

SUPEROXIDE DISMUTASES (SOD) AND NUTRITIONAL MODULATION AGAINST OXYGEN FREE RADICALS

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ABSTRACT: Superoxide dismutases are critical enzymes responsible for the elimination of superoxide radicals and are considered to be a key antioxidant in aerobic cells. Cellular consumption of oxygen is essential for oxidative phosphorylation during ATP generation in the mitochondria, yet this cellular metabolism also leads to the production of oxygen free radicals such as the reactive oxygen species (ROS), the superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). Accumulation of ROS results in cellular oxidative stress and cellular malnutrition, if not corrected, can lead to the damage of important biomolecular structures such as membranes lipids, proteins and DNA. Prolonged accumulation of high levels of free radicals in cells may cause irreversible cellular injury and cell death, and have been implicated in diseases such as cancer, Alzheimer's, Down Syndrome, cancer and premature aging. Nutritional modulation with foods that contain superoxide dismutases or aid in the covalent conjugation of superoxide dismutase have been found to increase the circulatory half-life and provide prolonged protection from partially reduced oxygen species known as oxygen free radicals.

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Enzymes and Toxicity

The ultimate regulators of metabolism are enzymes and their associated factors of trace elements, vitamins, hormones, and antimetabolites. Substances that act chemically to produce injury to organs and tissues of the body usually do so by two basic means: either by depressing or by stimulating the activity of the enzyme systems. Severe, acute effects such as destruction of cell membrane integrity by corrosive agents or protein coagulants, etc., are obvious exceptions. A single substance may have more than one pathway and site of action. Multiple pathways of action may be invoked simply by differing doses of the toxic agents which create oxidative stress or covalent bonding and oxygen radicals functioning as mediators of cell injury are the result of the following: (1) low doses may stimulate enzyme action; (2) high doses depress and inhibit the same or different enzyme systems. This is a characteristic action of most, if not all, toxic substances, including arsenic, benzene, chloroform, cobalt, fluoride, x-rays, vanadium, snake venoms, biofilms, and marine toxins and cellular toxins known as the "Maverick Compounds." A number of aspects of toxicity are shown in Figure 1.1,2,3

Systemic toxicity is, by and large, a matter of the activity of enzyme systems, either by inhibition or over stimulation (removal of a natural inhibitor system), all accomplished at the free-radical and oxygen radical level.

Substances display differing toxicities and have selective sites of action because different substances affect, to differing degrees, the various metabolic compartments and, thus, raise or lower the level of "observed toxicity." Different substances have differing chemical affinities for tissue sites and cellular organelles as shown in Figure 2.4

Potentiation and synergism, the enhanced toxicity of two or more simultaneously acting substances, can be explained by the action of one preventing the elimination or the metabolism of the other, wholly or in part, thus maintaining elevated systemic levels of the toxic agent, resulting in an observed toxicity greater than the additive toxicity of the combined components (Figure I).

Antagonistic action is explained by one component preventing, wholly or in part, the toxic action of another. This occurs when one component induces or supplies additional amounts of a critical enzyme system or factor that is being attacked by another component, the net result being to greatly reduce or even completely eliminate cellular toxicity. A similar mechanism appears to explain the antagonism of ethyl alcohol for methyl alcohol

toxicity. In this case the liver alcohol dehydrogenase preferentially attacks ethyl alcohol, thus slowing down or preventing the oxidation of methyl alcohol to neurotoxic metabolites (Figure 1).

It is important to realize that most of the metabolic activity of the body is a result of the activity of enzymes, which are biological catalysts formed by living cells throughout the body. Consequently, it is reasonable that the bulk of all toxic mechanisms should involve interference in some way with normal enzyme activity.

All enzymes have a basic protein structure composed of 20 or more amino acids grouped in various chain arrangements in a three-dimensional structure. To perform, the myriad of metabolic reactions of the body requires an estimated million diverse enzymes. This diversity of structure and function makes any simple classification inadequate. However, just as the major types of metabolism and detoxication were classified (Figure 3), so can the major metabolic reactions catalyzed by enzymes be classified.

Various Classes of Enzymes

The enzymes that perform oxidation-reduction reactions constitute one of the larger groups. The oxidases, which reduce the inhaled oxygen carried throughout the body by hemoglobin and myoglobin, reduce oxygen directly. One of the most important of these is cytochrome oxidase. Other important oxidases are xanthine oxidase with riboflavin as a prosthetic group, the polyphenol oxidases, with copper as prosthetic group, and tyrosinase responsible for the oxidation of tyrosine to the dark melanin pigment.

