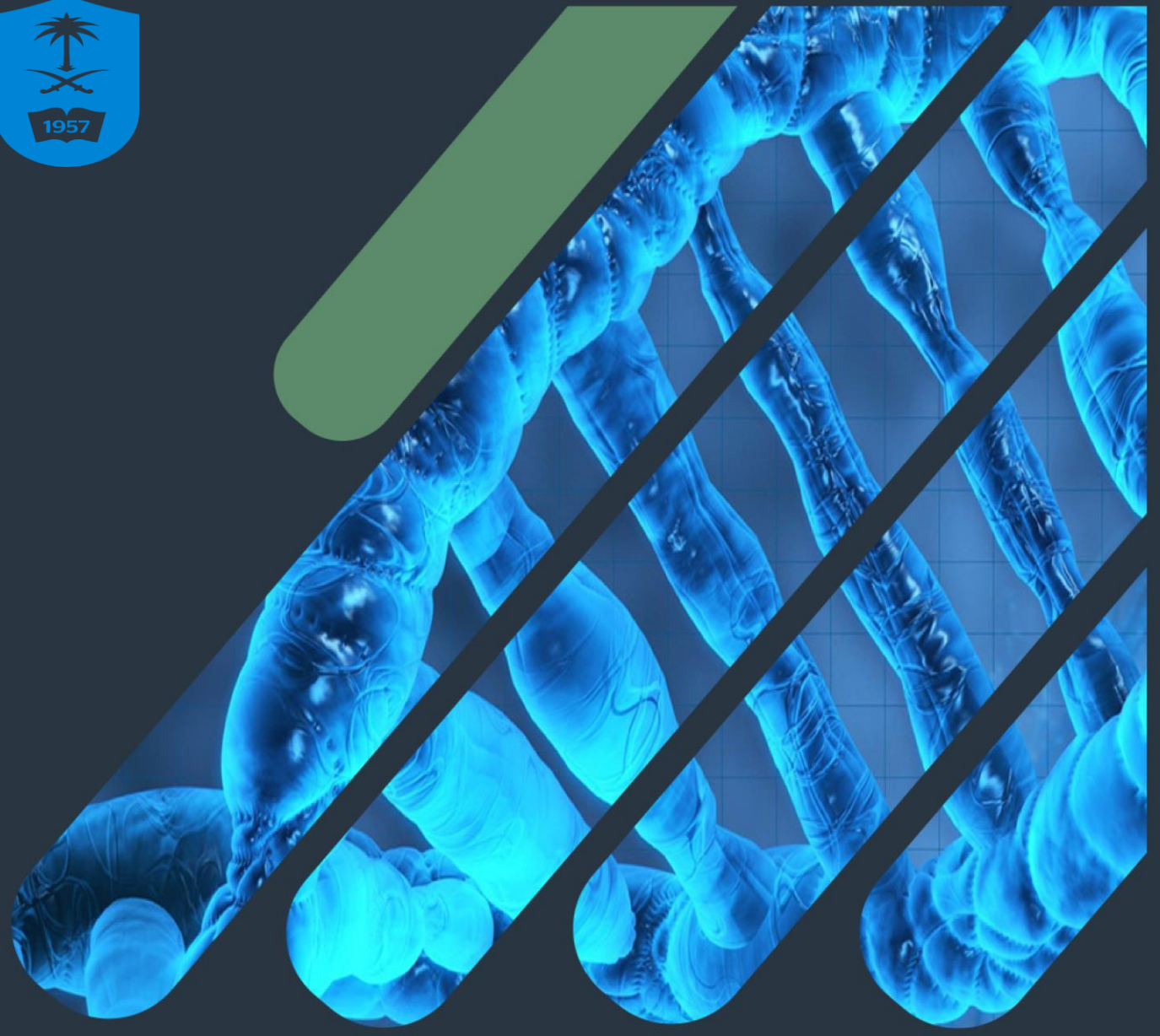
Biochemistry

Oxidative Stress



Stars can’t shine without darkness ..

Revised by
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- Important.
- Extra Information.
- Doctors slides.
- Doctors notes.

Biochemistry team 436

Objectives:

By the end of this lecture the students will be able to:

- Define oxidative stress .
- Understand the harmful effects of oxidative stress to the cell and its diseases .
- List the types, sources and effects of Reactive Oxygen Species (ROS) .
- List various antioxidants in the body .
- Understand the role of glutathione system in detoxifying oxidants in the body .
- Discuss how G6PD deficiency leads to oxidative stress .
- Understand the role of Reactive Nitrogen Species (RNS) in contributing to oxidative stress .
- Correlate the role of oxidative stress to pathogenesis of atherosclerosis

Dr.Sumbul recall information which will make the lecture easier to understand:

- **Oxidative stress are:** Oxygen species attack cells, oxygen and nitrogen free radicals.
- **ETC:** is an oxidative phosphorylation and generation of this oxidative species but we don't have the oxidative stress always , we have antioxidant machinery , there is transferring of electrons and the final acceptor is O2 which gets converted into water.
- Some of the antioxidants are vitamins such as C, E and A
- When this balance is disturbed between oxidative stress (generation of reactive species) and antioxidant machinery that will lead to the oxidative stress of the body.
- **Reduction of water:**

O2 receives 4 electrons and get reduced to water.
When this water is being reduced the O2 is reduced.

