

Hydrogen Peroxide: A review of a scientifically verifiable omnipresent ubiquitous essentiality of obligate, aerobic, carbon-based life forms

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Abstract

Electronically modified oxygen derivatives (EMODs), such as superoxide anion and hydrogen peroxide, carry out highly sophisticated intra, inter and extra cellular signaling roles that are essential for normal biochemical functioning. Hydrogen peroxide is freely diffusible through cell membranes, can not be excluded from cells and is required for normal operation of many enzymes that maintain and promote health. Hydrogen peroxide can act as an enzymatic substrate or activate or inhibit redox sensitive enzymes. Large health related organizations, such as the American Cancer Society, continue to disregard and deride the potential beneficial involvement and contribution of EMODs, especially hydrogen peroxide, in the treatment of any disease condition, inclusive of cancer. Their position of disdain is not supported by an exhaustive review of the medical and scientific literature. Although hydrogen peroxide has on occasion been a proposed mutagen, oncogenic transformation primarily leads to an increase of cellular EMODs that renders these same cells selectively vulnerable to additional EMOD production via EMOD induced apoptosis or necrosis. Legitimate medical health agencies are obliged to embrace the widespread scientific supportive findings regarding prooxidant hydrogen peroxide existent in the literature and encourage in depth scientific investigation into prooxidant EMOD disease protection and prevention. Scientific fact must supplant unsupported skepticism or flawed conclusions based on outdated data. While emphasizing hydrogen peroxide, this review is aimed at presenting the scientific facts regarding prooxidant EMOD health applications and cancer therapy. Based on the scientific literature currently available, EMOD use has significant potential benefits in the treatment of a wide range of human pathophysiologies. To deny the crucial role of hydrogen peroxide and prooxidant EMODs in normal metabolic processes and disease protection is to deny scientific truth.

INTRODUCTION

It is perhaps amongst the greatest biochemical wonders of evolution that the most crucial and widespread small molecular weight EMODs, which are purportedly the alleged "enemies within," are ever present and essential occupants of the most sensitive biochemical control systems within obligate aerobic cells. They carry on continual cross talk with their chemical kin. The naïve notion (promulgated by the free radical theory), that EMODs are inherently toxic, is counterintuitive to very basis of the evolutionary concept itself. After all, following oxygen's arrival, they evolved with the cell, by the cell and for the cell. Logic and biochemical principles dictate that careful EMOD modulation could serve as a safe means of advancing disease protection or reversal and health promotion.

However, even with hydrogen peroxide, which is an

abundant permeating cellular mediator, nonspecific inhibition or augmentation of its activities may lead to homeostatic derangements by exogenous or endogenous over- or under-production.

REFUTE THE OLD UNPROVEN ALLEGATION: FREE RADICALS ARE BAD, ANTIOXIDANTS ARE GOOD

In discussing the history of oxidants, such as hydrogen peroxide, respected free radical research pioneer, Barry Halliwell, said that, "In the beginning, it was simple: we said free radicals are bad, antioxidants are good." However, much has changed and over half a century later, these unfounded charges remain unproven. Further, the free radical theory, upon which this was based, has failed to be verified by the scientific method because of its lack of predictability. In fact, recent findings from randomized controlled trials

(RCTs) have repeatedly failed to support the free radical theory and some trial results have even shown harmful effects and increased risk of mortality from antioxidant use.

Following the 1992 US National Cancer Institute research for testing of beta carotene, Halliwell, from the National University of Singapore, said, "It was a shock. It (beta carotene) not only did no good but had the potential to do harm." Subsequently, an expert panel convened by the National Institutes of Health has concluded that there is no evidence to recommend beta carotene supplements for the general population, and strong evidence to recommend that smokers avoid it.

The story was the same with vitamin E, in which recent studies have been almost universally "disappointing." Further, in 2005, Dr. Edgar Miller of the Johns Hopkins Medical Institutions, published a meta-analysis review of 19 studies in *Annals of Internal Medicine* showing that vitamin E increased overall mortality.¹ Even though vitamin E appeared to be a good antioxidant in vitro, there is now serious scepticism that it acts in the same manner in vivo.²

Following a large RCT on vitamin C, Halliwell said, "Vitamin C is another disappointment. People are still trying to defend it, but you don't get an effect on free radical damage unless you start with people with a vitamin C deficiency. I think it is a lost cause."

Yet, the prevailing prejudice against EMODs is manifestly illustrated in the November 2003 issue of *Readers Digest*. In quoting Dr. Bruce Ames, a biochemist at the University of California at Berkley, he stated, "free radical oxidation doesn't just rise with aging--it causes it. The more that mitochondria 'leak' free radicals (i.e., oxygen radicals, EMODs), the more those radicals end up damaging the mitochondria, which in turn leak even more free radicals." In bold print, the article states, "The ultimate irony: The thing we need most to live--oxygen--is what's killing us." Statements such as this, which appear in both the lay press and in scientific publications, point out the currently accepted dogma which states that oxygen and its radicals are highly toxic, even lethal. To the contrary, the overall data shows that these conclusions are, at best, dubious.

HISTORY OF THE FLAWED FREE RADICAL THEORY

In Science in 1954, Rebeca Gerschman and her colleagues first introduced the notion that free radicals are toxic agents.

Gerschman published a paper entitled, "Oxygen poisoning and X-irradiation: a mechanism in common." This paper led to the supposed link between oxygen free radicals and cellular damage, which was later used as the basis for the free radical theory of aging and oxidative stress. In 1956, over 53 years ago, Dr. Denham Harman proposed the free radical theory of aging. The free radical theory of aging simply argues that aging results from the accumulated damage generated over time by EMODs (oxygen free radicals).³

Harman's theory stated that the aging process resulted from the "stochastic" or random accumulated damage caused by EMODs (reactive oxygen species, ROS), many of which are products of normal cellular metabolism.⁴⁻⁷

The mitochondrial electron transport chain (ETC) is an integral player because up to 10% of the reducing equivalents from NADH so-called "leak" to form superoxide anions and H₂O₂, although this is admittedly one of the highest "guestimates" of superoxide production by the ETC.⁸ The commonly quoted range for EMOD ETC production is between 1-5%. The negative opinions regarding EMODs have changed from seeing reactive oxygen species (ROS) and redox states as only sources of damage, to viewing them as integral components in signal transduction.⁹

EMODs and the redox state act as messengers in the intricate regulation of gene expression in development, growth, and apoptosis and there is widespread scientific support for the emerging perspective that EMODs are signaling molecules crucial in numerous cellular functions that are under precise control.

EMODS AND CANCER THERAPY

Cancer treatments using hydrogen peroxide have been around for decades and have been referred to as some of the following: hyperoxygenation, oxymedicine, oxidative therapy, bio-oxidative therapy and oxydology. The older literature based the use of hydrogen peroxide therapy on the work of Otto Warburg, M.D. (two time Nobel Prize in Medicine recipient), who believed that cancer cells grew best and primarily in an environment with hypoxic conditions. A simplistic view of Warburg's advocates was that the administration of hydrogen peroxide, which is an oxygen-rich solution, would restore the proper oxygen balance and selectively attack and kill cancer cells. The website for the University of California, San Diego Medical Center (Moore's Cancer Center) states, "According to the

American Cancer Society (ACS), there is no scientific evidence that hydrogen peroxide is a safe, effective or useful cancer treatment.” (Accessed 9-4-09).

A review of the scientific literature is essential to evaluate the accuracy or veracity of the ACS assessment, regarding the use of hydrogen peroxide for therapeutic purposes. Such a review rebuts their assertion.

Even though there has been “the poisoning of the oxidative watering hole” by the free radical theory for over a half century, world orthodoxy is now acknowledging that EMODs, inclusive of hydrogen peroxide, are of crucial benefit and play a central role in pathogen and neoplasia protection and cellular signaling.¹⁰

HYDROGEN PEROXIDE (HO)

How could the magnanimous wisdom of evolution produce a ubiquitous, omnipresent, allegedly highly damaging, powerful, toxic agent such as the accused hydrogen peroxide molecule, which, by design, is freely mobile and highly permeable through biological membranes, enabling its diffusion out of and within the cell from any intracellular production site? Such a case would defy scientific and evolutionary logic.

Hydrogen peroxide (H₂O₂) is a well-documented and essential component of aerobic living cells. It plays crucial roles in host defense and oxidative biosynthetic reactions. In addition there is growing evidence that at low levels, H₂O₂ also functions as a signaling agent, particularly in higher organisms. All aerobic organisms studied to date, from prokaryotes to humans, appear to tightly regulate their intracellular H₂O₂ concentrations at levels favorable to healthy homeostasis, i.e., 10⁻⁹ to 10⁻⁷M.¹¹

Well defined biochemical pathways involved in the response to exogenous H₂O₂ have been described in both prokaryotes and yeast. In animals and plants, many regulated enzymatic systems generate H₂O₂. In addition oxidation-dependent steps in signal transduction pathways are being uncovered, and evidence is accumulating regarding the nature of the EMOD type(s) involved in each of these pathways. Application of physiologic levels of H₂O₂ to mammalian cells has been shown to stimulate biological responses and to activate specific biochemical pathways in these cells.¹²

Oxidation serves as our first line of defense and occurs with the respiratory burst, which should more properly be referred

to as a “peroxide spike,” secondary to spontaneous or enzymatic dismutation of the superoxide anion.¹³ Ergo, our lives are sustained, at least in part, by an innate “prooxidant-cure” and during times of need, to fight invaders or tumors, we rely on this “peroxide-spike” or “Vis medicatrix naturae” (The Healing Power of Nature). Thus, hydrogen peroxide is a formidable and influential orthomolecular agent.

