BIOCHEMICAL ASPECTS OF FREE RADICALS IN HEALTH AND 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 Free Radical unstable and extremely reactive,
- Paired electrons in opposite spin are most stable,
- Free Radical tries to stabilize itself by attacking molecules with high electron density;

- Successful attack creates another unstable Free Radical that also tries to stabilize itself, thus starting a Chain Reaction that may damage molecules and cell structures;
- **Free Radicals are part of a group of Oxidants:**
  - **Reactive Oxygen Species (ROS),**
  - **Reactive Nitrogen Species (RNS)** - NO.
- **All Free Radicals are members of Reactive Oxygen Species group, BUT**
- **All Reactive Oxygen Species are not Free Radicals;**

What are Reactive Oxygen Species (ROS)?

- ROS is 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

- Superoxide radical (**$O_2^{-\cdot}$**),
- Hydroxyl radical (**·OH**),
- Per-hydroxyl radical (**HOO·**),
- Peroxyl radical (**·ROO**),
- Singlet Oxygen, ($^1\Delta_g O_2$),
- Alkoxyl radical (**RO·)**,
- Hydrogen Peroxide (**H_2O_2**),
- Nitric Oxide (**NO·**), etc.

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

- **PRO-OXIDANTS:** Chemicals and Reactions capable of producing **ROS** in cells and tissues;

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

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

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

Examples of natural Pro-oxidants production:

- **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**

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

Examples cont....

- Electron Transport Chain in Mitochondria,
- 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 & other components emitted by factories and motor vehicles,
- Passive cigarette smoke,
- Pesticides,
- Insecticides,
- UV- radiation from the Sunrays; etc.

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

- **Pro-oxidants are neutralized by Anti-oxidants,**

- **What are Anti-oxidants?**
- **Anti-oxidants:** compounds or reactions that:
    - **Disposing of ROS,**
    - **Scavenging ROS,**
    - **Suppressing formation of ROS, or**
    - **Opposing the actions of ROS;**
    - **Quench ROS,**

- Most **Anti-oxidants** 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 in one of four groups**:

➢ **Enzymes present in tissues**: Examples:

- Superoxide Dismutase (**SOD**);
  - **MnSOD** (in the mitochondria),
  - **CuZnSOD** (in the cytosol),
- 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);
  - Beta-Carotene (Carotenoids);
  - Lycopene, Lutein, Resveratrol,
  - Bio-flavonoids;
  - N-Acetyl Cysteine (NAC);
  - Selenium (Se);
  - Zinc (Zn), etc.

➢ **Antioxidant Quenchers**: Examples

- Cellular proteins that bind pro-oxidant minerals (iron, copper and others)
- **Transferrin** – iron transport protein,
- **Ferritin** – iron storage protein,
- **Metallothionein** – minerals and heavy metals (Zn/Cu/Cd/Hg)
- **Ceruloplasmin** – copper transport and storage,

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

- **This can be explained briefly in 4 sequential steps:**
- **1.** 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**)

- **2. Superoxide Dismutase** (**Anti-oxidant**) in RBC coverts the Superoxide ($\mathbf{O_2^-}$) formed to **Hydrogen Peroxide** (**Pro-oxidant**) and Oxygen,

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

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

$2H_2O_2$ + 2GSH ========➔ $2H_2O + O_2$ + GSSG

NB:

- {**GSH: Reduced Glutathione**}
- **{GSSG: Oxidized Glutathione**}

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

  GSSG + NADPH + H^+ ======➔ 2GSH + $NADP^+$

- NB: ***NADPH*** *is obtained from* ***G-6-PDH*** *reaction in the* ***HMP-pathway***,

- The following are essential for protection of RBC from damage by pro-oxidants (Superoxide & H_2O_2):
  - **Superoxide Dismutase (Anti-oxidant),**
  - **Reduced Glutathione (GSH: Anti-oxidant),**
  - **Glutathione Peroxidase,**
  - **Trace element Selenium (Se: Anti-oxidant)**,
  - **Formation of NADPH (G-6-PDH reaction)**,