Closely related in action are the dehydrogenases, which catalyze the removal of hydrogen, and thus "oxidize" organic molecules. As body oxidations generally (respiration) proceed in this manner, there are several highly specific dehydrogenases. All cellular respiration involves three major classes of dehydrogenases: 1) pyridine-linked dehydrogenases, which require a dinucleotide as coenzyme, 2) flavin-linked dehydrogenase, which contain flavin nucleotide, and 3) the cytochromes, which contain an iron-porphyrin ring system. More than 150 of the pyridine-linked dehydrogenases are known. One of these, glucose -6-phosphated dehydrogenase (G-6-PD), features prominently as the key system in rendering an individual hypersusceptible to hemolytic hazardous materials.⁵ A genetic deficiency in G-6-PD can make a person susceptible to incurring a hemolytic crisis from exposure to such hazardous materials by either blocking the action of certain components of the G-6-PD system in the red cells or by the chemical's utilizing the hydrogen critically needed for cell respiration, resulting in loss of red cell integrity, and consequent cell lysis.

Another large and diversely acting group is the hydrolytic enzymes, chief among which are the phosphatases, which hydrolyze esters of phosphoric acid. These enzymes are involved in all catabolic (destructive) and anabolic (synthetic) reactions of the cells. Other representative hydrolytic enzymes are the esterases, such as liver esterases and pancreatic esterases. Others in this group are those that hydrolyze protein structures, proteolytic enzymes that break the common peptide bond of these structures. This group is further comprised of more specialized enzymes, the peptidases, the carboxyl- and aminopeptidases, so named because of action on peptides with adjacent carboxyl (COOH) or amino (NH₂) groups; those that hydrolyze glycosidic linkages, the carbohydrases, which act on polysaccharides and glycosides.

The decarboxylases are a widespread group composed of keto-acid decarboxylase, which is responsible for the liberation of the end product of metabolism, carbon dioxide. Amino acid decarboxylases are responsible for the formation of amines by carbon dioxide liberation from amino acids. In the chain of metabolic end reactions, oxidative delaminating enzymes remove the amino group from these toxic amines, be they endogenous or of foreign origin, resulting in reduced toxicity, liberation of the end product, ammonia, and its excretion of the urine. Some of the ammonia, however, is transferred to other substances by transferases. These transferases can also transfer other groups such as methyl, phosphate, and amino groups.

The above classes of enzymes, with other enzymes such as superoxide dismutases and other enzymes not classified, represent all the metabolic catalysts the body can muster to handle foreign chemical structures. As these structures may vary from closely similar to remotely related to the natural substrates of these enzymes, it is not difficult to see that destruction of a foreign toxic substance can range from nearly complete to scarcely perceptible.

Enzymatic Action

Enzymatic actions occur throughout the body without restriction to any particular organ site, although the liver cells perform a major portion of the metabolic activity of the body. Similarly active, however, but less diversified, are the enzymes in the lung, kidney, intestine, brain and nervous tissue, and bone. For this, it may be inferred that enzymatic mechanisms may occur with the enzyme situated at cell surfaces or within the cell itself. Although the activity of enzymes, in normal circumstances, occurs within or on cells which are inaccessible for measurement (except as biopsied tissue), toxic injury to cells may result in enzyme release in proportion to the injury into the blood and body fluids where they can be measured and serve as biologic indicators of exposure and/or response. 6,7

In "metabolizing" a foreign substance, it is important to observe that the enzyme is merely performing a function that it normally performs in metabolizing natural foodstuffs; no special enzymes exist to metabolize toxic substances. Although "drug-metabolizing" enzymes are commonly mentioned, this does not mean that the body develops a new class of enzymes in response to the administration of a drug, genetic material/drug, herbal remedy, nanofood, far infrared, fungal therapy or any other technology, however, these technologies, may act to induce larger amounts of enzyme activity within the individual body. 8,9,10

Enzyme Characteristics

It is now recognized that certain enzymes, heretofore considered homogenous in composition and in action, may consist of several distinct components, each still acting, however, on the same substrate; these components are called isoenzymes, or isozymes. Superoxide dismutases are of this category of enzymes and will be discussed in more detail later in this paper. Isozyme components can differ in number and activity, depending on the tissue of origin, e.g. lactic acid dehydrogenase has as many as five different isozymes, depending upon whether originating from the heart, kidney, liver, or lung.