- When something gives off the electrons we call it **"oxidant" and it's the reducing agent (it gets oxidized)**
- When something takes the electron **it's reduced and it's called the oxidative agent**
- When reduction of O2 to water is happening it requires 1 molecule of O2 which requires 4 electrons
- Sometimes the reduction is not complete "partial reduction" so it receives one electron so it won't make water it will make free radicals (reactive O2 species)
- If it's 4 it's a neutral molecule (water) but if it's partial reduction of O2 it will lead to the production of reactive O2 and that **can cause oxidative damage**.
- Reactive oxygen species can also have nitrogen reactive species which **can cause oxidative damage**

Dr.Sumbul recall information which will make the lecture easier to understand:

Recall what you studied in chemistry:

S arpital

P arpital the electron is present as pairs so if you have electrons which are not present as pairs and that electron become highly reactive and that is what is present in the free radicals so when that happens this electron tries to find a pair if you have a free radical species it will try to attack a protein, lipid and the other molecules to get that electron so when it attacks it will try to take an electron so it will oxidize to take the other things

If the lipid was in the cell it will cause damage to the cell

If it was a protein such as an enzyme the enzyme will get loss and that's how it attacks the DNA

Affecting the genes at the basic level which will complicate to cause cell death

Reduction is the gain of hydrogen or loss of oxygen

Recall what you studied in foundation:

- Xomonophosphate shunt the role of it is:

1- Generation of NADPH (reducing Co-enzymes and it's required in the glycolysis).

Other cells have other enzymes and can make NADPH even if their HMP is not working but when it comes to your RBCs the only way to generate NADPH is by HMP pathway

2- production of 5 carbon molecules (ribose -5- phosphate) the only way to produce it is by xomonophosphate and it's required in the synthesis of nucleotide

Dr.Sumbul recall information which will make the lecture easier to understand:

Recall what you studied about NO in respiratory block:

- It's a vasodilator that prevents the contraction of smooth muscles.
- It's produced in the endothelium.
- It increases the **cyclic GMP** production by the action of the **enzyme guanylate cyclase** which activates **protein kinase G** and that acts on calcium channels and then close the Ca channels so they can not get inside the smooth muscle cells so the contraction can not start.

Recall what you studied in MSK block:

G6PD deficiency is common in saudi arabia

Oxidative stress:

We don't have it always because we have natural antioxidants in our bodies like vitamins.

We have the oxidizing molecule that are being produced which are the reactive oxygen species and they are the reactive nitrogen species and the body has its own antioxidants machinery (the different sets of enzymes) we call them scavenger molecules like these Vitamins or Carotene so they can eat up these free radicals so if there is imbalance it will lead to oxidative stress

❖ A condition in which cells are exposed to excessive levels of :

- **Reactive Oxygen Species (ROS)**
- **Reactive Nitrogen Species (RNS)**

❖ **Cells are unable to neutralize their deleterious effects with antioxidants .**

❖ Oxidative stress is implicated in:

✓ Aging ✓ CAD ✓ Atherosclerosis

❖ Cellular imbalance of oxidants and antioxidants damages:

- DNA
- Proteins
- Lipids

Targets of ROS

❖ **Diseases due to Oxidative stress:**

- Inflammatory diseases (rheumatoid arthritis)
- Atherosclerosis
- CAD (Coronary Artery Disease)
- Obesity
- Cancer
- G6PD deficiency hemolytic anemia

Reactive Oxygen Species (ROS):

- Incomplete reduction of oxygen to water produces ROS.

  ROS normally is produced by the body and then utilized after doing their job

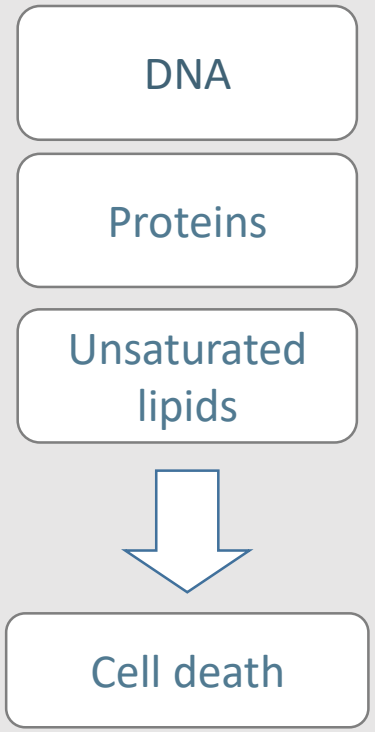
- ROS are continuously formed:
  - As byproducts of aerobic metabolism.