Disruption of the delicate balance between prooxidants and antioxidants has been implicated in the pathophysiology of many chronic diseases, such as atherosclerosis, cancer, diabetes, strokes, arthritis and cataract formation¹⁴ Unfortunately, far too frequently, we have been “radically misled,” regarding the overstated toxicity of EMODs. (see “The Howes Selective World Library of Oxygen Metabolism,” available at www.thepundit.com).^{15,16}

Prooxidants are fundamental for life and the concentrations of electronically modified oxygen derivatives (EMODs) are important signaling agents, possibly extending to virtually all cellular processes. Oxygen is an important signal in all major aspects of stem cell biology including proliferation and tumorigenesis, cell death and differentiation, self-renewal, and migration.¹⁷ “Redox signaling” is achieved by discrete, localized redox circuitry rather than by so-called “oxidative stress.”¹⁸

This realization represents a significant departure from traditional views that prooxidant EMODs are simply harmful by-products of normal oxidative metabolism or only a tool through which phagocytes accomplish antimicrobial action. With this paradigm shift comes the challenge of understanding how EMOD production is regulated and localized within cells in both normal and pathological circumstances. Current evidence supports a sustaining role for EMODs and a generalized “injury” and protective response in tissues and organs.

HYDROGEN PEROXIDE OVERVIEW

The literature illustrates the wide variety of cells and the distribution of EMOD production in the living/breathing cell. This alone demonstrates their low toxicity and the important role of EMODs in aerobic cells and their wide distribution is a counter argument to their supposed pernicious activity. Noted biochemist, Barry Halliwell, said that, “Over a year, a human body makes 1.7 kilograms or 3.74# of EMODs, which is a conservative estimate.” This begs the question, “Thus, how toxic are EMODs?”

O_2^- and H_2O_2 are cellular signaling molecules and they change the behavior of proteins as diverse as transcription factors and membrane receptors by virtue of their ability to undergo redox reactions with the proteins with which they interact, converting -SH groups to disulfide bonds and changing the oxidation states of enzyme-associated transition metals.

PREVALENT EMOD PRESENCE

EMOD activity has been detected in a wide variety of different cells including mesangial cells, oocytes, Leydig cells, thyroid cells, adipocytes, tumor cells, red blood cells and platelets. O_2^- and H_2O_2 are manufactured by many cell types, encompassing fibroblasts, endothelial and vascular smooth muscle cells, neurons, ova, spermatozoa and cells of the carotid body. Superoxide anions and hydrogen peroxide also participate in the induction of hyperactivated motility and the acrosome reaction.¹⁹

The carotid body is an organ located at the bifurcation of the common carotid artery that measures the oxygen tension of the blood and it produces hydrogen peroxide on a continual basis.²⁰

Halliwell claims that the gastrointestinal tract, especially the stomach, with its highly acidic environment, is constantly generating reactive oxygen species from food. "Every time you drink a cup of coffee it's a dilute bowl of hydrogen peroxide," says Halliwell. The hydrogen peroxide is there because of the presence of the antioxidants – "antioxidants" is really just another way of saying reducing agent, which can react with oxygen in the water to produce hydrogen peroxide.²¹

There is a surprising number of proteins whose operation depends upon the redox state of the cell and includes the general transcription factors NF-kappa B and AP-1 (jun/fos), as well as several transcription factors that induce the synthesis of proteins that protect against so-called oxidative stress (e.g., soxR, soxS, oxyR).

Yet, some have come to view oxygen as a dangerous gift: indispensable for energy production but the alleged cause of damage that accumulates slowly over a lifetime.²²

PEROXIDE, THE TERRIBLE

Some say that under normal healthy conditions, 90% of H_2O_2 is generated as a toxic by-product of the mitochondrial electron transport chain (ETC) respiratory activity.^{23,24}

In the past, some believed that H_2O_2 , which is long lived and highly biomembrane permeable, must be immediately neutralized at the site of production to prevent diffusion throughout the cell or to the extracellular space.²⁵ Specific enzyme systems exist expressly for this purpose. These H_2O_2 neutralizing anti-oxidant enzymes are catalase (E.C. 1.11.1.6) and glutathione peroxidase (GPx, E.C. 1.11.1.9) with GPx responsible for 91% of H_2O_2 consumption.²⁶

Even more alarming, some believe that, if allowed to accumulate, H_2O_2 will diffuse from its site of production and generate hydroxyl radical ($\bullet OH$), which is the most damaging and chemically reactive radical formed by cellular metabolism. They believe that the hydroxyl radical will indiscriminately destroy everything it encounters.²⁷⁻²⁹

The hydroxyl radical is believed to be principally responsible for the cytotoxic effects of oxygen in animals.²⁹

HYDROGEN PEROXIDE'S CRUCIAL ROLE IN NORMAL CELLULAR FUNCTION

Oxygen is the ultimate electron acceptor and reacts with all elements in the periodic table except the noble gases, which have no known biological function. The mitochondrial ETC is not perfect and up to 5% of electrons fail to combine with oxygen to produce water.²⁷ These so-called "leaked" electrons combine directly with molecular oxygen in the immediate vicinity, instead of the next carrier in the chain, to form the superoxide ($O_2^{\bullet -}$) radical.³⁰

Likely, complex I and III, of the ETC, are the source of so-called electron leakage leading to the eventual intracellular generation of hydrogen peroxide.^{31,32} This ETC "leakage" or metabolic reduction of triplet O_2 during cellular respiration produces superoxide anion (O_2^-), which is spontaneously or enzymatically dismutated to the prooxidant, H_2O_2 , within mitochondria by the enzyme superoxide dismutase (SOD).²⁷

H_2O_2 has a pervasive presence in cells and is continuously being produced by the plasma membrane, cytosol and different subcellular organelles including mitochondria, peroxisomes, endoplasmic reticulum, nucleus and by almost 100 enzyme systems.^{23, 25}

Studies have determined that H_2O_2 is a small, diffusible, and ubiquitous molecule that can be synthesized, modified and/or destroyed rapidly in response to external stimuli, it meets all of the important criteria for an intracellular messenger, and H_2O_2 is now firmly established as a

ubiquitous intracellular messenger under subtoxic conditions.³³⁻³⁶

As previously mentioned, metabolic reduction of triplet O₂ during cellular respiration produces superoxide anion radical (O₂⁻), which is spontaneously or enzymatically dismutated to the prooxidant, non-radical, H₂O₂. Varying cell types produce low levels of O₂⁻ and H₂O₂ in response to a variety of extracellular stimuli, including cytokines (TGF- β 1, TNF- β , and IL), peptide growth factors (PDGF; EGF, VEGF, bFGF, and insulin), the agonists of heterotrimeric G protein-coupled receptors (GPCR; angiotensin II, thrombin, lysophosphatidic acid, sphingosine 1-phosphate, histamine, and bradykinin), and shear stress. Research continues to uncover additional important sources of hydrogen peroxide in aerobic cells.

Hydrogen peroxide (H₂O₂) is present in exhaled breath and condensate and is produced by airway epithelia. Additionally, H₂O₂ is a vital substrate for the airway lactoperoxidase (LPO) anti-infection system. Duox is the major NADPH oxidase expressed in airway epithelia and therefore a contributor of H₂O₂ production in the airway lumen.³⁷

Although specific levels of ubiquitous H₂O₂ have been debatable, animals and humans have between 5.0 and 41 microM for aqueous humor and 115 and 187 microM for urine.^{38, 39}

Again, this begs the question, “Just how toxic are EMODs?”

It was believed that oxidative stress was the hallmark of asthma and increased levels of oxidants, such as H₂O₂ were considered a marker of the inflammatory process. In contrast to this notion, current studies suggest that hydrogen peroxide serves a role in suppressing both mucus production and airway hyper-responsiveness.⁴⁰

Hydrogen peroxide production has also been found in the nucleus of epithelial cells and it could convey redox signals altering gene expression.^{41, 42} H₂O₂ is a natural orthomolecular substance and has been detected in serum and in intact liver.⁴³

In mouse pancreatic beta cells, H₂O₂ hyperpolarizes the cell membrane coupled with an increase of cell membrane conductance.⁴⁴

Moreover, it has recently been shown that H₂O₂

- increases intracellular Ca²⁺
- decreases the ATP/ADP ratio
- and inhibits glucose-stimulated insulin secretion from isolated mouse islets. 45

HO AS AN INSULIN MIMETIC

Long ago, it was demonstrated that polyamines are able to exert insulin-like effects in fat cells through the production of hydrogen peroxide.⁴⁶

Hydrogen peroxide is now known to cause the reversible inhibition of protein tyrosine phosphatases (PTP) in cells, thereby strengthening insulin signaling. It has also been shown that production of hydrogen peroxide chemically in cells acts as an insulin mimetic. H₂O₂ improves glucose utilization in diabetics. Membrane receptors and transporters, including the insulin receptor and receptors for certain neurotransmitters, are regulated by the redox state of the cell.

Pancreatic β -cells are extremely sensitive to oxidative stress because of the low expression and activity of antioxidant enzymes. The GSH/GSSG (reduced/oxidized glutathione) ratio in islets is low compared with other tissues.⁴⁷

A 10-6-09 article by Tony Tiganis in the journal Cell Metabolism shows that “mice that lacked the antioxidant enzyme Gpx1 were less likely to develop insulin resistance - - an early sign of diabetes -- than normal mice. But when they treated the enzyme-deficient mice with an antioxidant, they lost this advantage and become more diabetic.” Tiganis said, “Our work suggests that antioxidants may contribute to early development of insulin resistance, a key pathological hallmark of type 2 diabetes.”

