

Reactive Oxygen Species and the Regulation of Gene Expression, Part One

How the Yin and the Yang of Free Radicals (ROS) May Impact the Naturopathic *Vis Medicatrix Naturae*

By Nita Bishop, ND

The newer research on flavonoids is opening up a window of understanding about free radicals.

There is a documented paradox in the roles of how the reactive oxygen species (ROS) function in the body. Evidence through research is mounting that suggests that ROS are not just "injurious byproducts of cellular metabolism," but also are essential participants in cell signaling and regulation.¹

Convincing evidence that tissue injury by pretreatment of antioxidants actually is beneficial has not been thoroughly established. Recent findings provide evidence of a key role of ROS in both intracellular signaling and intercellular communication processes involved in maintaining

the homeostasis, the *vis*, if you will. *Vis Medicatrix Naturae* is the cornerstone of naturopathic medicine. It is the "life force" and represents a natural yin-yang balance to life which must be delicately maneuvered when working with a patient.

Bastyr colleague John Neustadt, ND, recently published an article in the April/May 2006 issue of *Integrative Medicine Clinician's Journal*, which discusses mitochondrial cell dysfunction. He states, "Some research is emerging that is pointing to a deeper function for antioxidants – that is, they may not primarily function to quench free radicals, but might in fact exert many of their beneficial effects by altering cell signaling and genetic expression."²

Regarding the topic of free radicals in the body, we still ask the question, which came first, the chicken or the egg? Are free radicals the cause of cell death (apoptosis) or are they the "smoke after the fire," i.e., actually the consequence of damage?³

We associate the random acts of oxidation on cellular components causing cytotoxicity as one of the primary factors of pathological disease. However, as Dr. Neustadt cites, there is evidence accumulating that suggests ROS might act as signaling molecules for the initiation and execution of the apoptotic death program. The signaling appears to be targeted at specific metabolic and signal transduction cellular components along the way to the nucleus. So, the enzymatic generation of ROS might, in fact, be the cell's way to establish signaling molecules to regulate and downregulate cellular processes.⁴

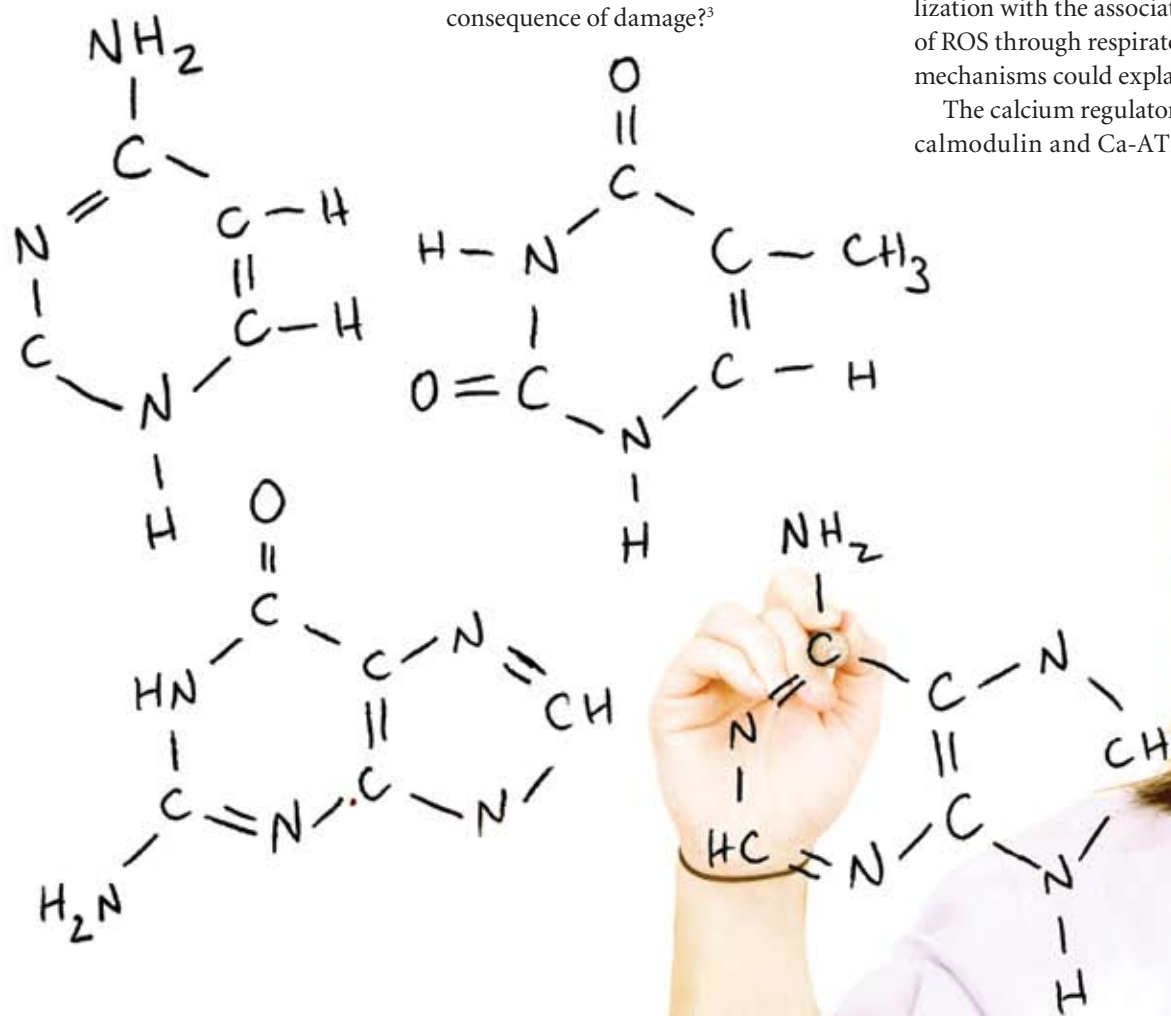
There are multiple theories on the actual mechanisms; oxidatively modified proteins assume unfolded tertiary structures that aggregate, so the area of thermodynamically unstable proteins, as targeted sensitive sites causing downregulating of the energy metabolism, is being studied. Alterations in intracellular calcium levels to ATP utilization with the associated generation of ROS through respiratory control mechanisms could explain this.