  Not just by the oxidative phosphorylation that is one place where lots of reactive oxygen species are produced and a lot of enzymes when they are doing their Normal function like xanthine oxidase (which Participates in purine metabolism - gout) which Produces hydrogen peroxide

  - Through reactions with drugs and toxins. +radiation
  - When cellular antioxidant level is low.
  - Creating oxidative stress in a cell.

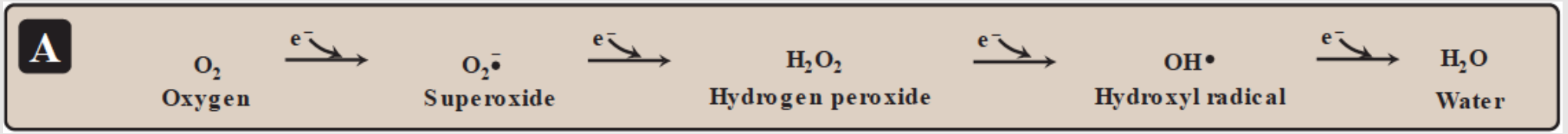
- Cells have protective antioxidant mechanisms that neutralize ROS.

✓ ROS can damage:



Reactive Oxygen Species (ROS)

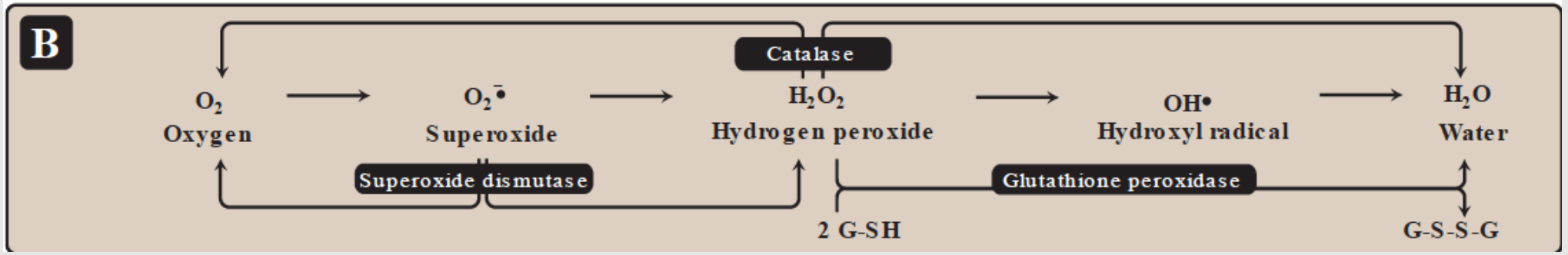
Formation of reactive intermediates from molecular oxygen. e-= electrons



- This is showing you the O2 is receiving an electrons
- The final product is water
- The dot means the electron
- the negative means it received an electron.
- O2 is not paired and that's why it's a free Radical
- H2O2 is not a free radical but it is a reactive oxygen species that can cause oxidative damage.
- Hydroxyl radical is the most reactive radicals.
- Hydroxyl radical is more reactive than H2O2 but the good thing that it's short lived.
- Free radicals are more reactive than hydrogen peroxide

Reactive Oxygen Species (ROS)

Actions of antioxidant enzymes. G-SH= reduced glutathione. G-S-S-G= oxidized glutathione