OTHER ROLES FOR PEROXIDE

Hydrogen peroxide is only one of the many components that help regulate the amount of oxygen getting to cells. Its presence is vital for many other functions as well. It is required for the production of thyroid hormone and sexual hormones. Even the synthesis of thyroxin depends on the requirements of a H₂O₂ substrate for thyroid peroxidase and it stimulates the production of interferon.

A role for EMODs in controlling oxygen-sensitive channel function in excitable cells has been demonstrated previously. Hydrogen peroxide is capable of activating potassium

transport pathways in excitable cells and in alveolar epithelial cells. These data suggest that EMODs and the hydroxyl radicals, formed from O₂ in close vicinity to the cell membrane, play an important role in the oxygen-dependent activation of the K⁺-Cl⁻ co-transporter.⁴⁸

H₂O₂ is produced by the autooxidation of ascorbic acid (vitamin C) and catecholamines, such as dopamine, norepinephrine and serotonin.⁴⁹

Amazingly, H₂O₂ is generated at a rate of 1.36 +/- 0.2 microM/h (3.9 +/- 0.6 nmol.h-1.g Hb-1), and a steady-state red blood cell concentration of H₂O₂ which is approximately 2 x 10⁻¹⁰ M. Kinetic comparisons of H₂O₂ production and oxyhemoglobin autooxidation (which generates O₂⁻ that dismutates to H₂O₂) suggests that the latter is the main source of H₂O₂ in red blood cells.⁵⁰

Apparently, many articles and agencies are unaware of peroxide's prime importance because studies have shown that the addition of exogenous H₂O₂, or its intracellular production in response to receptor stimulation, affects the function of various proteins, including protein kinases, protein phosphatases, transcription factors, phospholipases, ion channels, and G proteins.

EMODs can induce cellular senescence and apoptosis and can therefore function as anti-tumorigenic species.⁵¹

Another 100+ articles related to hydrogen peroxide's varied roles, including apoptosis, can be found at:
<http://www.caspases.org/showcitationlist.php?keyword=hydrogen%20peroxide%20h2o2>,

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HO CAN DILATE ARTERIES

H₂O₂ can dilate blood vessels in the heart and brain. Mechano-sensitive mechanisms that are sensitive to deformation, pressure, stretch, and wall shear stress elicit release of NO and H₂O₂, resulting in reactive dilation of isolated coronary arterioles.⁵²

On June 23, 2009, the Medical College of Wisconsin received a four year, \$1.5 million grant from the National Institutes of Health's National Heart, Lung and Blood

Institute to study the role of naturally produced hydrogen peroxide in controlling human blood flow. Dr. David Gutterman's lab has observed a unique relationship between dilation in heart blood vessels from patients with coronary artery disease and the endothelial production of hydrogen peroxide and thus far, the association of vessel dilation with mitochondrial hydrogen peroxide has only been reported in human hearts.

EMOD INDUCED APOPTOTIC CANCER CELL DEATH

Cancer accounts for nearly 25% of all human deaths and no definitive cure is available as yet.⁵³ The most common treatment modalities promising a cure appear to be a combination of radiotherapy and chemotherapy and these methods, in part, utilize the reactivities of prooxidant EMODs, including hydrogen peroxide. Radiation exposure leads to the hydrolysis of water, thereby generating EMODs, which initiate chemical peroxidative processes that destroy biomolecules.⁵⁴

The postulated mechanism of action for many forms of chemotherapy, radiation therapy, photodynamic therapy, ozone therapy, hyperbaric oxygen therapy, intravenous mega-dose of vitamin C, the Howes singlet oxygen cancer therapy system and hydrogen peroxide therapy is the generation of electronically modified oxygen derivatives (EMODs). The production of EMODs leads to the stimulation of various signaling pathways, and in particular, stress-responsive signal transduction pathways are strictly regulated by the intracellular redox state.^{55,56}

HIGH LEVEL ACUTE HO TREATMENT OF VARIOUS CELLS IN VITRO LEADS TO APOPTOSIS.

However, some investigators believe that hydrogen peroxide can cause apoptosis via a non-apoptotic pathway. Recent studies in a variety of cell types have suggested that cancer chemotherapy drugs induce tumor cell apoptosis in part by inducing formation of reactive oxygen species (EMODs). Investigators demonstrated that, at least in B lymphoma cells, chemotherapy-induced apoptosis occur using a mechanism that does not involve oxidants. Hydrogen peroxide, which reportedly kills cells by a non-apoptotic pathway, caused increases in both protein and lipid oxidation.⁵⁸

HYDROGEN PEROXIDE AND HYDROXYL

RADICAL ANTINEOPLASTIC CYTOTOXICITY

The cytotoxicity of the antineoplastic quinones doxorubicin, mitomycin C, and diaziridinylbenzoquinone for the Ehrlich ascites carcinoma can be significantly reduced or abolished by the antioxidant enzymes catalase and superoxide dismutase, the hydroxyl radical scavengers dimethyl sulfoxide, diethylurea, and thiourea, and the iron chelators deferoxamine, 2,2-bipyridine, and diethylenetriaminepentaacetic acid. Furthermore, treatment of intact tumor cells with doxorubicin, mitomycin C, and diaziridinylbenzoquinone required hydrogen peroxide, iron, and intact tumor cells. These results suggest that drug-induced hydrogen peroxide and hydroxyl radical production has a role in the antineoplastic action of redox active anticancer quinones.⁵⁹

PHOTODYNAMIC THERAPY (PDT) AND SINGLET OXYGEN

Photodynamic therapy (PDT) is a novel approach for destruction of malignant cells and involves the administration of nontoxic dyes known as photosensitisers (PS) either systemically or topically, followed by illumination of the lesion with visible light (usually red).⁶⁰ The PS absorbs the light, and in the presence of oxygen, transfers the energy, thereby producing cytotoxic oxygen species (either singlet oxygen or oxygen radicals, i.e., EMODs).^{61, 62}

This reportedly leads to a rapid tumoricidal response mediated by both direct tumor cell toxicity and photodamage to the involved microvasculature and cellular structure.⁶³

Investigations have demonstrated that apoptotic and necrotic pathways are both involved in PDT-mediated cell death.^{60, 64}

A wide distribution of early response genes, genes associated with signal transduction pathways and cytokine expression, as well as stress response genes, are activated by PDT and primarily singlet oxygen.⁶⁵⁻⁷¹

EMODS CAN HAVE COMPLEX INTERACTIONS

Interestingly, EMODs can react and interact to produce a family of products. For example, superoxide can react with itself to produce hydrogen peroxide or it can undergo univalent reduction to form hydrogen peroxide. Superoxide can react with the hydroxyl radical to produce singlet oxygen. Superoxide can also react with nitric oxide (NO) (also a radical) to produce peroxynitrate (OONO). Singlet oxygen can react with superoxide to produce hydrogen

peroxide. Hydrogen peroxide can react with hypohalous acid to produce singlet oxygen. Hydrogen peroxide, in the presence of metal ions, can react to form a hydroxyl radical (HO[•]) and the hydroxide ion (HO⁻). Ozone can react with water to form hydrogen peroxide. Methylene blue and superoxide dismutase produce H₂O₂. Ascorbate can react with singlet oxygen and produce hydrogen peroxide.

Examples of the cross reactivity of EMODs illustrate the generation of a family of EMODs from the more basic EMOD agents (superoxide, hydrogen peroxide and singlet oxygen).

A ROLE FOR HYDROGEN PEROXIDE IN THE PRO-APOPTOTIC EFFECTS OF PHOTODYNAMIC THERAPY

Although the first EMOD formed during irradiation of photosensitized cells is almost invariably singlet molecular oxygen, ¹O₂^{*}, other EMODs have been implicated in the phototoxic effects of photodynamic therapy. Among these are superoxide anion radical (O₂^{-•}), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH[•]). Investigators studied the role of H₂O₂ in the pro-apoptotic response to PDT in murine leukemia P388 cells. A primary route for detoxification of cellular H₂O₂ involves the peroxisomal enzyme catalase. Inhibition of catalase activity by 3-amino-1,2,4-triazole led to an increased apoptotic response. PDT-induced apoptosis was impaired by addition of an exogenous recombinant catalase analog (CAT-skl) that was specifically designed to enter cells and more efficiently localize in peroxisomes. A similar effect was observed upon addition of 2,2'-bipyridine, a reagent that can chelate Fe(+2), a co-factor in the Fenton reaction that results in the conversion of H₂O₂ to the hydroxyl radical (OH[•]). These results provide evidence that formation of H₂O₂ during irradiation of photosensitized cells contributes to PDT efficacy.⁷²