Many enzymes have additional specificity requirements, in that they require a metal or a vitamin, or both, as cofactor(s) or activator(s). For example, the enzyme cacaroxylase that splits carbon dioxide from certain organic acids, requires vitamin B1 and magnesium ions as necessary constituents before it can function. If these subcomponents were not present in one or more area of the enzyme the enzyme would not function and be observed by the clinician as inactive, inhibited, or depleted.

Because enzymes are proteins, they exhibit the physical and chemical properties of proteins. They undergo denaturation 1) by heat, as in burns (a photon type source); 2) by marked changes in acidity or alkalinity as affected, for example, by contact with corrosive agents, biofilms, cigarette smoke, illegal drugs, chemotherapeutic agents or mycotoxins 8; or 3) by chemical denaturing agents, such as urea in high concentrations. These agents alike cause structural, configurational changes, and crystallization in tissues/cells in the protein, and the characteristic specificity is lost, and with it the catalytic activity of the enzyme.

Enzyme activity can be inhibited in a number of ways. For example, among the enzymes requiring a specific metal as activator, any agent that will displace or render inactive the metal will render the enzyme inactive to the degree that the metal was rendered inert or removed from the enzyme. Certain metals with similar spatial requirements for the specific metal required by the enzyme may do this. Certain poisonous metals such as beryllium are believed to act in this way. Cyanide may combine with the iron of an iron-dependent enzyme (i.e. Fe-SOD) and inactivate or inhibit the enzyme.

A third way by which enzyme activity is inhibited is by accumulation of the product of the enzyme's activity. This is one of the natural ways by which body enzyme activity is regulated and is known as metabolite inhibition. A prime example of this is exposure to MSG and aspartame where the cellular connective glue of the outer membrane of the mitochondria becomes unglued from the cytoplasm of a cell, thus causing increased obesity.¹¹

The fundamental aspects of enzyme activity with respect to toxicity may be summarized as follows. Enzymes combine with the toxicity substance. This combination may result in partial or complete inhibition of enzyme activity or the enzyme may act on the toxic substance more or less incompletely, possibly with the production of even more toxic substances, but generally with production of degraded, less toxic substances. If the enzyme whose activity is blocked is a critical one; there may be slowing down of some vital function, resulting in alteration of cellular constituents in amount or type, even in cell death.

Superoxide Dismutases and Oxygen Radicals as Mediators of Toxic Cell Injury

Superoxide dismutase (SOD) is a critical enzyme responsible for the elimination of superoxide radicals and is considered to be a key anti-oxidant in aerobic cells. Cellular consumption of oxygen is essential for oxidative phosphorylation during ATP generation in the mitochondria, yet this cellular metabolism also leads to the production of reactive oxygen species (ROS, including the superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). SOD is made up of isoenzymes that have functional metal groups for its catalytic action within the cell and externally. Many pictures taken with GRASP software show that there is an electrostatic field around an active site of the enzyme superoxide dismutase, which controls oxygen toxicity by converting the superoxide radical to less dangerous forms. These electrostatic fields may be a combination of Brownian dynamics, covalent bonding with valence charges, and super-radiant states as associated with cooper pairs in quantum biophysics. 12,13,14

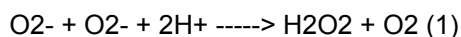
Superoxide dismutase (SOD) may be in the form of copper/zinc superoxide dismutase (Cu/Zn-SOD), manganese superoxide dismutase (Mn-SOD), and iron superoxide dismutase (Fe-SOD)¹⁵. Recent genome applications in enzymomics and proteomics has shown that there may be additional isoenzymes in the form of selenium superoxide dismutase (Se-SOD) and nickel superoxide dismutase (Ni-SOD), even cadmium superoxide dismutase (Cd-SOD) has been associated with this enzyme. The latter having a high valence charge (cadmium) that may play a negative role in the destruction of the free oxygen radical mechanisms within the cell. 16