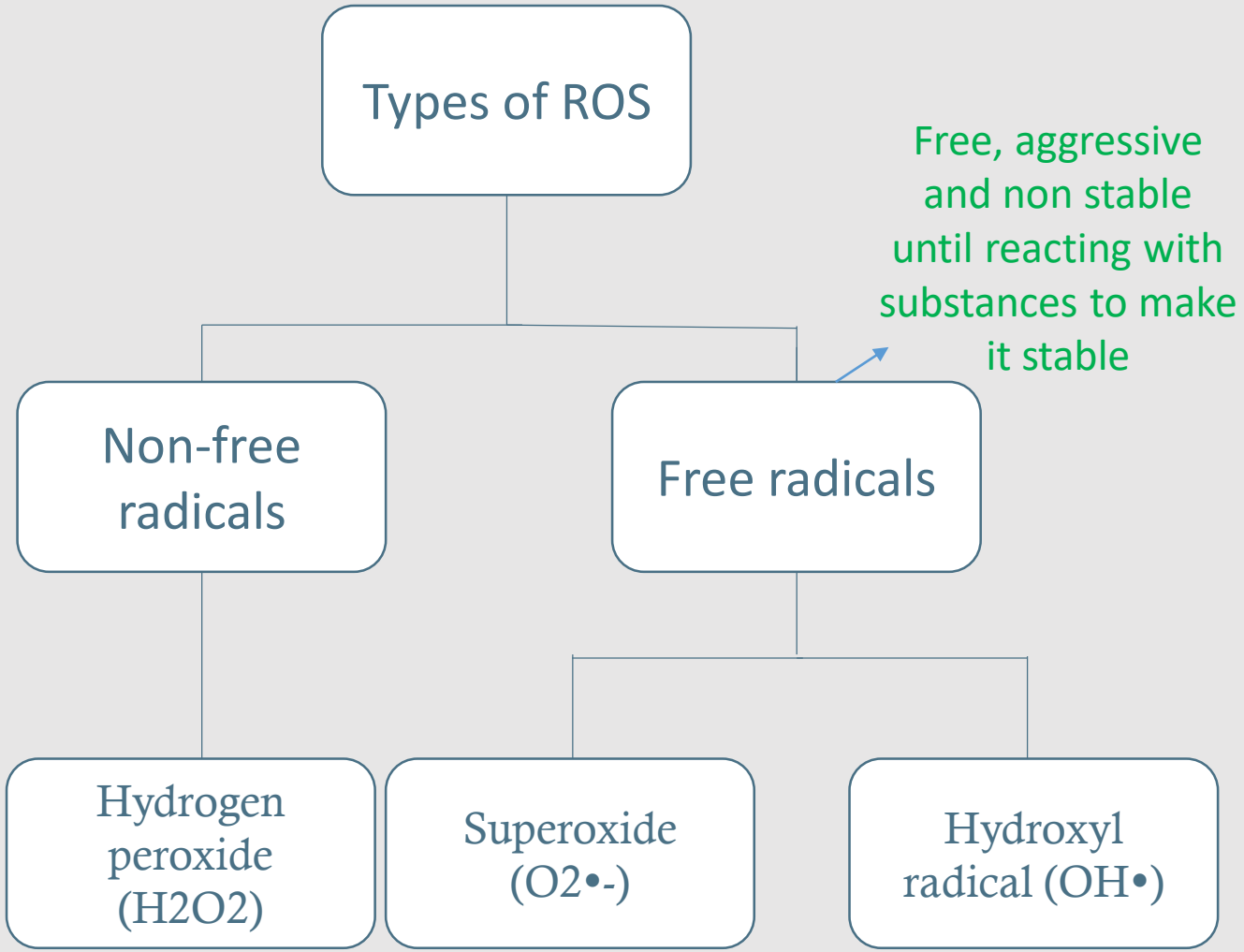


- This picture shows the antioxidant machinery that's there in the system.
- H_2O_2:can be taking care of by **catalase enzyme** which will break it down into oxygen and water so 2 neutral molecules
- **Glutathione peroxidase:** is going to take care of the hydrogen peroxide and It's short lived

Superoxide dismutase : it can take care of superoxide free radical which will attach on it and produce the o2 and H_2O_2

- They change the structure of the molecule they react with to abnormal structure causing damage and dysfunction
- So incomplete reduction of O_2 leads to ROS Formation

Types and sources of ROS



Cells have protective antioxidants mechanisms that neutralize ROS

Sources:

- Aerobic metabolism.
- Partial reduction of molecular oxygen in ETC.
- Ingestion of drugs, toxins, chemicals.

Antioxidants

Utilization of oxidants

Enzymes:

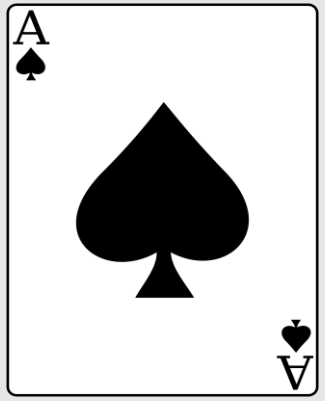
- Superoxide dismutase
- Catalase
- Glutathione system

Imbalance between oxidants and antioxidants in the cells can result in the development of many diseases including atherosclerosis.

Selenium is an anti oxidant trace element ..