HO, O AND OH. ARE INVOLVED IN PHOTOTOXIC TUMORICIDAL ACTION

Researchers estimated the participation of EMODs, other than singlet oxygen (¹O₂^{*}), in the antitumor effect of PDT with hematoporphyrin derivative (HPD) as well as determined the ability of photoexcited HPD to the formation of protein peroxides that are regarded as a new form of EMOD. Experiments indicated that H₂O₂ and oxygen radicals could mediate the tumoricidal action of HPD-PDT; they found that photosensitization of EAC cells with HPD leads to the formation of significant amounts of H₂O₂,

superoxide (O_2^-) and hydroxyl (OH \cdot) radicals, which along with $^1O_2^*$ were involved in photoinactivation of the cells in vitro. Their data showed that in EAC cells subjected to HPD-PDT, the generation H_2O_2 , O_2^- and OH \cdot could be largely mediated by: (i) an increase in the activity of xanthine oxidase (XOD), due most probably to the conversion of xanthine dehydrogenase (XDH) to XOD via a Ca^{2+} -dependent proteolytic process as well as oxidation of SH groups in XDH; and (ii) photooxidation of some cellular constituents (proteins). Another interesting finding of their studies was that in tumor cells subjected to HPD-PDT the Fenton-like reactions could play an important role in the generation of OH \cdot , and that cell-bound Cu/Zn-superoxide dismutase as well as catalase can protect tumor cells against the phototoxic action of HPD. They clearly demonstrated the ability of photoexcited HPD to the generation of protein peroxides in tumor cells. Studies suggest that $^1O_2^*$ is the main agent responsible for the generation of protein peroxides in EAC cells treated with HPD-PDT, although other EMODs (H_2O_2 , O_2^- and OH \cdot) were also implicated in this process.⁷³

Photofrin® is a purified form of hematoporphyrin derivative; it is a photosensitizer used in the treatment of cancer. Upon exposure to light it produces singlet oxygen, a highly electrophilic species that initiates oxidations that lead to cell death.^{74, 75}

Interestingly, singlet oxygen reacts readily with ascorbate, producing hydrogen peroxide.^{76, 77}

UBIQUITOUS AND OMNIPRESENT HO

The biochemistry of H_2O_2 is also, to a considerable extent, the biochemistry of the superoxide anion. Superoxide anion (O_2^-) converts rapidly to H_2O_2 either spontaneously or with the help of one of the superoxide dismutase enzymes (i.e., CuZnSOD, EcSOD, MnSOD, etc.). Since many of the EMODs are rapidly converted and/or transformed into other EMOD types, it also serves as a source of a large family of EMOD agents.

The two primary metabolic EMODs are O_2^- and H_2O_2 . O_2^- is the primary stoichiometric precursor of H_2O_2 but considerable H_2O_2 can be produced directly and not via the superoxide anion.

Chance, et al. reviewed the metabolism of H_2O_2 in mammalian systems.¹¹

Though controversial, many papers still state that the mitochondria are the major cellular source of hydrogen peroxide. The amount of H_2O_2 produced by brain mitochondria is up to 5% of the amount of O_2 consumed. H_2O_2 may be produced directly as a product of biological oxidations, or it may be produced by the dismutation of superoxide. The relative estimates of subcellular sources of H_2O_2 are as follows:

- Endoplasmic reticulum (mixed function oxidations) 45%
- Peroxisomes (metal-catalyzed oxidations) 35%
- Mitochondria (oxidative phosphorylation) 15%
- Cytosol (xanthine oxidation) 5%

These EMOD agents are formed continuously in all aerobic cells, either via oxygen energy metabolism, through reactions with drugs or toxins or via metabolism of fatty acids.

In 1999, Juan and Buettner calculated the steady state (ss) levels to be as follows:

- $[H_2O_2]_{ss}$ in red cells is 10-10 M
- $[H_2O_2]_{ss}$ in mitochondrial membrane is 10-8 M
- $[H_2O_2]_{ss}$ in liver cells is 10-8 M
- $[O_2]_{ss}$ is 10-5 M, much higher than $[H_2O_2]$
- $[O_2^-]_{ss}$ in cells is 10-10 M

An additional source of O_2^- and H_2O_2 is the NADPH oxidase families (NOX and DuOX), which are found on various cellular membranes. Once thought to be restricted only to phagocytes, stimulated H_2O_2 production is now known to occur in almost all cells through NOX and/or DuOX activities. Thus, H_2O_2 has been accused of contributing to pathology through its reaction with transition metals that produce hydroxyl radicals via the Fenton reaction. Yet, even though it is not discussed, two hydroxyl radicals can combine to form hydrogen peroxide. This could well represent another salutary pathway for peroxide formation and pathogen and neoplasia defense.

H_2O_2 is involved in the generation of hypohalous acids through catalysis by myeloperoxidases and lactoperoxidase.

These oxidizing acids are capable of killing microorganisms and allegedly causing tissue damage during inflammation. Ubiquitous H_2O_2 acts as a secondary messenger in signal transduction through its reaction with key proteins containing critical cysteine residues.^{78, 79}

Yet, the antioxidant, cysteine, itself can undergo autoxidation and form H_2O_2 . This is somewhat analogous to the prooxidant EMOD activity of ascorbic acid.

The most common prooxidant in vivo is H_2O_2 and under inflammatory conditions, it is abundantly formed by dismutation of O_2^- released from activated phagocytes and other enzymatic systems,⁸⁰ but physiologically also by intracellular NADPH oxidases.⁸¹

Although some H_2O_2 appears to be produced constitutively, receptor-mediated H_2O_2 formation appears to be more common. Typical examples are TNF α -induced mitochondrial O_2^- formation,⁸² or cytoplasmic increase of H_2O_2 upon growth factor receptor stimulation.⁸³ Nonetheless, H_2O_2 is argued to be the most common prooxidant in vivo.

In a study on synergism between tumor necrosis factor- α and H_2O_2 , levels of cellular toxicity were found. With PC12 tumor cells, TNF α toxicity was seen at >50 ng/ml, and that of H_2O_2 at > 150 microM. However, when together, sub-lethal levels (25 ng/ml TNF α and 30 microM H_2O_2) induced toxicity.⁸⁴

Please remember that the chemistry of superoxide is also the chemistry of hydrogen peroxide. Once activated, phagocytes produce large quantities of superoxide, on the order of $10\text{ nmol}\cdot\text{min}^{-1}\cdot 10^6\text{ neutrophils}^{-1}$ during the oxidative burst.⁸⁰

The rate of superoxide production in vascular cells is thought to be ~1-10% of that in leukocytes.^{85, 86} Basically, all cells continuously form O_2^- and submitochondrial particles generate O_2^- at a rate of $4\text{-}7\text{ nmol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$.¹¹

Superoxide anion can be viewed as an innate pathway for hydrogen peroxide production. Thus, EMODs are intentionally being generated to serve as salutary cellular products intended to help regulate critical metabolic and reproductive mechanisms.⁸⁷ These prooxidant EMODs stimulate numerous transcription factors as well as signaling cascades via activation of kinases and inhibition of tyrosine phosphatases.

Under physiological conditions, the intracellular production

of EMODs does not alter the redox state of cells which have large reserves of reducing agents, notably reduced glutathione, as well as extremely effective antioxidant defense mechanisms, such as SOD, catalase, and peroxidases. This allows agonist-induced increases in EMODs to function as second messengers by limiting their effecting time and space in a manner similar to other well-known intracellular signals, such as cyclic AMP or nitric oxide.⁸⁸

Differing from O_2^- that is charged, hardly permeable, and extremely short-lived, H_2O_2 is uncharged, relatively longer-lived, and freely diffusible. This property makes H_2O_2 an ideal signaling molecule. Clearly, this illustrates the great importance of H_2O_2 in combination with other EMODs, in the regulation of cellular homeostasis and redox status. Under basal conditions, human cells produce about 2×10^9 (2 billion) O_2^- and H_2O_2 molecules per cell per day.⁸⁹

EMODs are recognized as controlling key steps in cellular signal transduction cascades.^{90, 91} EMODs can reversibly control gene expression and regulation at non-cytotoxic doses.⁹² More specifically, there is evidence that hydrogen peroxide, H_2O_2 , can modulate cellular functions through altering signal transduction in many cell types, including endothelial cells (ECs), vascular smooth muscle cells (VSMC), and T cells.⁹³⁻⁹⁷

In fact, H_2O_2 is now widely recognized as a ubiquitous intracellular messenger, under subtoxic conditions.^{23, 34, 35, 36, 98-100} As of 2000, at least 127 genes and signal transducing proteins had been reported to be sensitive to reductive and oxidative (redox) states in the cell.⁹² That number, as of 2009, now exceeds 200.

EMODS KILL CANCER

Otto Warburg was the primary scientist to implicate oxygen in cancer.¹⁰¹ Anaerobic metabolism is favorable to many pathogenic organisms and hypoxia is the predominant condition within neoplastic cells. Hypoxia has been closely associated with pathological processes.¹⁰² Requiring adequate oxygen levels, many anticancer drugs and radiation kill cancer cells by inducing prooxidant EMOD apoptosis.¹⁰³

High level acute H_2O_2 treatment of various cells in vitro leads to apoptosis, with the involvement of NADPH oxidase isoforms and Src family kinases.¹⁰⁴

The chemotherapeutic agents doxorubicin, mitomycin C,

etoposide and cisplatin are superoxide generating agents and consequently hydrogen peroxide producers.¹⁰⁵

Bleomycin and doxorubicin are agents shown to produce prooxidant oxygen agents.¹⁰⁶

In reactions involving Fe(II) and oxygen, a so-called “activated” bleomycin species is generated that damages DNA through free radical intermediates.¹⁰⁷

Superoxide and hydrogen peroxide can also react with Fe(II) or Fe(III) bleomycin, respectively, to produce the activated form of the drug. DNA damage from bleomycin and ionizing radiation is similar in both induction and repair.¹⁰⁸ Several other anti-cancer drugs are known to bring about their tumoricidal actions by an EMOD dependent mechanism. A majority of the studies reported that adriamycin, mitomycin C, etc., augment EMOD production (superoxide anion and hydrogen peroxide) and lipid peroxidation in vitro and in vivo.¹⁰⁹

The anti-estrogen tamoxifen, increasingly used alongside other breast cancer therapies, has also been shown to induce oxidative stress (EMOD production) within carcinoma cells in vitro.¹¹⁰

