July 2003 Issue | Woody R. McGinnis, MD Ashland, Oregon

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Welcome to *Functional Medicine Update* for July 2003. As I mentioned last month, a principal focus of this two-month period will be to look at laboratory methods for assessing functional status. The available information on this topic could consume an entire year's discussion on *FMU.* A number of new functional tests have emerged, from noninvasive scanning technologies in radiology, to radioimmune and monoclonal assays, to specific types of genomic, proteomic, or metabolomic assays. Because practitioners may not be able to put some of these new tests into use in daily clinical practice, I will continue to discuss functional assessment from a clinician's perspective. We will look at tests that might prove to be cost-effective without overutilization.

Clearly, for any one patient, thousands of dollars of tests are available, and under ideal circumstances we might want to run all of them. It would be as though the patient was a research project, and we wanted to know everything there was to learn about him or her. In most real cases, however, we must distill our investigation down to the small cluster of tests with the greatest probability of pointing the way to individualized therapy for that patient, or what we call personalized functional medicine.

Interpreting Test Results

Last month I discussed various types of traditional blood tests and analytes that might be useful for interpretation of function. Most of these traditional laboratory tests are built on the appearance of histopathology. They are not designed to assess function. Sometimes we encounter individuals who, although they fall within the broad normal range for a specific analyte, may have a functional disorder. A test result at the edge of the ideal range may not suggest the presence of good health, but a trajectory toward disease.

One has to be cautious in considering narrowing the range of a test without actually knowing what he or she is doing and having good data to support it. The traditional standard blood tests found in a chemistry screen such as SMAC are designed to evaluate pathology within a normal population. Therefore, two standard deviations from the mean is generally used to indicate where that normal population resides as it pertains to the presence or absence of an analyte assessed by the chemistry screen. If you start to shade the data, however, or limit the range into an "idealized range," what are you idealizing it around? What data support that idealizing? How much variability do you have in any one test? If you tested an individual over time, randomly or on a daily basis, you might find the range was greater than the ideal range. On one day, the person might be considered ideal, and on another day he or she would not, based on the variability of the test and biological variation.

History of the Chemistry Screen

These are important concerns. The development of the chemistry screen such as the SMAC emerged from work done in the 1950s at the National Institutes of Health, and later in public health service. Researchers evaluated the relative range of tests like glucose, blood urea nitrogen, creatinine, and liver enzymes. It is interesting to examine those studies. Many of those patients in the 1950s have been followed serially, year by year, or every few years for decades, and the trends of changes in these analytes and disease have been evaluated.

An examination of recent publications on the relative relationship of individuals with chemistry screen analytes within the normal range and the tendency of these individuals to develop disease as they get older suggests it is not the data at a certain point that determines a person's relative risk. It is the trend in data over time, or the trajectory, that actually reveals the risk. The concept of trajectory is very important. It suggests change in organ system function. A single point on a curve, such as a glucose number on a certain day in July, 2003, may not be nearly as important as where that number was a year ago or three years ago, and where it will be a year from now. The trajectory of fasting glucose is what tells us something about calculating variations of the function of the individual.

Challenge Testing

In functional medicine, biochemical testing is most useful when it is applied in some type of challenge testing mode or serial testing over a period of time with reproducibility studies on the individual. This type of testing may reveal a trend toward elevated levels or reduced levels of specific substances. That is one reason why challenge testing is often desirable in functional laboratory evaluation. You may not have the luxury of testing the patient repetitively over time. Let's say a gentleman just stops by your office and wants to know why he feels bad. You don't have data from three years ago, and you obviously don't have future data, so you have only that point in time in which you are going to do the test.

Challenge testing can help you get some understanding of the relative functionality of the individual's organ or organ system. An example is the difference between the fasting blood sugar test and the oral glucose tolerance test. A patient's fasting blood sugar level, for example, could be relatively normal. Putting that person under a glucose load by having him or her ingest a high amount of simple carbohydrate, however, places demand on physiological reserve. Now you may see a significant difference in functional reserve as it pertains to the way he or she normalizes blood sugar over time. The symptoms may then appear. The individual may get shaky or sweaty, or develop a headache or whole-body fatigue. Symptoms may develop on the postprandial glucose tolerance test that you might not see on the fasting blood sugar analysis.