Vitamins:

- Vitamins A, C, E →
- β-Carotene

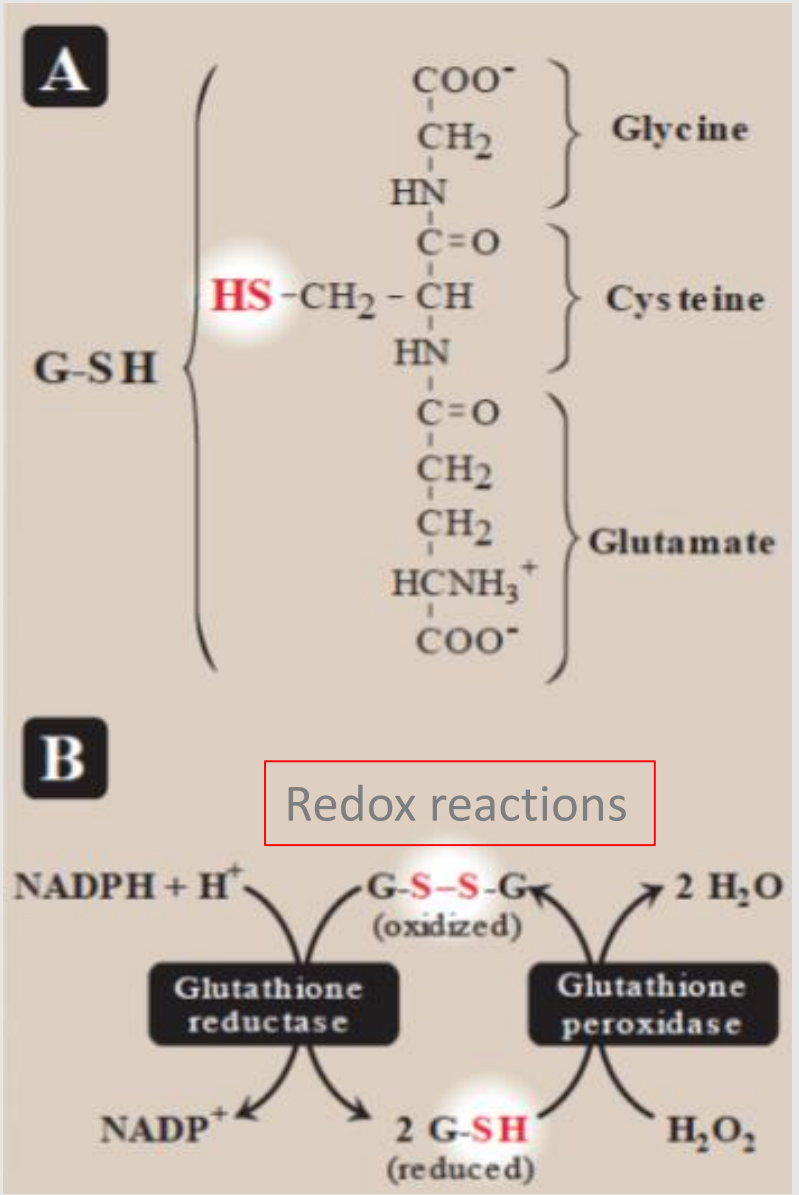


Glutathione system:

- ✓Present in most cells
- ✓Chemically detoxifies H_2O_2
- ✓Catalyzed by glutathione reductase
- ✓Produces NADPH that reduces H_2O_2

You need an enzyme called **glutathione reductase** to convert NADP+ to NADPH then it will be oxidized to **GSSG** to reduce glutathione and the advantage of this to reduce **G-SH** which can take care of the hydrogen peroxide and this's the antioxidant machinery which is present in the RBCs.
So in this system you need glutathione reductase and glutathione peroxidase .

بعد ما حولنا الـ H2O2، إلى نتائج أقل ضررا على الخلية ، نحتاج نرجع (G-SH) زي ماكان عشان نستخدمه مرة ثانية ، كيف؟ عن طريق (Glutathione reductase) اللي راح يأخذ هيدروجينز من NADPH ويحوله من Oxidized form إلى Reduced form اللي ينفعنا ونحتاجه ..



G6PD deficiency

- Leads to NADPH deficiency . Because you need the enzyme to produce it.1- ATP synthesis is going to be defected. 2-they are not going to protect the RBCs from oxidative damage so if the oxidative damage continues because of the free radicals the cellular proteins DNA and lipids get oxidized. they get targeted by this free radicals and that leads to the death of RBCs which cause hemolytic anemia for G6PD deficiency.
- Cells are unable to reduce free radicals.
- Oxidation of cellular proteins is increased causing impaired cell functions.