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

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

- Balance between **Pro-oxidants : Antioxidants** is essential,
- Shift in balance in favour of Pro-oxidants occurs 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 to low intake of Selenium,
  - Reduction in the [GSH],
    - GSH is tri-peptide: **γ-glutamyl-cysteinyl-glycin**e
    - **Methionine** is required for biosynthesis of GSH in cells and tissues,

- 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 higher than the amount of available **Antioxidants** in cells and tissues,

- **Oxidative Stress**: general term used to describe a state of damage caused by ROS (Pro-oxidants),
- **Oxidative Stress** can damage specific molecules, compounds or entire cell, tissue or organ;

What are the effects of ROS on Cells and Tissues?

- Damage to cells & 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:
- Some chronic and degenerative diseases
  - diabetes, cataracts, Alzheimer's disease, cancers, cardiovascular disease, aging, Arthritis, etc…
- Other diseases,
  - Kidney disease, Lung dysfunction (smoking),Colitis, Pancreatitis, drug reactions, etc….
- **Cardiovascular disease & Cancer are most common conditions associated with Free Radical Damage**;

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, thus the system is unable to detoxify Superoxide;
- Excess Superoxide in cells react non-specifically causing damage to DNA and cell membranes;

G-6-PDH deficiency and Haemolytic Anaemia

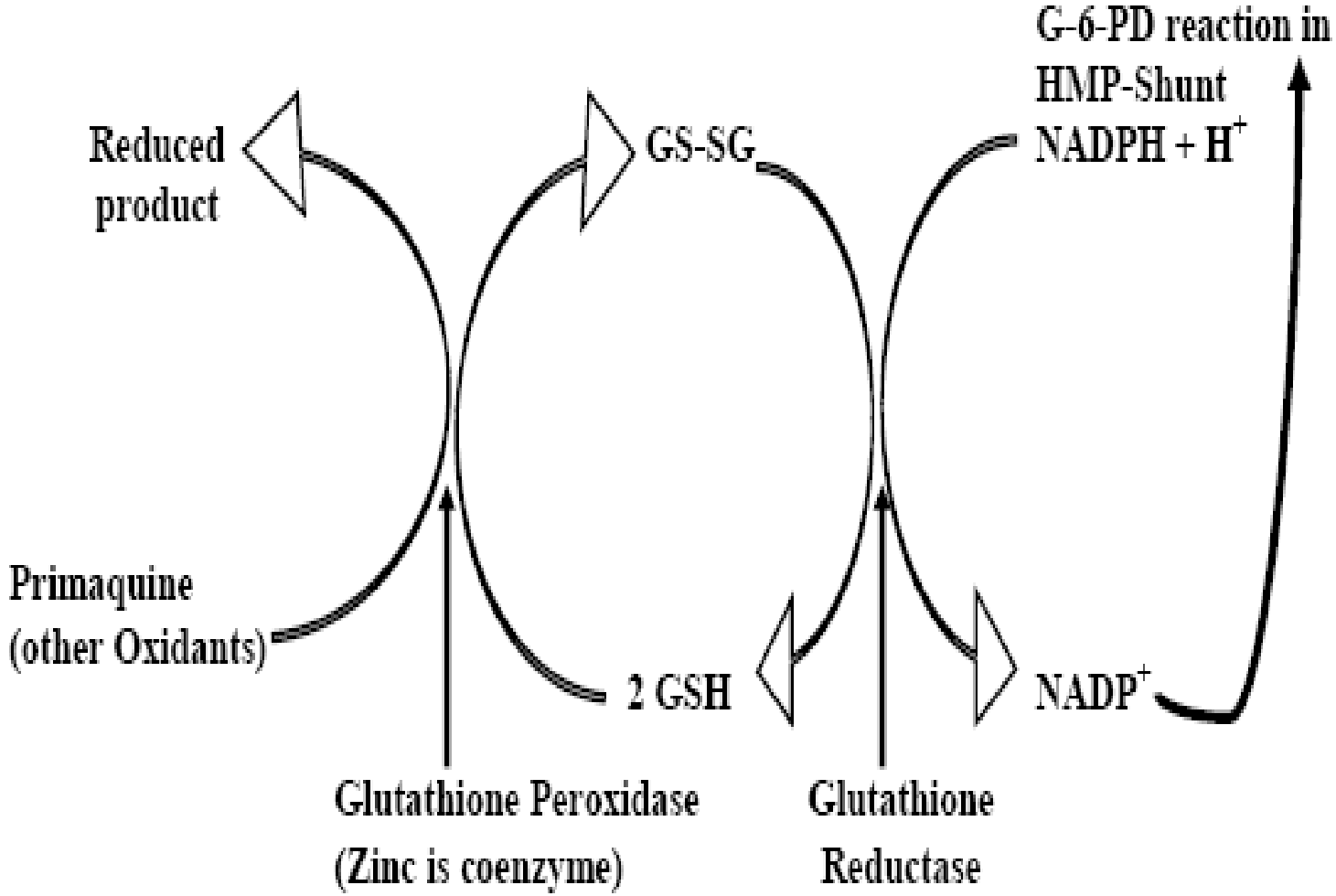
- **G-6-PDH:** formation of **NADPH** in HMP-shunt
  - Major pathway for NADPH used in protection of RBC membrane and Hepatocytes,
- Major function of **NADPH** in RBC is for production of **GSH** required for removal of **ROS** formed during O_2 transport;
- Individuals deficient or with low levels of **G-6-PDH** may develop Haemolytic Anaemia,

