**Biospectra : Vol.8(1), March, 2013, pp 11-46**
*An International Biannual Refereed Journal of Life Sciences*

**Animal Sciences**

ISSN : 0973-7057

# On the formation of some reactive oxygen species versus Health hazards

**Umapati Sahay[a], R.P. Singh[b], Anita Jha[c], Manjula Kumari[d] Hem Srivastav[e] & A.P.V. Khalkho[f]**

[a]*Dept of Zoology, R.U., Ranchi, Jharkhand, India

[b]Dept. of Zoology, Marwari College, Ranchi, Jharkhand, India

[c]Dept. of Zoology, Gardani Bagh, Govt. Girls' College, Patna, Bihar, India

[d]Dept. of Botany, Gardani Bagh, Women's College, Patna, Bihar, India

[e]Ex-Research Scholar, Zoology Dept., R.U., Jharkhand, India

[f]Women's College, Chaibasa, K.U., Jharkhand, India

*Received , 15th December, 2012 ;Revised: 15th January, 2013*

**Abstract :**High concentration of $O_2$ in the body becomes toxic and leads to tissue damage. Oxygen toxicity involves oxygen free radicals or reactive oxygen species commonly called ROS. ROS are byproducts of normal metabolism. Oxygen is essential for metabolism, in which molecular oxygen is completely reduced, the final product being $H_2O$; yet if reduction is incomplete, a number of reactive radicals are formed such as $O^{\cdot}_2^-$ (super oxide), $H_2O_2$ (hydrogen peroxide), $O^{\cdot}H^-$ (hydroxyl radical). In addition, $1O_2$ (singlet oxygen), $HOO^-$ (hydroperoxy radical), $ROO^-$ (lipid peroxide), $NO^-$ (nitrogen monoxide radical) and $ONOO^-$ (peroxinitrite) too are important from biological point of view.

These free radicals are short lived, highly reactive and have a capacity to damage biomolecules, cells and tissues and are also capable of generating new radicals by chain reactions. There are two categories of radicals –

1. A free radical contains one or more unpaired electrons (Exp. $O^{\cdot}_2^-$, $O^{\cdot}H^-$, $R^{\cdot}OO^-$) but
2. Non free radical derivatives (without unpaired electrons) are $H_2O_2$, $1\ O_2$.

The latter category also comes from biological systems. These radicals cause cellular damage leading to cellular injury (damages to carbohydrate, lipids, protein, RNA and DNA) and cause cell death. Excessive generation of ROS results into oxidative stress leading to pathological disorders such as cardiovascular, neurological, pulmonary, dermatological, diabetic condition, skeletal and neoplastic diseases. Redox active toxicants cause oxidative stress.

The authors have described and compiled informations of these radicals, their good and bad aspects with special reference to environmental pollutants (asbestos, crystalline silica, coal, cigarette smoke, agricultural dust, inorganic dust and metal ions). They have also dealt with Lung epithelial lining versus ROS, Ischemia versus ROS, NF-kB versus ROS, inactivation of $P_{53}$ (transcriptional activator) in the light of works done by various authors.

The ROS, their formation and consequences have been explained with the help of suitable diagrams.

**Key words : Reactive oxygen species, ROS ($O^{\cdot}_2^-$, $H_2O_2$, $O^{\cdot}H^-$, $1O_2$, $HOO^-$, $ROO^-$, $NO^-$, $ONOO^-$ etc.), NF-kB, Ischemia**

## INTRODUCTION

Reactive Oxygen Species are byproducts of normal metabolism, capable of causing cellular damage leading to cell death and tissue injury (Freeman *et al*, 1982)[1]. Our body in order to neglate or minimize cellular damage, possess enzymatic and non-enzymatic antioxidants. Plants do not have antioxidants.

Reactive Oxygen Species (ROS) includes hydroxyl radicals ($O^{\cdot}H$), superoxide anion radical ($O^{\cdot}_2$), peroxyl radical ($ROO^{\cdot}$), Alkoxyl radical ($R^{\cdot}O$), thiyl radical ($R^{\cdot}S$)

*Corresponding author :
Phone: 9934157570
E-mail : sahayumapati@gmail.com

and oxides of nitrogen $N^{\cdot}O$, $N^{\cdot}O_2$. Free radicals contain one or more unpaired electrons whereas non free radicals are without unpaired electrons ($H_2O_2$, $1O_2$ etc.).

Non radicals include hydrogen peroxide ($H_2O_2$)$1O_2$, and hypochloride (HOCl). ROS and non radicals are associated with the production of certain diseases.

There is a balance between the production of ROS and antioxidants in human body. Any imbalance (i.e., excessive generation of ROS) results into an oxidative stress* and leads to disease **(Fig.-1)**.

During normal metabolism, free radicals (a natural byproduct of metabolic activity) are formed.

These free radicals are electrically charged molecules that attack our cells, tearing through cellular membrane and damage nucleic acids, proteins and enzymes inside the cell. They neutralize viruses, bacteria, environmental factors like pollution and radiations; but if produced in excess or when antioxidants are not available, generate **OXIDATIVE STRESS**, the oxidative stress in turn causes cells to lose their structure, function leading to destruction (oxidative damage) **(Fig. 1)**.

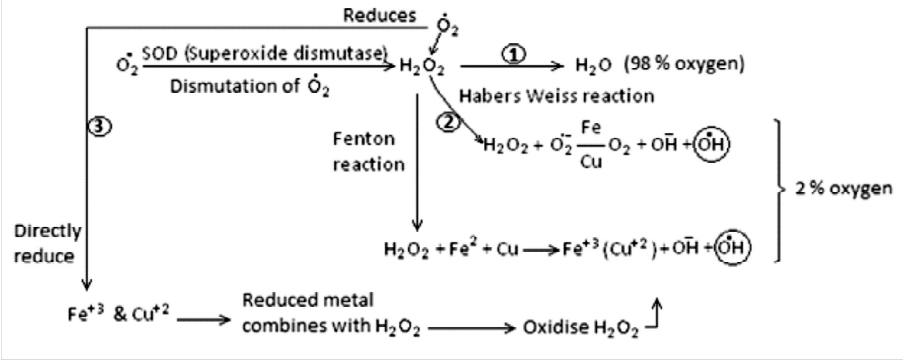
Antioxidants on the other hand reduce oxidative damage caused by free radicals (Frie, 1994[2]; Halliwell & Gutteridge, 1999[3]) for example vitamin C is a potent free radical fighter (Krohausen *et al* 1989[4]). Its concentration in blood stream and tissues is high. It is a primary ingredient of collagen and bind cells to form the tissues and has a role in the manufacture and defence of connective tissue. Vitamin C neutralize free radicals Mishra and Fridovich, 1972[5], is an aldose reductase inhibitor and prevents tissue damage caused by sorbitol **(Fig. 2)**.

Exposure to redox active toxicants (asbestos, crystalline silica, coal, cigarette smoke, agricultural dust, inorganic dust and metal ions) leads to changes in the pro-oxidant level which utilizes antioxidants thereby causing oxidative stress and cell injury, which in turn leads to *pneumoconiosis*** and *carcinogenesis****.

In chronic exposure to redox active toxicants there is an influx of inflammatory cells into the lungs which promotes the production of ROS and leads to the production of pulmonary *neoplasm*****, Cantin *et al* (1987)[6]; Oury *et al* (1964)[7], finally leads to tumour.

1. *Mitochondrial c-oxidase:*

Mitochondrial c-oxidase in a state of normal oxygen tension (in human body) turns, more than 98 % oxygen through a four electron catalytic reduction and forms water, however remaining 2 % of oxygen undergoes sequential incomplete reduction and forms long lived reactive species via Habers Weiss reaction as under:




**Haber's Weiss and Fenton reaction**

* attacks by free radicals is collectively called oxidative stress.

Free radicals are electrically charged particles or molecules, that attack our cells, tearing through cellular membrane and damage nucleic acids, proteins and enzymes inside the cells.

** Any lung disease due to permanent desposition of substantial amount of particulate matter in the lungs.

*** Production of cancer.

**** Abnormal growth in which cell multiplication is uncontrolled and progressive (benign/malignant).

$H_2O_2$ is produced from catalyzed SOD (superoxide dismutase) in the absence of upregulation of catalase.

$O^{\cdot}_2$ directly reduces $H_2O_2$ (generated from the simultaneous dismutation of $O^{\cdot}_2$, to $O^{\cdot}H$, $O_2$ and $OH^-$ (Haber's Weiss reaction). $O^{\cdot}_2$ also reduces trace elements $Fe^{+3}$, sometimes $Cu^{+2}$ and generates $O^{\cdot}_2{}^-$. Reduced metal reacts with $H_2O_2$ and get it oxidized with release of $O\bar{H}$ and $O^{\cdot}H$ (Fenton reaction). Thus in Fenton reaction $O^{\cdot}_2{}^-$, $H_2O_2$ and $Fe^{+3}$ are involved **(Fig. 3)**.

(1)*ROS in lung epithelial lining:*

In lung epithelial lining fluid, the concentration of reactive oxygen species (ROS) are low due to the presence of

(1)SOD (Cu, Zn SOD) – found in cytoplasm and peroxysomes.

(2)Mn SOD – present in mitochondria.

(3)Catalase – in peroxysomes.

(4)Antioxidant vitamin E.

(5)High concentration of reduced glutathione and ascorbate Cantin *et al* (1987)[6].

In the lung matrix however, extracellular SOD (Ec-SOD) scavenges extracellular $O^{\cdot}_2{}^-$ Oury *et al* (1994)[7].

The reactivity of $O^{\cdot}H$ is very high, its action is non specific, its reactivity is confined to few molecular radii from its origin.

(2)*Ischemia and ROS:*

In ischemia, decreased perfusion, low Oxygen tension and trauma, xanthine dehydrogenase is converted into xanthine oxidase (also released from liver and intestine). This xanthine oxidase binds to pulmonary epithelium and becomes a locus for the production of reactive $O^{\cdot}_2{}^-$ species Weinbroun *et al* (1995)[8]. When xanthine combines with molecular oxygen in the presence of xanthine oxidase, it produces partially reduced oxygen species.

(3)*ROS with the help of NADH oxidase*:

Neutrophils and macrophages migrate to lung under the proinflammatory cytokines and release ROS with the help of NADPH oxidase.

ROS is of two types:

*1. Exogenous ROS:* It is produced in many circumstances and situations, for example —

a) In asthamatics, Mattoli *et al* (1991)[9] found increased level of inflammatory cells (monocytes) and ROS.

b) Fulkerson *et al* (1996)[10] found ROS production during cystic COPD (chronic obstructive pulmonary disease), ARDS (acute respiratory distress syndrome).

c) ROS is produced by stimulated *macrophages* and *polymorphonuclear leucocytes (neutrophil)* when people are exposed to pollutants Sibille *et al* (1990)[11].

d) Cross *et al* (1991)[12] observed that in inhaled ozone, nitrogen dioxide, automobile exhaust, cigarette smoke evoke the production of ROS.

e) Pritchard *et al* (1996)[13] opine that particles such as residual oil, fly-ash and air dust containing ionizable concentration of transition metals via phagocytosis by macrophages too, generate ROS.

f) Transition metal containing dust (in vivo exposure) help in the release of ROS, Berg *et al* (1996)[14].

g) Industrially derived silica and asbestos react with lipid and protein of airways cells and generate ROS Vallyathan *et al* (1988)[15] and Brody *et al* (1988)[16].

h) When lung diseases are treated $N^{\cdot}O$ reacts with oxygen and superoxide anion radical and forms peroxynitrite Fulkerson *et al* (1996)[10]. **(Fig. 4).**

*2. Endogenous ROS*: It is also produced in different situations, such as **(Fig. 5)** —

a) Gatti *et al* (1993)[17]; Shooji *et al* (1995)[18] opine that in response to pro-inflammatory cytokines (narcosis factor α-TNFα) ROS is produced in airways epithelial cells.

b) TNF-α regulates gene expression and the activities of xanthine oxidase upregulation (an abundant enzyme in epithelial cells) which generate superoxide anion radical and $H_2O_2$ as it meets the oxygen in airways epithelial cells. Interferon γ, IL-1 adds to the effect of xanthine oxidase up-regulation, Kooji *et al* (1992)[19]; Pfeffer *et al* (1994)[20].

This has been observed by Rahman *et al* (1995)[21] in human epithelial cell type II (A 549) and the cell in order to protect itself from oxidant burden (ROS), converts cellular glutathione to its oxidised form.

(2)Oxidative enzymes such as NADPH and cycloxygenase which are associated with airways epithelial cells adds further burden of ROS, Ramacle *et al* (1995)[22].

(3)In response to inflammatory cytokines in airways epithelial cells i NoS (found in epithelial cells too produce $N^{\cdot}O$.

Through electron transport reactions in mitochondria

of stimulated epithelial cells forms reactive species, Shooji *et al* (1995)[18]; Schulzeosthoff *et al* (1993)[23].

Whether ROS is exogenous or endogenous, in all cases ROS leads to the development of oxidative stress which leads to cytotoxic effect.

However, oxidative stress is protected by non-enzymatic antioxidants (vit. C, vit. E, β-carotene, uric acid and thiols). Carotenoids and vit.-C quenches radical in non-lipid cellular compartments, uric acid quenches O˙H, singlet oxygen, hypochlorous acid, oxoheme oxidants and hydroperoxyl radical also stops oxidative damage of protein and nucleobases, immobilizes oxidant species produced via Fenton reaction and which are intracellular oxidants Ames *et al* (1981)[24]; Davis *et al* (1986)[25].

Mucous removes O˙H, $H_2O_2$ according to Cross *et al* (1984)[26]; Grisham *et al* (1987)[27].

Intracellular oxidant burden is reduced by bilrubin (a free radical scavenger), heme oxigenase I (protein) catalyzes the production of bilrubin from Heme according to Stocker *et al* (1981)[28], Keyse *et al* (1989)[29] and Schubert *et al* (1991)[30]. **(Fig. 6)**.

Airways epithelial cells possess enzymes, such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHPx). Out of these SOD dismutes $O^{\cdot}{}_2^-$ and produce $H_2O_2$ which is processed further by CAT or glutathione cycle.

In the cytosol and nucleus of epithelial cells CuZnSOD is found, whereas in mitochondria MnSOD is found Hassan *et al* (1990)[31] which is upregulatd by ROS and cytokines such as TNF α, IL-I and IL-6 and by inflammatory minerals Wong *et al* (1988)[32]; Ono *et al* (1992)[33]; Persinger *et al* (1993)[34]; Duan *et al* (1993)[35]; Janssen *et al* (1994)[36] and Visner *et al* (1990)[37].

$H_2O_2$ is consumed by catalase and glutathione peroxidase (GSHPx). Glutathione oxidation reduction cycle reduces $H_2O_2$ and degrades lipid peroxides and products of lipooxygenase catalysed reactions Freeman *et al* (1982)[1]. (**Fig. 6)**.

In general, it can be said that pro-inflammatory cytokines force Neutrophils and macrophages to migrate to lung and release ROS with the help of NADPH oxidase, Babior (1994)[125].

Chrysolite asbestos cause production of ROS ($O^{\cdot -}$) in human alveolar macrophage Perkins *et al* (1991)[38] and in guinea pig alveolar macrophage Kella *et al* (1990)[39].

Crocidolite, amosite, chrysotile asbestos and silica exposure produce ROS (measured by electron spin resonance ESR spectrum).

$O^{\cdot -}_2$ produced opens up $Ca^{2+}$ channel, Kella *et al* (1990)[39].

Chen *et al* (1998)[40] opine that ROS is activated by cytokines, viruses, protein kinase C with activators and immunological stimuli. ROS is also activated by SOD by the process of dismutation of $O^{\cdot}{}_2^-$ and generation of $O^{\cdot}\bar{H}$ which in turn activate NF-kβ (nuclear factor kappa transcription factor which plays an important role in inflammatory and immune responses). NF-kβ is also produced by crocidolite induced alveolar macrophage cell line and from primary alveolar cells, Vallyathan (1998)[41] which is time and dose dependent.

NF-kβ regulates TNFα (tumour necrosis factor α) which is a proinflammatory cytokine. It also activates cytokine receptor Siebenlist *et al* (1994)[42]; Baeurele *et al* (1994)[43].

NF-kβ is inhibited by antioxidants like *N-acetyl cysteine* and *pyrrolidine dithiocarbamate* (PDTC) Shreak *et al* (1992)[44] and metal chelators *deforaxamine* which block metal generated O˙H from $H_2O_2$.

The production of TNF-α is inhibited by oxygen radical scavengers. Inhibition of NF-kβ nuclear translocation by SN 50 has been observed.

Cell permeable inhibitory polypeptide or sequence specific *oligonucleotide* which hits *TNF-α* binding site of NF-kβ and attenuates the effect of asbestos on TNF-α production. **(Fig. 7).**

Vallyathan *et al* (1998)[41] opine that ROS produce DNA strand break (detected by alkaline unwinding assay).

ROS is capable of bringing oxidative modification of DNA base, sequence changes, poly ADP ribosylation, activation of kinases and proto onchogenes, inactivation of suppressor gene, Weissmann *et al* (1996)[45]; Halliwell *et al* (1990)[46].

Some of the reactive species are dealt here separately:

***N˙O (Nitric oxide)***

Nitric oxide is a membrane permeable free radical which is synthesized from terminal guanodin nitrogen of *L-arginine* with the help of N˙O synthase – Marletta (1993)[47]. **(Fig. 9).**

The enzyme N˙O synthase (NOS) exists in three isoforms **(Fig. 8)** which expresses itself in different cell types according to Moncada *et al* (1991)[48] and Nathan (1992)[49]. Three different genes are responsible for the transcription of the three isoforms which catalyze the oxidation of L-arginine to L-citrulline, Steuhar *et al* (1992)[50] and Nathan *et al* (1994)[51] in the presence of co-factors like NADPH, FAD (Flavin adenine dinucleotide), Flavin mononucleotide (FMN), Calmodulin heme and tetrahydrobiopterin.

According to Nathan *et al* (1994)[51] the NOS isoform differ in:-

i) Calcium dependence,
ii) tissue distribution,
iii) amino acid sequence and
iv) regulation of expression and function.

The endothelial isoform of NOS are **(Fig. 8)**:

1. NOS (e NOS or $NOS_3$) – 135 k Da
2. Neuronal NOS (n NOS or NOS) – 150-160 k Da

The above two are constitutive NOS (cNOS) and require calcium for expression Snyder (1992)[52]; Gibaldi (1993)[53] and Bredt *et al* (1991)[54].