Utilization of oxidants

HMS or mentose phosphate pathway you have G6P that is acted upon by the enzyme G6PD and that's how its producing NADPH.
This is one of the early steps of HMP pathway.
And as a byproduct you are making NADPH which is going to be used by glycolysis in the RBCs at the same time this NADPH can be utilized in the antioxidants machinery in the RBCs which is the glutathione system so if there is oxidative stress and that could be because of certain drugs or infections and lead to the production of H2O2

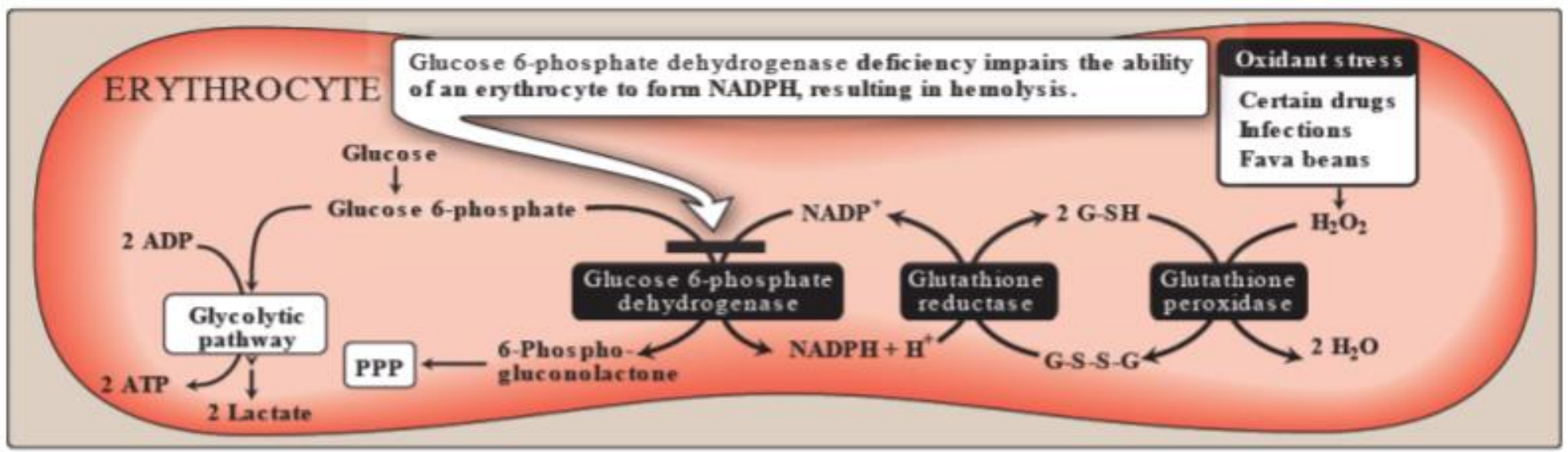


Figure 13.10
Pathways of glucose 6-phosphate metabolism in the erythrocyte. NADP(H) = nicotinamide adenine dinucleotide phosphate; G-SH = reduced glutathionine; G-S-S-G = oxidized glutathionine; PPP = pentose phosphate pathway.

Effects of ROS

1. Lipid peroxidation (polyunsaturated fatty acids) the preferable target is where you have the double bonds because you are sharing the electrons between 2 atoms they are not so tightly held together by the atom itself so they are softer target for this free radicals the more double bond you have the more lipid peroxidation will happen. So if you eat unsaturated fatty acid in your diet you are protecting your body from free radicals.
2. DNA damage
3. Protein denaturation
4. Cytoskeletal damage
5. Chemotaxis
6. Cell signaling effects
7. Release of Ca2+ from intracellular stores
8. Altered vascular tone
9. Increased endothelial cell permeability

(that's why there is usually an inflammation and edema with oxidative stress)

Nitric oxide (NO)

Endothelial-derived relaxing factor (EDRF)

- ✓ NO with Oxygen or Superoxide will give :
  1. Nitrates
  2. Nitrites
  3. Peroxynitrite (O=NOO-)
- ✓ Peroxynitrite is one of Reactive Nitrogen Species (RNS)

NO is a gas with short half-life (3-10 seconds)