Molecular oxygen, necessary for the survival of aerobic organisms, is a diradical in its ground state. Despite oxygen's radical nature, its reactivity is surprisingly low. The two unpaired electrons of molecular oxygen possess the same spin. Thus, the reaction of oxygen with electron donors to form covalent bonds is kinetically unfavored and very slow; however, oxygen can readily participate in one-electron reductions.² This is very important in cellular metabolism because approximately 90% of the oxygen consumed by humans is used by the cell's mitochondria. Mitochondrial oxidative stress, caused by oxygen free radicals that are produced when faulty electron transfer occurs at any point of the electron transport chain, has been linked to conditions such as Alzheimer's, heart disease, and cancer. Cells immobilize oxygen free radicals in the mitochondria by using the metabolic enzyme, superoxide dismutase (SOD), which simultaneously reduces and oxidizes (dismutation) superoxide free radical to form hydrogen peroxide and oxygen. Hydrogen peroxide is then converted into water and oxygen by catalase enzymes.¹⁷

The stepwise reduction of oxygen to water produces a variety of potentially toxic intermediates. The addition of a single electron produces the superoxide anion. In turn, a second electron reduces superoxide to the peroxide ion. At physiological pH, the peroxide ion exists primarily as hydrogen peroxide. Addition of the third electron splits the oxygen-oxygen bond to form the hydroxyl radical (and a hydroxide anion that is essentially water). Addition of the final electron to the hydroxyl radical yields another molecule of water.²

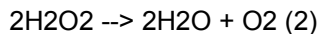
These partially reduced, and thereby activated, forms of oxygen are continuously generated in all aerobic cells as a result of oxidative processes, both autocatalytic and enzymatic. Superoxide anion and hydrogen peroxide are produced by various oxidases (e.g. xanthine oxidase and cytochrome P-450) as well as by the redox cycling of electron transport carriers, thiols, and catecholamines. Activated phagocytes also generate superoxide anions and hydrogen peroxide. The formation of these activated oxygen species is, in turn, related to the bactericidal activity of these cells and probably also contributes to the development of inflammation in tissues where these cells accumulate as seen in Syndrome X and morbid obesity.¹⁸ By contrast, cytochrome C oxidase mediates the complete reduction of oxygen to water. It is a well coupled system and does not represent a source of activated oxygen under normal conditions, especially since the pH of water is 7.0 and any other significant increase between 7.1 – 7.9 would result in a balancing affect upon cellular acidosis as when exposed to organophosphates, carbamates and later stages of cancer as seen in early experiments with eccelerated water and Genie Spa Spheres.¹⁹

Aerobic organisms have developed numerous mechanisms which protect the cell from the physiological generation of activated oxygen. However, when the generation of such species over whelms the cell's ability to detoxify them, cell injury can result. The superoxide dismutases dispose of superoxide anions. As illustrated in Equation 1, these metal-containing enzymes catalyze the dismutation of two molecules of the superoxide anion to give hydrogen peroxide and dioxygen.



In eukaryotic cells, there are two distinct superoxide dismutase (SOD) enzymes, a copper-zinc containing enzyme found in the cytosol and in manganese-containing enzyme found in the mitochondria. The SODs present in prokaryotic cells contain either iron or manganese at the active site. Obligatory anaerobes have no SOD activity. SOD activity can be induced under conditions of excess oxygen radical formation. For example, pulmonary SOD can be induced by hyperbaric oxygen or far infrared administration.^{20,21} This induction has been related to a protection of the organism from the deleterious effects of oxidative stress, which untreated will cause diseases such as cancer, Alzheimer's, chronic fatigue, fibromyalgia, and multiple sclerosis.^{18,22} In turn, inhibition of SOD with, for example disulfiram, is known to sensitize a cell to oxidative stress. Disulfiram is used not only as a drug but a weed killer, too.

The hydrogen peroxide produced by the action of superoxide dismutases and other oxidative processes can be detoxified by two different systems. The enzyme catalase converts hydrogen peroxide to water.



This reduction of hydrogen peroxide by catalase occurs without the intermediate formation of the hydroxyl radical. Catalase is a homoprotein that is found almost exclusively within peroxisomes. Similar to the inhibition of superoxide dismutase, inhibition of catalase with aminotriazole potentiates oxidative stress cell injury.