Types of Challenge Tests

One might look at a range of types of fasting as challenge protocols for examining different aspects of physiological function. Several studies have looked at a detoxification function by challenging the individual with a specific substance whose detoxification pathways are well described. The caffeine clearance test, for example, looks at the rate at which caffeine is cleared, or acetylated, and then excreted. The glycine challenge test employs sodium benzoate as an orally consumed challenge substance. Urinalysis of the glycine conjugate, which is the conjugated metabolite of benzoic acid, indicates phase 2 conjugation. The acetaminophen challenge test examines the relationship of metabolic breakdown products in the urine from acetaminophen, which is metabolized through some phase 1 and phase 2 detoxification reactions.

Functional medicine employs a number of challenge protocols to assess relative organ reserve. I believe the use of challenge tests will increase as we start getting into functional genomic and functional proteomic analysis. It is one thing to know a person has a specific SNP (single nucleotide polymorphism). It is another thing to know how the expression of the SNP contributes to metabolism under the conditions in which the person is living.

Functional Genomic and Functional Proteomic Testing
You might ask what happens if you have an apoE4 characteristic, which in the double allele case is associated with increased risk to cardiovascular disease and Alzheimer's dementia. You might ask if this apoE4 characteristic is contributing in a harmful way. You would tie that genotypic test together with a challenge to see how it was responding to the challenge, how those genes contribute to health. That might be something like a saturated fat challenge to look at oxidative stress after challenge.

We can envision a number of new tests to evaluate functional changes related to challenging the physiology and looking at gene and protein expression patterns. We could then examine their effects on metabolism to make a correlation between genotypic susceptibility and phenotypic outcome.

The Future of Functional Testing
That is a forecast of the direction in which functional testing may be going as we move into the future. We may be doing more and more evaluations of reserve and gene expression. This reserve concept derives from the concept Dr. James Fries developed regarding organ reserve, the compression of morbidity, and natural death.[1]
We have frequently stressed the importance of maintaining organ reserve. We can lose organ reserve if we don't practice appropriate lifestyle habits. On the other hand, it can be regained if we follow an intervention program that restores function and organ reserve.[2]

The functional testing I am describing fits well with Dr. Fries' concept of compression of morbidity, natural death, and the maintenance of organ reserve.

Three Current Areas of Testing
I would like to discuss three areas of testing to show how this concept would be applied. First are oxidants and antioxidants and their relationship to chronic disease. How and why would we look at certain biomarkers for evaluating oxidative stress? This is an emerging area of increasing interest and implication as we learn more about the relationship of oxidative injury to various types of pathologies we later see as chronic degenerative disorders.

On side II of this month's *FMU*, we will have an insightful presentation by Dr. Woody McGinnis on the relationship of oxidative injury to various neurological disorders. Assessing oxidative injury and antioxidant sufficiency is not just an esoteric concept. It may have important relationships to clinical management.

Trace Mineral Assessment (includes both essential minerals and potentially toxic minerals. How do we evaluate.)
The second area of assessment I want to discuss is trace mineral intake. This their status, and what are some good laboratory methods that stand the test of specificity, accuracy, and precision? Third, I want to talk about phytochemicals, and the increasing interest in the range of thousands of compounds in various

foods that modulate gene expression and function. These substances include isoflavones, lignans, flavonoids, flavonols, and the whole range of terpenes—monoterpenes, diterpenes, triterpenes, and tetracyclic compounds related to steroid chemistry. I will discuss how these substances are assessed.