Oxygen levels are important since radiotherapy and photodynamic therapy generate oxygen radicals within the carcinoma cell. Thus, tumor hypoxia is a therapeutic concern since it can reduce the effectiveness of radiotherapy, some O₂-dependent cytotoxic agents, and photodynamic therapy.¹¹¹

HO INCREASES DOXORUBICIN KILL OF BLADDER TUMOR CELLS

Investigators determined whether the cytotoxicity of doxorubicin hydrochloride would be enhanced by adding hydrogen peroxide as a source of oxygen free radicals. Mouse bladder tumor cells (MBT-2) were grown in RPMI 1640 medium and treated with various concentrations of doxorubicin hydrochloride for 2 hours. They observed a dose dependent inhibition of MBT-2 cell growth after exposure to doxorubicin hydrochloride. Exposure to doxorubicin and hydrogen peroxide resulted in greater cell growth inhibition than exposure to either agent alone. The effects of hydrogen peroxide on cell proliferation were reversed by pre-incubation with alpha-tocopherol. As a source of oxygen free radicals, hydrogen peroxide enhances the antiproliferative effect of doxorubicin hydrochloride on a mouse bladder tumor cell line.¹¹²

HO INCREASES SULINDAC KILL OF SQUAMOUS CELL CARCINOMA

A skin squamous cell carcinoma (SCC-25) cell line was utilized and treated with sulindac prior to exposure to tert-butyl hydroperoxide (TBHP) or hydrogen peroxide for 2 hours. The combination of sulindac and TBHP enhanced the killing of the skin cancer cells. Sulindac combined with TBHP leads to markedly increased levels of intracellular EMODs in SCC cells.

A small group of patients with actinic keratoses (AKs), were treated with the combination of sulindac and hydrogen peroxide gels. The results revealed that 60% of the treated AKs responded to therapy by exhibiting a decrease in size or becoming not visible to the naked eye. In addition, 50% of the treated AKs showed no residual AK on histopathology specimens after skin biopsy at the end of the study. Researchers concluded that the combination of sulindac and TBHP or H₂O₂ significantly enhances the killing of SCC cells.¹¹³

THE BAYLOR GROUP PEROXIDE EXPERIENCE

In the 1960s, a group of investigators at Baylor Medical School (the Baylor group) conducted ground breaking studies with hydrogen peroxide in the treatment of a wide range of disease conditions. Various investigators have studied the value of H₂O₂ and shrinking the size of tumors,¹¹⁴ and have studied treatment advantages and increased tumor cytotoxicity by the use of regional H₂O₂ infusion.¹¹⁵

Earlier work indicated that hydrogen peroxide, a secretory product of mononuclear phagocytes,¹¹⁶ accounts for a considerable portion of their nonphagocytic lysis of tumor cells in at least three circumstances: when certain secretagogues were added, when antitumor antibody was present, or when the tumor cells were coated with eosinophil peroxidase.¹¹⁷

Many clinical and experimental applications of hydrogen peroxide have been demonstrated by the Baylor group. In over 300 patients regional intra-arterial hydrogen peroxide has potentiated the effect of radiation therapy in situations of malignancy involving the head, neck, pelvis and retro-peritoneum.¹¹⁸

Increased localization of radioactive isotopes in malignant tumors has been achieved by regional and intra-arterial infusion of hydrogen peroxide.^{119, 120}

Granulocytes also secrete H_2O_2 , which may participate in their cytotoxic effects in a variety of conditions. Preformed or enzymatically generated H_2O_2 , with or without a peroxidase, lyses tumor cells, which was shown by Nathan's group and others.¹²¹⁻¹²⁷

Reports have suggested that hydrogen peroxide released by mononuclear phagocytes and neutrophils may extend the antimicrobial, antitumor, and oxidant-injury activities of these cells to adjacent tissues.^{128, 129}

Nathan devised a nontoxic way to deliver hydrogen peroxide to sites of malignancy in vivo and to test its antitumor efficacy. Glucose oxidase was chosen for this purpose because its substrates, glucose and oxygen, are abundant in the body fluids and because its sole products are H_2O_2 and gluconic acid.¹³⁰ Glucose oxidase was coupled covalently to polystyrene microspheres (GOL) produced H_2O_2 . Injection i.p. prolonged the survival of mice by 27% after injection of 106 P388 lymphoma cells in the same site, consistent with destruction of 97.6% of the tumor cells. Placing mice for several hours in 100% O_2 , the probable rate-limiting substrate for GOL, afforded a 42% prolongation of survival from P388 lymphoma, consistent with destruction of 99.6% of the tumor cells. A single injection of preformed H_2O_2 readily killed P388 cells in the peritoneal cavity, but only at doses nearly lethal to the mice. In contrast, GOL had very little toxicity. Thus, an H_2O_2 -generating system confined to the tumor bed exerted clear-cut antitumor effects with little toxicity to the host.

Studies demonstrated that a combination of sub-lytic concentrations of chemically generated NO and H_2O_2 leads to death of murine lymphoma cells, in part, via induction of apoptosis.¹³¹ In vitro studies have suggested that a reaction of nitric oxide (NO) gas and H_2O_2 produces singlet oxygen (which is the primary cytotoxic agent in PDT) or hydroxyl radicals.^{132, 133} Nevertheless, this has not yet been demonstrated in cell cultures.

OTHERS ARGUE THAT EMODS MAY INHIBIT APOPTOSIS

In the past, the main focus of the importance of EMODs in oncology was that these agents were capable of inducing DNA damage, which could theoretically lead to cellular proliferation and a predisposition to cancer.

Even though an overwhelming accumulation of intracellular EMODs can create an oxidatively stressed environment

leading to necrosis, a slight increase is a stimulus for cellular proliferation.^{134, 135} Burdon et al interpreted this to mean that sublethal oxidative stress promotes cell proliferation in vitro, with both superoxide and hydrogen peroxide stimulating growth. Proliferation in response to hydrogen peroxide may be due to the activation of mitogen-activated protein kinases (MAPKs). HeLa cells treated with hydrogen peroxide undergo a sustained activation of all three MAPK pathways: extracellular signal related protein kinase; c-Jun amino-terminal kinase/stress-activated protein kinase; and p38.

However, Howes believes that a more logical explanation may be that an EMOD insufficiency "allows" for cell proliferation.¹⁵

Theoretically, the carcinogenic process in animal models involves initiation and promotion. Allegedly, the production of EMODs and hydrogen peroxide occurs with several known tumor promoters, including 12-O-tetradecanoylphorbol-13-acetate (TPA), okadaic acid (OA), thapsigargin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and H_2O_2 , as well as peroxisome proliferators, steroidal estrogens, phenobarbital, chlordane and aroclor. However, their specific mechanism of action is evasive and speculative.¹³⁶

Severe oxidative stress leads to apoptosis. Conversely, persistent oxidative stress at sublethal levels may cause resistance to apoptosis. The induction of programmed cell death by EMODs is dependent on p53 in both mouse and human cell lines.¹³⁷ Current studies indicating a possible mechanism whereby H_2O_2 could promote tumor formation is sparse.¹³⁸ Further, many of these studies were carried out using non-physiological levels of H_2O_2 , which can have cytotoxic activity.

However, this is in contrast to the work of others, who state that O_2^- and H_2O_2 do not react with DNA bases at all.¹³⁹ Yet, the hydroxyl radical (OH \cdot) generates a variety of products from all four DNA bases and this pattern is used as a diagnostic "fingerprint" of OH \cdot attack.¹⁴⁰

Singlet oxygen selectively attacks guanine to produce the 8-hydroxyguanine (8-OHG), which is also used as an index of oxidative damage to DNA and it can be measured as the nucleoside, 8-hydroxydeoxyguanosine (8-OHdG).

Some investigators believe that H_2O_2 may act as a "genotoxicant" or "epigenetic" agent and act as a promoting agent. However, even if hydrogen peroxide could cause

DNA damage, peroxide is at best a very weak mutagen in mammalian cells.¹³⁶

Some endogenous DNA damage arises from intermediates of oxygen reduction that either attack the bases or the deoxyribosyl backbone of DNA. Yet, the study of oxidative DNA damage and its role in carcinogenesis is still controversial.¹⁴¹

The International Agency for Research on Cancer (IARC) has determined that hydrogen peroxide is not classifiable as to its carcinogenicity to humans. Even though accusations against H₂O₂ have been wide spread, there is no verified data, in man, that H₂O₂ in any way causes or promotes cancer in vivo. The WHO-IARC said, "There is inadequate evidence in humans for the carcinogenicity of hydrogen peroxide."