The enzyme glutathione peroxidase also catalyzes the reduction of hydrogen peroxide to water. Reduced glutathione is used as the source of reducing equivalents necessary to drive this reaction. In this process, two molecules of glutathione are oxidized to yield one molecule of glutathione disulfide (GSSG). Glutathione disulfide is efficiently reduced to glutathione by glutathione reductase. NADPH serves as the source of the reducing equivalents. The glutathione peroxidase-reductase system seems to be the first line of defense against hydrogen peroxide, and catalase is a secondary system. Catalase is also a specific defense against hydrogen peroxide formed within the peroxisomes.

Glutathione peroxidase is found in the cytosol of most cells as well as within the mitochondria. Glutathione peroxidase also shows considerable activity toward organic hydroperoxides, converting them to their corresponding alcohols. By contrast, catalase will only catabolize hydrogen peroxide. The cytosolic and mitochondrial forms of glutathione peroxidase are dependent upon the metal selenium for activity. It is not surprising that selenium deficiency has been demonstrated to exacerbate oxidant-stress injury²³ and when selenium and vitamin E are depleted in a tumor cell the cell is carcinogenic.²⁴ It is very important to note that SODs within the cell may be found to be interdependent upon the fat soluble vitamin such as vitamin E vs. the water soluble vitamin C as found on the outer cell membrane. Their common link being the phospholipidation of the cell, especially the skin cells where compounds may be absorbed within 25 seconds into the circulatory system vs. 10 seconds for dimethylsulfoxide (DMSO) and olive oil.²⁵

The importance of glutathione reductase is emphasized by the observation that inhibition of this enzyme also potentiates the cytotoxicity of oxidative stress. With the inhibition of glutathione reductase, oxidized glutathione is not converted to reduced glutathione, a result that limits the effectiveness of the peroxidase by limiting the supply of glutathione. The chemotherapeutic alkylating agent, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) inhibits glutathione reductase without effect on glutathione peroxidase and catalase. This compound sensitizes a number of cell types to the toxicity of hydrogen peroxide and other organic hydroperoxides. It should also be noted that, during severe oxidative stress, NADPH levels are markedly depleted, and effect related to the consumption of NADPH by glutathione reductase. Such a depletion may exert a substrate-level inhibition of the reductase. Thus, the NADPH depletion that results from oxidative stress may actually sensitize the cell to the oxidative stress. Superoxide dismutase and catalase provide antioxidant protection by inhibiting the formation of the hydroxyl radical as found in free oxygen radicals.

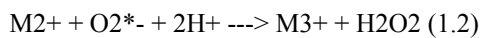
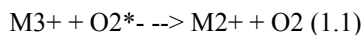
The Future of Superoxide Dismutases and Nutritional Modulation

NASA principal investigator, Dr. Gloria Borgstahl, formerly of the University of Toledo, and now of the University of Nebraska, has successfully crystallized *E. coli* manganese superoxide dismutase (MnSOD), antioxidant enzymes that are homologous to those found in cellular mitochondria, on the International Space Station during the period of December 2001 to April 2002. Several of the MnSOD crystals grown on ISS were 80 times greater in crystal volume than earth-grown crystals. Diffraction resolutions were observed providing significantly improved data obtained from crystals grown in

earth laboratories. An exciting result was that the MnSOD crystals grown on ISS were suitable for neutron studies and time-Laue studies-methods that require large, perfect crystals. With the neutron examples the researchers hope to be able to obtain the, never-before-seen, three-dimensional structure of the hydrogen's on each amino acid of the protein and thereby be able to answer the unsolved questions concerning the source of these hydrogens in this reaction mechanism. With the time-resolved Laue experiments, the team will be able to generate the superoxide substrate within the crystals with a laser pulse and thus film the "movie" of the enzyme converting it to the products peroxide and water. The enzyme MnSODs in the body is important, and in-depth study of their structure is not to the ability to understand their true function, but these experiments may lead to new therapeutics for the treatment of various degenerative diseases.¹⁸

Superoxide dismutases (SODs) are antioxidant metalloenzymes catalyzing the redox disproportion in the (dis)mutation of superoxide radical, $O_2^{\cdot-}$ as previously discussed in Equation (1).