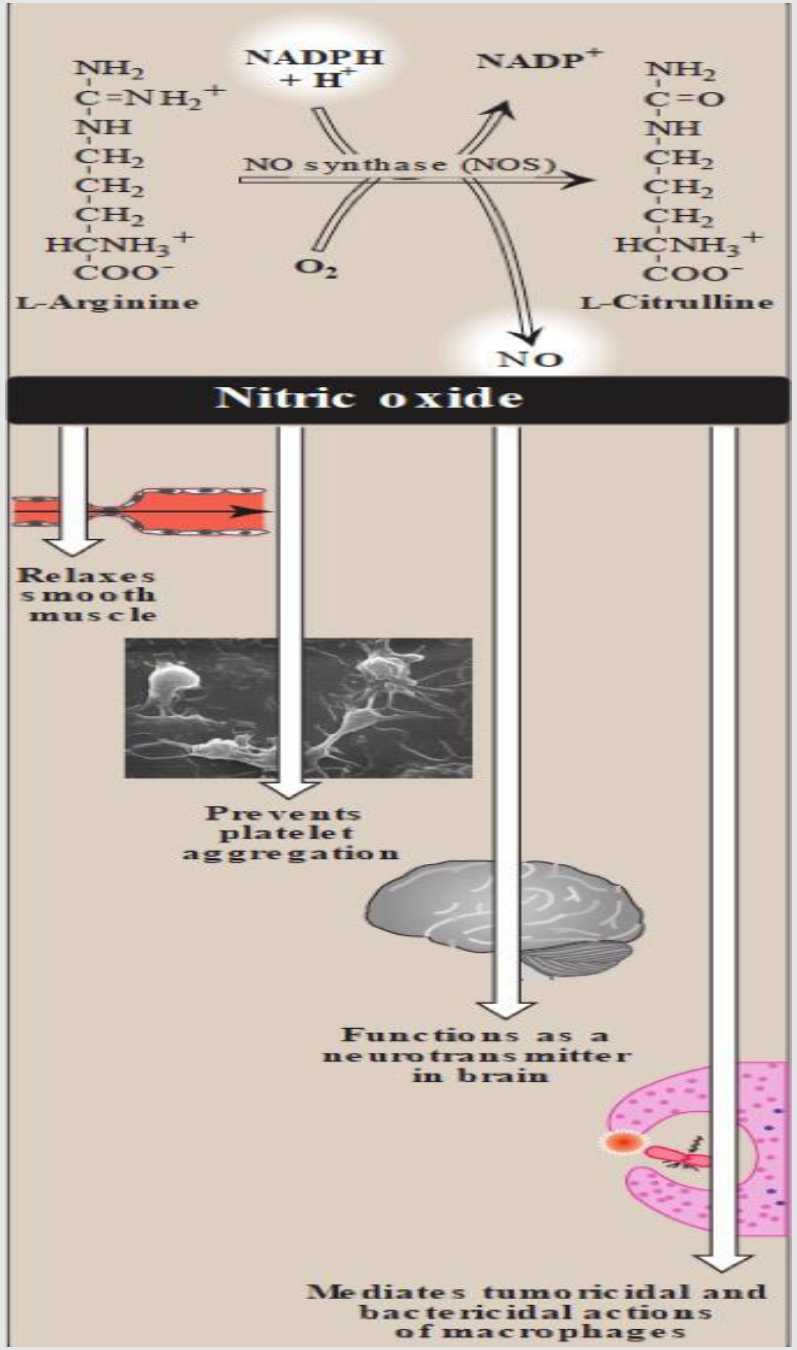
Causes vasodilation by relaxing vascular smooth muscle

Nitric Oxide

NO is produced by nitric oxide synthase from L-arginine

L-Arginine + NADPH + O2 make NO and L-Citrulline

Synthesis and some of the actions of nitric oxide (NO). NADPH = reduced nicotinamide adenine dinucleotide phosphate. [Note: Flavin mononucleotide, flavin adenine dinucleotide, heme, and tetrahydrobiopterin are additional coenzymes required by NOS.]



Nitric Oxide they are synthesized in different locations by different enzymes

Type 1

- n-NOS in the neural tissue (neurotransmission) in both central and peripheral nervous system

Type 2

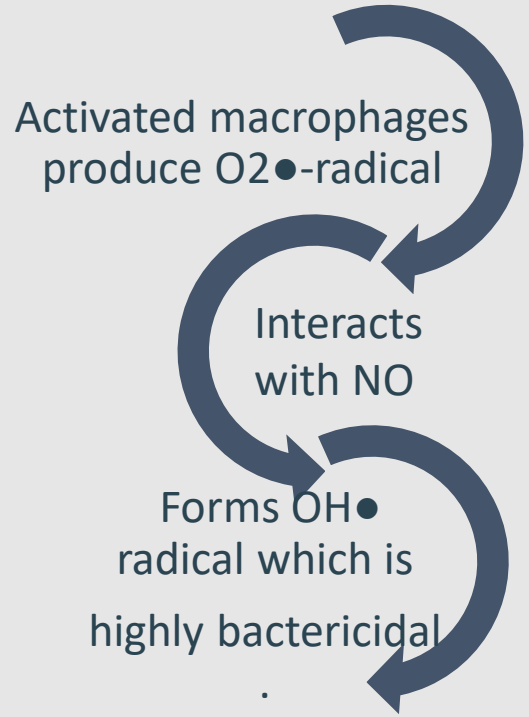
- e-NOS in endothelium (vaso-relaxation)

Type 3

- i-NOS I for inducible in macrophages, neutrophils (infection)
- Normally in macrophages the I-NOS activity is very low when it gets activated by TNF-alpha, IL-6 and the bacterial micropolysacharide so it gets activated when there is infection or tumor etc …
- ✓ No synthesis increases so the enzyme is induced
- i-NOS activity (normally low) increased by infection and pro-inflammatory cytokines . Most reactive O2 free radicals

Type 4

- b-NOS (bacterial) not present in the human being because it's protective for the bacteria itself so if you target it you develop .