3. i NOS or $NOS_2$ – 130 k Da – is generally expressed in response to cytokines [IL] - Iβ, TNF.α, INF-γ and bacterial lipopolysacchrides Nathan (1992)[49]; Lorsback *et al* (1993)[55]; Billar *et al* (1992)[56] and or is expressed in certain cells by immunologic or inflammatory stimuli Hibbs *et al* (1987)[57], Steuhr *et al* (1991)[50], Grisham *et al* (1992)[58], Mc Call *et al* (1989)[59], Curran *et al* (1989)[60]; Schulz *et al* (1992)[61], Werner – Felmarer *et al* (1990)[62] and Bensley *et al* (1991)[63]. It has been implicated in *Endotoximia**, inflammatory bowel disease, neurological disorders and *atherosclerosis***. **(Fig. 8).**

$NOS_2$ is also formed from synovial fibroblasts of patients suffering from *arthritis****. It has been found that $NOS_2$ is inhibited by the absence of TGF-β (transforming growth factor $B_1$) and that its absence cause lethal inflammatory condition.

Disrupted generation of TGF-β leads to over production /expression of $NOS_2$ which ultimately produce N˙O leading to the death of SJL mouse Imai *et al* (1993)[64]; Gaillard *et al* (1992)[65]; and Marletta *et al* (1993)[47] found that *Eicosanoid* reduce expression of $NOS_2$ and results into the formation of N˙O.

Activation of $NOS_2$ leading to the production of N˙O is also brought about by cytokines, microbial products and immune complexes which act as activators.

**N˙O helps in many ways for example (Fig.14)**:

i) It develops resistance to tumours and microbes.
ii) According to Genaro *et al* (1995)[66] when N˙O is produced in small amounts, it modulates β-cell function by modifying bcL-2 level and apoptosis.
iii) Darius *et al* (1992)[67]; Kubes *et al* (1991)[68] found that N˙O modifies adhesiveness and chemotaxis of polymorpho-nuclear neutrophils and monocytes.
iv) Stadler *et al* (1993)[69] found that N˙O modulates prostaglandin $E_2$ formation.
v) According to Magrinet *et al* (1992)[70] and Panjabi *et al* (1992)[71] N˙O regulates blood pressure and vascular tone, neurotransmission, controls cellular growth and differentiation and also modulates learning.
vi) Stimulates Guanyl cyclase after binding (via c GMP; c - GMP gated channels, c – GMP dependent kinases and photo-diesterage. It also controls cell function. **(Fig. 10).**
vii) N˙O binds with high affinity to iron heme of haemoglobin (Hb) and myoglobin (Mb). Hb and Mb are quenchers of N˙Os action and forms nitrosylhaemoglobin (NOHb) – this is detected by electro-paramagnetic resonance (EPR).
viii) N˙O increase conjunctivital blood flow and plays a vital role in retinal ischemia /degenerative diseases.
ix) According to Bredt *et al* (1991)[54]; Pollock *et al* (1991)[72], N˙O activates soluble guanyl cyclase which produce intracellular signaling molecule c-AMP, decreases intracellular calcium and release smooth muscle cell. **(Fig. 10).**
x) Combines with $O^{\cdot}_2^-$ in mitochondria and forms peroxynitrite $ONOO^-$ which stimulates $Ca^{2+}$ release

*Endotoximia - presence of endotoxin in blood.

**Atherosclerosis - where atheromas containing cholestrol lipid and lipophages are formed within the intima and inner/ media of large & medium size arteries.

*** Arthritis - inflammation of joints

from mitochondria but maintains Dy (mitochondrial membrane potential). **(Fig. 11).**

xi) Stimulate specific calcium release pathway from mitochondria due to oxidation of thiol in mitochondria, Beckmann *et al* (1990)[73] and in variety of cells Muijsers *et al* (1997)[74]. **(Fig. 11, 12, 13).**

**N˙O on the other hand shows some bad effects. (Fig. 15)** These are:-

i) According to Brown *et al* (1994)[75]; Carr *et al* (1990)[76]; Schweizer *et al* (1994a)[77], Richter *et al* (1994)[78], Cleeter *et al* (1994)[79]; Brown *et al* (1995)[80] and Takehara *et al* (1995)[81] N˙O inhibits respiration as observed in isolated enzymes, submitochondrial particles, mitochondria, hepatocytes, brain nerve terminals and astiocytes.

ii) Inhibits platelet aggregation and secretion according to Stamler *et al* (1991)[82]; cell proliferation and lymphocyte formation Moncada *et al* (1993)[83] at relevant concentration.

iii) Deenergizes mitochondria using respiratory substrate like pyruvate and malate, ascorbate + tetramethyl phenylenadiamine (not deenergized if ATP is used in transient inhibition of c-oxidase).

iv) According to Tamir *et al* (1995)[84] N˙O participates in the pathogenesis of spontaneous myositis* in **SJL** mouse.

v) Brown *et al* (1995)[80]; Richer *et al* (1995)[78] found that N˙O inhibits c-oxidase at physiologic cellular oxygen pressure (c-oxidase is sensitive to N˙O below 30 μM concentration.

vi) Magrinet *et al* (1992)[70] and Claney *et al* (1995)[85] hold that N˙O destroys tissue/cells, can induce *cyclooxygenesis (Cox)*, cause pain, destroy protease inhibitors, increase the formation of IL-1, TNF-α NADPH activity in myloid cells.

vii) iNOS is implicated in *Endotoxemia*, inflammatory bowel disease can be activated, repressed by N˙O.

viii) In Ischemia** and reperfusion injury and in inflammatory sepsis elevated level of $O^{\bullet}_{2}^{-}$ (superoxide anion) and N˙O production takes place, Beckmann *et al* (1990)[73], Muijsers *et al* (1997)[74] found that N˙O and $O^{\bullet}_{2}$ make $ONOO^{-}$ in a variety of cells

ix) $ONOO^{-}$ according to Radi *et al* (1991)[86], oxidizes

a) Sulf hydryl group and initiates lipid peroxidation.

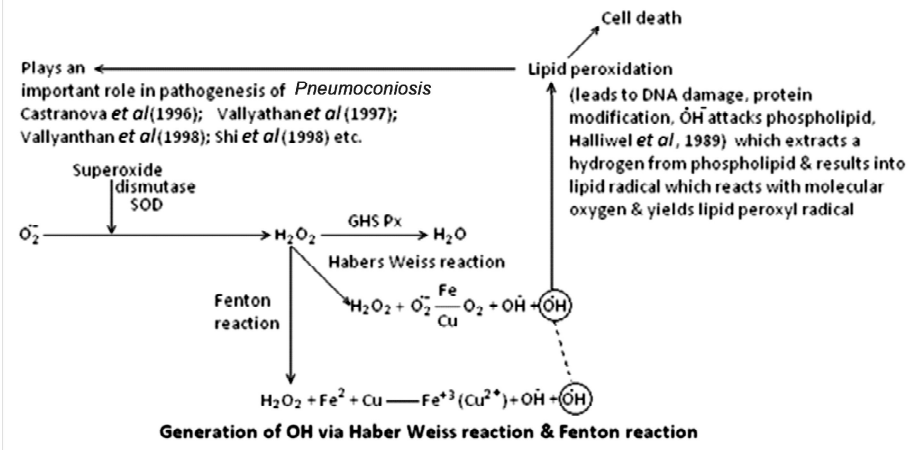
b) Nitrate phenolic ring of tyrosine residues of protein and forms nitrotyrosine (detected by anti-nitrotyrosine antibodies Ischiropaulus *et al* (1992)[87].

c) brings relaxation in coronary artery, Lieu *et al* (1994)[88] and pulmonary artery, Wu *et al* (1994)[89].

d) inhibits leucocyte endothelial interaction at nanomolecular concentration via inhibition of the upregulation of P-selection.

e) Naseem *et al* (1995)[90] found inhibition of platelet aggregation.

(f) brings reduction in the polymorphonuclear accumulation in ischemia* reperfused heart (due to nanomolecular concentration of $ONOO^{-}$).




**Generation of OH via Haber Weiss reaction & Fenton reaction**

* Myositis – inflammation of voluntary muscles.

**Ischemia – deficiency of blood in a part, due to functional constriction or actual obstruction of a blood vessel.

**$H_2O_2$:**

$H_2O_2$ is generated due to dismutation of $O^{\cdot}{}_2^-$. $O^{\cdot}{}_2^-$ reduces $H_2O_2$ and produce OH, $O\bar{H}$ and $O_2$ (Haber Weiss reaction). $O^{\cdot}{}_2^-$ also reduces trace metals ($Fe^{+3}$ and $Cu^{2+}$) and generates $O_2$**.** Reduced form of metal reacts with $H_2O_2$ and results into oxidized form of metal, $OH^-$ and $O^{\cdot}H$ as illustrated below. When SOD is catalysed, $H_2O_2$ is formed**,** which shows toxic effect in certain circumstances.

$O^{\cdot}H^-$ is nonspecific and highly reactive form confined to few molecular radii from its origin, attacks membrane phopholipid (Halliwel *et al* 1989)[91], extracts a hydrogen from phospholipid and results into lipid radical which combines with molecular oxygen and forms lipid peroxyl radical which in turn destroy membrane phospholipid and brings oxidative cell injury according to Vallyathan *et al* (1995)[92], Castranova *et al* (1996)[93]; i.e., brings changes in :-

a) Extra cellular release of protease, lytic enzymes, ROS and cytokines.
b) Amplification of inflammatory mediators and growth factor.
c) Upregulates and consumes antioxidant enzymes **(Fig. 16).**

Repeated cell injury activates fibroblastic proliferation and collagen formation ultimately leading to pulmonary fibrosis.

Oxidative injury is inhibited by catalase (an antioxidant) and formate. $O^{\cdot}H$ hydroxylate guanosine residues (dG) to 8-hydroxy 2'-deoxyguanosine (8-OH dG) according to Diazarolu (1991)[94].

$O^{\cdot}H$ is also produced in various ways. Some of these are: **(Fig. 16).**

i) Inhalation of particulate matters, or phagocytosis produce $O^{\cdot}{}_2^-$ which on dismutation yields $H_2O_2$. $H_2O_2$ via Fenton reaction in the presence of transition metal (ferrous or cuprous ions) produce $O^{\cdot}H$. $H_2O_2$ is capable of crossing the cell membrane by diffusion whereas $O^{\cdot}{}_2^-$ crosses the cell membrane through anion channels (Fig. 3). The reactivity of $O^{\cdot}{}_2^-$ and $H_2O_2$ differ considerably compared to each other.
ii) According to Vallyathan *et al* (1992)[95]; Kadriiska *et al* (1997)[96] and Castranova *et al* (1996)[93] inhalation of toxic pollutants undergo phagocytosis and generate $O^{\cdot}H$.
iii) Fractural quartz instigates the production of $O^{\cdot}H$.
iv) According to Vallyathan *et al* (1998)[41]; Shi *et al* (1988)[97] surface radical in freshly ground silica upon contact with aqueous solution too generate $O^{\cdot}H$.
v) $O^{\cdot}H$ plays an important role in silica induced NF-kβ activation Sun *et al* (1996)[98], bring oxidative injury, cause deletion and point mutation which results into oncogene activation **(Fig. 16).**

**Activation of NF-kβ (Nuclear factor Kappa β)**

NF-kβ plays an important role in inflammatory and immune responses once it is activated along with the activation of oncogenes, cytokine receptors, cell adhesion molecules, growth factors opines Sibenlist *et al* (1994)[42]; Baeuerlle (1994)[43].

Immunological stimuli, protein kinase activators, cytokines, viruses and ROS activate NF-kβ, Chen *et al* (1998)[99]. SOD (by promoting the dismutation of $O^{\cdot}{}_2^-$ generate $O^{\cdot}H$ radical) increase the activation of NF-kβ. $O^{\cdot}H$ radical (one of the reactive oxygen species) is a potent activator of NF-kβ.

There are compound which inhibit NF-kβ activation. These are:-

(i) Catalase, (ii) $O^{\cdot}H$ specific scavenger sodium formate, (iii) anti-oxidants like N-acetyl cysteine and pyrrolidine dithiocarbamate, (iv) metal chelator deforaxamine.

NF-kβ regulates the production of TNF-α which is dose and time dependent. Vallyanthan *et al* (1998)[41] found that crocidolite asbestos induces the production of TNF-α whereas SN-50 (cell permeable inhibiting peptide or sequence specific oligonucleotide) directed against TNF-α binding site of NF-kβ attenuated the effect of asbestos on the TNF-α production. **(Fig. 17).**

The silica particles as well as exposure to chrysolite produce ROS which is enhanced by Fe (II) and $H_2O_2$.

ROS attacks on deoxyribose purine and pyrimidine bases, induces oxidative modification of DNA (base damage), sequence change in DNA, poly ADP ribosylation, activates kinases and protooncogenes and activates tumor suppressor gene P53 (having 10 cysteine residues).

$O^{\cdot}H$ of ROS leads to deletion and point mutation which results into oncogene activation. **(Fig. 16).** Dizdarrolu (1991)[94] found that $O^{\cdot}H$ in isolated DNA, induce DNA damage as it brings hydroxylation of dG (guanosine residues) resulting into 8-OHdG (8-hydroxy-2'-

deoxyguanosine) measured by HPLC with electrochemical detection and this, therefore, leads to point mutation (base damage) ultimately leading to mispairing. **(Fig. 16).**

***Inactivation of $P_{53}$ (Transcriptional activator)***

Gene $P_{53}$ is a transcriptional activator which serves as tumour suppressor, has a role in human carcinogenesis Harris (1993)[100] and Harris *et al* (1993)[101].

In 50 – 60 % of human lung cancers mutated $P_{53}$ is found. Mutated $P_{53}$ possess long life and is found in higher concentration in cancer cells and preneoplastic cells. Double strandd DNA break or other types of DNA damage activate $P_{53}$ according to Harris (1993)[101] and Harris *et al* (1993)[102].

Gansange *et al* (1997)[102] observed $G_1$ arrest in proliferating fibroblasts and accumulation of $P_{53}$ and Cdk inhibitor I WAFI/CIP 1.

$P_{53}$ – a transcriptional activator has certain works to do. These are:

i) Controls the expression of several genes that control growth inhibitory and apoptotic pathway.
ii) Serves as tumour suppressor.
iii) Has 10 cysteine residues in it.
iv) In reducing environment it is active.
v) In oxidizing environment (prooxidant state) it undergoes conformational change and becomes wild $P_{53}$ **(Fig. 19)** – a mutant.

In 50 % of the human tumours $P_{53}$ is defective that is the reason tumours are aggressive and resistant to treatment, on introduction of active $P_{53}$ genes in such tumour cells, the tumour regress.

**Peroxynitrite ($OONO^-$) –** a potent oxidizing and nitrating species **(Fig. 20)**

According to Beckmann *et al* (1990)[73] *peroxy-nitrite* is a potent oxidant generated from $N^{\bullet}O$ and $O^{\bullet}_2^-$.

$OONO^-$ formation has been shown in:

(i) rheumatoid arthritis,
(ii) shock associated endotoxemia,
(iii) silica induced lung injury,
(iv) neurotoxicity,
(v) endothelial injury and colonic inflammation, Beckmann *et al* (1994)[103]; Darley – Usmar *et al* (1995)[104] and Proyor *et al* (1995)[105],
(vi) photoreceptors in the ganglion cell layers and blood vessels of retina, Wu *et al* (1997)[106].

**$OONO^-$ oxidises:**

(i) sulfhydryl group and instigate lipid peroxidation.
(ii) Nitrate phenolic rings of tyrosine residues of protein and forms nitrotyrosine, Haddad *et al* (1994)[107].
(iii) Produces vascular relaxation in isolated coronary, Liu *et al* (1994)[88] and pulmonary arteries Wu *et al* (1994)[89].
(iv) Inhibits platelet aggregation Naseem *et al* (1995)[90].
(v) Inhibits leukocyte endothelial interaction in vitro and vivo by inhibiting the upregulation of P-selectin, Lefer *et al* (1997)[108].
(vi) At nano-molar concentration of $OONO^-$ reduces polymorphonuclear accumulation in ischemic reperfused rat hearts.
(vii) $OONO^-$ along with $N^{\bullet}O$ lowers intra ocular pressure in eye and increase blood flow.
(viii) Plays a role in occular inflammation Bellot *et al* (1996)[109] and Allen *et al* (1996)[110] and $OONO^-$ is also involved in uveitis* Allen *et al* (1998)[111].
(ix) Decays under acidic condition to produce hydroxyl like free radical Beckmann *et al* (1990)[73].
(x) Increase in 3-nitrotyrosine due to nitration of tyrosine by $OONO^-$ has been seen in the lung of patients with sepsis and in animals with acute lung injury.
(xi) $OONO^-$ can also S-nitrosylate glutathione and other thiols which has cardioprotective and cytoprotective effects Lefer *et al* (1997)[108].
(xii) $OONO^-$ is capable of reacting with other proteins and alter their function for example $OONO^-$ and $N^{\bullet}O$ both inactivate the activity of catalase. Venkatraman *et al* (1994)[112] observed in MRL mice overproduction of $OONO^-$, $O^{\bullet}_2^-$ and $N^{\bullet}O$ with depletion of catalase. As a result of this lipid peroxidation was increased with *increased oxidative stress.*
(xiii) $OONO^-$ is an efficient oxidant of thiols Radi *et al* (1991)[86]. Activation and expression of NOS results into the formation of $OONO^-$. It has been implicated in certain diseases like acute *endotoxemia*, *inflammatory* bowel disease, neurological disorders and *artherosclerosis*. It contributes to the $N^{\bullet}O$ mediated biological effects Lin *et al* (1995)[113].
(xiv) $O^{\bullet}_2^-$ combines in mitochondria with $N^{\bullet}O$ and forms $OONO^-$ which stimulates $Ca^{2+}$ release from mitochondria without disturbing the Dy.

(xv) $OONO^-$ inhibits Acotinase and can stimulate the specific $Ca^{2+}$ release pathway from mitochondria by oxidizing some vicinal thiols in mitochondria.

(xvi) $OONO^-$ induces apoptosis which is concentration and time dependent Lin *et al* (1995)[113]. Cells die by apoptosis or necrosis which is concentration dependent Bonfoco *et al* (1995)[114].

(xvii) $OONO^-$ is capable of reacting with other proteins and alter their function for example $ONOO^-$ and $N^{\bullet}O$ both inactivate the activity of catalase. Venkatraman *et al* (1994)[112] observed in MRL mice overproduction of $OONO^-$, $O^{\bullet}{}_2^-$ and $N^{\bullet}O$ with depletion of catalase. As a result of this lipid peroxidation was increased with *increased oxidative stress.*

(xviii) As mentioned earlier $ONOO^-$ nitrates phenolics, including tyrosine and tryptophan residues in several proteins Beckmann *et al* (1992)[115].