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

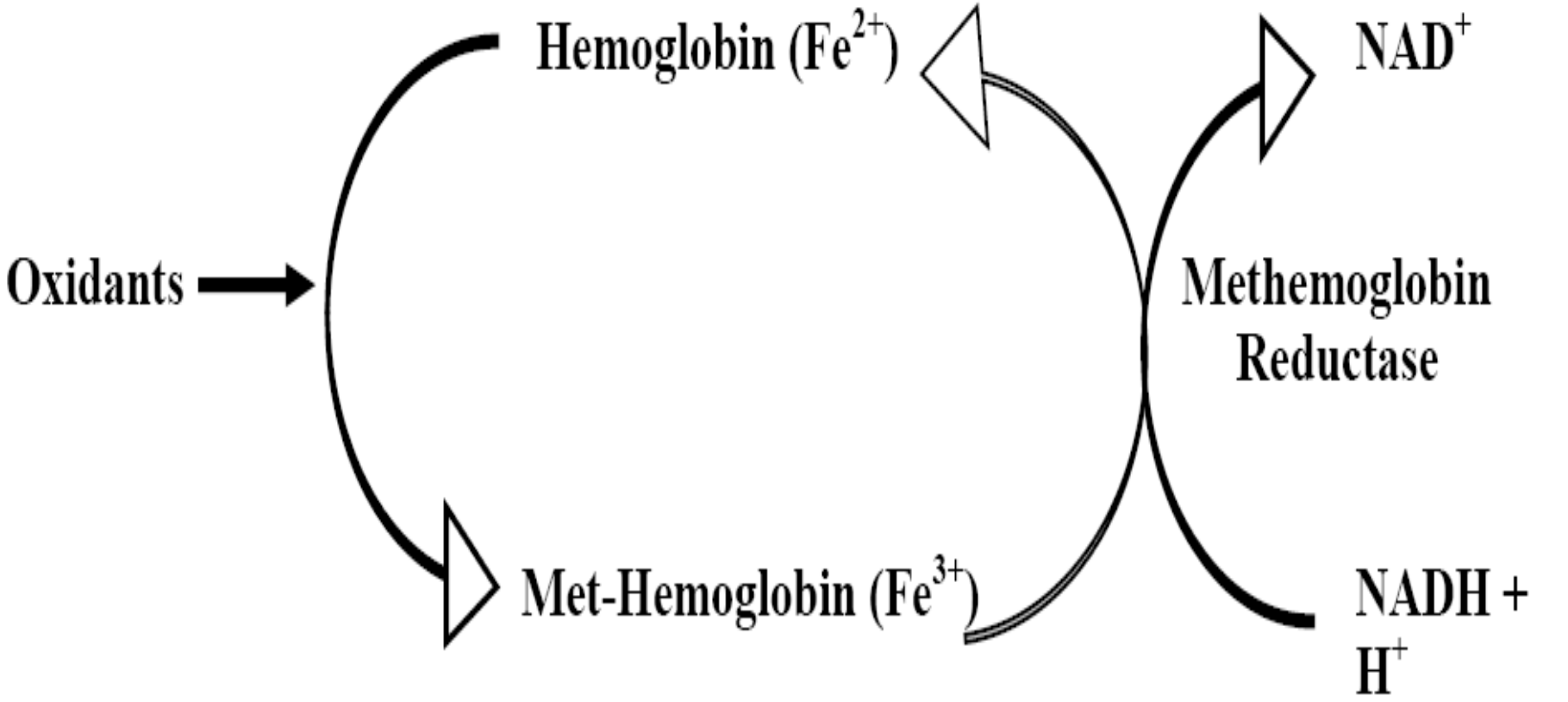
- Consumption of any of these may lead to increased production of **ROS** (**Pro-oxidants**) in tissues;

