BIOCHEMICAL ASPECTS OF FREE RADICALS, ROLE IN DISEASES – An Overview

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M Med Part I

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What is a Free Radical?

- **Free Radical**: any atom or molecule that contains an Unpaired Electron in its Outer Electron Orbital;
- Free Radicals are Electron-Deficient Species,
  - Unpaired electron makes the Free Radical unstable and extremely reactive,
- Paired electrons in opposite spin are most stable,
- Free Radical try to stabilize itself by attacking other molecules or structures with high electron density,

- It creates another unstable Free Radical molecule that will also try to stabilize itself, thus starting a Chain Reaction that eventually damage cell structures and molecules;
- **Free Radicals are part of a group of Oxidants: Reactive Oxygen Species (ROS),**

- All Free Radicals are members of the Reactive Oxygen Species group, **but**
- All Reactive Oxygen Species are not Free Radicals;

What are Reactive Oxygen Species (ROS)?

- Reactive Oxygen Species a group made up of:
  - Free Radicals,
  - Reactive Anions containing Oxygen Atoms,
  - Molecules containing Oxygen atoms that can either produce Free Radicals or are chemically activated by Free Radicals;

Some Free Radicals and Reactive Oxygen Species in tissues

Some common Free Radicals and ROS in Human tissues:

- Superoxide radical (**$O_2^{-\cdot}$**);
- Hydroxyl radical (**·OH**);
- Per-hydroxyl radical (**HOO·**)
- Peroxyl radical (**·ROO**);
- Singlet Oxygen;
- Alkoxyl radical (**RO·)**;
- Hydrogen Peroxide (**H_2O_2**);
- Nitric Oxide (**NO·**), etc.

What are some of the sources of ROS (Pro-oxidants)?

- Chemicals and Reactions capable of producing **ROS** in cells and tissues are called **Pro-Oxidants**,

- Sources of ROS (Pro-oxidants) can be separated into two major groups:
  - **ROS produced naturally in human cells and tissues,**
  - **ROS produced externally** (Environmental pollutants),

Production of ROS in human tissues (Pro-oxidants in human cells and tissues):

- Pro-oxidants formed naturally in human tissues include:
  - **Superoxide Radical,**
  - **Hydrogen Peroxide,**
  - **Hydroxyl Radical,**

Examples of natural Pro-oxidants production include:

- **Reactions in Red Blood Cells**, Example:
  - Auto-oxidation of Hb to MetHb (about 3% of Hb in RBC are Auto-oxidized per day) resulting in the formation of **Superoxide radical**

$$O_2 + e^- \text{======} \rightarrow O_2^{-\cdot}$$

- Mitochondrial Respiratory Chain (Aerobic respiration),
- Microsomal Cytochrome P450 in metabolism of Xenobiotics (Phases 1 & 2 reactions): Biotransformation reactions,
- Cyclooxygenase reactions,
- Lipoxygenase reactions,
- Auto-oxidation of Catecholamines,
- Xanthine Oxidase reactions,
- Beta-oxidation of fatty acids,
- Stimulation of Phagocytosis by Pathogens,
- Metabolism of Arginine, etc.

Some external source of ROS (Pro-oxidants)

Environmental pollutants:-

- Smoke and other components emitted by factories and motor vehicles,
- Passive cigarette smoke,
- Pesticides
- Insecticides,
- UV- radiation from the Sunrays;

How are Pro-oxidants (ROS) disposed of or neutralized in the body?

- **Anti-oxidants** either dispose of or neutralize ROS,
- **Anti-Oxidants**: compounds and reactions capable of:
  - Disposing of ROS,
  - Scavenging ROS,
  - Suppressing formation of ROS, or
  - Opposing the actions of ROS;
- Most **Antioxidants** are **electron donors**, thus they react with **Pro-oxidants** to form products that are harmless to the tissues;

Give some examples of Anti-oxidants?