HYPEROXIA

Under conditions of hyperoxia, mitochondrial EMOD generation increases as a linear function of the oxygen tension.¹⁴² Oxygen is critical to aerobic metabolism, but excessive oxygen (hyperoxia) can cause cell injury and death. An oxygen-tolerant strain of HeLa cells, which proliferates even under 80% O₂, termed "HeLa-80," was derived from wild-type HeLa cells ("HeLa-20") by selection for resistance to stepwise increases of oxygen partial pressure. Unpredictably, antioxidant defenses and susceptibility to oxidant-mediated killing do not differ between these two strains of HeLa cells. However, under both 20 and 80% O₂, intracellular reactive oxygen species (EMODs) production is significantly (~2-fold) less in HeLa-80 cells. In both cell lines the source of EMODs is evidently mitochondrial. Although HeLa-80 cells consume oxygen at the same rate as HeLa-20 cells, they consume less glucose and produce less lactic acid. Most importantly, the oxygen-tolerant HeLa-80 cells have significantly higher cytochrome c oxidase activity (~2-fold), which may act to deplete upstream electron-rich intermediates responsible for EMOD generation. Indeed, preferential inhibition of cytochrome c oxidase by treatment with n-methyl protoporphyrin (which selectively diminishes synthesis of heme a in cytochrome c oxidase) enhances EMOD production and abrogates the oxygen tolerance of the HeLa-80 cells. Thus, it appears that the remarkable oxygen tolerance of these cells derives from tighter coupling of the electron transport chain and reduced EMOD production.¹⁴³

VITAMIN C

The ultimate agent to treat cancer would be cytotoxic only to tumor cells and non-toxic to normal cells. Vitamin C has been theorized to meet these requirements but has been criticized by conventional medicine in favor of more powerful and toxic chemotherapeutic agents.¹⁴⁴ Riordan found that at a dose of 7.04 mg/dl, vitamin C is completely toxic to cancer cells while being completely non-toxic to normal cells. Only at eight times the dose needed to kill cancer cells does vitamin C become toxic to normal cells. This reveals its considerable clinical potential.¹⁴⁵

Metabolically, vitamin C produces dehydroascorbate (DHA), an oxidant. Normal cells take in DHA, which is then converted to ascorbate and H₂O₂, by an oxidation/reduction (redox) electron transfer. Benade et al at the National Cancer Institute found that, in Ehrlich ascites carcinoma cell cultures, vitamin C selectively destroyed cancer cells by generating excess intracellular H₂O₂.¹⁴⁶

It has been observed for a long time that ascorbic acid and ascorbic acid salts are preferentially toxic to tumor cells, which were thought to be related to intracellular generation of hydrogen peroxide.¹⁴⁷⁻¹⁴⁹ It is theorized that cancer cells are less able than normal cells to neutralize H₂O₂ because they are deficient in catalase. Dr. Agus et al reported that cancer cells have extra glucose channels that rapidly bring in glucose and excess DHA.¹⁵⁰

Cancer cells are defective in that they cannot fully distinguish between glucose and DHA. This may explain why vitamin C is safe in large doses for normal cells but toxic to cancer cells. The good results of Cameron and Hoffer with humans confirm the National Cancer Institute lab tests.

Mark Levine's group published a study on line for PNAS on September 12, 2005, with results showing that, "Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues." Human lymphoma cells were studied because of their sensitivity to ascorbate (EC50 of 0.5 mM) and suitability for evaluating mechanisms. Extracellular, but not intracellular, ascorbate resulted in cell death, which occurred by apoptosis and pyknosis/necrosis. Cell death was independent of metal chelators and absolutely dependent on H₂O₂ formation.¹⁵¹

Investigators stated that it was not known why it killed

cancer cells but not normal cells. They felt that it was possible the hydrogen peroxide caused damage that was repaired in normal cells but not in sensitive cancer cells. The main mechanism thought to be responsible for this is the lack or relative deficiency of catalase in tumor cells.¹⁵²

Therefore, it takes a smaller amount of H₂O₂ to reach or “trigger” apoptosis. This is the point of selectivity for toxicity to cancer cells, wherein there is no harm to normal cells. Interestingly, there is a reported 10- to 100-fold greater content of catalase in normal cells than in tumor cells.¹⁴⁶

Humans lack gulonolactone oxidase, which is necessary to synthesize vitamin C and H₂O₂ is produced as a by-product in the process. It is incredibly ironic, that in the synthesis of one of the most touted of all of the antioxidants, ascorbate, that “dreaded H₂O₂” is generated stoichiometrically on a molecule per molecule basis. This illustrates the fact that H₂O₂ is very important in maintaining homeostasis within the cell and as a secondary messenger and that antioxidants and prooxidants may be considered to be flip sides of the same redox coin.

HO INDUCED APOPTOSIS IN HUMAN GASTRIC CANCER CELLS

Investigations were made into the molecular mechanism by which ascorbic acid (AA) induces apoptosis in human gastric cancer cells, AGS cells. High concentration (more than 5mM) of AA increased cellular iron uptake by increasing transferrin receptor (TfR) expression and induced AGS cell apoptosis which was inhibited by catalase. Interestingly, p38 mitogen-activated protein kinase (MAPK) inhibitor inhibited the upregulation of TfR and increased cell survival by AA. TfR-siRNA-transfected cells reduced apoptosis by AA. H₂O₂ increased TfR expression in AGS cells. Taken together, investigators concluded that high concentration of AA, through H₂O₂, induces apoptosis of AGS cells by p38-MAPK-dependent upregulation of TfR.¹⁵³

HO REGULATION OF T CELLS

The immune system is vital to protect us against infectious agents (bacteria, viruses, fungi, protozoans and cancer). Patients with T Cell immunodeficiencies are prone to infections and to certain types of cancers, especially leukemias and lymphomas.

There is evidence that T cells themselves produce H₂O₂ upon stimulation of their antigen receptor.^{154, 155} A potential source for the unique production of H₂O₂ is the T cell receptor itself.

This proposal comes from studies with isolated antibodies, which have the ability to catalyze a light-dependent reaction between molecular oxygen and water that leads to the production of H₂O₂.^{156, 157}

These events occur in all antibodies, regardless of source or antigenic specificity. The reaction is initiated by singlet oxygen that reacts with H₂O to ultimately produce H₂O₂ via intermediates such as H₂O₃ and ozone.¹⁵⁸

HO SAFETY

The Food & Drug Administration (FDA) in Federal Regulation Vol. 46, Number 6, Jan 9, 1981, in effect gave the food industry a green light to use hydrogen peroxide in the “Aseptic” packaging process. The FDA has further ruled that hydrogen peroxide can be used in the processing of cheese and related cheese products (part 133), eggs and egg products (part 160), and as an anti-microbial agent in whey processing. They have also ruled it to be used in cleaning and healing mouth injuries and as a mouthwash (1% to 3% food grade H₂O₂).

As previously stated, “The International Agency for Research on Cancer (IARC) has determined that hydrogen peroxide is not classifiable as to its carcinogenicity to humans. Even though accusations against H₂O₂ have been wide spread, there is no verified data, in man, that H₂O₂ in any way causes or promotes cancer in vivo.” The WHO-IARC said, “There is inadequate evidence in humans for the carcinogenicity of hydrogen peroxide.”

Information from the Hazardous Substances Data Bank (HSDB), a database of the National Library of Medicine’s TOXNET system (<http://toxnet.nlm.nih.gov>) indicates in general, ingestion, ocular or dermal exposure to small amounts of dilute hydrogen peroxide will cause no serious problems.

In 5 persons who accidentally drank 50 mL of a 33% H₂O₂ solution (not the readily available 3%), symptoms included stomach and chest pain, retention of breath, foaming at the mouth and loss of consciousness. Later, motor and sensory disorders, fever, micro-hemorrhages and moderate leukocytosis were noted. Still, all recovered completely within 2-3 weeks.¹⁵⁹ Yet, it may rarely be the cause of accidental death.¹⁶⁰

A review by Howes found a total of 13 deaths due to the accidental or intentional use of H₂O₂ in the entire history of

recorded medical literature available on the internet.¹⁶¹

Compare the record of safety with hydrogen peroxide with that of pharmaceutical drugs, which kill 12 per hour, every hour of the day, for 365 days a year (106,000/year). A 2000 report published in the Journal of the American Medical Association by Barbara Starfield, M.D., MPH reported that drugs kill over 106,000 annually, as a conservative estimate.¹⁶¹

Pravda has discussed the possible role of hydrogen peroxide in the induction of ulcerative colitis secondary to non-physiological concentrations of peroxide used in peroxide enemas.¹⁶²

Caveat: Some references do not provide access to the original article or to an abstract. Most of the references available to Howes on the internet do not go beyond 1979. Thus, there may be cases of which I am unaware. Many of the articles concerning ingestion or infusion of peroxide are non-conclusive. Clinical histories are incomplete and documentation is scanty. One thing for certain is that many cases of over-zealous or accidental ingestion of concentrated hydrogen peroxide (20-40%) have had a surprisingly uneventful recovery. Actually, the ingestion or infusion of 3% H₂O₂ has resulted in very few patients who developed serious complications or severe outcomes.

A retrospective review of all exposures reported to a regional poison center over a 36 month period and found that of 95,052 exposures reported, 325 (.34%) were due to hydrogen peroxide. The pediatric population (< 18 years) accounted for 71% of hydrogen peroxide exposures and ingestion was the most common route of exposure (83%). Nausea and vomiting were the most common symptoms secondary to ingestion. Ocular and dermal exposures to dilute solutions resulted in transient symptoms without permanent sequelae. While most exposures by all routes resulted in a benign outcome (no effect or minor effect), there was a trend toward more severe outcomes in those who ingested a concentration greater than 10% (p = 0.011).¹⁶³ (Division of Emergency Medicine, University of Utah School of Medicine, Utah Poison Control Center, Salt Lake City).

Reports have described levels of H₂O₂ over 50 μM as being cytotoxic for a wide range of plants and animal cells in vitro, but is dependent upon many factors such as, pH, media used, cell type used, length of exposure, etc. Paradoxically, acatalasemia in humans appears to produce no significant

phenotype, nor does “knockout” of glutathione peroxidase in mice except under conditions of “abnormally high oxidative stress.” This is contrary to the teachings of the free radical theory and consistent with Howes’ Unified Theory.¹⁶⁴

DISCUSSION

It is important to reemphasize the fact that antioxidants have repeatedly failed to prevent, control or reverse cancer and a host of so-called oxidative stress diseases. Thus, the free radical theory lacks predictability and consequently, according to the scientific method, it is unfounded. It is inexcusable that researchers continue to incriminate EMODs as only deleterious, noxious or mutagenic agents. In vitro studies may have little resemblance to the events occurring in living/breathing cells, which have considerable EMOD levels omnipresent.