The calcium regulatory proteins calmodulin and Ca-ATPase are

selectively oxidized or nitrated *in vivo* during both aging and oxidative stress. Is this energy-producing metabolic process being downregulated as an adaptive response to the generation of ROS? Is the mitochondria the main intracellular source of ROS?

As naturopathic physicians, we emphasize that the primary organ of detoxification is the liver. The primary organ for excretion or elimination is the kidney. What the liver does in Phase I and Phase II (biotransforms toxins from fat-soluble to water-soluble compounds before the kidneys can eliminate them) is key. The first phase of detoxification produces abundant amounts of free radicals. These are buffered by intrahepatic and extrahepatic detoxification systems. As naturopathic physicians, we use systems such as detoxification, hydrotherapy, adequate nutrient/vitamin/mineral supplementation, foods from the Brassica family (kale, brussels sprouts), changes in lifestyle, stress reduction and increased exercise to assist detoxification.⁵

Constant exposure to occupational and/or environmental toxins can exhaust the buffering intrahepatic and systemic mechanisms. We conclude that cellular toxicity contributes to



most, if not all, chronic diseases, and a naturopathic practitioner will consider this component when faced with inexplicable symptom clusters, such as fatigue, malaise, lack of energy, frequent colds and nonspecific lowered immune responses (food allergies, etc.). Even in immune disease processes such as AIDS, the toxic load with respect to the immune responses plays a significant role.⁶ However, we have a similar process occurring on a microscopic level within the cellular matrix, which is not as well-understood, but should receive equal emphasis.

A 1994 meta-analysis by Linde, et al., of 105 published studies on cellular detoxification, supported the efficacy of this classic approach. This review is published in *Human & Experimental Toxicology*. An interesting note: One of the lead authors, Dr. Wayne Jonas, is former director of the National Center for Complementary and Alternative Medicine at the National Institutes of Health. A clinical study, completed in the summer of 2000 and reported in the *International Journal of Integrative Medicine* (Nov/Dec 2000), incorporated the serum thiol analysis as a measure of DNA repair in a 48-day protocol of homeovitic cellular detoxification for chemicals, heavy metals and latent viruses. These researchers also evaluated the immune-enhancing activity of cellular detoxification by measuring glutathione (GSH) levels, NK (natural killer cell activity) and antioxidant capacity. All three of these outcome parameters were significantly increased ($p < .05$), indicating the beneficial effects on immune function and also on DNA repair.⁶