- Anti-oxidants can be put into one of three groups:
- **Enzymes present in tissues**: Examples:
  - Superoxide Dismutase (SOD);
  - Glutathione Peroxidase;
  - Catalase;
  - Myeloperoxidase, etc.
- **Compounds present in tissues**: Examples:
  - Reduced Glutathione (GSH);
  - Sulfhydryl (-SH) group,
  - Alpha-Lipoic Acid;
  - Ubiquinone (Co Q 10);
  - Thioredoxin, etc.

- **Some essential nutrients**: Examples:
  - Vitamin C (Ascorbic acid);
  - Vitamin E (d-alpha-tocopherol);
  - Carotenoids;
  - Bioflavonoids;
  - N-Acetyl Cysteine (NAC);
  - Selenium (Se);
  - Zinc (Zn), etc.

How does Anti-oxidants protect RBC from damage by Pro-oxidants (ROS)?

- In RBC Oxygen can convert Hb to MetHb with production of Superoxide (Pro-oxidant)

$$\mathbf{Hb\text{-}Fe^{2+} + O_2 \Longrightarrow MetHb\text{-}Fe^{3+} + O_2^-}$$

(**Superoxide**)

- Superoxide Dismutase (Anti-oxidant) in RBC coverts the Superoxide (O_2^-) formed to Hydrogen Peroxide (Pro-oxidant) and Oxygen,

$$\mathbf{O_2^- + O_2^- + 2H^+ \Longrightarrow H_2O_2 + O_2}$$

- GSH (Anti-oxidant) in RBC reacts with H_2O_2 (Pro-oxidant) to form H_2O and Oxidized Glutathione (GSSG) in a reaction catalysed by **Glutathione Peroxidase** that requires trace element **Selenium** (Anti-oxidant),

$$\mathbf{2H_2O_2 + 2GSH \xrightarrow{\quad\quad} 2H_2O + O_2 + GSSG}$$

- GSSG formed, reacts with **NADPH** to form **GSH** and $NADP^+$ in a reaction catalysed by **Glutathione Reductase**,

$$\mathbf{GSSG + NADPH + H^+ \xrightarrow{\quad\quad} 2GSH + NADP^+}$$

- To prevent damaging effects that accumulation of Superoxide radical and Hydrogen Peroxide would have inflected on RBC the following are essential:
  - **Superoxide Dismutase (Anti-oxidant),**
  - **Reduced Glutathione (Anti-oxidant),**
  - **Glutathione Peroxidase**
  - **Trace element Selenium**,

- GSH also protects RBC from oxidation of **–SH** groups in Haemoglobin molecules,

What is the relationship between Pro-oxidants and Anti-oxidants in tissues?

- Balance between **Pro-oxidants : Antioxidants** is essential,
- Shift in balance in favour of Pro-oxidants occur when:
  - Production of **ROS** increases due to Stress, or
  - Intake of P**ro-oxidants** with no corresponding increase in intake of **Anti-oxidant,**

- Tissue levels of Anti-oxidants are diminished, by:
  - Inactivation of SOD,
  - Reduced activity of Glutathione Peroxidase due low intake of Selenium,
  - Reduction of GSH, or
  - Reduction of any other Anti-oxidant such as:
    - NADPH,
    - Ascorbic acid,
    - Vitamin E,
    - Carotenoids, etc.
- Consequence of a shift in favour of Pro-oxidants is **Oxidative Stress,**

What is Oxidative Stress?

- **Oxidative Stress** occurs when rate of formation of **Pro-oxidants** is unbalanced in proportion to Antioxidants,
- Oxidative Stress: general term used to describe a state of damage caused by ROS (Pro-oxidants),
- Oxidative Stress can damage specific molecules or the entire organism,

What are the effects of ROS on Cells and Tissues?

- Main damage to cells and tissues results from ROS-induced alteration of macromolecules and structures;

ROS are capable of:

- Disruption of membrane integrity by reacting with Proteins and Polyunsaturated fatty acids,
- Causing alterations in Membrane Fluidity and Permeability,
- Inducing changes on Membrane Receptors,

- Damaging Proteins by Oxidation of -SH groups,
- Stimulation of Phospholipases and Hydrolases,
- Inhibition of Na-K-ATPase, Ca-ATPase, Adenylate Cyclase,
- Inhibition of other Channels and Pumps that are vital to cell metabolism,
- Damaging Nucleic acids by breaking DNA strands and modifying Nucleotide bases,

What are some of the diseases causes by ROS?