We begin by looking into the antioxidant/oxidant connection and determining what biomarkers we might use for evaluating oxidative stress. All cells in the body are chronically exposed to oxidants. We are aerobic, oxygen-breathing organisms. Some of that oxygen is converted into oxidants like superoxide, hydroxyl radical, and hydrogen peroxide. These agents can damage tissues and increase the relative rate of tissue injury. A number of clinical conditions are associated with increasing oxidative injury to proteins, lipids, and nucleic acids. These conditions include cardiovascular disease, various forms of cancer, cataracts, age-related macular degeneration, and even neurodegenerative disorders like Parkinson's disease, Alzheimer's disease, and various dementias. They might also include, as we will learn on side II of this month's *FMU*, autism and schizophrenia.

A large body of research has developed, particularly over the last decade, that has investigated the role of the oxidant/antioxidant duality in maintaining balance in functionality, or tipping the balance toward dysfunction that later becomes a chronic degenerative disorder.[3] This oxidant/antioxidant theme, which has evolved to be a major discussion point, both in the general public as it relates to nutrients, and in the medical community, is at the forefront of the way we look at the origin of many of these disorders from a molecular perspective.

Antioxidants and the Reduction/Oxidation Process

The antioxidant nutrients are those that soak up or defuse the higher energy oxidants—superoxide, hydroxyl radical, hydrogen peroxide, and singlet oxygen. These are four major activated forms of oxygen the body produces that can accelerate the rate of oxidative injury to proteins, carbohydrates, lipids, and nucleic acids. The antioxidant nutrients have different levels of electromotive potential—or reducing potential—based on their structure, their place in the body, where they reside in the cell, or if they occur in the plasma. They have differing abilities and specificities for uncoupling or chemically modifying or reducing an oxidant into a less injurious form. Whenever there is reduction, there is oxidation. Whenever there is oxidation, there is reduction.

Vitamin C and Glutathione

It is important to recall that when ascorbic acid, vitamin C, is used as an antioxidant, it is oxidized itself. Oxidation of a protein, for instance, is prevented by having adequate levels of vitamin C present at that site to soak up an oxidant like hydroxyl radical. In so doing, vitamin C is converted to dehydroascorbyl radical. Ascorbyl radical can in itself be damaging if it is not quenched to its reduced form back to ascorbate.

You need something like glutathione to convert ascorbyl radical back to ascorbic acid. As vitamin C is converted from its radical form back to vitamin C, glutathione is converted to its glutathione disulfide form. Glutathione disulfide then depletes the glutathione reserves of the body, and it has to be reactivated through an enzyme system that is glutathione reductase. Oxidation of glutathione requires glutathione peroxidase, a selenium-containing enzyme, and the reduction of glutathione disulfide back to glutathione requires glutathione reductase, which requires vitamin B2-derived FAD reducing factor.

ATP and Mitochondrial Function

The ultimate driver of this process is ATP. We must have adequate levels of reducing power, which comes from mitochondrial function.

If your mitochondrial energy production is compromised, your electron transport efficiency is compromised, your reducing power is compromised, and your body cannot recharge its antioxidants effectively. This is an important theme, because often we think of antioxidants as working infinitely long in the body. However, the higher the oxidative stress, the more substances like glutathione are depleted, and the greater the necessity there is for reducing them back to their active form with reducing factors like ATP or going through FADH2. It is the oxidation/reduction couple. Every time there is an oxidation, there has to be a reduction, and the system needs to be in balance to maintain proper function and reserve.

Antioxidant Reserve

You might think of the body as having an antioxidant reserve. An antioxidant reserve would be like a buffering capacity of the body, cell, or tissue to withstand oxidative exposure. When that oxidative reserve is depleted, just like the blood buffer system, where depletion would result in rapid changes in pH, by changing the oxidative reserve and depleting it, the oxidative potential of the cell can change rapidly, and in so doing it can create oxidative stress.

The oxidative stress signals, real electrochemical signals produced within cells, can shift the way the genes are expressed. The genes take their expression messages, in part, from the electropotential of the cell. As that electro or reducing potential of the cell is shifted, expression of certain genes may be upregulated. In some cases, the upregulation can trigger apoptotic changes in cells, meaning cell death. This cell suicide mechanism steps in, saying in effect that this cell is injured, so we had better get rid of it. Then the cell undergoes apoptosis.