Increased i-NOS activity → free radicals → oxidative stress

Oxidative stress and atherosclerosis :

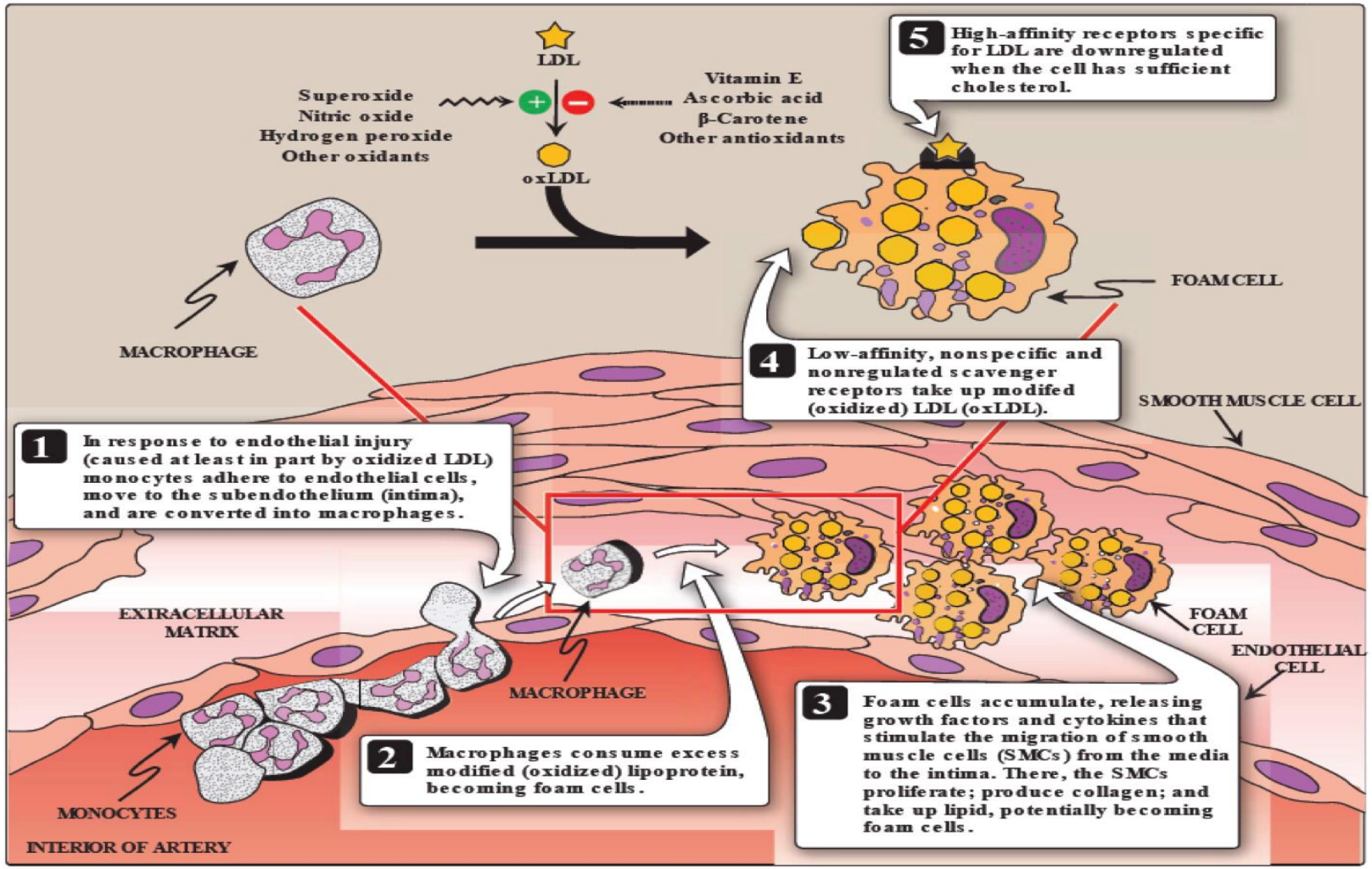


Figure 18.22
Role of oxidized lipoproteins in plaque formation in an arterial wall. LDL = low-density lipoprotein.

Extra explanation for memory refreshing:

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

Quiz

SAQ

https://www.onlineexambuilder.com/oxidative-stress/exam-144499

MCQ's

https://www.onlineexambuilder.com/oxidative-stress-saq-s/exam-144511

Helpful video

https://www.youtube.com/watch?v=dlZ5ROca0KI&feature=youtu.be

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THANK YOU

PLEASE CONTACT US IF YOU HAVE ANY ISSUE

- Review the notes

- https://www.youtube.com/watch?v=dlZ5ROca0KI&feature=youtu.be

- Lippincott's Illustrated Reviews: Biochemistry, 6th E

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