- ROS are involved in several diseases; Examples:
  - Arthritis and other Inflammatory Diseases,
  - Kidney disease,
  - Lung Dysfunction (effect of smoking)
  - Cataracts,
  - Colitis, Pancreatitis;
  - Skin Lesions, and Aging
  - Drug reactions,
- Cardiovascular disease and Cancer are the most common conditions associated with Free Radical Damage;

What is the specific role of ROS in some diseases?

Toxicity of Oxygen to Premature Infants:

- Premature infants are ventilated with high conc. of O_2 to compensate for their immature lung development;
- Breathing conc. O_2 over a prolonged period is dangerous because of increased production of **SUPEROXIDE** (**$O_2^{-\cdot}$**),
- Premature infants are susceptible because their capacity to produce **Superoxide Dismutase (Antioxidant)** is not fully developed; they are unable to detoxify Superoxide;
- Excess Superoxide in cells can react non-specifically causing damage to DNA and cell membranes;

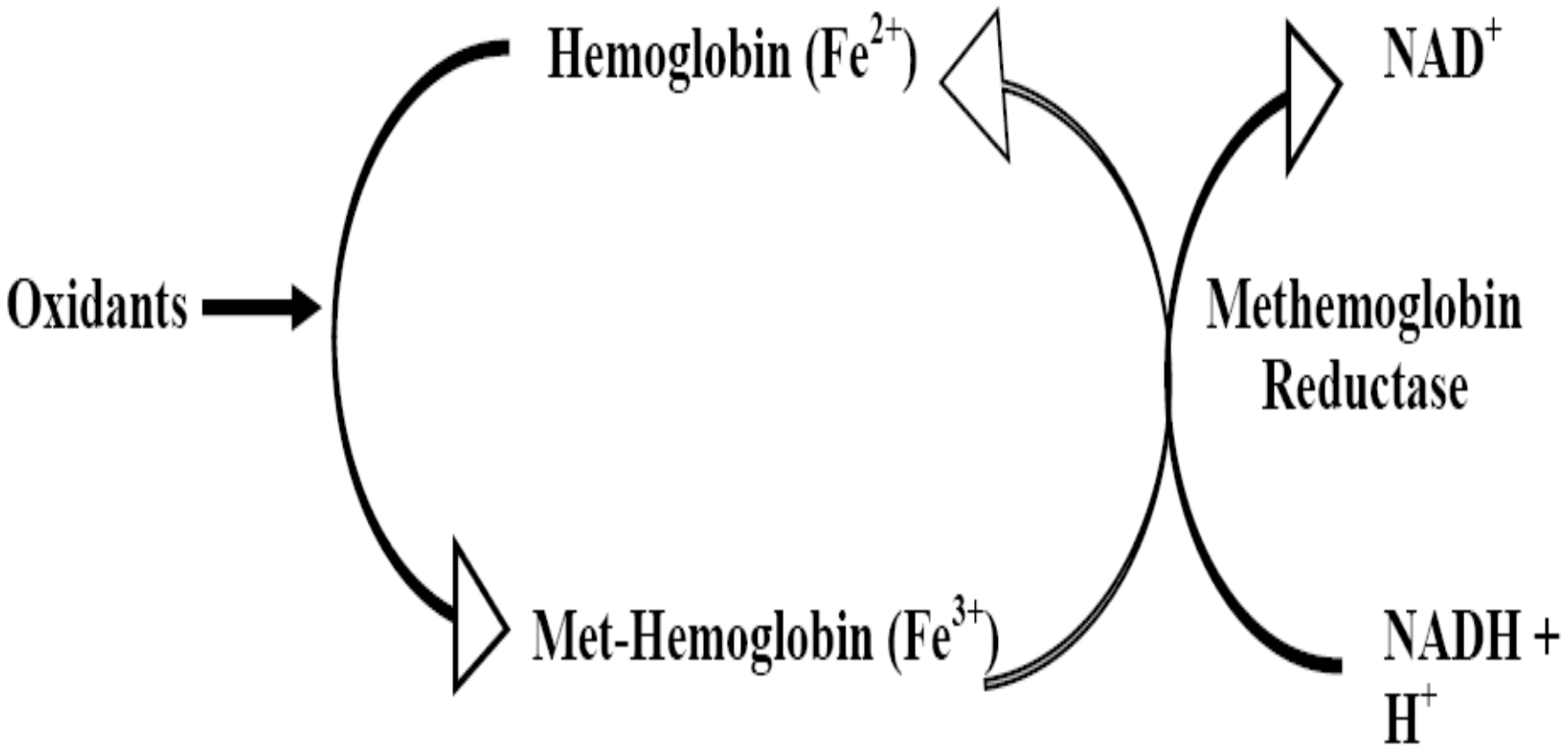
G-6-P DH deficiency and Haemolytic Anaemia