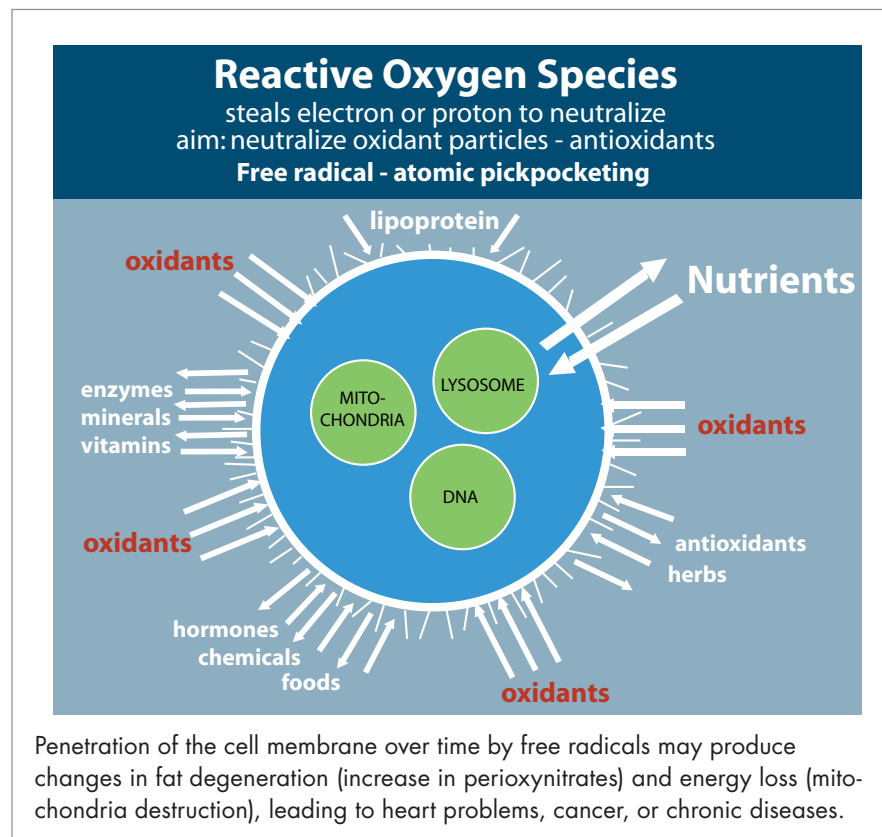
In review, naturopathic physicians know there are three areas where the cell can go wrong:

- redox
- pH
- altered mineral status

Oxidative stress might be broadly defined as imbalance between oxidant production and the antioxidant capacity of the cell to prevent oxidative injury. Oxidative stress has been implicated in a large number of diseases. However, the pathobiology is not entirely clear, largely due to a lack of understanding of the mechanisms by which ROS function in both normal and pathological disease states. We know that at some point in evolution, oxygen metabolism became the basis of life. Obtaining energy from oxygen through the formation of ATP along the mitochondrial respiratory chain is necessary for more highly organized life forms, but the resulting inflammation-promoting radicals produced in the process must be rendered harmless. It's a double-edged sword. You need energy to maintain homeostasis, but at some point, you have to cool down the "nuclear reactor" as it is forming ATP from oxygen.

Here is the dilemma: How do you produce essential biomolecules necessary in the regulation of cellular functions, yet disarm the toxic byproducts of metabolism produced in the formation of ATP so they do not harm the body? Our bodies do something similar during Phase I and Phase II in the liver.

Our science tries to quantify what is going on within a cell with a cause-effect analytical approach, and yet the key might be related to appropriate concentrations of ROS produced – the yin/yang effect.



Reactive Oxygen Species and Regulation of Gene Expression

We have a well-accepted premise that there are two types of cellular signals, extracellular and intracellular. Recent research indicates a new twist: Ligand receptor interactions generate both intracellular and extracellular ROS.