(xix) Bovine aortic endothelial cells Kobzik *et al* (1993)[116]; rat alveolar macrophages Ischiropolous *et al* (1992)[87] and human neutrophils Carreras *et al* (1994)[117] also produce $ONOO^-$.

(xx) Gow *et al* (1996)[118] and Denicola *et al* (1996)[119] have reported that at physiologic concentration of $CO_2$ and bicarbonate, $OONO^-$ reacts vigorously via the formation of the nitrosoperoxycarbonate anion ($O=N\text{-}OOCO_2^-$) and increases the yield of nitration. $ONOO^-$ attacks biological targets even in the presence of antioxidants Van der vilet *et al* (1994)[120].

(xxi) Crow *et al* (1995)[121] observed that $ONOO^-$ and $N^{\bullet}O$ modify the thiols of transcription factors used in the DNA bending. For example zinc finger protein.

(xxii) Zu *et al* (1998)[122] observed that asbestos inhalation induces iNOS in lung inflammation and epithelial cells resulting into the formation of $OONO^-$ (the significant amount of nitrotyrosine) a marker of $OONO^-$ was observed by Zu and his colleagues in the lung of rats exposed to chrysolite and crocidolite.

(xxiii) Saleh *et al* (1997)[123] too observed significant amount of nitrotyrosine and iNOS in macrophages, neutrophils and alveolar epithelial cells of patients with idiopathic pulmonary fibrosis and opined that possibly this is due to $OONO^-$ causing fibrosis.

(xxiv) Broaddus *et al* (1996)[124] found that asbestos induces apoptosis of human and rabbit pleural mesothelial cells by $OONO^-$.

## CONCLUSION

The generation of free radicals is due to normal biological processes or cellular metabolism or due to environmental pollutants. ROS may be produced exogenously in asthamatics, during chronic obstructive pulmonary disease, in acute respiratory syndrome, when people are exposed to pollutants, due to inhalation of ozone, nitrogen dioxide, automobile exhaust, cigarette smoke, residual oil, flyash and air-dust containing ionisable concentration of transition metals, from industrially derived silica and asbestos when react with lipid and protein of airways cells and when lung diseases are treated with $N^{\bullet}O$.

Endogenous ROS are produced in airways epithelial cells in response to proinflammatory cytokines (necrosis factor $\alpha$-TNF $\alpha$) during xanthine oxidase upregulation, due to oxidative enzyme NADPH and cyclooxigenase in airways epithelial cells, through electron transport reactions in mitochondria of epithelial cells.

In either case of elevated level of ROS leads to oxidative stress, which has cytotoxic effects — pathological disorders (cardiovascular, neurological),

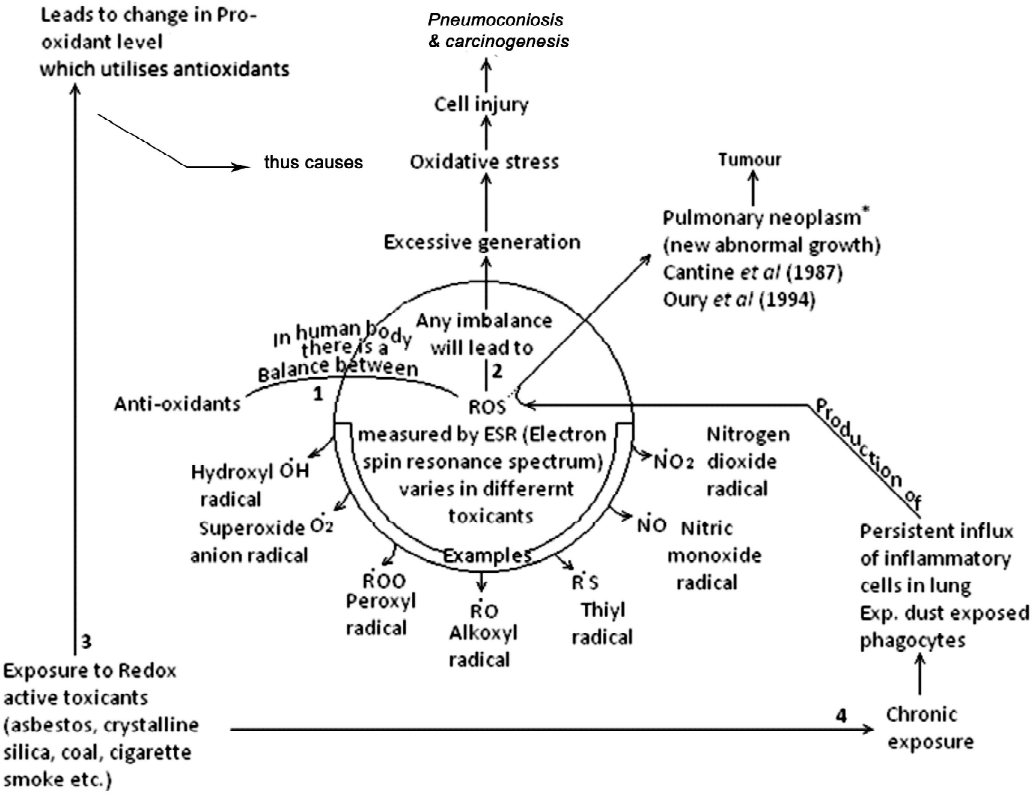



**Fig. 1. Types of ROS & its role in *Pneumoconiosis, Carcinogenesis* & Pulmonary *neoplasm***

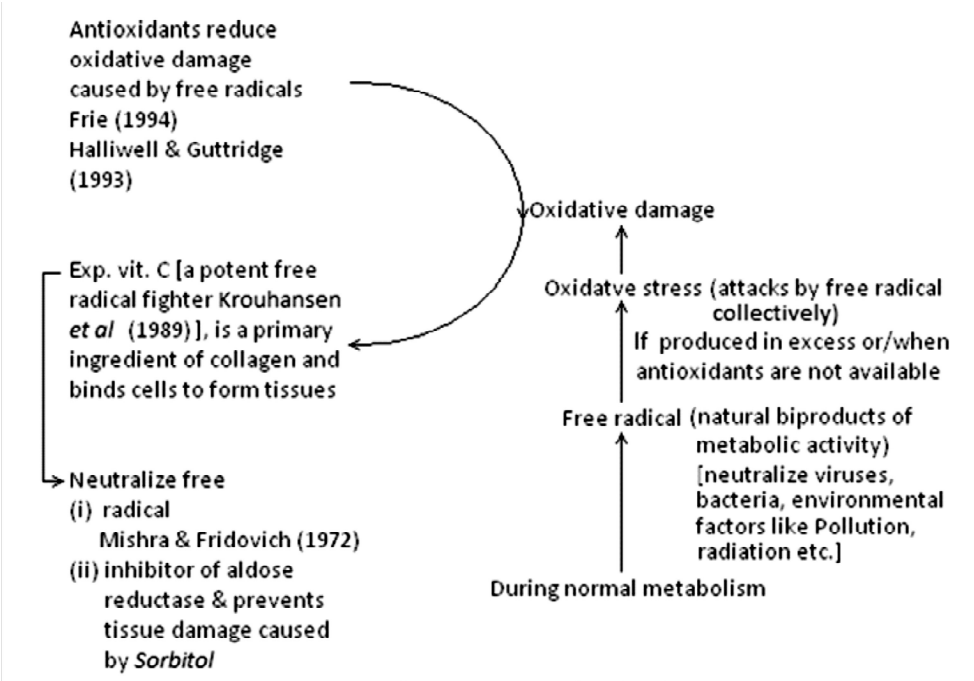



**Fig. 2. Oxidative damage & role of antioxidants like vit. C.**

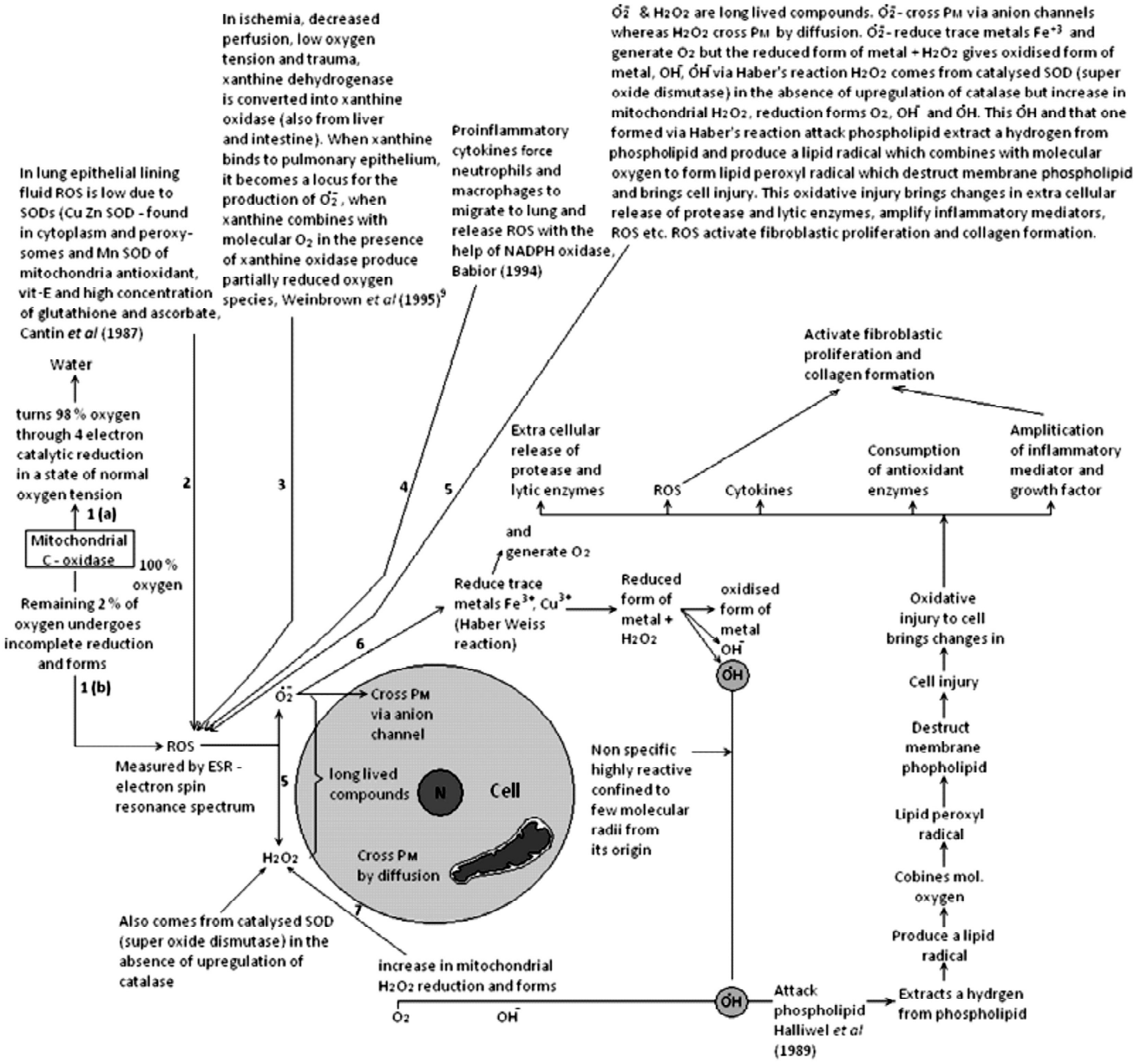



**Fig. 3. Fate of mitochondrial C-oxidasse in normal oxygen tension, Haber's & Fenton reaction & the role of $\dot{O}H$ in producing oxidative injury to cell**