It is generally accepted that in all SODs the metal ion (M) catalyzes dismutation of the superoxide radical through a cyclic oxidation reduction mechanism:



The four classes of SODs are known, distinguished by the metal prosthetic groups: Cu/Zn, Fe, Mn and Ni. Fe- and Mn-SODs constitute a structural family.^{26,27} Fe- and Mn- SODs are unequally distributed throughout the kingdoms of living organisms and are located in different cellular compartments.^{28,29,30,31} In particular, Fe-SOD is found in obligate anaerobes and aerobic diazotrophs (exclusively), facultative aerobes (exclusively or together with Mn-SOD). In the cytosol of cyanobacteria, in the chloroplast stroma of higher plants, in the protozoa, kelp, Yamatoshinjo and Oaky Smoky (Cu/Zn-SOD, Fe-SOD, Mn-SOD)^{TM 32,33,34} Fe-SOD and Mn-SOD form some organisms (e.g. Escherichia coli) exhibit almost absolute metal specificity,³⁵ while other enzymes, such as "cambialistic" SOD from Propionibacterium shermanii, are captive with either metal.³⁶ Fe- and Mn-SODs occur as homodimers or homotetramers.

The 3-D structures of several Fe-SODs have been determined. ^{36,37,38,39,40} The monomers fold into two domains. The N-terminal domain consists of two long antiparallel helices. The C-terminal domain contains a central beta-sheet formed by three antiparallel beta strands with 4-6 surrounding helices. The iron atom is liganded by two residues from each of the N-terminal helices and two residues from the loops in the C-terminal domain.

The active site iron is pentacoordinate, with the metal ligands (N x epsilon) of three conserved His residues, O x delta of the conserved Asp residue of a water molecule) arranged in distorted trigonal bipyramidal geometry, which opposite of the tetrahedral molecule of water. The first His residue and a solvent molecule fill the two axial positions. In the azide-FeIII - SOD complex, the iron becomes hexacoordinate with distorted octahedral geometry (similar to the fat soluble Vitamin E), with azide coordinated trans to ASP ligand.⁴¹ Table 1 lists the mononuclear iron environment residues in known 3D structures with their PDB Code and Reference.

Superoxide dismutases (SODs) have been found in various nutritional sources such as Jerusalem artichoke powder and Bifidobacterium. ^{42,43} Juice Plus + Vineyard Blend, foods grown in red clay soils, Yamatoshinjo and hemp oil. ^{44, 34,45} The use of these nutritional modulators have shown significant risk to oxidative stress via the superoxide radical as they have maintained cellular integrity and reduced cellular sensitization as individual whole foods with an individual's meals.

Summary

Cells are continuously exposed to a variety of oxidative process which could potentially lead to cellular injury or death. As a result, aerobic organisms possess effective defense mechanisms against oxidative stress as associated with the superoxide radical and improper nutritional modulation that would reduce superoxide dismutases (SODs) in the cell and thereby circumvent toxic cell injury. These protective mechanisms fall into two broad categories: (1) those which prevent the initiation of lipid peroxidation and (2) those which prevent its propagation.

Superoxide dismutase and catalase provide antioxidant protection by inhibiting the formation of the hydroxyl radical. Chelation of the ferric iron necessary for the formation of the hydroxyl radical and direct removal of this species by radical scavengers (e.g., mannitol) are also protective. The generation of species capable of initiating peroxidation may exceed the capacity of effectively remove them. Thus, there are other defenses to prevent the uncontrolled propagation of lipid peroxidation and oxidative stress as associated with free oxygen radicals/hydroxyl radicals. These include water-soluble

antioxidants, such as ascorbic acid (vitamin C) and reduced glutathione, and fat-soluble antioxidants, most notably alpha-tocopherol (vitamin E). It appears that vitamins E and C and glutathione combine in some as yet poorly defined cycle to donate hydrogen atoms to lipid and/or peroxy radicals, thereby preventing further propagation of the lipid peroxidation, which was initiated by free oxygen radicals.

The metabolism of toxic substances as associated with hazardous materials by mixed function oxidation or other mechanisms leads to irreversible cell injury through mechanisms that have been related either to the covalent binding of reactive metabolites, to changes in protein thiols, to alterations in intracellular calcium homeostasis, or to the formation of partially reduced oxygen species. The relative roles that each of these mechanisms plays in any particular example of toxic cell injury remains controversial and a subject of continuing investigation through the advancement of new analytical technologies/equipment. In the case of the various forms of superoxide dismutases, “quickened” advances in science, chemistry and wave genetics 46 will show that these metalloenzymes will play a major character role in the explanation of “creation” through the variable of true far infrared sources, superluminal radiance, the effects of Cooper pairs upon Eccelerated™ intelligent water and the role of the white worm hole found in black holes as recently found in the DNA molecule. These future researchers will be the Indigo and Crystal children – our grand children with their little pet black rabbit with pink ears and a white spot named Ben as it hops down a white worm hole in the quantum biophysics of the human cell. Time and Eternity will show all of us the wonders that will amaze the original Creator of us all through the marvels of science, spirituality, art and medicine.