How plants use H₂O₂. Because hydrogen peroxide is made of two atoms of hydrogen and two atoms of oxygen, it's less stable and readily enters into oxidative reactions. Dr. Farr first characterized the oxidative effects of hydrogen peroxide in humans in 1984. H₂O₂ induction genes have been directly shown to be activated by oxidation in prokaryotes/bacterial studies. In a plant cell, the generation of H₂O₂ in response to various pathogens elicits localized cell death and *limits the spread of the pathogen*; we have a more systemic response involving the induction of defense genes regulating plant immunity. A recent study has shown that H₂O₂ also oxidizes two conserved cysteines to form *intramolecular disulfide linkages* that trigger the activation of this transcription factor, changing its conformation! This is changing the expression of DNA.

Mitochondria might be key in this

process. Work with isolated mitochondria have been shown to produce H₂O₂ from dismutating superoxide radicals. Two different sites were reported to shuttle single electrons to oxygen out of the normal respiratory sequence. The mechanism of how this is done is still controversial. However, the use of non-invasive detection methods shows that ROS formation will not occur unless there are changes in the physical state of the membrane/lipid fluidity. Is the mitochondria the main ROS generator in the cell?⁷

exact roles ambiguous. We don't know whether the relationship involves direct or indirect attack on the matrix components' synthesis. However, some studies show intracellular signaling pathways are redox sensitive and ROS are involved in the regulation of production of some biochemical factors involved in cartilage degradation and joint inflammation. A review of the literature seems to support the concept that ROS are not only deleterious agents involved in cartilage degradation, but they also are involved in intracellular signaling mechanisms.¹⁰ Naturopathic physicians and other natural health practitioners must endeavor to think multidimensionally.

The body is a nonlinear, highly intermeshed open energetic system. As Bruce Lipton states in "The Biology of Consciousness," the type of information and input into the keyboard of the body makes the difference. In the living system, *information is the most suitable energy carrier for setting off both near and distant intercellular reciprocal effects. This corresponds with the aim of the organism to keep itself WHOLE and BALANCED. Information is the basis used for maintaining the balance of the organism.*

Intracellular ROS synthesis is regulated by various signaling devices, including hormones, cytokines and growth factors. The mechanisms of both extracellular signaling and intracellular signaling in the body are as follows:

In extracellular signaling:

1. changes in ion concentration trigger
2. GTP-binding regulatory proteins, and cause
3. activation of receptor kinases (enzyme-linked receptors).

Downstream signaling will be delivered by second messengers. Once the information gets into the cell, how is it carried to the nucleus? **Intracellular signaling involves:** cAMP, Ca²⁺ and phospholipid metabolites. Ultimately, the intracellular signaling pathways lead to the activation of transcription factors that regulate the expression of specific sets of genes essential for diverse cellular functions. There is growing evidence that redox (reduction/oxidation) regulation might occur at multiple levels in the signaling pathways from *cell receptor* to *nucleus*.

ROS signaling occurs via two mechanisms:

1. alterations in intracellular redox state: GSH (thiols) primarily maintain the redox buffering capacity;
2. oxidative modifications in proteins.

High levels of reduced/oxidized GSH are maintained by the activity of selenium-dependent GSH peroxidase. Hydrogen peroxide (H₂O₂) works by breaking down the blood enzyme catalase, yielding water and singlet oxygen, and is a rich source of oxygen.¹¹

Another example of a pro/con relationship can be seen in reviewing the role of nitrous oxide in the human body. Low concentrations of NO = vasodilation. High concentrations of NO = microbial killing.⁸

A new and growing field of ROS research being explored centers around the redox signaling in hypertension. This could have positive effects on cardiovascular and renal disease. The kidney and vasculature are tissues rich in NADPH oxidase-derived ROS, which under certain conditions – increased superoxide anion and H₂O₂, plus reduced nitric oxide synthesis and a decrease of antioxidants – can cause human hypertension. Could specific, targeted therapies against free radicals by decreasing ROS generation and/or by increasing nitric oxide availability, plus antioxidants, be useful in preventing hypertensive end-organ damage?⁹

Antioxidant Therapy in the Management of Bone and Joint Diseases

Many practitioners are now looking at free radicals in bone and cartilage research. ROS seem to emerge as major factors, along with mechanical stimuli, cytokines and growth factors. As we study ROS production involved in joint diseases such as osteoarthritis and rheumatoid arthritis, we find their