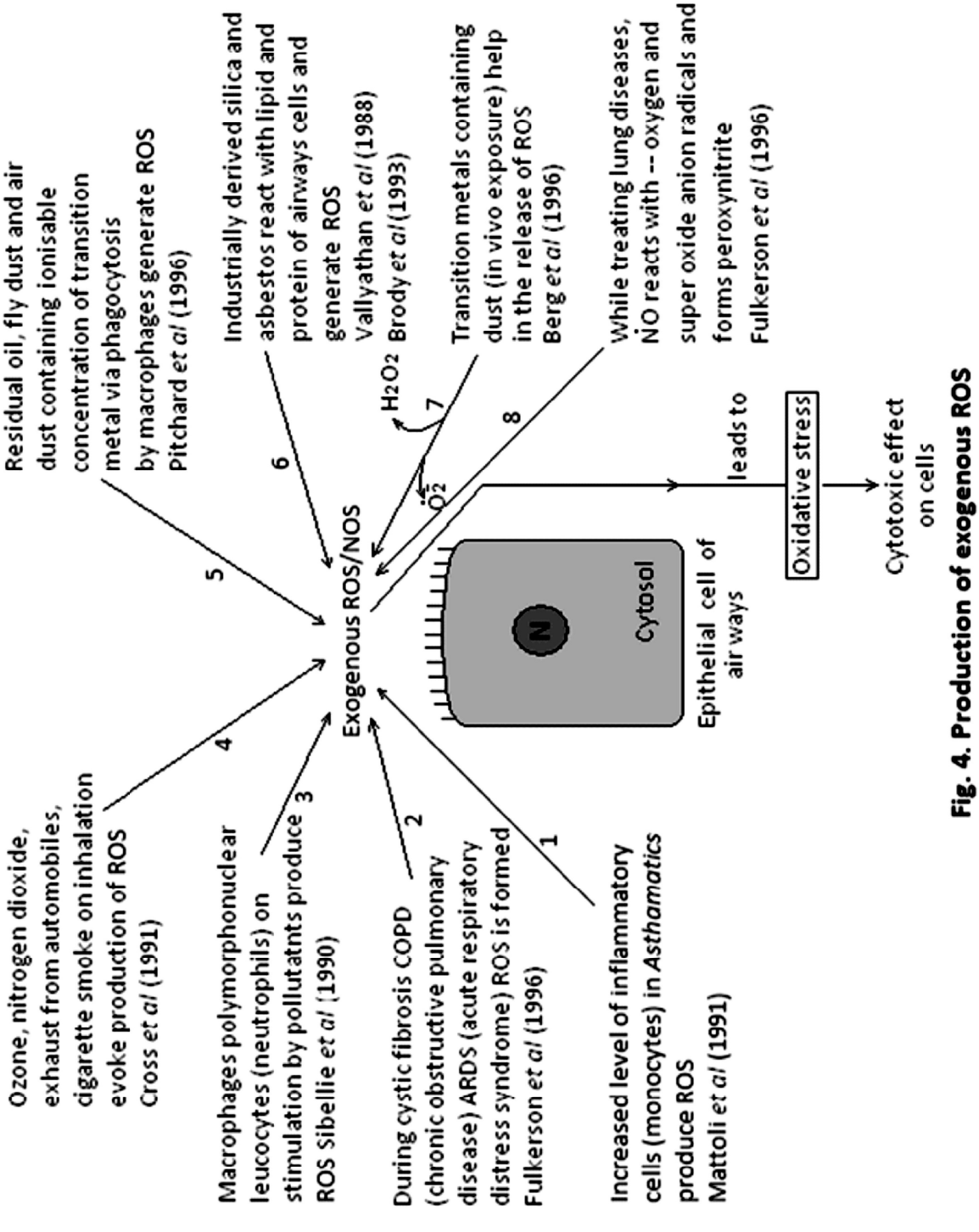



**Fig. 4. Production of exogenous ROS**

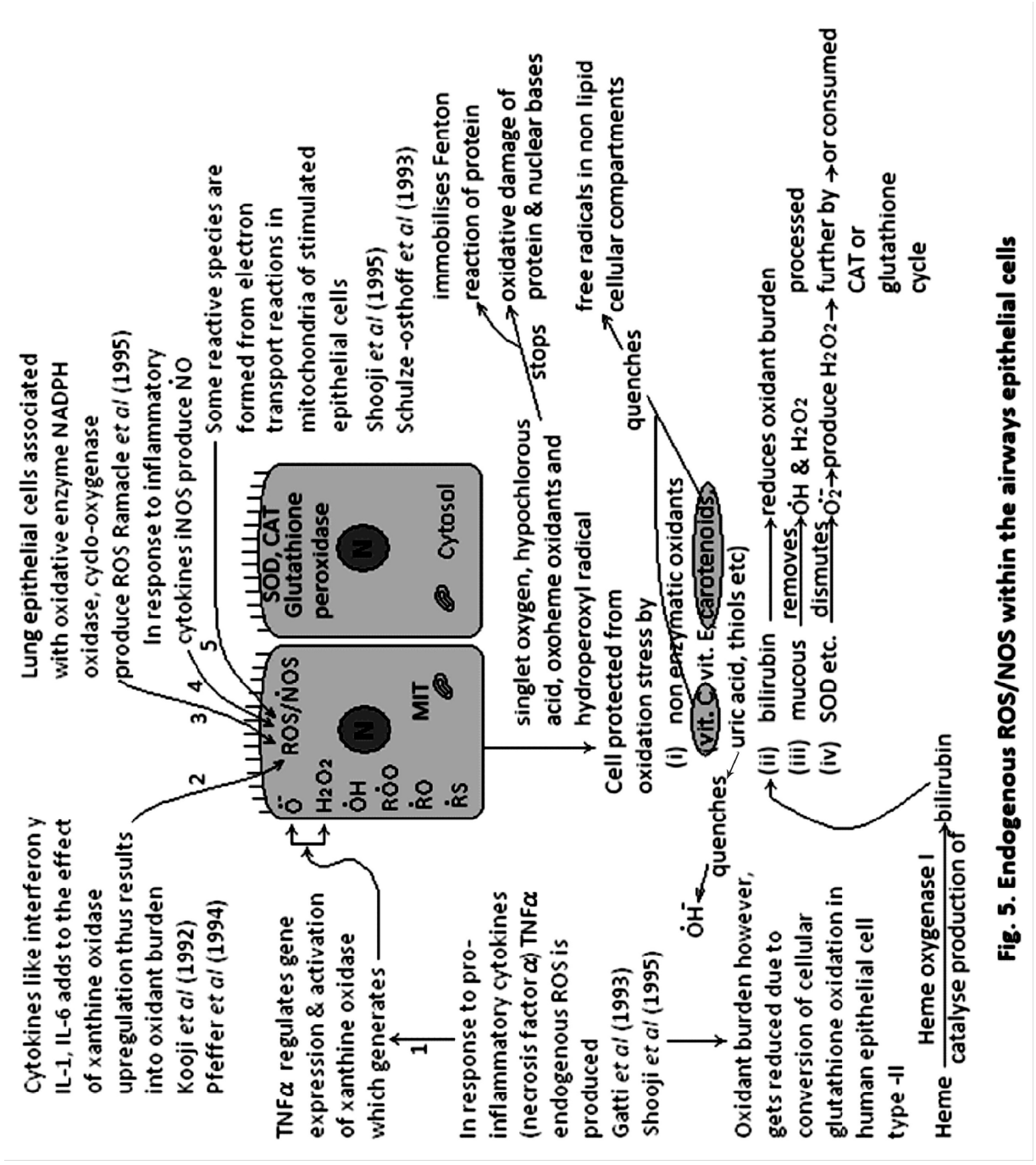



**Fig. 5. Endogenous ROS/NOS within the airways epithelial cells**

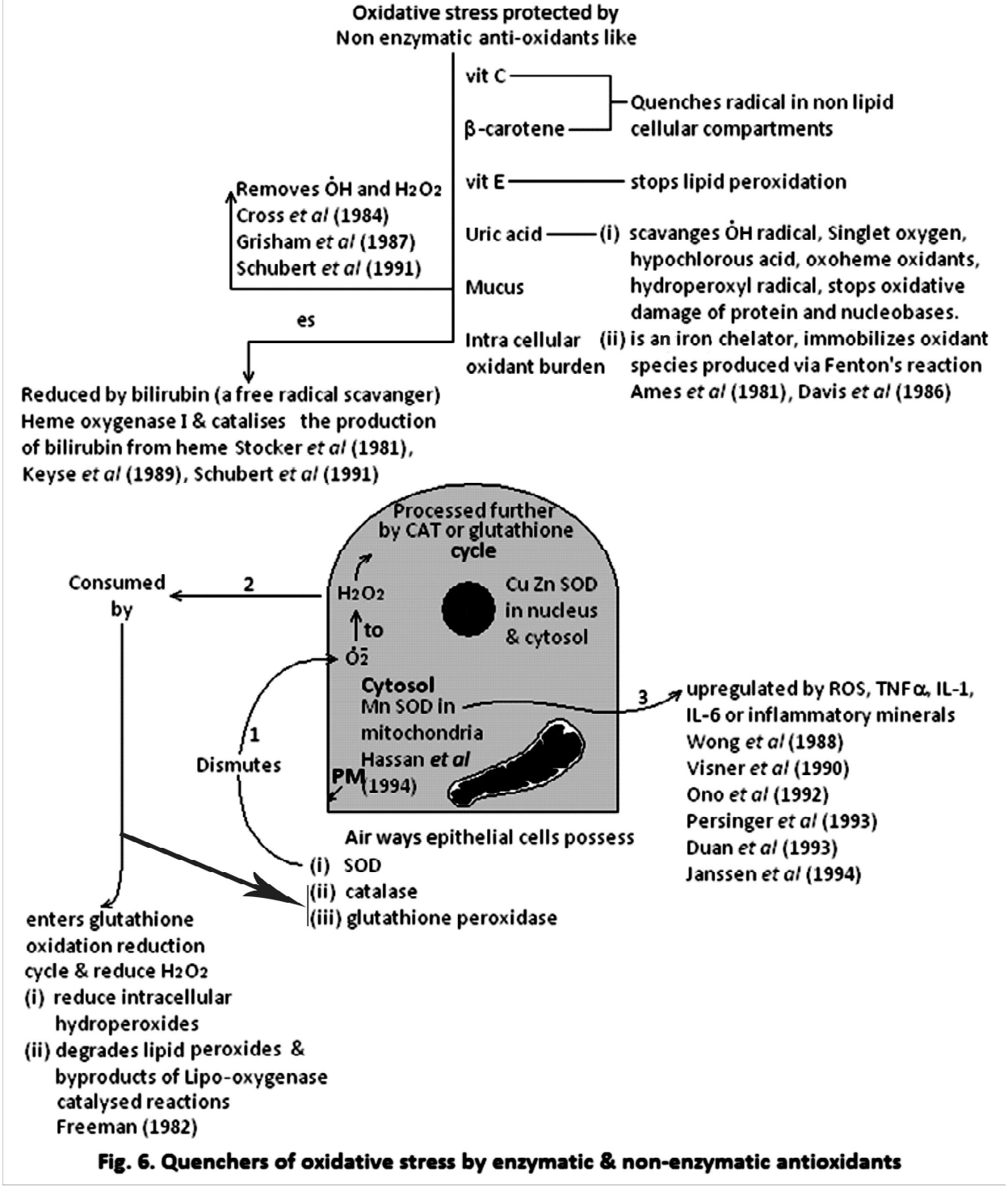



**Fig. 6. Quenchers of oxidative stress by enzymatic & non-enzymatic antioxidants**

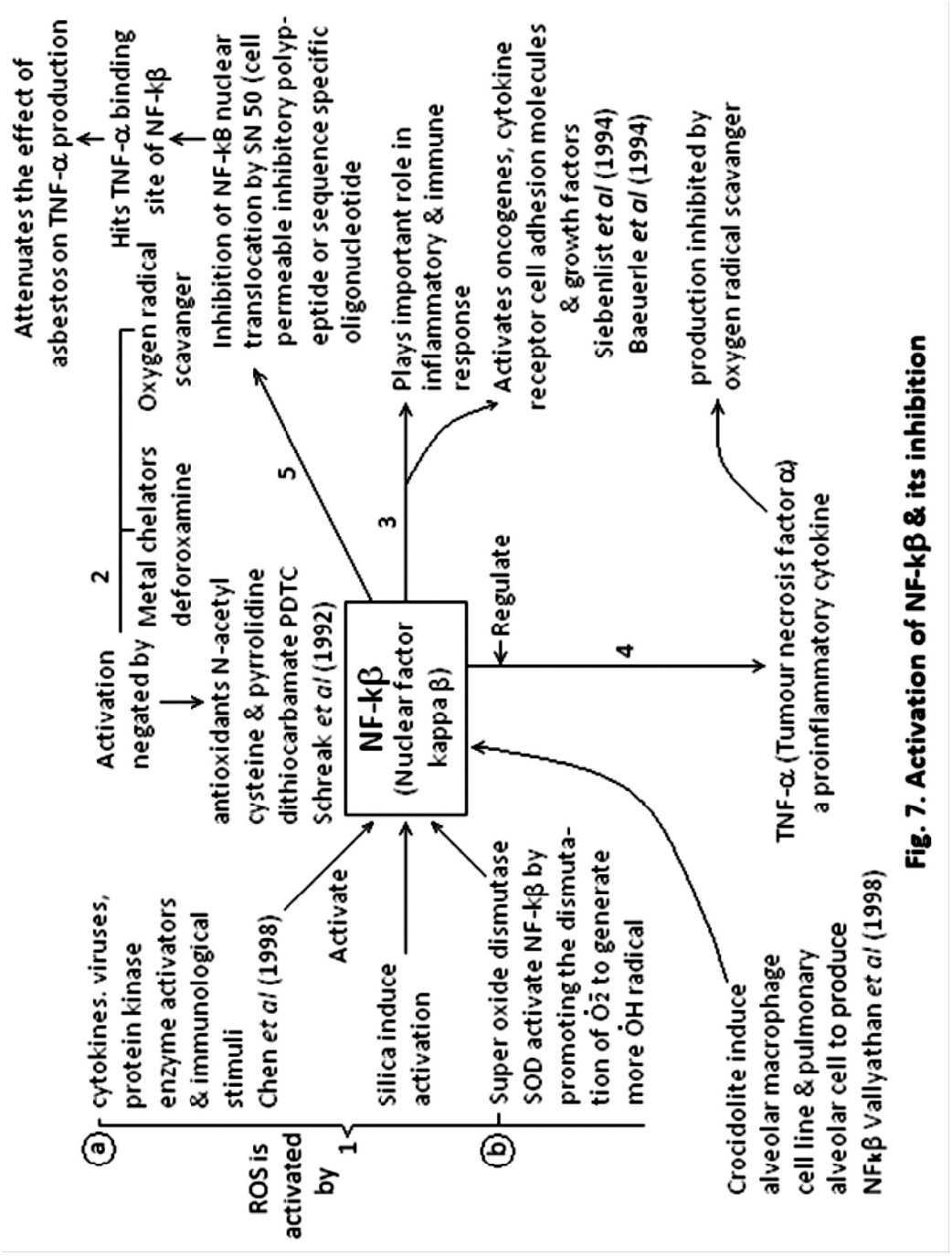



**Fig. 7. Activation of NF-kβ & its inhibition**

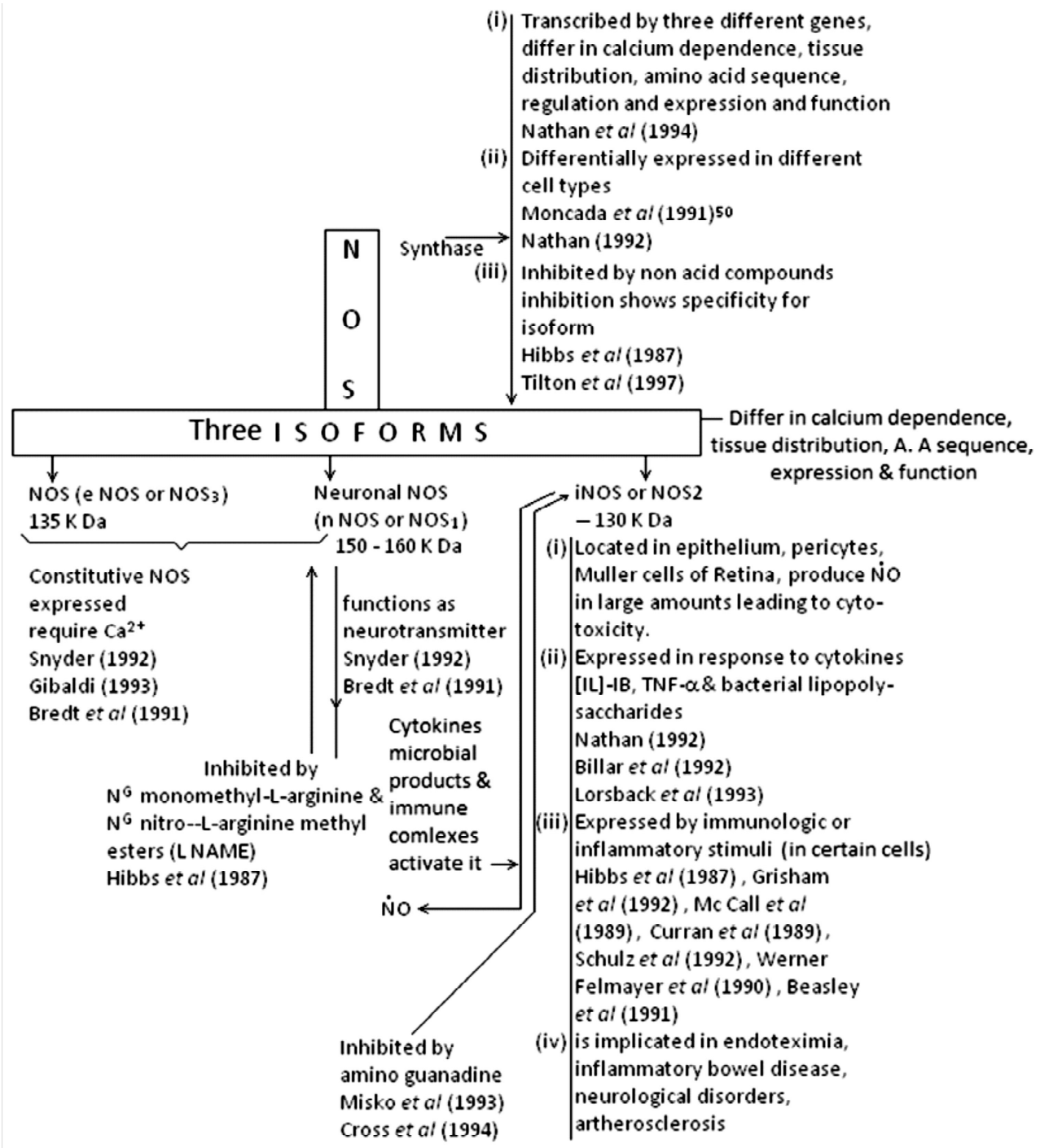



**Fig. 8. Isoforms of NOS / functions & inhibition**

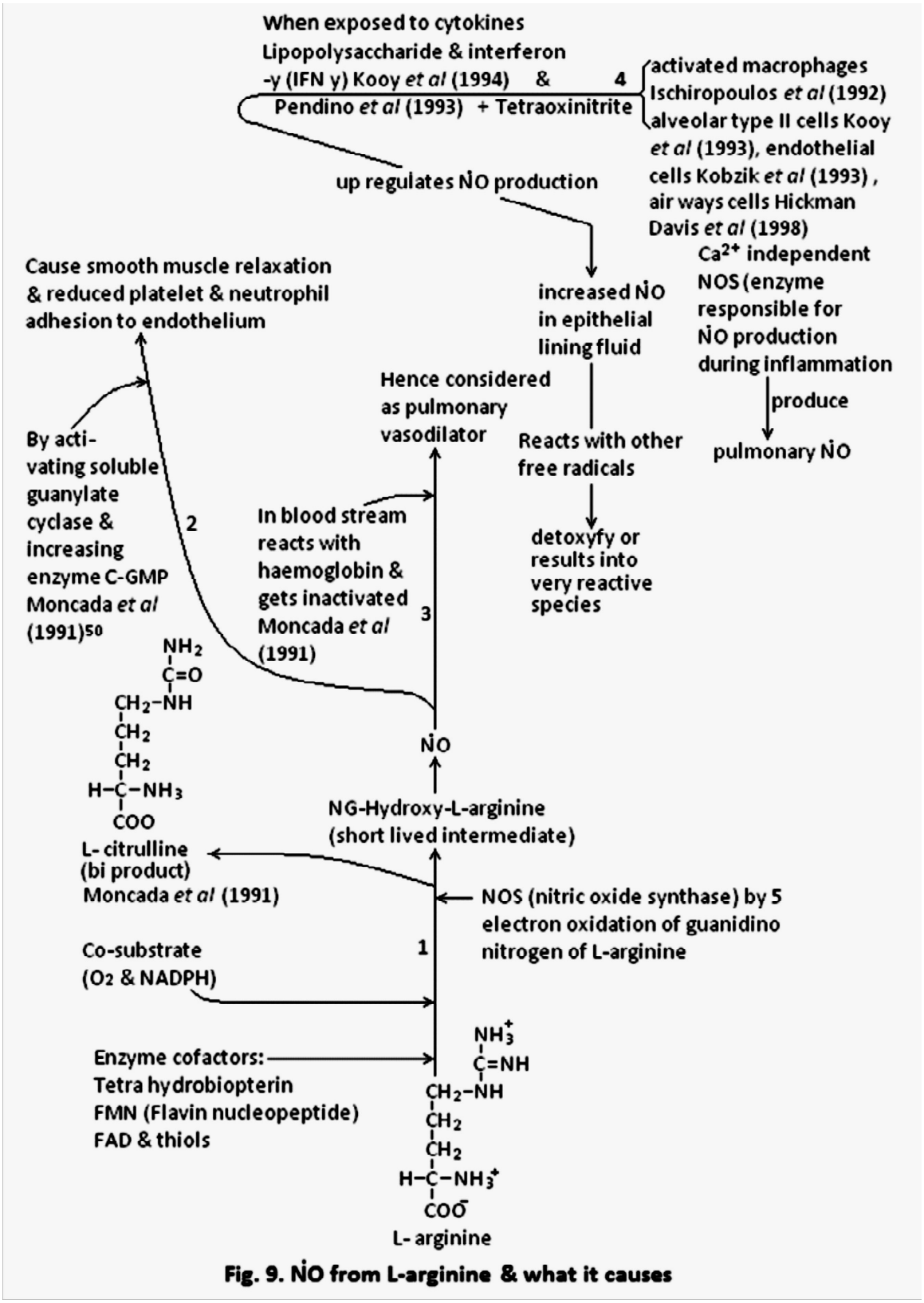



**Fig. 9. ṄO from L-arginine & what it causes**

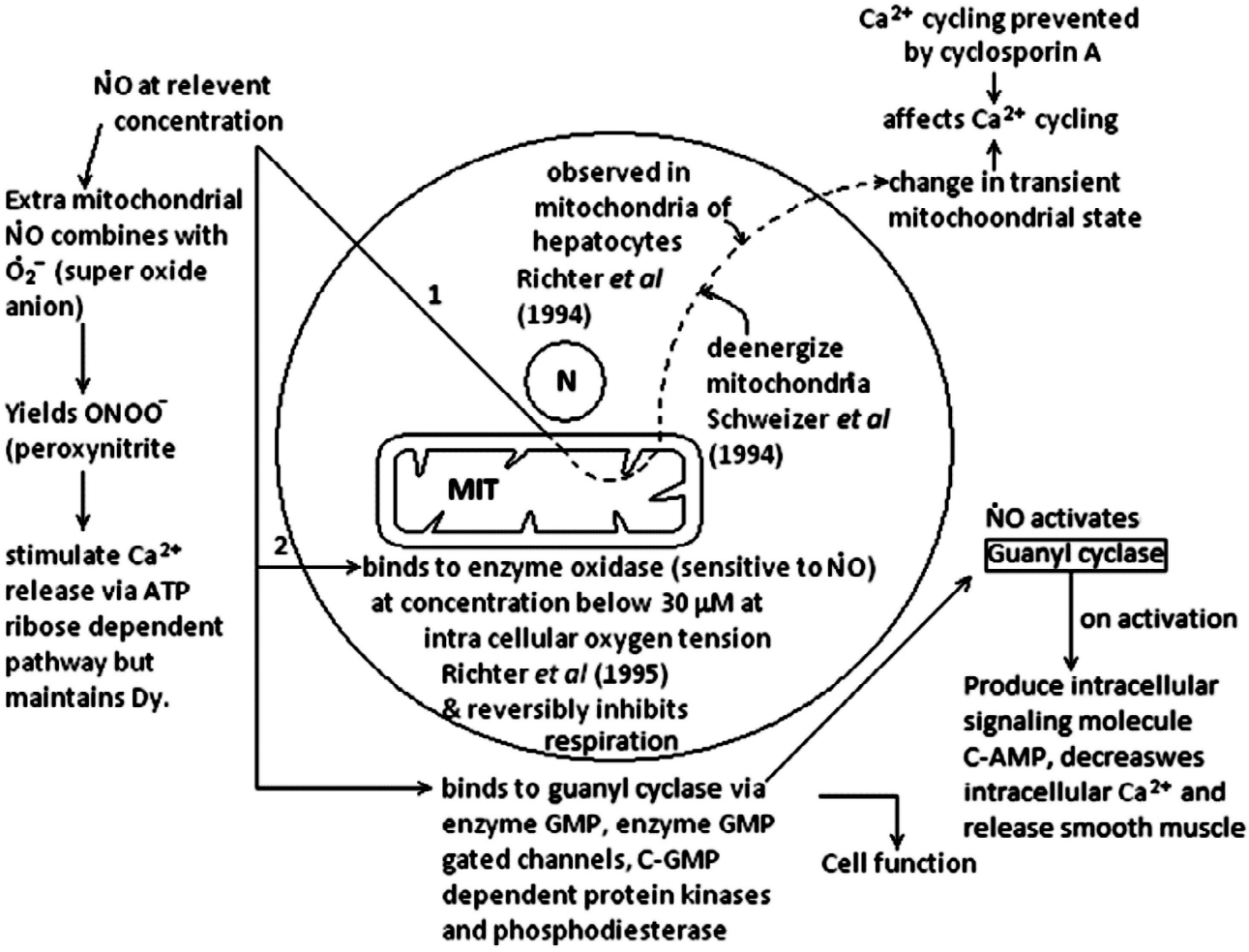
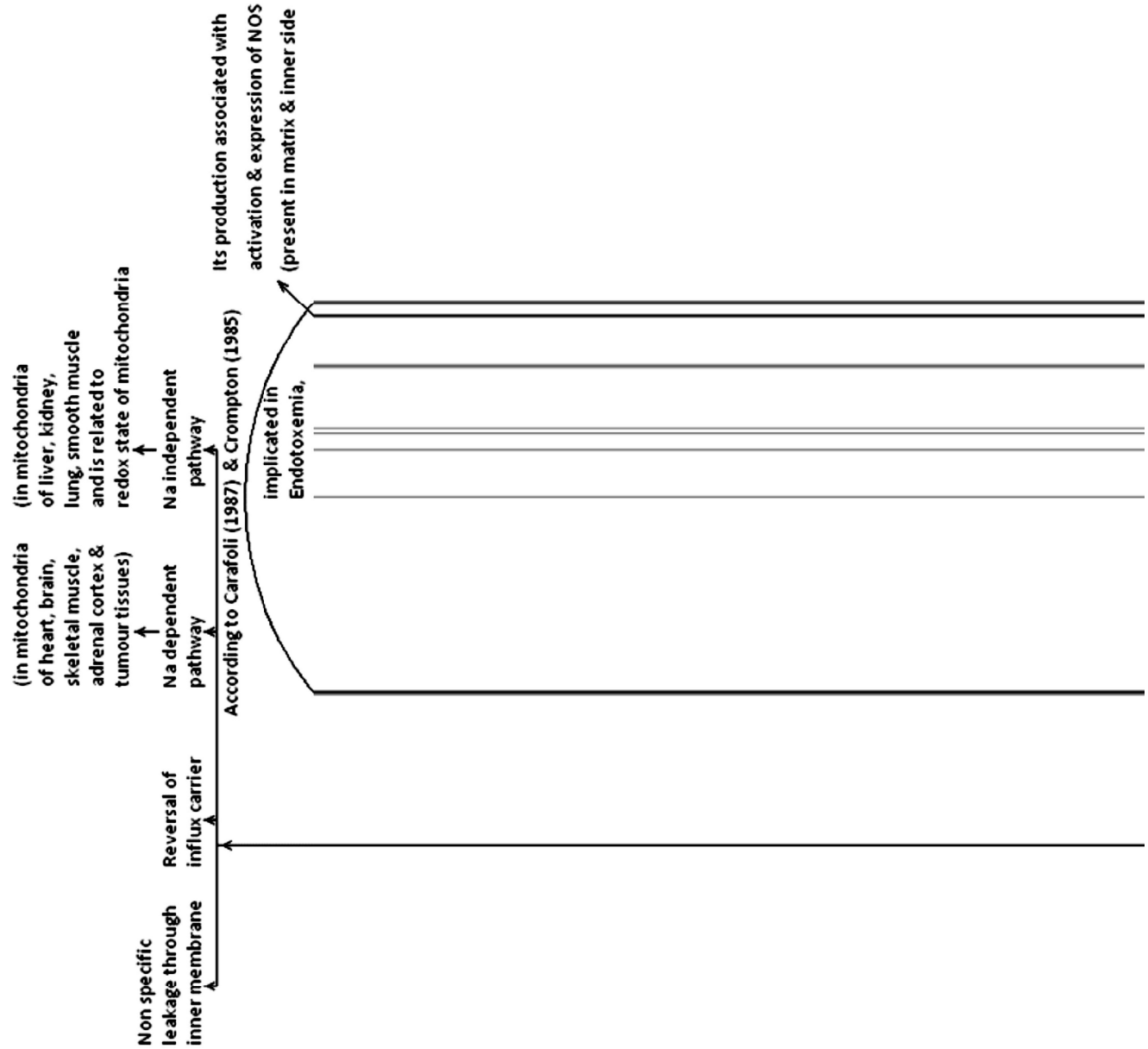