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TABLE 1-1: A partial list of known extrinsic mutagenic agents.

Taken from Levitan, Max and Ashley Montagu. Textbook of Human Genetics. Oxford University Press. New York. © 1971, pgs. 671 & 672.

I. Radiations

1. Ionizing, X-rays, alpha particles, beta particles, gamma rays
Neutrons (various speeds), cosmic rays
2. Non-ionizing, ultraviolet light (0.014-0.315 u),
near-visible light (0.320-0.400 u)

II. Temperature changes

Heat, heat shocks, cold shock

III. Chemicals

1. Compounds related to DNA or RNA bases adenine (purine), 2-amino purine (purine analogue), 5-bromouracil (pyrimidine analogue), caffeine (purine), 2,6-diamino purine (purine analogue), theobromide (purine analogue), formaldehyde (known to react with purines and pyrimidines), nitrous oxide (known to react with purines and pyrimidines) deoxyribonuclease (DNA metabolic enzymes)
2. Alkylating agents (mustard gases and related compounds), nitrogen mustards, sulfur mustards, ethylene oxide, ethyl methyl sulfates, halogenated and not, diethyl and dimethyl sulfate, diepoxybutane
3. Acridine dyes, acridine orange, acriflavine, proflavine
4. Carcinogens, 1,2,5,6 dibenzanthracene, methyl cholanthene, benzpyrene, beta-naphthylamine.
5. Inorganic salts, copper sulfate, ferrous chloride, manganous chloride
6. Organic acids, acetic acid, carbolic acid (phenol) and related compounds, formic acid, and lactic acid.
7. Inorganic acids, boric acid
8. Others, ammonia, colchicines, hydrogen peroxide, necrosine, neutral red (in the presence of light), sodium desoxycholate, triazine, urethane and certain other carbamates.

TABLE 2-2: Fungal Mycotoxin Postulated Diseases.

Taken from http://www.mold-help.org/definition_of_fungalbionics.htm and www.doctorfungus.com © July 15, 2002.

COLCHICINE-RESPONSIVE

Acute Gouty Arthritis	Alcoholic Cirrhosis
Familial Mediterranean Fever	Mollaret's Meningitis
Belchet's Syndrome	Psoriasis
Thrombocytopenic Purpura	Chronic Lymphocytic Leukemia
Amyloidosis North African	Leukocytoclastic Vasculitis
Sarcoid	Arthritis

GRISEOFULVIN-RESPONSIVE

Atherosclerosis (Angina)	Systemic Sclerosis
Raynaud's Syndrome/Disease	Shoulder-Hand Syndrome

ALLOPURINOL-RESPONSIVE

Sarcoidosis	Oxalate Nephrolithopathy
Idiopathic Respiratory	Distress Syndrome/Newborns
Rheumatoid Arthritis (some)	Calcium Pyrophosphatopathy
Hyperlipidemia	Inflammatory Bowel Disease

COLCHICINE PREVENTS IN EXPERIMENTAL ANIMALS

Atherosclerosis	Casein Induce Amyloidosis	Cushing's Disease
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NYSTATIN-RESPONSIVE

Psoriasis	Inflammatory Bowel Disease
Hyperactivity Syndrome	Multiple Sclerosis

Duchenne's Muscular Dystrophy

KETOCONAZOLE-RESPONSIVE

Inflammatory Bowel Disease	Disseminated Vascular Coagulation
Idiopathic Female Infertility	Precocious Puberty in Boys
Hyper-Low Density	Lipoproteinemia
Hyperaldosteronism	Prostate Carcinoma

Note: The anti-fungal nature of colchicines and allopurinol has been fully documented.

TABLE 3-3: Food from farmers, middlemen, and retail outlets in Bangkok, Thailand.

Note: Surface was sterilized prior to fungal study. Taken from Pilt JL, Hocking AD, Bhudhasamai K, Miscamble BF, Wheeler EKP: The Normal Mycoflora of Commodities from Thailand, part 1 Nuts and Oilseeds. International Journal Food Microbiology 20:211-226, 1993.