- **G-6-P DH** catalyses formation of **NADPH** in HMP-shunt,
- Major means of producing NADPH for protection of RBC membrane and Hepatocytes,
- Major function of **NADPH** in RBC is for production of **GSH (Antioxidant)** required for removal of **ROS** formed during O_2 transport;
- Individuals who are either deficient or have low levels of **G-6-P DH** may develop Haemolytic Anaemia,

- It may be more critical if these individuals consume:
- PRIMAQUINE (Primaquine-sensitive haemolytic anaemia)
- Sulphonamides,
- Chemicals, such as, Naphthalene,
- Legumes (broad beans - Vicia faba) or
- Some specific Food Additives...

- Biochemical basis for the haemolytic anaemia is that the consumption of any of these materials leads to increased production of **ROS** (**Pro-oxidants**) in the cells;

Diagram showing formation of Met-Hemoglobin by Oxidants and action of Methemoglobin Reductase that converts MetHb back to Hb



Non-enzymatic action of Ascorbate (Vit C) in conversion of MetHb to Hb. Dehydroascorbate is passed out in urine. Role of Reduced Glutathione (GSH) in the conversion of Dehydroascorbate to Ascorbate, and actions of enzymes involved are shown. NADPH is from G-6-Phosphate Dehydrogenase reaction in HMP-shunt.