**Fig. 10. Deenergization of mitochondria & $Ca^{2+}$ cycling**


Non specific leakage through inner membrane
Reversal of influx carrier
(in mitochondria of heart, brain, skeletal muscle, adrenal cortex & tumour tissues)
Na dependent pathway
(in mitochondria of liver, kidney, lung, smooth muscle and is related to redox state of mitochondria
Na independent pathway
According to Carafoli (1987) & Crompton (1985)
implicated in Endotoxemia,
Its production associated with activation & expression of NOS (present in matrix & inner side

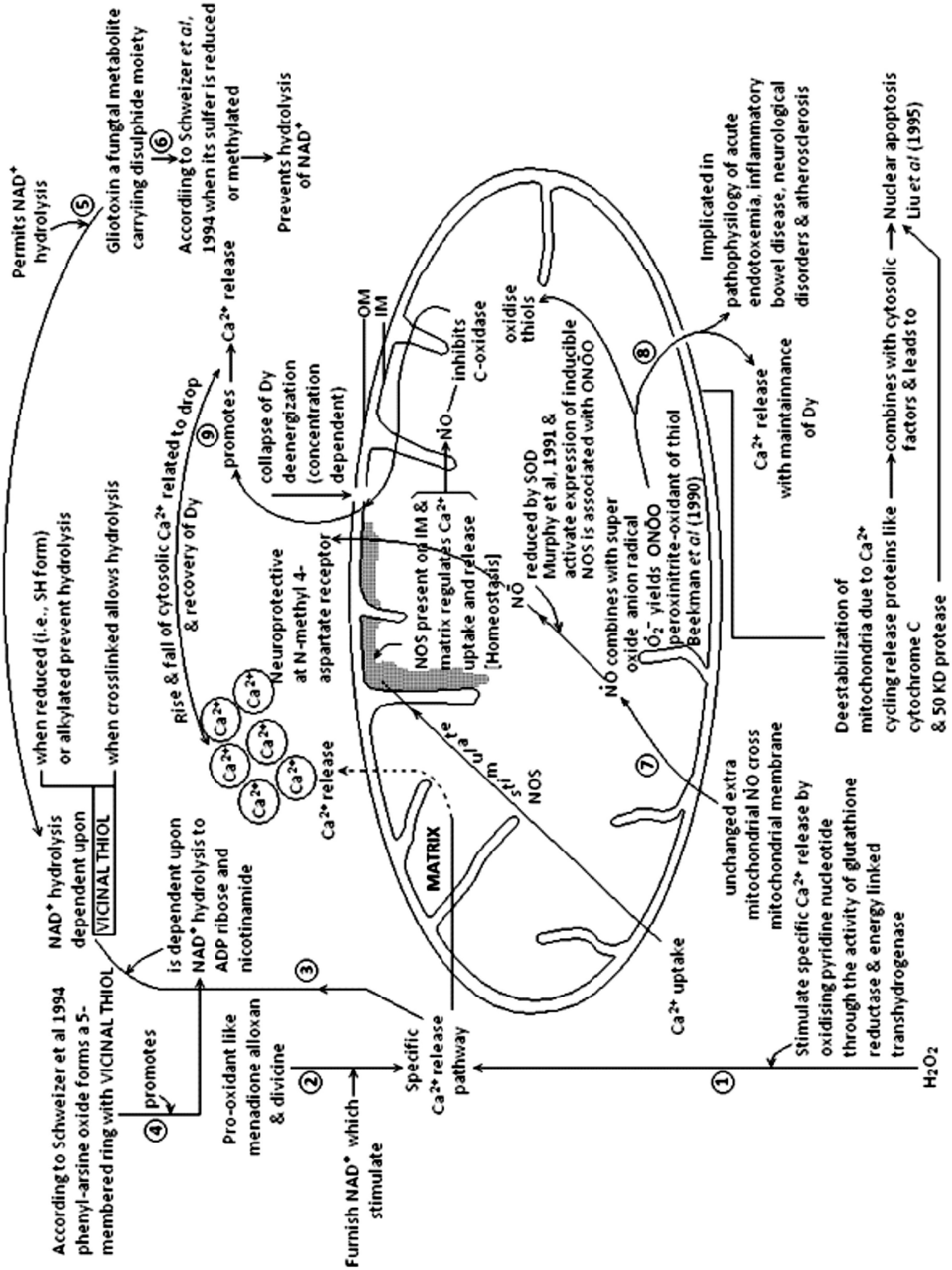



**Fig. 12. $Ca^{2+}$ release from mitochondria is under the control of $NAD^{+}$ hydrolysis which in turn is controlled by VICINAL THIOLS**