Previously, investigators suggested that anything which served as an antioxidant was good and anything which oxidized something else was bad. That has repeatedly been proven to be untrue. All antioxidants can serve as prooxidants of greater or lesser reactivity. Further, H₂O₂ is now well recognized as an important and widespread second messenger for all aerobic cells. Based on scientific investigations, implying or giving the false impression that the presence of H₂O₂ is categorically bad is another example of unfounded and erroneous reporting.

RETHINKING THE FREE RADICAL THEORY

Any critical evaluation of EMODs must address the misconceptions propagated by the free radical theory of oxidative stress and aging. Hard data has yielded the following:¹⁵

- high levels of the antioxidant bilirubin cause kernicterus and permanent brain damage
- the antioxidant β-carotene increases the rate of lung cancer development in smokers
- the antioxidant CoQ, ubiquinone, when deleted from the diet of *C. elegans*, increases its lifespan
- SOD/catalase mimetics decrease the lifespan of house flies
- the antioxidant α-tocopherol, vitamin E, increases the rate and number of heart attacks and strokes
- high levels of the antioxidant, uric acid, cause gout

and cardiovascular disease

- acatalasemic patients live basically normal lives

Many molecules are designed to accept and receive electrons as a natural part of their reactivity, especially the transition metals and the heme proteins. Oxygen's various modified derivatives, electronic configurations and states, are the primary agents that protect us from infections and neoplastic growths throughout our lives, from conception to death. Report after report shows that mitochondria play a crucial role in apoptosis.¹⁶⁵

A growing body of evidence favors the involvement of intracellular reactive oxygen species (EMODs) at some point during apoptotic execution.¹⁶⁶⁻¹⁷⁰ Apoptosis is carried out by a multistage chain of reactions in which EMODs act as triggers and essential mediators.^{171, 172} The level of lipid peroxidation in patients with cancer was significantly reduced compared with that in healthy control subjects.¹⁷³ The lower level of lipid peroxidation in the cancer patients may be indicative of low EMOD levels, which would allow for the development of cancer.

Even death signaling by anticancer drugs generally relies on positive input from the mitochondria, as is evidenced by the resistance of tumor cells over-expressing the death-inhibitory protein Bcl-2 that is localized to the membranes of mitochondria, endoplasmic reticulum, and nucleus.¹⁷⁴⁻¹⁷⁶ Repeatedly, the critical role of cellular redox status in the regulation of death signaling has been demonstrated.¹⁷⁷⁻¹⁸⁰

These findings become more important considering the critical role of the mitochondria during apoptosis and the fact that mitochondria have been implicated directly as a prime source of EMODs during drug-induced apoptosis.¹⁸¹⁻¹⁸³ As the mitochondria are a major source of intracellular EMODs, it is tempting to speculate that EMODs, such as H₂O₂, may function both upstream and downstream of the mitochondria. Tumor cells lacking Bax (Bax^{-/-}) are resistant to the effect of some anti-cancer drugs.¹⁸⁴

Analysis of subcellular distribution of Bax (in HCT116, HL60, and CEM cells) revealed that Bax redistributed to the mitochondrial fraction from the cytosol on exposure to H₂O₂, which could be significantly blocked by the H₂O₂ scavenger, catalase.

Recruitment of Bax to the mitochondria during apoptotic

signaling has been linked to the activation of upstream caspase 8 and caspase 8-mediated cleavage of the proapoptotic protein Bid. This is particularly true on ligation of death receptors, such as CD95 (Apo1/Fas). Additionally, H₂O₂ and anticancer drugs have been shown to up-regulate the expression of the CD95 receptor or its ligand (CD95L) in some systems.^{185, 186}

Investigators utilized the ability of certain anticancer drugs to increase intracellular production of EMODs, specifically H₂O₂.¹⁸⁷ Indeed, exposure of HCT116 Bax^{+/-} or HL60 cells to a novel anticancer compound C1 resulted in an increase in intracellular H₂O₂ and translocation of Bax to the mitochondria. This translocation of Bax was inhibited by catalase, thus establishing the critical role of intracellular H₂O₂ in mitochondrial recruitment during drug-induced apoptosis of tumor cells. These data indicate that Bax translocation triggered in tumor cells during drug (C1)-induced apoptosis was a direct result of intracellular H₂O₂ production, independent of the upstream caspase 8 or ceramide pathways.¹⁸⁷

CYTOSOLIC ACIDIFICATION

Cytosolic acidification is an early event in apoptosis and provides an intracellular milieu permissive for efficient death execution. In this regard, exposure of cells to H₂O₂ or drugs that trigger intracellular increase in H₂O₂ results in a significant drop in cytosolic pH.¹⁸⁷

Accordingly, signals that inhibit apoptotic acidification impede death signaling as demonstrated in a recent study.¹⁸⁸ Investigators' results provided strong evidence that the link between H₂O₂ and Bax translocation could be the drop in cytosolic pH brought about by exposure of cells to exogenous H₂O₂ or endogenous production of H₂O₂ on drug exposure. It is possible that this indicates the pivotal role of H₂O₂ in cancer cell killing or apoptotic execution.

This shows that apoptosis is likely initiated with EMOD production, especially H₂O₂. H₂O₂ is, for the most part, essential for cancer killing and a shift to an acidic intracellular environment may also aid in its tumoricidal activity and the production of other agents within the EMOD family. Studies demonstrated the ability of commonly used chemotherapeutic drugs vincristine and daunorubicin to trigger an early increase in intracellular H₂O₂.¹⁸⁸

Pro-oxidant intracellular milieu is a hallmark of many tumor cells and is believed to endow tumor cells with a survival

advantage over their normal counterparts.^{189, 190} It has been shown previously that maintaining a slightly elevated intracellular O_2^- promotes cellular proliferation¹³⁵ and inhibits apoptotic signaling.¹⁹¹ Fortunately, this is one specific feature which provides us with an opportunity to selectively kill cancer cells by increasing EMOD levels even further.

Many investigators have demonstrated the critical role of intracellular H_2O_2 in rendering the cytosolic milieu permissive for efficient apoptotic execution.^{169, 170, 192} Further, these data strongly support and underscore the critical role of H_2O_2 in creating a permissive intracellular milieu for efficient drug-induced execution of tumor cells.¹⁹³

DICUMAROL INCREASED EMODS KILLING HUMAN PANCREATIC CANCER CELLS

Dicumarol is a naturally occurring anticoagulant derived from coumarin that induces cytotoxicity and oxidative stress in human pancreatic cancer cells. Dicumarol

increased intracellular levels of superoxide (O_2^-), as measured by hydroethidine staining, and inhibited cell growth.¹⁹⁴ Mitochondrial production of EMODs mediates the increased susceptibility of cancer cells to dicumarol-induced cytotoxicity.¹⁹⁵

MnSOD Overexpression and Inhibition of H_2O_2 Removal Increases Cancer Cell Cytotoxicity

Overexpression of manganese superoxide dismutase (MnSOD) and inhibition of H_2O_2 removal, increases cancer cell cytotoxicity. Investigators hypothesized that increasing endogenous O_2^- production in cells that were pretreated with adenoviral MnSOD (AdMnSOD) plus 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) would lead to an increased level of intracellular H_2O_2 accumulation and increased cell killing. The cytotoxic effects of Adriamycin or radiation, agents known to produce O_2^- , were determined in MDA-MB-231 breast cancer cells pretreated with AdMnSOD plus BCNU both in vitro and in vivo. In vitro, AdMnSOD plus BCNU sensitized cells to the cytotoxicity of Adriamycin or radiation. In vivo, AdMnSOD, BCNU, and Adriamycin or ionizing radiation inhibited tumor growth and prolonged survival. Thus, agents that produce O_2^- in combination with AdMnSOD plus BCNU may represent a powerful new antitumor regimen against breast cancer.¹⁹⁶

MYELOPEROXIDASE INVOLVEMENT IN HO-INDUCED APOPTOSIS OF HL-60 HUMAN LEUKEMIA CELLS

Investigators examined the mechanism of H_2O_2 -induced cytotoxicity and its relationship to oxidation in human leukemia cells. The HL-60 promyelocytic leukemia cell line was sensitive to H_2O_2 , and at concentrations up to about 20-25 μ M, the killing was mediated by apoptosis. When HL-60 cells were incubated with methimazole or 4-aminobenzoic acid hydrazide, which are inhibitors of myeloperoxidase, they no longer underwent H_2O_2 -induced apoptosis.¹⁹⁷ This strongly supports the primary role of EMOD induced apoptosis in cancer cell cytotoxicity.

ANTITUMOR THERAPY VIA ENZYMATIC GENERATION OF HYDROGEN PEROXIDE

Investigators studied the antitumor activity of an H_2O_2 -generating enzyme, D-amino acid oxidase (DAO), and its conjugate with polyethylene glycol (PEG; PEG-DAO). To generate cytotoxic H_2O_2 at the tumor site, PEG-DAO was first administered i.v. to tumor-bearing mice. After an adequate lag time, the substrate of DAO, D-proline, was injected i.p. This treatment resulted in significant suppression of tumor growth.