- RBC of individuals with low or deficient G-6-PDH 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 membrane lipids leading to **Lysis** of membrane and consequently haemolysis,

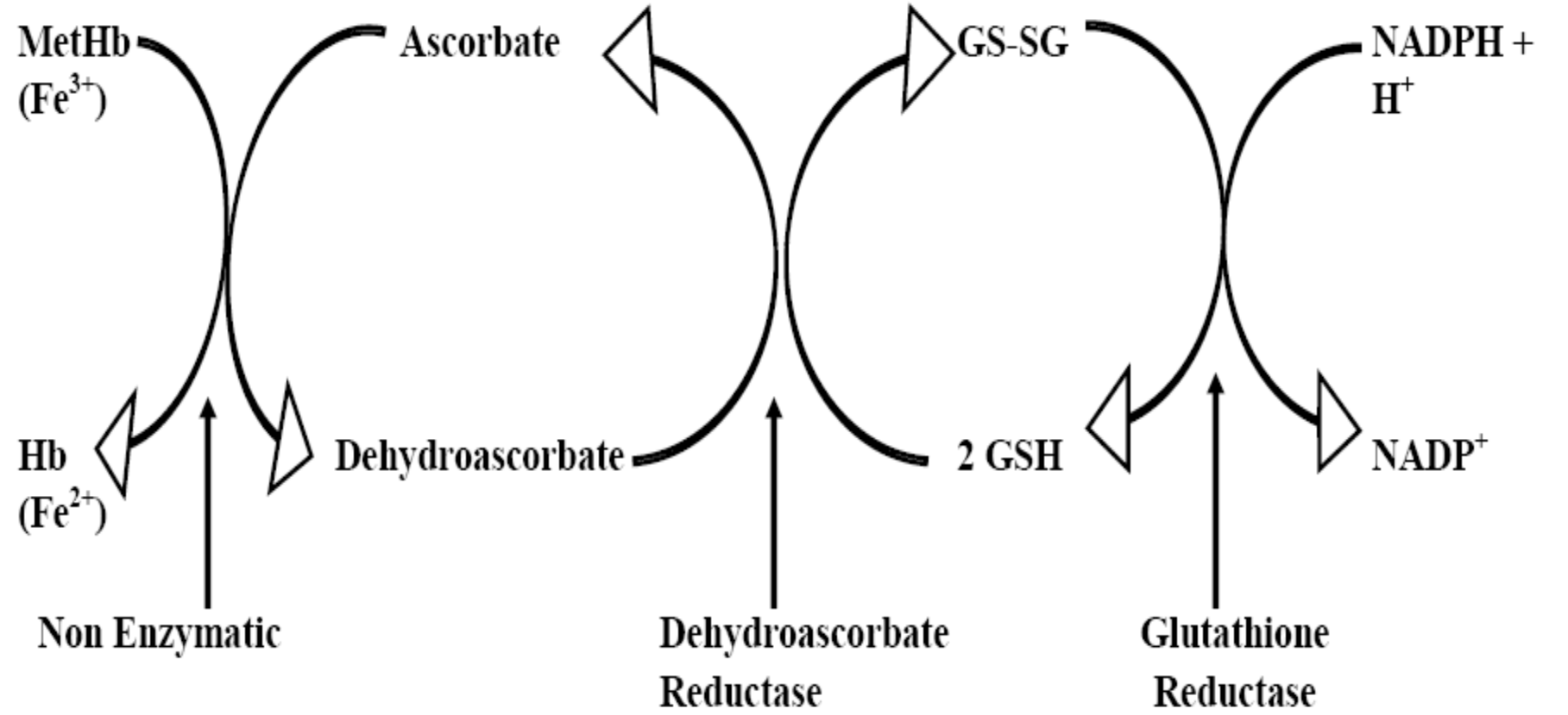
Antioxidant role of GSH in neutralizing Primaquine and other Pro-oxidants (ROS) in cells; GSSG formed is converted back to GSH. NADPH is from G-6-PD reaction in HMP-shunt.