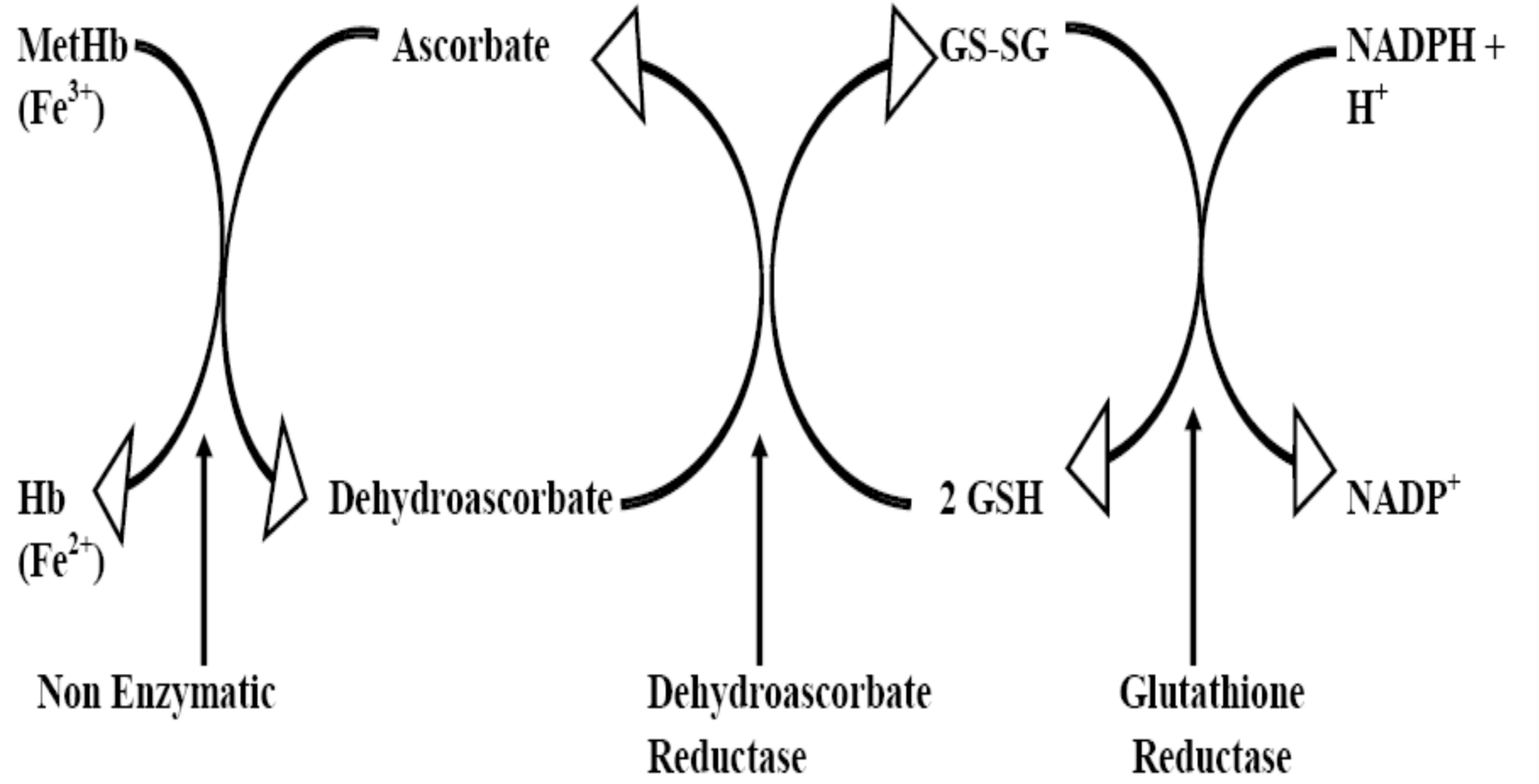
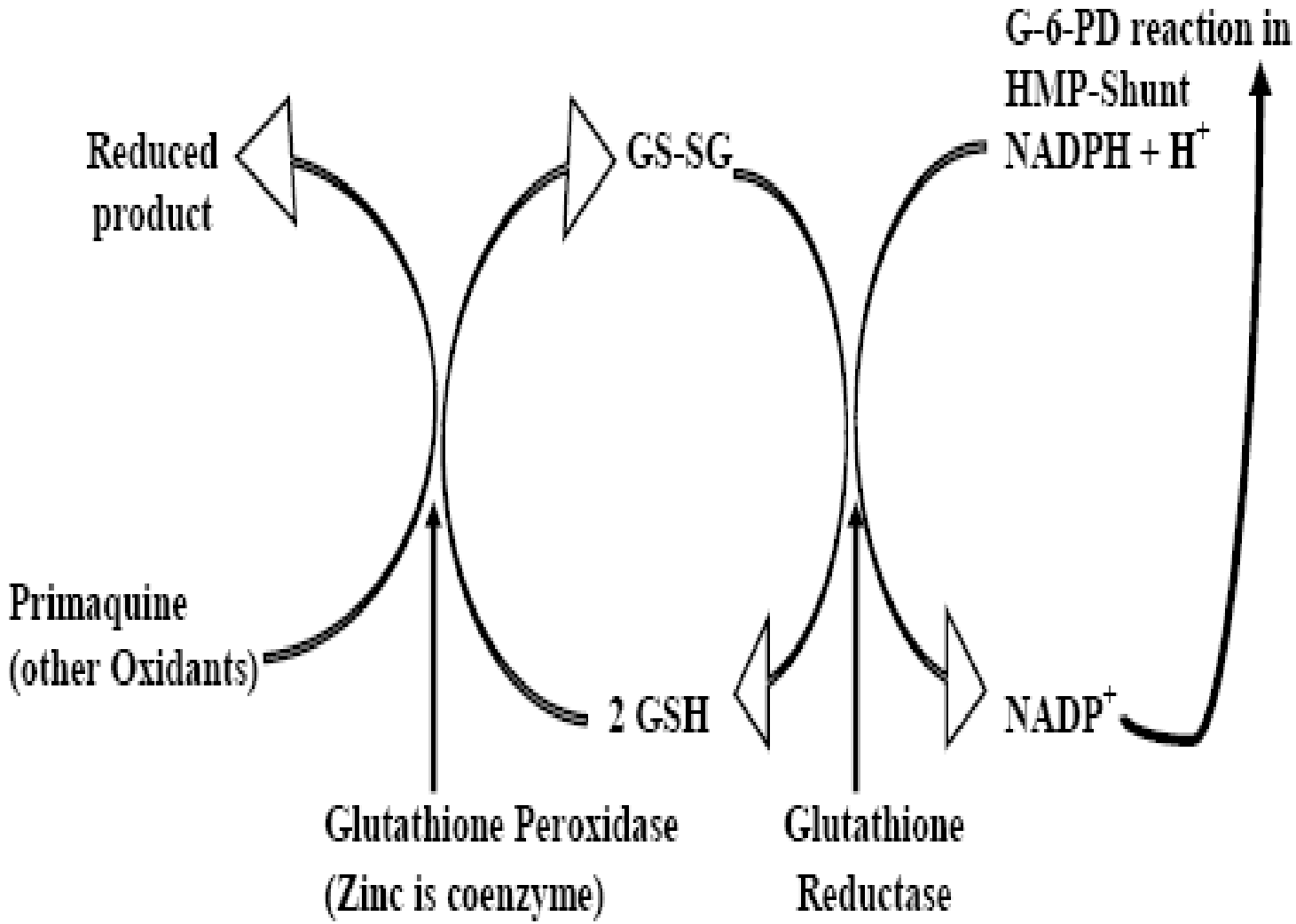