PEG-DAO thus delivered together with D-proline produces remarkable antitumor activity via extensive generation of H_2O_2 .¹⁹⁸

SOD OVER EXPRESSION INCREASES PEROXIDE LEVELS AND SUPPRESSES HUMAN PROSTATE CANCER CELLS

Investigators studied the role of the antioxidant enzyme manganese superoxide dismutase (MnSOD) in androgen-independent human prostate cancer (PC-3) cells' growth rate in vitro and in vivo. Production of extracellular H_2O_2 was increased in the MnSOD-overexpressing clones. Results are consistent with MnSOD being a tumor suppressor gene in human prostate cancer.¹⁹⁹

This supports the assertion that prooxidant EMODs, such as H_2O_2 , contribute to a continually functional oxidative protective system to curtail cancer growth. The increased SOD resulted in increased peroxide levels, which in turn suppressed tumor growth, via EMOD induced apoptosis.

INCREASED EMODS INCREASES CANCER CELL CYTOTOXICITY

Relative to normal cells, neoplastic cells demonstrate

increased sensitivity to glucose-deprivation-induced cytotoxicity. To determine whether oxidative stress mediated by O_2^- and hydroperoxides contributed to the differential susceptibility of human epithelial cancer cells to glucose deprivation, the oxidation of DHE (dihydroethidine; for O_2^-) and CDCFH(2) [5- (and 6-)carboxy-2',7'-dichlorodihydrofluorescein diacetate; for hydroperoxides] was measured in human colon and breast cancer cells (HT29, HCT116, SW480 and MB231) and compared with that in normal human cells [FHC cells, 33Co cells and HMECs (human mammary epithelial cells)]. HCT116 and MB231 cells were more susceptible to glucose-deprivation-induced cytotoxicity and oxidative stress, relative to 33Co cells and HMECs. HT29 cells were also more susceptible to 2DG (2-deoxyglucose)-induced cytotoxicity, relative to FHC cells. Overexpression of manganese SOD (superoxide dismutase) and mitochondrially targeted catalase significantly protected HCT116 and MB231 cells from glucose-deprivation-induced cytotoxicity and oxidative stress and also protected HT29 cells from 2DG-induced cytotoxicity. These results show that cancer cells (relative to normal cells) demonstrate increased steady-state levels of EMODs (reactive oxygen species; i.e. O_2^- and H_2O_2) that contribute to differential susceptibility to glucose-deprivation-induced cytotoxicity and oxidative stress. These studies support the hypotheses that cancer cells increase glucose metabolism to compensate for excess metabolic production of EMODs and that inhibition of glucose and hydroperoxide metabolism may provide a biochemical target for selectively enhancing cytotoxicity and oxidative stress in human cancer cells.²⁰⁰

EMODS ARE POSITIVE SIGNALS IN THE FRUIT FLY IMMUNE SYSTEM

The September 24, 2009 issue of the journal *Nature*, carried an article by Dr. Utpal Banerjee et al, UCLA's Jonsson Comprehensive Cancer Center researchers found much to their surprise, that in *Drosophila*, the common fruit fly, moderately elevated levels of EMODs are a good thing. Banerjee said, "These small molecules act as an internal communicator, signaling certain blood precursor cells, or blood stem cells, to differentiate into immune-bolstering cells in reaction to a threat. After the progenitor cells differentiate, the EMOD levels return to normal, ensuring the safety and survival of the mature blood cells."

Thus, he asks, "could excessive use of antioxidants deplete our immune systems?" Allegedly, reducing levels of reactive

oxygen is usually the goal, and what Banerjee found was surprising, in that when EMODs were taken away from the blood stem cells, they failed to differentiate into the immune-bolstering cells, called macrophages. On the other hand, when levels of EMODs were further increased by genetic means, the blood stem cells "differentiated like gang busters," Banerjee said, making a large number of macrophages.

The EMODs, Banerjee said, acted as a signaling mechanism that kept the blood stem cells in a certain state - when levels rose, it was a message to the cell to differentiate. Keeping their EMOD levels slightly elevated puts the cells on alert, sensitized and ready to respond to any threat quickly.

That work prompted the obvious question: If fruit fly blood stem cells and mammalian blood stem cells operate in the same way, is it a good thing for people to be taking antioxidants? Are antioxidants dulling the immune system and its ability to react to threats? It is interesting, however, that these types of blood progenitors in mammals also give rise to macrophages, Banerjee said.

Banerjee said, "If we find that those blood stem cells aren't primed to respond because the ROS levels are reduced, that would not be a good thing. Our findings raise the possibility that wanton overdose of antioxidant products may in fact inhibit formation of cells participating in innate immune response." Once again, this data emphasizes the crucial role of EMODs in aerobic cells.

<http://www.medicalnewstoday.com/articles/165268.php>
Accessed 9-25-09.

JUST TO ADD FURTHER COMPLICATIONS

Pro-senescent Effect of Hydrogen Peroxide on Cancer Cells and Tumor Suppression

Mild oxidative stress is known to induce premature senescence, termed stress-induced premature senescence (SIPS), in normal human diploid cells. Investigators determined whether mild oxidative stress would trigger SIPS in a human tumor cell line, human lung adenocarcinoma A549. The results showed that sublethal concentrations of H_2O_2 induced SIPS in A549 cells and consequently attenuated, but did not completely eliminate, the tumorigenicity of these cells. They next investigated the reasons for this incomplete impairment of tumorigenicity in A549 cells in SIPS. The results suggested that H_2O_2 treated A549 cells are composed of a heterogeneous cell population:

one is sensitive to H₂O₂ and the other is resistant or undergoes reversal; the latter reverted to their original tumorigenic form. The molecular mechanisms determining the cellular fate of tumor cells in SIPS should be identified in order to make use of SIPS and oncogene-induced senescence in tumor cells as methods of tumor suppression.

²⁰¹

INDIRECT EVIDENCE FOR EMOD INDUCED APOPTOSIS VIA ANTIOXIDANT STUDIES

The US is experiencing epidemics of cancer, diabetes, obesity and fatigue, which may be related to increased ingestion of antioxidant vitamins and dietary supplements, which are now commonly found as supplements or fortifiers of many foods and are aggressively marketed to an ever-growing segment of the population. These agents could be interfering with or modifying our continually operational prooxidant protective system.

Despite two decades of controversy regarding the use of dietary antioxidant supplementation during conventional chemotherapy and radiation therapy, questions remain about their efficacy and safety. However, on the basis of published randomized clinical trials, the use of supplemental antioxidants during chemotherapy and radiation therapy should be discouraged because of the possibility of tumor protection and reduced patient survival.²⁰²

Several new reports are raising concerns about the safety and efficacy of vitamin and mineral supplements in healthy individuals and cancer patients and survivors. Some experts see a need for further studies; whereas, others say that there are sufficient negative data to stop vitamin trials altogether.

²⁰³

Significant in vitro data exists showing that antioxidants can block EMOD-induced apoptosis for a wide variety of cancerous cell types, such as leukemia, lymphoma, retinoblastoma, myeloma, pheochromocytoma and human cancers of the breast, lung, pancreas, liver, colon, rectum and endometrium.²⁰⁴ This data can not be ignored.

However, it has recently been shown that EMODs may have an alternative activity, by modulating tumor cell signaling and that tumor cell signaling mediated by EMODs are readily reversible upon treatment with antioxidants. This emerging evidence may serve as bona fide signal transduction modifiers for cancer. A re-examination is warranted.²⁰⁵

However, in the words of one investigator, "If you suppress free radicals, you suppress programmed cell death."²⁰⁶

ONE FINAL NOTE

Philipp Niethammer, Harvard Medical School postdoctoral researcher and biologist, accidentally discovered while analyzing the severed tail of zebrafish that the hydrogen peroxide in their wounds appeared in bursts at the wound about 17 minutes before the leukocytes that were supposed to be producing them appeared too. On 6-4-09, ScienceNow reported that hydrogen peroxide summons reinforcements from the immune system, and more specifically white blood cells, which in turn aid with the healing process. Please view the video of peroxide migration in the wound of a zebrafish <http://www.youtube.com/watch?v=a7PJ8yXyPVU>. "Hydrogen peroxide marshals immune system." Accessed 10-9-09. This interesting video illustrates the rapid wound response and permeability of H₂O₂.

However, due to the complex nature of the interactions of EMODs and antioxidants within the body, it is difficult to clearly and definitively interpret the results of many experiments and observations.

CONCLUSION

Unarguably, EMODs are intricately, inextricably and crucially involved in cancer cell killing via their prominent role in apoptosis. Statements of the ineffectiveness in the killing of cancerous cells via hydrogen peroxide or other EMOD types are baseless, inaccurate and irresponsible. The lingering inaccuracies of the free radical theory must be countered by the obvious omnipresent and ubiquitous known salutary effects of the prooxidant EMODs. Their presence in steady state quantities testifies to their essential nature in healthy homeostasis and their low toxicity. EMODs, and especially hydrogen peroxide, are produced throughout the body in steady state levels on an as needed and when needed basis and serve to support the interrelated highly complex redox systems of the body. It is inconceivable that they only exist for pernicious purposes. Because of their relatively short half lives, their localized instantaneous concentrations can remain at low levels. Yet, their synthesis and availability can be called upon at any given moment to combat impending pathogens or neoplasia.

EMODs have bactericidal, fungicidal, virucidal and anti- protozoan and anti-neoplastic roles but also have far reaching cellular signaling control functions. The peroxide spike during the respiratory burst classically serves as a

protective role against infectious pathogens, as does EMOD induced apoptosis to combat neoplasia. Hydrogen peroxide is likely the most ubiquitous member of the family of EMOD agents. Its important and prominent biochemical role is ever expanding.

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