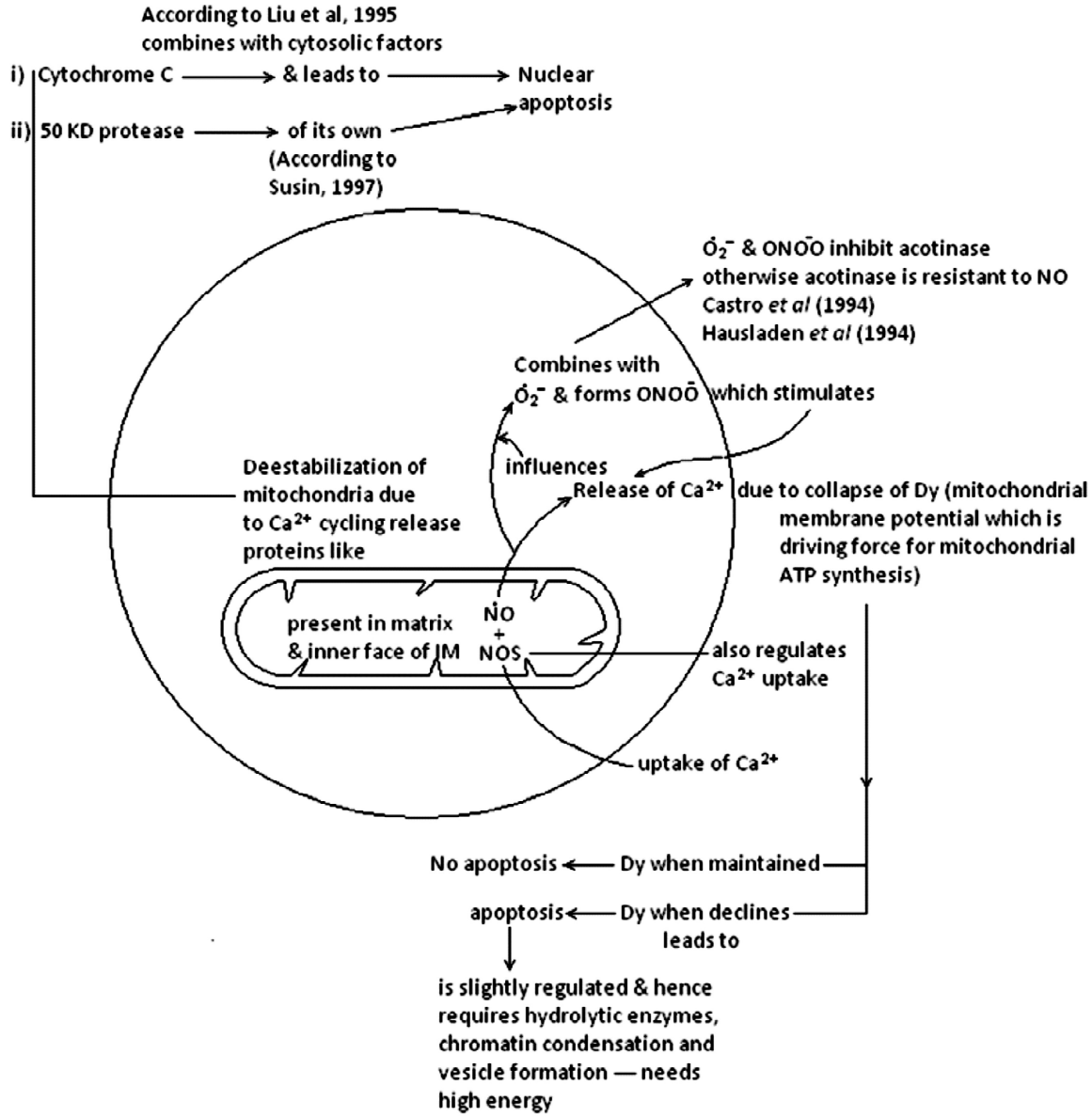



**Fig. 13. $Ca^{2+}$ uptake and consequences**

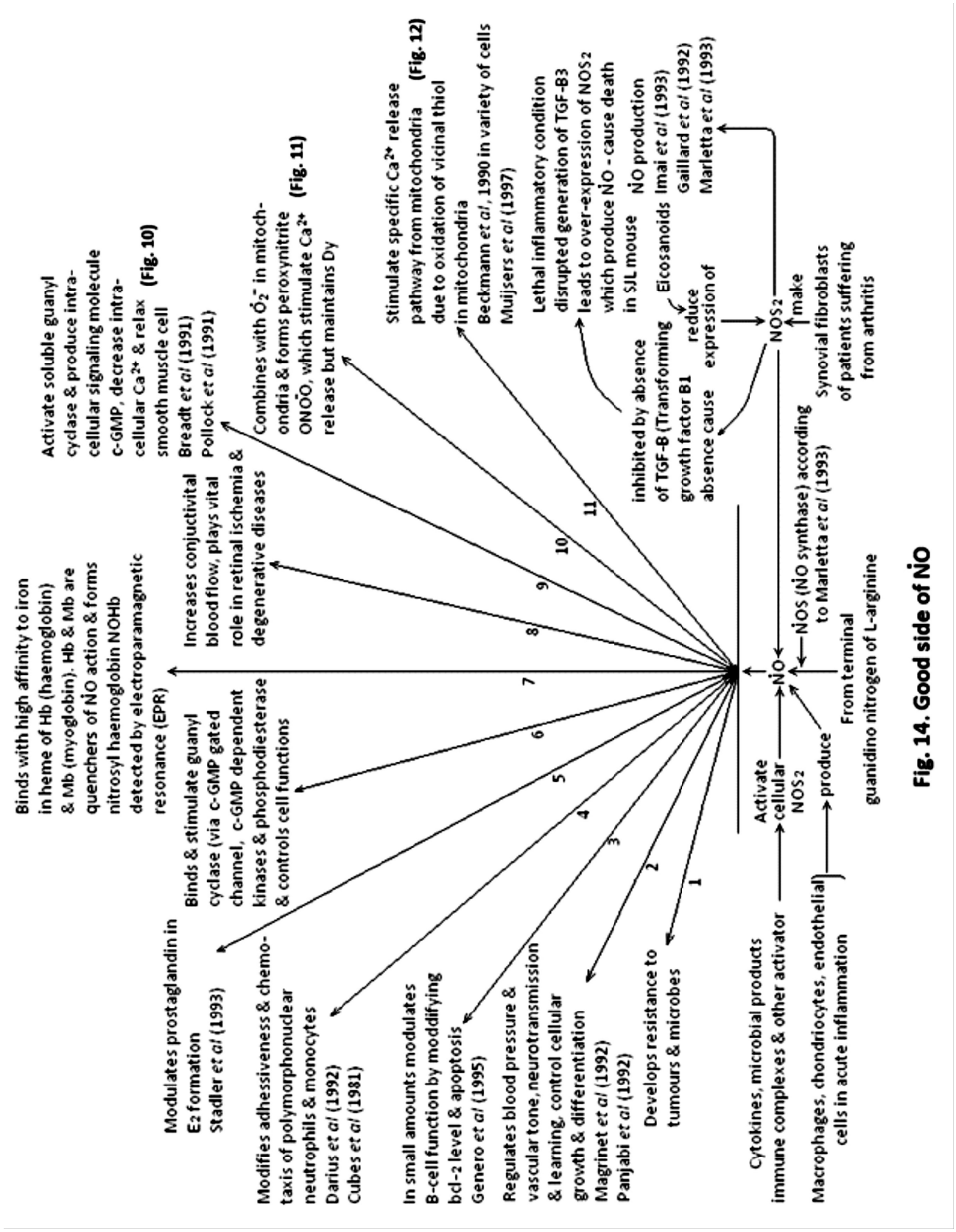



**Fig. 14. Good side of NO**

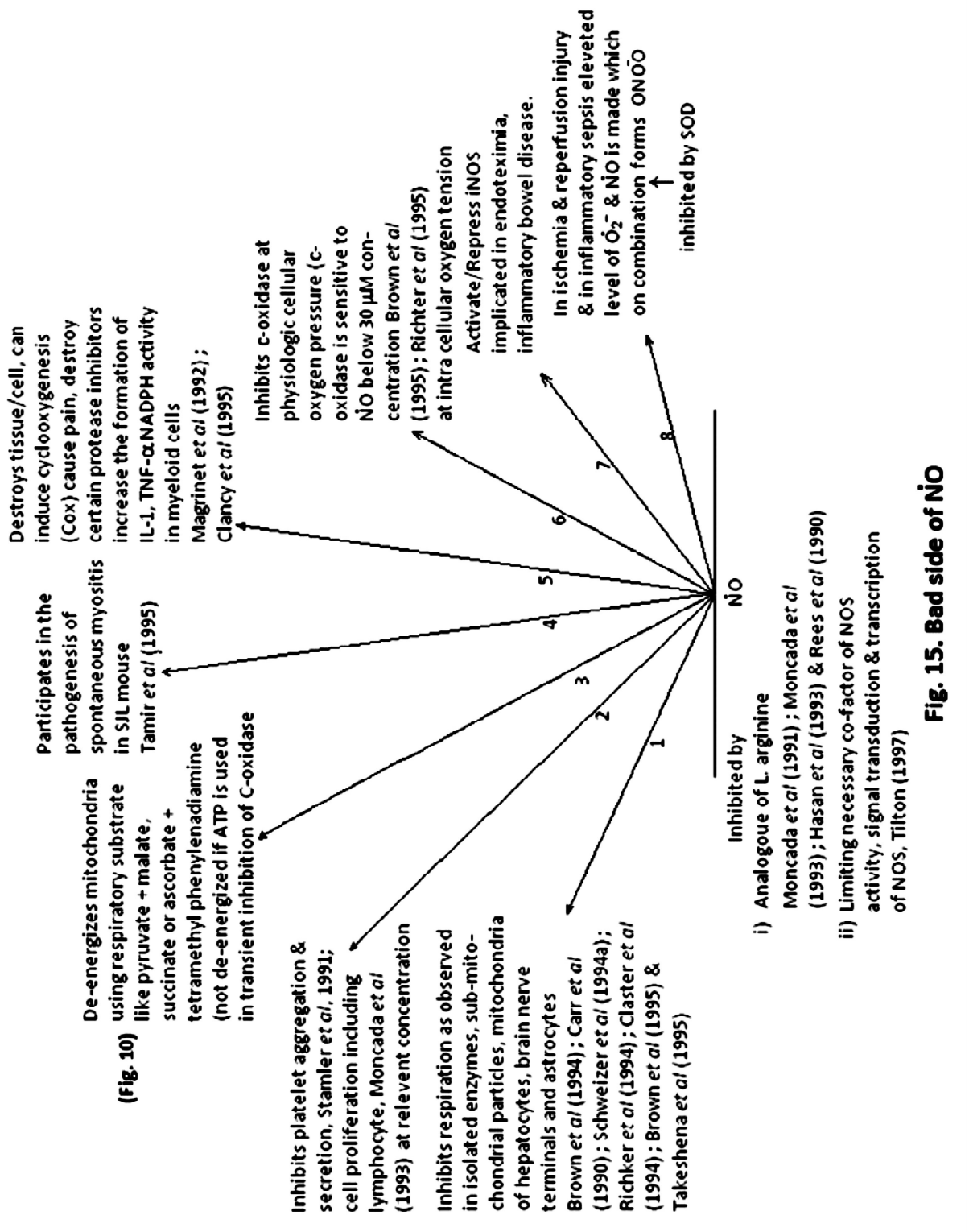



**Fig. 15. Bad side of ṄO**

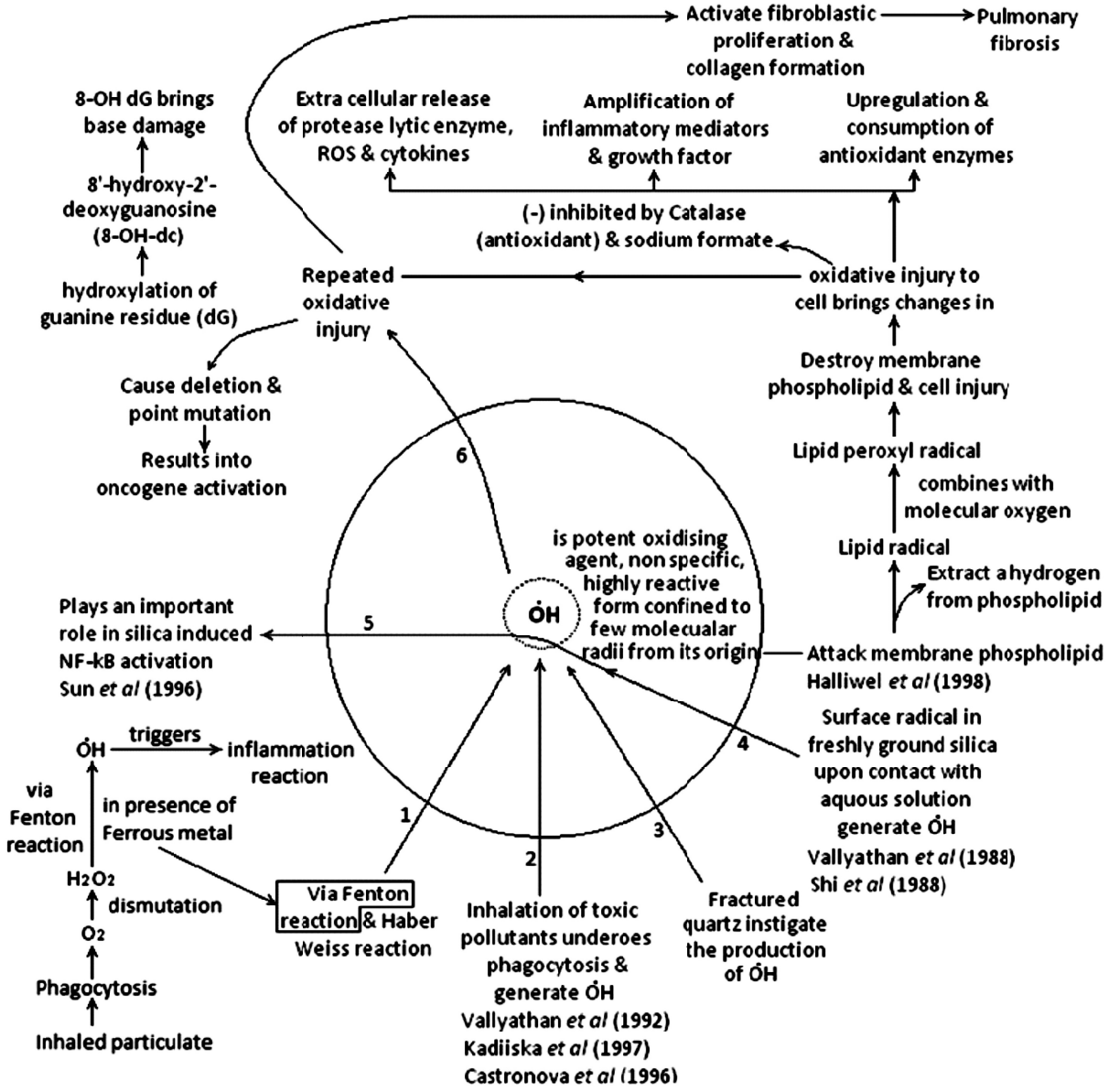



**Fig. 16. ȮH formation & Consequences**

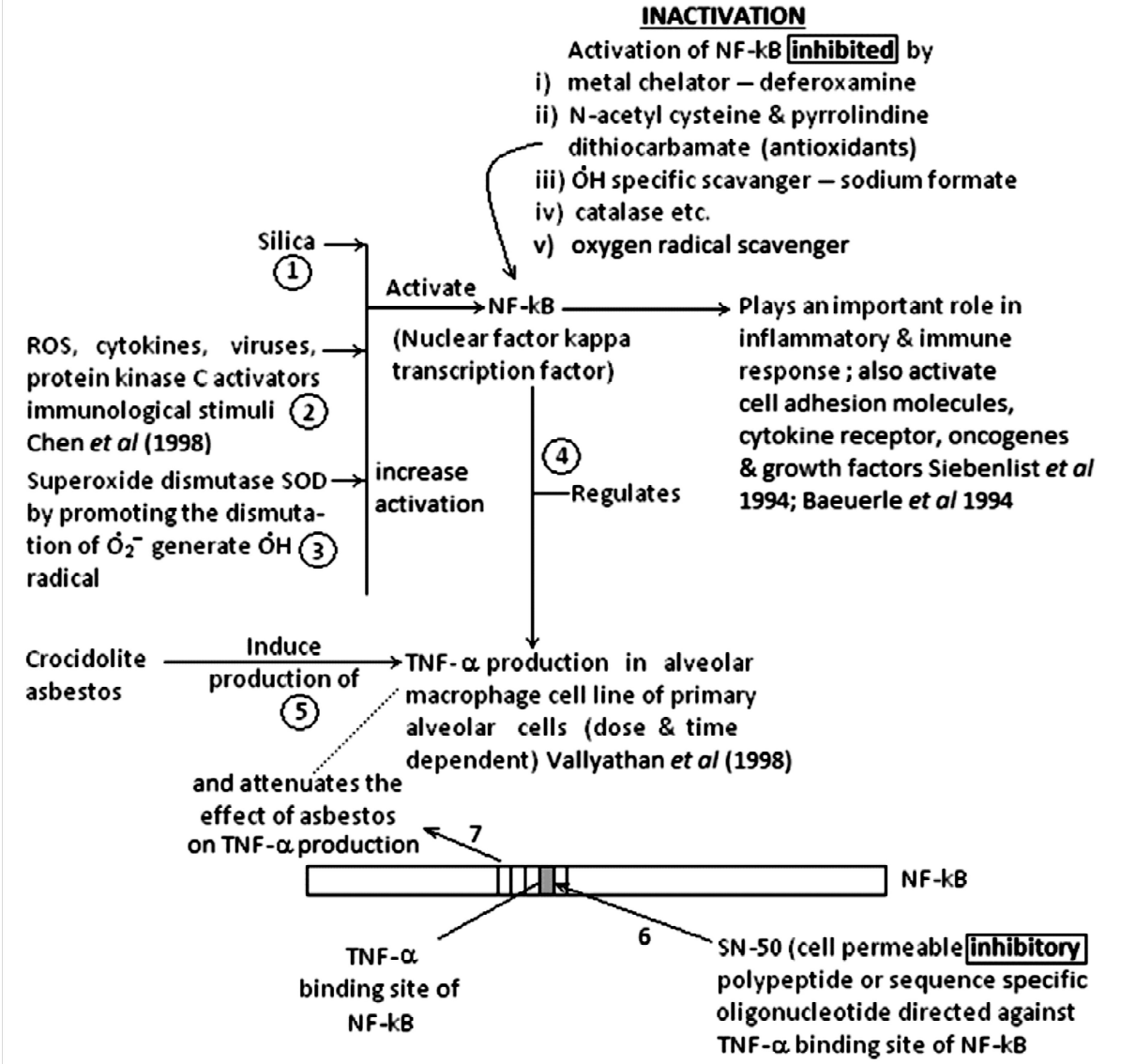



**Fig. 17. Activation & inactivation of NF kB**

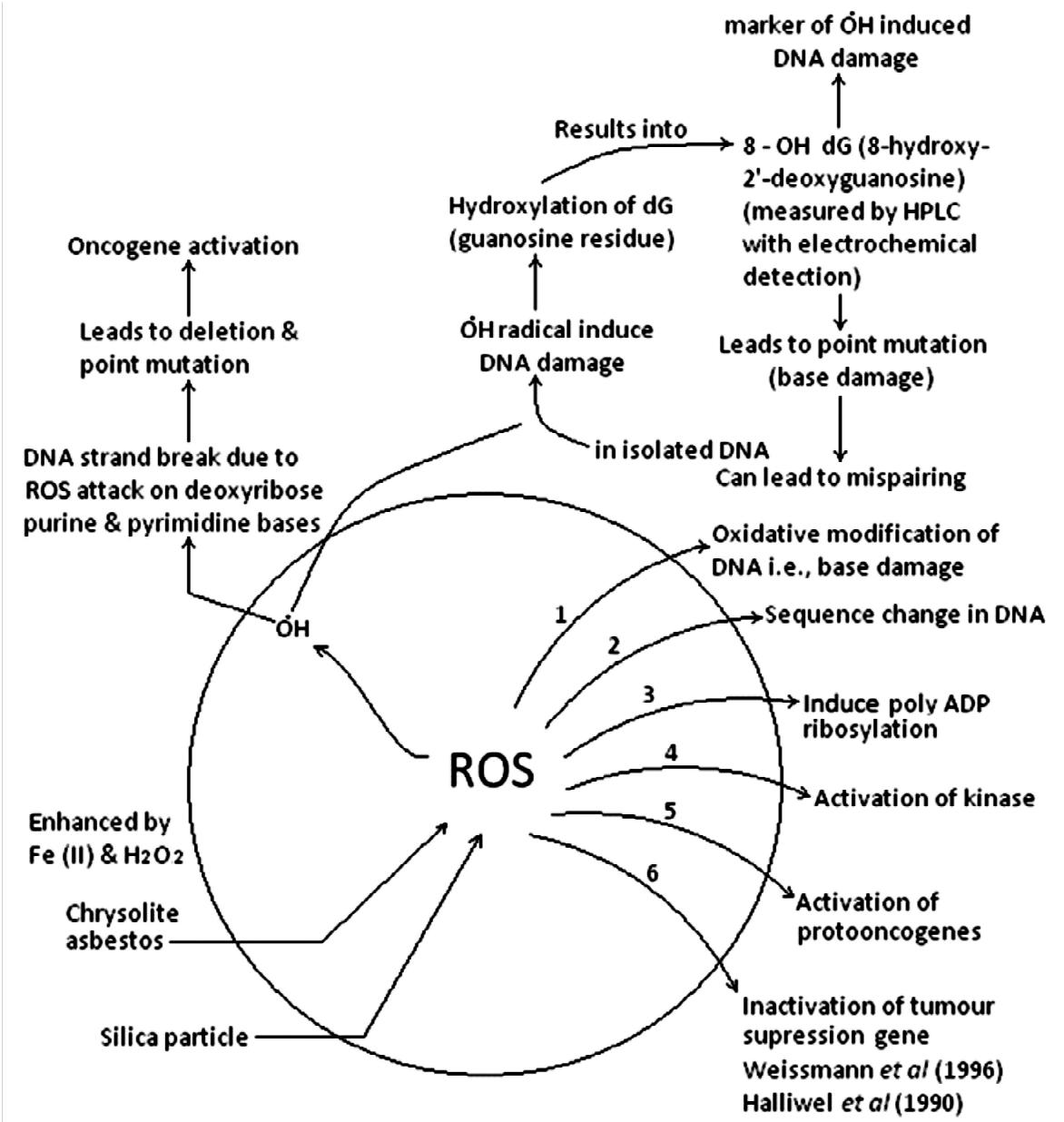



**Fig. 18. Consequences of ROS**

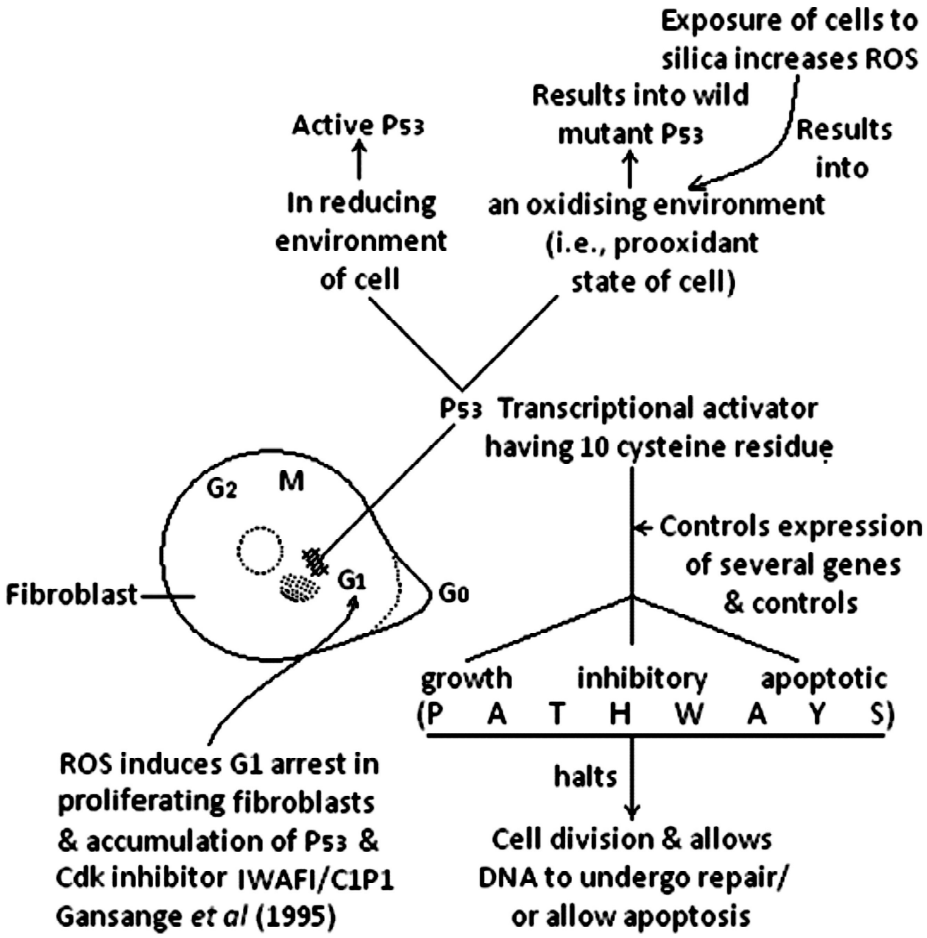



**Fig. 19. Mutant wild P53**

P53 - (Tumour supressor gene) is a transcriptional activator & controls:

(i) the expression of several genes which are responsible for growth inhibition and apoptic pathway