Diagram: Antioxidant role of Reduced Glutathione (GSH) in neutralizing Primaquine and other Pro-oxidants (ROS) in cells; GSSG formed is converted back to GSH. Note: NADPH is from G-6-Phosphate Dehydrogenase (G-6-PD) reaction in HMP-shunt.



- RBC of individuals with low or deficient G-6-P DH cannot generate sufficient **NADPH** to regenerate **GSH** from **GSSG**;
- This affects their ability to dispose of **ROS**,
- Accumulation of **ROS** in **RBC** causes damage to the membrane lipids leading to **Lysis** of the cell membrane and consequently haemolysis,

How important is Reduced Glutathione (GSH) in preventing cellular damage by ROS?

- Cellular level of **GSH** has been found to be low in many disease states indicating **Oxidative Stress** and **inadequate Antioxidant activity**;

- One suggestion is that maintaining and improving GSH levels may play significant role in combating some of these diseases;
- **How then can GSH levels in cells be improved?**

Ways to increase GSH levels in cells:

- GSH can be given as supplement, but it is poorly absorbed in GIT.
- **Cysteine** is main precursor for biosynthesis of **GSH** in cells, but Cysteine is an **unstable compound**,
  - **Cysteine** can be obtained from more stable compound **N-Acetyl Cysteine** (**NAC**), which is the best way to administer Cysteine,
- **NAC** is very effective in increasing **GSH** levels in cells;
- **Alpha-Lipoic Acid** and **Vitamin C** increase internal recycling of GSH, thus can increase GSH levels in cells;

- GSH is important in the normal functioning of Immune Cells,
- Low GSH levels have been associated with Impaired Immune Function,
- TNF-Alpha (a major pro-inflammatory Cytokine) impairs GSH production by several mechanisms, resulting in lowered GSH levels,

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