CORN

Acremonium siricium
 Aspergillus flavus
 Aspergillus niger
 Aspergillus tamaritii
 Aspergillus wentii
 Bipolaris maydis
 Chaetomium globosum
 Chaetomium funicola
 Chaetomium spp.
 Curvularia lunata
 Eurotium amstelodami
 Eurotium chevalieri
 Eurotium rubrum
 Fusarium moniliforme
 Fusarium proliferatum
 Fusarium semitectum
 Nigrospora oryzae
 Penicillium citrinum
 Penicillium pinophilum
 Penicillium raistrickii
 Phoma spp.
 Rhizoctonia solani
 Rhizopus oryzae
 Rhizopus arrhizus
 Trichoderma harzianum

PEANUTS

Aspergillus candidi
 Aspergillus flavus
 Aspergillus niger
 Aspergillus tamaritii
 Aspergillus wentii
 Chaetomium globosum
 Chaetomium funicola
 Chaetomium spp.
 Eurotium amstelodami
 Eurotium chevalieri
 Eurotium repens
 Eurotium rubrum
 Fusarium equiseti
 Fusarium semitectum
 Fusarium solani
 Lasiodiplodia theobromae
 Macrophomina phaseolina
 Nigrospora oryzae
 Penicillium aethiopicum
 Penicillium citrinum
 Penicillium funiculosum
 Penicillium glabrum
 Penicillium janthinellum
 Penicillium olsonii
 Rhizopus oryzae

TABLE 4-4: Mycotoxicoses in which Experimental and Epidemiological Data Suggesting Human Involvement, http://www.mold-help.org/definition_of_fungalbionics.htm and www.doctorfungus.com

DISEASE	SPECIES	FOOD/FEED	MYCOTOXIN
Gout/Hyper-uricemia	Fowl	Moldy Corn	Oosporein
	Fowl	Barley	Ochratoxin
	Chicks		Kojic acid
	Chickens		Oxalic acid
	Pigeons		Alloxan
	Rats		Yeast
	Primate		Aflatoxin
	Man		Cyclosporin
	Man		Penicillin
	Man	Beer/Wine/Bread	Multiple
Man	Meat Products	Multiple	
Man	Rye	Ergotamine	
Atherosclerosis/	Sheep		Sporidesmin

Hyperlipidemia	Man Primates		Cyclosporin Fumonisin Ergot
Cardiac Ischemia With Arrhythmias	Rabbit		Citreoviridin/ Penicillium
Hypertension	Man Rabbit		Alcohol T-2 Toxin
Multiple Sclerosis	Man		Ergot
Pulmonary- Hypertension	Swine		T-2 Toxin
Scleroderma	Man		Amanita
Diabetes	Man		Cryptococcus/ Alloxan
Crohn's Disease	Man	Fermentation	S.cerversisae
Lung Cancer	Man	Tobacco	Fusarium
Esophageal carcinoma	Man		Fusarium
Breast Cancer	Man	Fermentation	S. cerevisiae
Endometrial CA	Man		Fusarium
Colon CA	Man		Fusarium
Hepatocellular- carcinoma	Man	Cereals, grains Peanuts	Aspergillus
Hepatoma	Man		Aflatoxin
Cardiomyopathy	Man	Fermentation	Alcohol
Osteoporosis	Man	Fermentation	Alcohol
Alimentary toxic aleukia (ATA or septic angina)	Man	Cereals, grains (toxic bread)	Fusarium triachiodes
Dendroochio- toxicosis	Horse, Man	Fodder (skin contact, nhaled fodder particles)	Dendroochium toxicum
Kashin Beck Disease, 'Urov Disease'	Man	Cereal grains	Fusarium trichiodes
Stachybotryo- toxicsis	Man Horse Other Livestock	Hay, cereal grains, fodder (skin contact, inhaled haydust)	Stachybotris atra
Cardiac beriberi	Man	Rice	Fusarium
Ergotism	Man Animals	Rye cereal grains	Claviceps purpurea
Balkan-nephropathy	Man	Cereal, grains	Penicillium
IGA Nephropathy	Mice	Grains	Vomitoxin
Reye's Syndrome	Man	Cereal, grains	Aspergillus

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