(ii) halts cell division and allows DNA repair

(iii) permits apoptosis of damaged cells

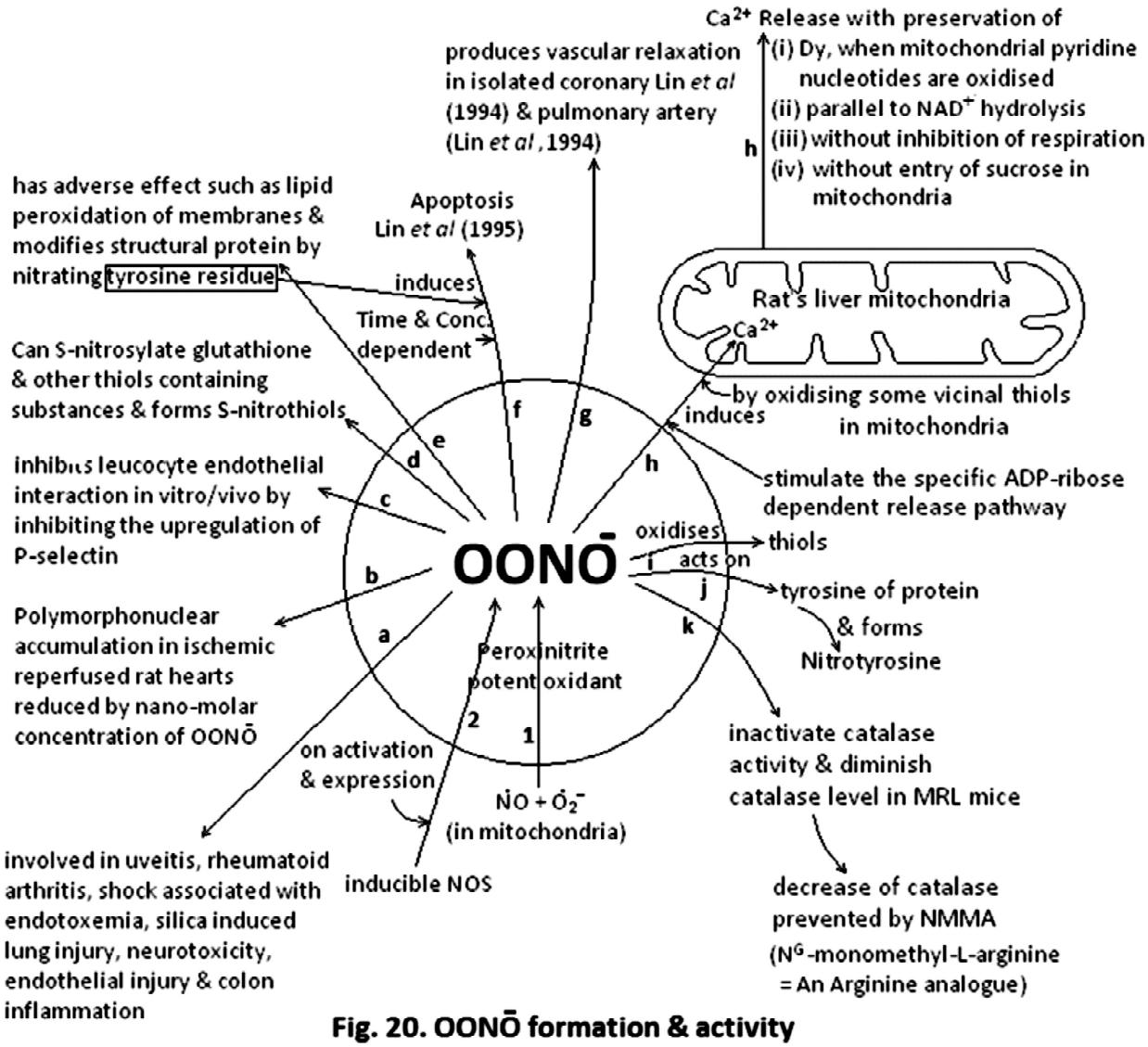



**Fig. 20. OONO⁻ formation & activity**

---

uveitis - inflammation of uveia
uveia - the iris, ciliary body and choroid together
arthritis - inflammation of joints
rheumatism - inflammation, metabolic derangement of connective tissue in joints attainded by pain

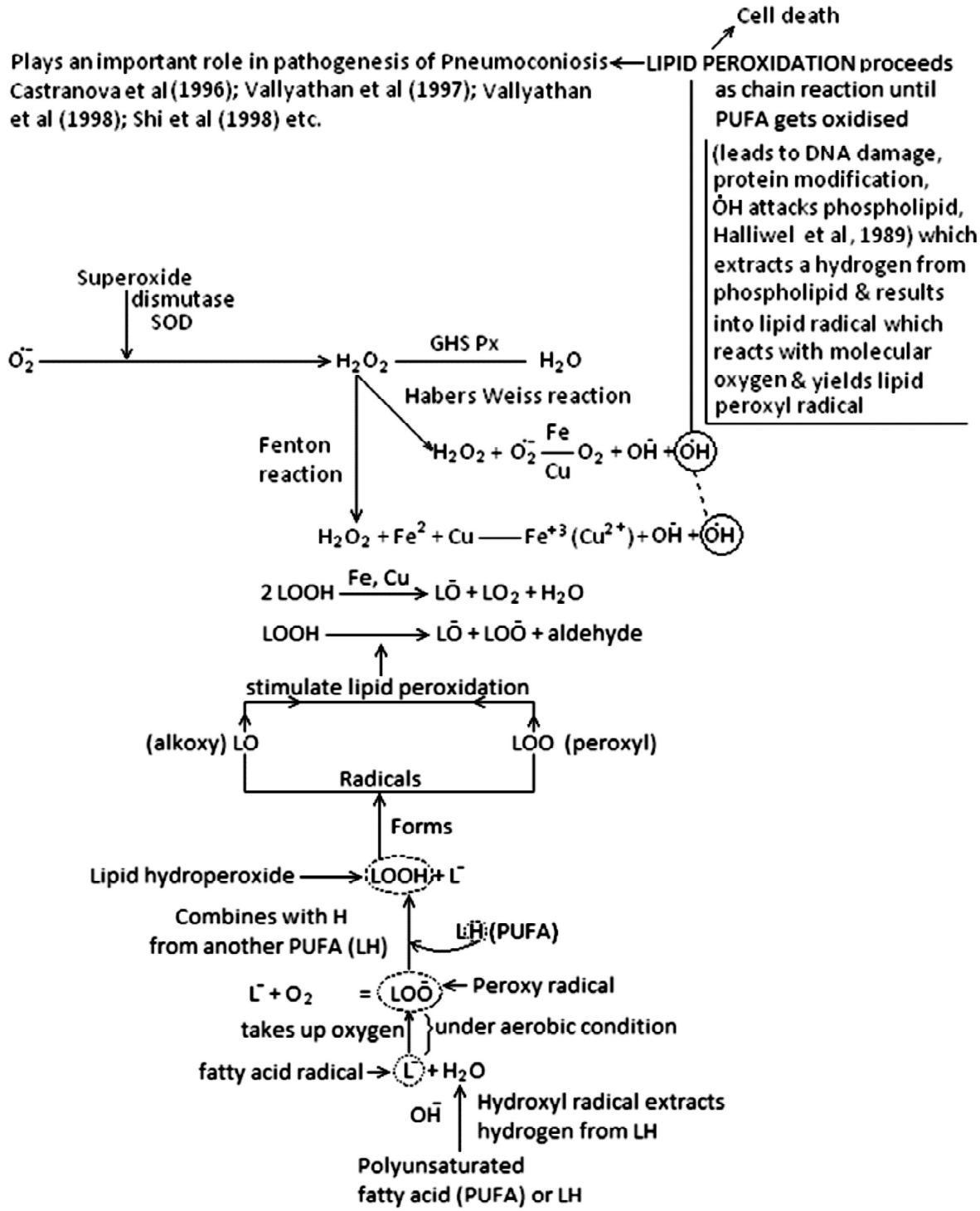



**Fig.21: Free radical - induced peroxidation of membrane lipid & Generation of ȮH via Haber Weiss reaction & Fenton reaction**

dermatological, diabetic condition, skeletal disorders, neoplastic diseases).

Scientists believe that before toxicity, ROS mediates signaling alteration and if changes in signaling is blocked by radical scavengers/antioxidants, toxicity could be blocked partially.

## REFERENCES

1. **Freeman, B.A. and J.D. Carpo. 1982**. Biology of disease free radicals and tissue injury. *Lab. Invest.* **47:** 412 – 426.

2. **Frie, B. 1994**. Reactive oxygen species and antioxidant vitamins: mechanism of action. *Am. J. Med.* **97:** 5 – 13.

3. **Halliwell, B. and J.M.C. Gutteridge. 1999**. Frèe radicals in Biology and Medicine. 3rd ed. New York Oxford University Press, 134 – 140.

4. **Krounhansen *et al* 1989**. Details not available.

5. **Mishra, H. and I. Fridovich. 1972**. The role of superoxide anion in the auto-oxidation of epinephrine simple Assay for superoxide dismutase. *J. Biol. Chem.* **247:** 3170.

6. **Cantin, A.M.; S.L. North; R.C. Hubbard and R.G. Crystal. 1987**. Normal alveolar epithelial lining fluid contains high level of glutathione. *J. Appl. Physiol.* **63:** 152 – 157.

7. **Oury, T.D.; L.Y. Chang; S.L. Marklund; B.J. Day and J.D. Crapo. 1994**. Immunocytochemical localization of extra-cellular superoxide dismutase in human lung. *Lab. Invest.* **70:** 889 – 898.

8. **Weinbroun, A.; V.G. Nielsen; S. Tan; S. Gelman; S. Matalon; K.A. Skinner; E. Bradley (Jr) and D.A.Parks. 1995**. Liver ischemia – reperfusion increases pulmonary permeability in rat: role of circulating xanthine oxidase. *Am. J. Physiol.* **268:** G 988 – G 996.

9. **Mattoli, S.; V.L. Mattose; M.A. Soloperto; L. Allegra and A. Fasoli. 1991**. Cellular and biochemical characteristics of broncholveolar lavage fluid in symptomatic non-allergic asthma. *J. Allergy. Clin. Immunol.* **87:** 794 – 802.

10. **Fulkerson, W.N. MacIntyre; J. Stamler and J. Carpo. 1996.** Pathogenesis and treatment of the adult respiratory distress syndrome. *Arch. Intern. Med.* **156:** 29 – 38.

11. **Sibille, Y. and H. Reynolds. 1990.** Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am. Rev. Res. Dis.* **141:** 471 – 501.

12. **Cross, C. and B. Halliwel. 1991**. Biological consequences of general environmental contaminants. In: The Lung Scientific Foundations (Crystal, R. West. J. Eds.) New York: Raven Press 1961 – 1973.

13. **Pritchard, R.; A. Ghio; J. Lehmann; D.W. Winsett; J.S. Topper; P. Parks; M.I. Gilmour; K.L. Drehr and D.L. Costa. 1996**. Oxidant generation and lung injury after particulate air pollutant exposure increase with the concentration of associated metals. *Inhal. Toxicol.* **8:** 457 – 477.

14. **Berg, I.; T. Schluter and G. Gercken. 1996**. Increase of bovine alveolar macrophage superoxide anion and hydrogen peroxide release by dusts of different origin. *J. Toxicol. Environ. Health.* **74:** 465 – 478.

15. **Vallyathan, V.; X. Shi; N.S. Dalal; W. Irr and V. Castranova. Gu. 1988**. Generation of free radicals from freshly fractured silica dust. *Annu. Rev. Respir. Dis.* **18:** 1213 – 1219.

16. **Brody, A. 1993**. Asbestos-induced lung disease. *Inviron. Health. Perspect.* **100:** 21 – 30.

17. **Gatti, S.; R. Laggioni; B. Echtenacher and P. Ghezzi. 1993**. Role of tumour necrosis factor and reactive oxygen intermediates in lipopolysacchride-induced pulmonary oedema and lethality. *Chi. Exp. Immumol.* **91:** 456 – 461.

18. **Shooji, Y.; Y. Uedono; H. Ishikua; N. Tekeyyama and T. Tanaka. 1995.** DNA damage induced by tumour necrosis factor – alpha in L 929 cells is mediated by mitochondrial oxygen radical formation. *Immunol.* **84:** 543 – 548.

19. **Kooji, A.; K. Bosch; W. Frederka and V. Noorden. 1992**. High levels of xanthine oxidoreductase in rat endothelial, epithelial and connective tissue cells. *Virchows Arch. B. Cell Path.* **62:** 143 – 150.

20. **Pfeffer, K.; T. Hueckstead and J. Hoidal. 1994**. Xanthenes dehydrogenase and xanthine oxidase activity and gene expression in renal epithelial cells: cytokine and steroid regulation. *J. Immunol.* **153:** 1789 – 1797.

21. **Rahman, I.; X. Li; K. Donaldson; D. Harrison and Mac Nee W. 1995**. Glutathione homeostasis in alveolar epithelial cells in vitro and lung in vivo

under oxidative stress. *Am. J. Physiol.* **269:** L 285 – L 292.

22. **Ramacle, J.; M. Raes; O. Moussaint, P. Renard and G. Rao. 1995**. Low level of reactive species as a modulator of cell function. *Mutat. Res.* **316:** 103 – 122.

23. **Schulze-osthoff, K.; A.C. Bakker; B. Vanhaesebroeck; R. Beyaert; W. Jacob and W. Fiers. 1992**. Cytotoxic activity of tumour necrosis factor is mediated by early damage of mitochondrial function. *J. Biol. Chem.* **267:** 5317 – 5323.

24. **Ames, B.; R. Cathcast; E. Schwiers and P. Hochstein. 1981**. Uric acid provides antioxidants defense in human against oxidant and radical caused aging and cancer: a hypothesis. *Proc. Natl. Acad. Sci. USA.* **78:** 6842 – 6862.

25. **Davis, K. and A. Seranian. 1986.** Uric acid – iron ion complexes. *Biochem. J.* **235:** 747.

26. **Cross, C.; B. Halliwel and A. Allen. 1984**. Uric acid iron ion complexes. *Biochem. J.* **235:** 747. ¾ 1984. Antioxidant protection: a function of tracheobranchial and gastro-intestinal mucous. *Lancet*. **1:** 1328 – 1329.

27. **Grisham, M.; C. Von Ritter; B. Smith; J. Lamont and D. Granger. 1987.** Interaction between oxygen radical and gastric mucin. *Am. J. Physiol.* **253:** G 93 – G 96.

28. **Stocker, R.; Y. Yamamoto; A. Mc Donagh; A. Glazer and B. Ames. 1987**. Bilirubin is an oxidant of possible physiological importance. *Science.* **235:** 1043 – 1046.

29. **Keyse, S. and R. Tyrrell. 1989**. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts UVA radiation, hydrogen peroxide and sodium arsenite. *Proc. Natl. Acad. Sci.* USA. **11: 787 – 791.**

30. **Schubert, J. and J. Wilmer. 1991**. Does hydrogen peroxide exist "free" in biological system? *Free. Radical. Biol. Med.* **11:** 545 – 555.

31. **Hassan, H. and J. Scandolios. 1990**. Superoxide dismutases in aerobic organisms. *New York: Wiley – Liss*. 175 – 199.

32. **Wong, G. and D. Goeddel. 1988**. Induction of manganese superoxide dismutase by tumour necrosis factor: possible protective mechanism. *Science.***242:** 941 – 944.

33. **Ono, M.; H. Khoda; T. Kawaguchi; M. Ohhira; C. Sekiya; M. Naomiki; A. Takeyasu and N. Taniguchi. 1992.** Induction of manganese superoxide dismutase by tumour necrosis factor, interleukin I and IL-6 in human hepatoma cells. *Biochem. Biophys. Res. Commun.* **182:** 1100 – 1107.

34. **Persinger, R. J. Marsh; Shull, S.; T.M. Janssen and B.T. Mossaman. 1993**. Differences in constitutive and inducible expression of antioxidant enzymes in rodent pleural mesothelial cells, tracheal epithelial cells and lung fibroblasts after exposure to hydrogen peroxide in vitro. *Am. Rev. Respir. Dis.* **147:** A 439.

35. **Duan, X.; K. Pinkerton and C. Plopper. 1993.** Increase of antioxidant enzyme in target site within the lung following long term ozone exposure. *Am. Rev. Respir. Dis.* **147:** A 441.

36. **Janessan, Y.M.W.; N.H. Heintz; J.P. Marsh; P.J.A. Borm and B.T. Mossman. 1994**. Induction of c-fos and c-jun protooncogenes in target cells of the lung and pleura by carcinogenic fibres. *Am. J. Cell. Mol. Biol.* **11:** 522 – 530.

37. **Visner, G.; W. Dougall; J. Wilson; I. Burr and H. Nick. 1990**. Regulation of manganese superoxide dismutase by lipopolysaccharide, interleukin I tumour necrosis factor. Role in the acute inflammatory response. *J. Biol. Chem.* **265:** 2856 – 2864.

38. **Perkin, R.C.; R.K. Schewle and A. Holian. 1991**. In vitro bioactivity of asbestos for human alveolar macrophage and its modification by Ig G. *Am. J. Cell. Mol. Biol.* **4:** 532 – 537.

39. **Kella, B.; R.F. Hamilton; R.K. Scheule and A. Holian. 1990.** Role extra-cellular calcium in chrysolite asbestos stimulation of alveolar macrophages. *Toxicol. Appl. Pharmecol.* **104:** 130 – 138.

40. **Chen, F.; Y. Lu; L.M. Demers; X. Shi; V. Vallyathan and V. Catronova. 1998**. Role of hydroxyl radical in silica induced NF-KB activation in macrophages. *Ann. Chin. Lab. Sci.* **28:** 1 – 13.

41. **Vallyathan. Val; Shi Xianglin and V. Castronova. 1998.** Reactive oxygen species: Their relation to Pneumoconiosis and carcinogenesis. *Environ. Health. Perspect.* **106 (supplem 5):** 1151 – 1155.

42. Sibenlist, U.; G. Franzoso and K. Brown. 1994.

Structure, regulation and function of NF-KB. *Annu. Cell. Biol.* **10:** 405 – 455.

43. **Baeurele, P.A. and T. Henkel. 1994.** Function and activation of NF-KB in the immune system. *Annu. Rev. Immunol.* **12:** 141 – 179.

44. **Shreak, R.; B. Meier; D.N. Mannel; W. Droge and P.A. Baeuerle, P. 1992.** Dithiocabamaate as potent inhibitor of nuclear factor kB activation in intact cell. *J. Exp. Med.* **175:** 1181 – 1194.

45. **Weismann, H. and B. Halliwell. 1996.** Damage to DNA by reactive oxygen species and nitrogen species: role in inflammatory diseases and progression of cancer. *Biochem. J.* **313:** 17 – 29.

46. **Halliwell, B. and J.M.C. Gutteridge. 1990.** Role of free radicals and catalytic metal ions in human disease: an overview. *Methods. Enzymol.* **186:** 1 – 85.

47. **Marletta, M.A. 1993**. Nitric oxide synthase structure and mechanism. *J. Biol. Chem.* **268:** 12231 – 12234.

48. **Moncada, S.; R.M. Palmer and A. Higgs.1991**. nitric oxide physiology, pathophysiology and pharmacology. *Pharmacol. Rev.* **43:** 109 – 142.

49. **Nathan, C. 1992**. Nitric oxide as a secretary product of mammalian cells. *FASEBJ.* **6:** 3051 – 3064.

50. **Steuhar, D.J.; H.J. Cho; N.S. Kwon; F. Weise and C.F. Nathan. 1991**. Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD and FMN – containing flavoprotein. *Proc. Natl. Acad. Sci. USA.* **88:** 7773 – 7777.

51. **Nathan, C. and Xie, Q.W. 1994.** Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.* **269:** 13725 – 13728.

52. **Snyder, S.H. 1992.** Nitric oxide: first in a new class of neurotransmitters. *Science.* **257:** 494 – 496.