Formation of Met-Hb by Oxidants and action of Methemoglobin Reductase that converts MetHb back to Hb



Non-enzymatic action of Ascorbate in conversion of MetHb to Hb: Dehydroascorbate is passed out in urine. Role of GSH in conversion of Dehydroascorbate to Ascorbate, and actions of enzymes involved are shown.



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

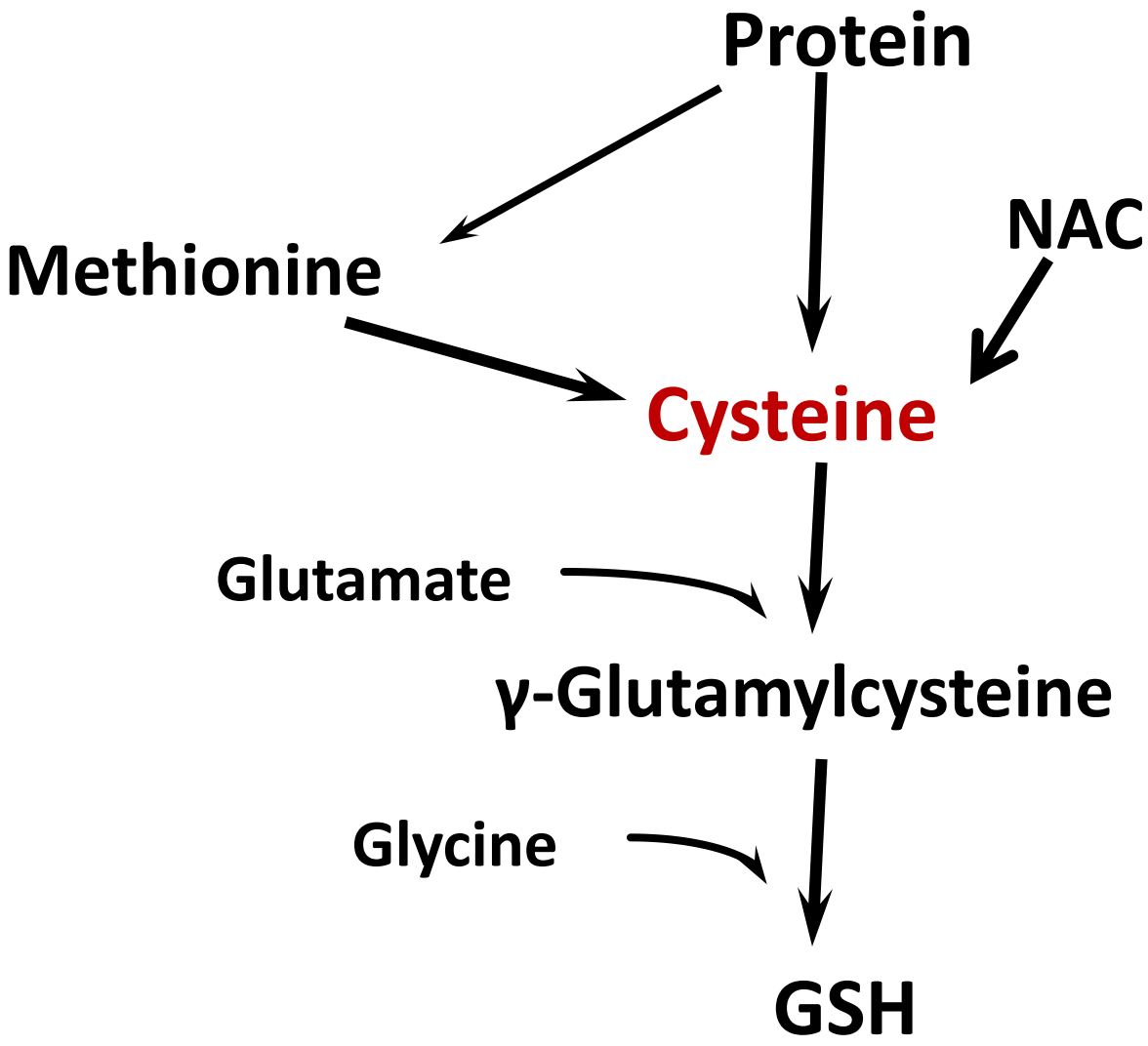
- Cellular level of **GSH** is usually low in many disease states indicating **Oxidative Stress** and **inadequate Antioxidant activity**;

- 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,
  - Figure shows how GSH can be produced in cells;
- **NAC** is very effective in increasing **GSH** levels in cells;

Biosynthesis of Reduced Glutathione (GSH)



NAC: N-Acetyl Cysteine

- **Alpha-Lipoic Acid** and **Vit C** increase internal recycling of GSH, they increase GSH levels in 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,

REFERENCES

- Pramod J, Singh S, Singh J. Role of Free Radicals and Antioxidants in Human Health and Disease. IJCRR. 2013; 5(19): 14-22.
- Textbook of Biochemistry with Clinical Correlations 4th Edition. Edited by Thomas M. Delvin. Chapter on Steroid Hormone.
- Harper's Illustrated Biochemistry 26th Edition; 2003; Ed. By R. K. Murray et. al.
- Biochemistry, By V. L. Davidson & D. B. Sittman. 3rd Edition.
- Hames BD, Hooper NM, JD Houghton; Instant Notes in Biochem, Bios Scientific Pub, Springer; UK.
- VJ Temple Biochemistry 1001: Review and Viva Voce Questions and Answers Approach; Sterling Publishers Private Limited, 2012, New Delhi-110 – 020.
- G Beckett, S Walker, P Rae, P Ashby, Lecture Notes: Clinical Biochemistry 7th Ed. 2008, Blackwell Publishing, Australia.
- WWW.sumeria.net/oxy/reactive.html
- WWW.ephca.com/fry-ucci.htm
- WWW.bw.uu.nl/mcb/IOS/Default.htm