53. **Gibaldi, M.1993.** What is nitric oxide and why are so many people studying it? *J. Clin. Pharmacol.* **33:** 488 – 496.

54. **Bredt, D.S.; P.M. Hwang; C.E. Glatt; C. Lowenstein; R.R. Read and S.H. Snyder. 1991**. Cloned and expressed nitric oxide synthase structure resembles cytochrome P-450 reductase. *Nature.* **351:** 714 – 718.

55. **Lorsback, R.B.; W.J. Murphy; C.J. Lowenstein; S.H. Snyder and S.W. Russell. 1993.** Expression of the nitric oxide synthase gene in mouse macrophages activated for tumour cell killing. *J. Biol. Chem.* **268:** 1908 – 1913.

56. **Billar, J.M.; R.A. Hofman; R.D. Curran; J.M. Langrehe and R.L. Simmons. 1992**. A role for inducible nitric oxide biosynthesis in liver in inflammation and in the allogeneic immune response. *J. Lab. Clin. Med.* **120:** 192 – 197.

57. **Hibbs, J.B. Jr.; R.R. Taintor and Z. Vavrin. 1987**. Macrophage toxicity, role of L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science.* **235:** 473 – 476.

58. **Grisham, M.B. and T. Yamada. 1992.** Neutrophils, nitrogen oxide and inflammatory bowel disease. *N.Y. Acad. Sci.* **664:** 103 – 115.

59. **Mc Call, T.B.; N.K. Boughton – Smith; R.M.J. Palmer; B.J.R. Whittle and S. Moncada. 1989**. Synthesis of nitric oxide from L-arginine by neutrophils. *Biochem. J.* **261:** 293 – 296.

60. **Curran, R.D.; T.R. Billar; D.J. Steuhr; K. Hofman and R.L. Simmons. 1989.** Hepatocytes produce nitrogen oxides from L-arginine in response to inflammatory products from Kupffer cells. *J. Exp. Med.* **170:** 1769 – 1774.

61. **Schulz, R.; E. Nava and S. Moncada. 1992**. Induction and possible biological relevance of a $Ca^{2+}$-independent nitric oxide synthase in the myocardium. *Br. J. Pharmacol.* **105:** 577 – 580.

62. **Werner – Felmayer, G.; E.R. Werner; D. Fuchs; A. Hausen; G. Reinegger and O. Trenz. 1990**. Tetrahydrobiopterin-dependent formation of nitrite and nitrate I murine fibroblasts. *J. Exp. Med.* **172:** 1599 – 1607.

63. **Bensley, D.; J.H. Schwartz and B.M. Brenner. 1991**. Interleukin – 1 induces prolonged L-arginine dependent cycle guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. *J. Clin. Invest.* **87:** 602 – 608.

64. **Imai, Y.; H. Kolb; and V. Burkart. 1993**. Nitric oxide production from macrophages is regulated by arachidonic acid metabolites. *Biochem. Biophys. Res. Commune.* **197: 105 – 109.**

65. **Gaillard, T.; A. Mulsch; H. Klein and K. Decker. 1992**. Regulation of Prostaglandin $E_2$ of cytokine-elicited nitric oxide synthesis in rat liver

macrophages. *Boil. Chem. Hoppe-seyler.* **373:** 897 – 902.

66. **Genero, A.M.; S. Hortelano; A. Alvarez; C. Martineza and L. Bosca. 1995**. Spleenic b-lymphocyte programmed cell death is prevented by nitric oxide release through mechanisms involving sustained Bch-2 level. *J. Cli. Invest.* **95:** 1884 – 1890.

67. **Darius, H.; L. Grodzinsca and J. Meyer. 1992**. The effect of nitric oxide donors molsidomine and SIN-1 on human polymorphonuclear leukocyte function in vitro and vivo. *Eur. J. Clin. Pharmacol.* **43:** 629 – 633.

68. **Kubes, P.; M. Suzuki and D.N. Granger. 1991**. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. USA.* **88:** 4651 – 4655.

69. **Stadler, J.; B.G. Harbrecht; M. Disilvio; R.D. Curran; M.L. Jordon; R.L. Simmons and T.R. Billar. 1993.** Endogenous nitric oxide inhibits the synthesis of cyclooxigenase products and interleukin-6 by rat Kupffer cells. *J. Leukoc. Biol.* **53:** 165 – 172.

70. **Magrinet, G.; S.N. Mason; P.J. Shami and J.B. Weinberg. 1992.** Nitric oxide modulation of human leukemia cell differentiation and gene expression. *Blood.* **80:** 1880 – 1884.

71. **Panjabi, C.J.; D.L. Laskin; D.E. Heck and J.D. Laskin. 1992**. Production of nitric oxide by murine bone marrow cells. Inverse correlation with cellular proliferation. *J. Immunol.* **149:** 2179.

72. **Pollock, J.S.; J.A. Forstermann; T.D. Mitchell; H.H.H.W. Warner; H.H. Schmidt; M. Nakane and M. Murad. 1991.** Purification of a soluble isoform of guanylyl cyclase-activating factor synthase. *Proc. Natl. Acad. Sci. USA.* **88:** 10480 – 10484.

73. **Beckmann, J.S.; T.W. Beckmann; J. Chen; P.A. Marshall and B.A. Freman. 1990**. Apparent hydroxyl radical production by peroxinitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA.* **87:** 1620 – 1624.

74. **Muijsers, R.B.; G. Folkerts; P.A. Henricas; G. Hashjin Sadeghi and F.P. Nijkamp. 1997**. Peroxinitrite, a two faced metabolite of nitric oxide. *Life. Sci.* **60:** 1833 – 1845.

75. **Brown, G.C. and C.E. Cooper. 1994**. Nanomolar concentration of nitric oxide reversely inhibits synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett.* **356:** 295 – 298.

76. **Carr, G.J. and S.J. Ferguson. 1990.** Nitric oxide formed by nitrite reductase of *Paracoccus denitrificans* is sufficiently stable to inhibit cytochrome oxidase activity and is reduced by its reductase under aerobic conditions. *Biochem. Biophys. Acta.* **1017:** 57 – 62.

77. **Schweizer, M. and C. Richter. 1994a**. nitric oxide potently and reversibly de-energizes mitochondria at low oxygen tension. *Biochem. Biophys. Res. Commun.* **204:** 169 – 175.

78. **Richter, C.; V. Govadze; R. Laffranchi; R. Schlapbact; M. Schweizer; M. Suter; P. Walter and M. Yaffee. 1995.** Oxidants in mitochondria: from physiology to diseases. *Biochem. Biophys. Acta.* **1271:** 67 – 74.

79. **Cleeter, M.W.J.; J.M. Cooper; V.M. Darley-Usmar; S. Moncada and A.H.U. Schpira. 1994.** Reversible inhibition of cytochrome oxidase, the terminal enzyme of mitochondrial respiratory chain by nitric oxide. Implications for neurogenerative diseases. *FEBS. Lett.* **345**: **50 – 54.**

80. **Brown, G.C.; J.P. Bolanos; S.J.R. Heales and J.B. Clark. 1995.** Nitric oxide produced by activated astrocytes rapidly and reversibly inhibits cellular respiration. *Neurosci. Lett.* **193:** 102 – 204.

81. **Takehara, Y.; T. Kanno; T. Yoshioka; M. Inoue and K. Utsumi. 1995.** Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide. *Arch. Biochem. Biophys.* **323:** 27 – 32.

82. **Stamler, J.S. and J. Loscalzo. 1991.** The antiplatelet effects of organic nitrates and related nitroso compounds in vitro and in vivo and their relevance to cardiovascular disorders. *J. Am. Coll. Cardiol.* **18:** 1529 – 1536.

83. **Moncada, S. and A. Higgs. 1993**. The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* **329:** 2002 – 2012.

84. **Tamir, S.; T. Derojaswalker; A. Gal; A.H. Weller; X.T. Li; J.G. Fox; G.N. Wogan and S.R. Tannenbaum. XXXX.** Nitric oxide production in

relation to spontaneous â-cell lymphoma and myositis in SJL mice. *Cancer. Res.* **55:** 4391 – 4397.

85. **Claney, R.M. and S.B. Abramson. 1995**. Nitric oxide, a novel mediator of inflammation (Review). *Proc. Soc. Exp. Biol. Med.* **210:** 93 – 101.

86. Radi, R.; J.S. Beckman; K.M. Bush and B.A. Freeman. 1991. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.* **266:** 4244 – 4250.

87. **Ischiropoulos, H.; L. Zhu; J.S. Beckman .1992.** Peroxynitrite formation from macrophage derived nitric oxide. *Arch. Biochem. Biophys.* **298:** 446 – 451.

88. **Liu, S.; J.S. Beckman and D.D. Ku. 1994**. Peroxynitrite, a product of superoxide and nitric oxide, produces coronary vasorelaxation in dogs. *J. Pharmacol. Exp. Thar.* **268:** 1114 – 1121.

89. **Wu, M.N.; K.A. Pritchard; K.M. Kaminski; R.P. Fayngersh; T.H. Hintze and M.S. Molin. 1994**. Involvement of nitric oxide and nitrosothiols in relaxation of pulmonary arteries to peroxinitrite. *Am. J. Physiol.* **266:** 42108 – 42113.

90. **Naseem, K.M. and K.R. Bruckdorfer.1995**. Hydrogen peroxide at low concentrations strongly enhances the inhibitory effect of nitric oxide on platelets. *Biochem. J.* **310:** 149 – 153

91. **Halliwel, B. and J.M.C. Gutteridge. 1989.** Lipid oxidation: a radical chain reaction. In: Free Radicals in Biology & Medicine. 2nd Ed. New York. Oxford University Press. 159.

92. **Vallyanthan, V.; V. Castronova; D. Pack; S. Leonard; J. Shumaker; A.F. Hubbs; D.A. Shoemaker; D.M. Ramsey; J.R. Pretty and J.L. Mahawin *et al.* 1995**. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation: potential role of free radicals. *Am. J. Respir. Crit. Care. Med.* **152:** 1003 – 1009.

93. **Castronova, V.; W.H. Pailes; N.S. Dalal; P.R. Miles; L. Bowman; V. Vallyanthan; D. Pack; K.C. Weber; A. Hubbs; D. Schwegler-Berry *et al.* 1996.** Enhanced pulmonary response to inhalation of freshly fractured silica as compared with aged dust exposure. *App. Occup. Environ. Hyg.* **11:** 937 – 941.

94. **Dizdarolu, M. 1991**. Chemical determination of free radical-induced damage to DNA. *Free. Radic. Biol. Med.* **10:** 225 – 242.

95. **Vallyanthan, V.; J.F. Mega; X. Shi and N.S. Dalal. 1992.** Enhanced generation of free radicals from phagocytes induced by mineral dusts. *Am. J. Respir. Cell. Mol. Biol.* **6:** 404 – 413.

96. **Kadriiska, M.B.; R.P. Mason; K.L.Dresher; D.L. Costa and A.J. Ghio. 1997**. In vivo evidence of free radical formation in the rat lung after exposure to an emission source air pollution particle. *Chem. Res. Toxicol.* **10:** 1104 – 1108.

97. **Shi, X.; N.S. Dalal and V. Vallyanthan. 1988**. ESR evidence for the hydroxyl radical formation in aqueous solution suspension of quartz particles and its possible significance to lipid peroxidation in silicosis. *J. Toxicol. Environ. Health.* **25:** 237 – 245.

98. **Sun, Y. and L. W. Oberley. 1996**. Redox regulation of transcriptional activators. *Free. Radic. Biol. Med.* 21335 – 21348.

99. **Chen, F.; Y. Lu; L.M. Demers; X. Shi; Y. Rajanasakul; V. Vallyathan and V. Castronova. 1998**. Role of hydroxyl radical in silica induced NF-kâ activation in macrophages. *Ann. Clin. Lab. Sci.* **28:** 1 – 13.

100. **Harris, C.C. 1993**. $p^{53}$: at the crossroads of molecular carcinogenesis and risk assessment. *Science.* **262:** 1980 – 1981.

101. **Harris, C.C. and M. Hollstein. 1993.** Clinical implications of the $P^{53}$ tumour suppressor gene. *N. Engl. J. Med.* **329:** 1318 – 1327.

102. **Gansange, S.; F. Gansange; H. Gause; B. Poch; M.H. Schoengerg and H.G. Berger. 1997**. The induction of apoptosis in proliferating human fibroblasts by oxygen radicals is associated with a $p^{53}$ **and** $p^{21}$ WAI1C1P1 induction. *FASIB Lett.* **404:** 6 – 109.

103. **Beckman, J.S. J. Chen; H. Ischiropoulos and J.P. Crow. 1994.** Oxidative chemistry of peroxynitrite. *Methods. Enzymol.* **233:** 229 – 240.

104. **Darley-Usmar, V.; H. Wiseman and B. Halliwel. 1995.** Nitric oxide and oxygen radicals: a question of balance. *FEBs Lett.* **369:** 131 – 135.

105. **Proyor, W.A. and S. Padmaja. 1995**. The chemistry of peroxinitrite a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.* **268:** L699 – L722.

106. **Wu, M.N.; K.A. Pritchard; P.M. Kaminski; R.P. Fayngersh; T.H. Hintze and M.S. Wolin. 1994**. Involvement of nitric oxide and nitrosothiols in relaxation of pulmonary arteries to peroxinitrite. *Am. J. Physiol.* **266:** H 2108 – H 2113.

107. **Haddad, I.Y.; G. Pataki; P. Hu; C. Gillani; J.S. Beekman and S. Matalon. 1994**. Quantitation of nitrotyrosine level in lung sections of patients and animals with acute lung injury. *J. Clin. Invest.* **94:** 2407 – 2413.

108. **Lefer, D.J.; R. Scalia; B. Campbell; T. Nossuli; R. Hayward; M. Salamon; J. Grayson and A.M. Lefer. 1997**. Peroxinitrite inhibits leukocyte endothelial cells interaction and protects against ischemia reperfusion injury in rats. *Lab. Chin. Invest.* **99:** 684 – 689.

109. **Bellot, G.L.; M. Palmero; C. Garcia-Cabanes; R. Spi; C. Hariton and A. Orts. 1996**. Additive effect of nitric oxide and prostaglandin-$E_2$ synthesis inhibitors in endotoxin-induced uveitis in the rabbit. *Inflamm. Res.* **45:** 203 – 208.

110. **Allen, J.B.; M.C. Mc Gahan; Y. Ogaura; D.C. Sellon; B.D. Clark and L.N. Fleisher. 1996**. Intravitreal transforming growth factor - $b_2$ decreases cellular infiltration in endotoxin-induced ocular inflammation in rabbits. *Curr. Eye. Res.* **15:** 95 – 103.

111. **Allen, J.B.; King Teresa and Christopher Privalle. 1998.** Nitric oxide and peroxinitrite production ocular inflammation. E*nviron. Health. Perspect.* **106 (5):** 1145 – 1149.

112. **Venkatraman, J.T.; B. Chandrashekar; J.D. Kim and G. Fernandes. 1994**. Genotype effects on the antioxidantenzyme activity and m-RNA expression in liver and kidney tissues of autoimmune-prone MRL/$Mp^{JL}$pr mice. *Biochem. Biophys. Acto.* **1213:** 167 – 175.

113. **Lin, K.T.; J-Y Xue; M. Nomen; B. Spur and Py-K Wong. 1995**. Peroxinitrite-induced apoptosis. *J. Biol. Chem.* **270:** 16487 – 16490.

114. **Bonfoco, E.; D. Kraine; M. Ankarcrona; P. Nicotera and S.A. Lipton. XXXX**. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell neuron. *Proc. Natl. Acad. Sci. USA.* **92:** 7162 – 7166.

115. **Beckman, J.S.; H. Ischiropoulous; L. Zhu; M. Vander Woerd; C. Smith; J. Chen; J. Harrison; J.C. Martin and M. Tsai. 1992.** Kinetics of superoxide dismutase and iron-catalyzed nitration of phenolics by peroxynitrite. *Arch. Biochem. Biophys.* **298:** 438 – 445.

116. **Kobzik, L.; D.S. Bredt; C.J. Lowenstein; J. Drazen; B. Gaston; D. Sugarbacker and J.S. Stamler. 1993**. Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localisatine . *Am. J. Prespir. Cell Mol. Biol.* **8:** 371 – 377.

117. **Carrears, M.C.; G.A. Pargament; S.D. Catz; J.J. Poderoso and A. Boveris. 1999**. Kinetics of nitric oxide and hydrogen peroxide production and formation of peroxinitrite duringthe respiratory burst of human neutrophils. *FEBS. Lett.* **341:** 65 – 68.

118. **Gow, A.; D. Duran; S.R. Thom; H. Ischiropoulous. 1996.** Carbon dioxide enhancement of peroxynitrite-mediated protein tyrosine nitration. *Arch. Biochem. Biophys.* **333:** 42 – 48.

119. **Denicola, A. B.A. Freeman; M. Trujillo and R. Radi. 1996**. Peroxynitrite reaction withcarbon dioxide /bicarbonate: kinetics and influence on peroxynitrite-mediated oxidation. *Arch. Biochem. Biophys.* **433:** 49 – 58.

120. **Vander Vilet, A.; M.L. Hu; C.A. O'Neill; C.E. Cross and B. Halliwel. XXXX.** Interactions of human blood plasma with hydrogen peroxide and hypochlorous acid. *J. Lab. Clin. Med.* **124:** 701 – 707.

121. **Crow, J.P. , Beekman , J.S. and J.M. Mclord. 1995.** Sensitivity of the essential zinc thiolate moiety of yeast alcohol dehydrogenase to hypochloride and peroxinitrite. *Biochemistry*. **34:** 3544 – 3552.

122. **Zhu, Sha; Machelle Manuel; Shogo Tanaka; Nonghoon Choe; Elliot, Kagan and Sadis Matalon. 1998**. Contribution of Reactive oxygen and nitrogen species to particulate-induced lung injury. *Environ. Health. Perspect.* **106(5):** 1157 – 1163.

123. **Saleh, D., Barnes, P.J. and Giaid, A. . 1997**. Increased production of the potent oxidant peroxynitrite in the lung of patients with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care. Med.* **155:** 1763 – 1769.

124. **Broaddus, V.C.; Yang, L., Scavo, L.M. , Ernst , J.D. and Boylan,A.M. . 1996**. Asbestos induces apoptosis in human and rabbit pleural mesothelial cells via reactive species. *J. Clin. Invest*. **98:** 2050 – 2059.

125. **Babior, B.M. 1994.** Activation of the respiratory burst oxidase. *Environ. Health Perspect.* **102 (Supplem 10)**: 53 -56.

* * *