Slowing Apoptosis

In post-mitotic tissue like the heart, brain, or muscle, it is not a good thing to lose cells at a rapid rate, particularly after infancy and youth when a lot of new tissue is produced. As a 40- or 50-year-old individual, you want to slow the rate of untoward apoptosis in the brain, heart, and muscles. Oxidative injury can increase apoptotic loss of cell mass in those particular organs. That leads to loss of organ reserve. I hope you can see the connection between early-stage oxidative injury, later-stage loss of antioxidant potential, and the triggering of the cell suicide mechanism, apoptosis, in particular tissues which leads to loss of organ reserve.

Nutrient-derived antioxidants play important roles in shuttling around the electrons involved with oxidation or reduction so they do not “jump up and catch the curtains on fire.” The curtains are the membranes of the cell. They are highly polyunsaturated, fatty acid-rich regions of the cell that can be easily oxidized and converted into rancidified fats. These oxidized lipids undergo various kinds of chemical reactions to produce aldehydes and carboxylic acids, such as nonenal and malondialdehyde (MDA). These are breakdown products from the polyunsaturated fatty acids, the omega 3 and omega 6 fatty acids in our body that can create reactive chemical substances that can further participate in injury to proteins, nucleic acids, and other parts of the cell.

Stemming the Oxidation Storm with Antioxidants

There is dog-chasing-its-tail potential when an oxidative storm occurs in a cell. It might more closely resemble a pyrotechnic display of Fourth of July fireworks. Chemistry of a firework is the so-called free

radical chemistry—1 begot 2, 4, 8, 16, 32, 64, 128, 256, 512, and away you go as the sky explodes. Exploding free radical chemistry in the heart, lungs, brain, or eyes is not good. This is tissue pathology. Something is needed to quench, check, or interrupt the process of free radical pathology. That is where antioxidants play such an important role.

The antioxidants are not just the enzymes I mentioned, such as glutathione reductase, glutathione peroxidase, catalase, and superoxide dismutase. Other nutrients come from the complex diets we have historically consumed. Carotenoids, for example, are the orange-red pigments in plants. Phenolic and polyphenolic compounds are frequently shades of blue, such as in berries. Chlorophyll-like substances occur in green plants. Various terpenoid substances are like essential oils that are monoterpenes or diterpenes. These are complex signaling substances in various plants. Triterpenes are cyclized together into plant sterols such as stigmasterol, campesterol, and isoflavone-like compounds.

Antioxidant Functions

Various families of substances have antioxidant potential. These antioxidants are quite specific in the way they work. Just because a substance in the test tube appears to be a good antioxidant does not mean it will function in the same way in the cell or the body. These substances are often membrane-linked. They are found in specific locations in cells, and they have unique personalities. We need to exercise caution in extrapolating from *in vitro*antioxidant capabilities to cell antioxidant capabilities.

The best test is to evaluate what is going on in the body. We have consumed a diet that is rich in multiple antioxidants—flavonoids, proanthocyadins, terpenoids, isoflavones, tocopherols such as vitamin E, ascorbate, and carotenoids. All of these substances work together in an orchestrated way to trap oxidative radicalsWith that bit of background information, we can get into discussing actual assessment. How do we assess what is going on in the body related to oxidants and antioxidants? That topic is still evolving. Obviously, I can’t answer that question unequivocally. We have, however, witnessed the emergence of new laboratory methods that can at least assess the aspect of the balance between oxidants and antioxidants.

Helmut Sies first used the term “oxidative stress” in the 1980s. This interesting phrase captures the concept of imbalance between oxidants and reductants. The oxidative/reductive balance of the cell has been tipped in such a way as to increase the oxidative rate and begin to have injurious effects on tissues. If we consider oxidation as a fire and the cinders that result from that fire as the debris left behind as the result of oxidative combustion, what would we need to look at for examining tissue injury?

Measurement of Lipid Peroxides—The TBARS Test

One of the things most commonly looked at is called the malondialdehyde-active, or MDA-active, substances. Generally, this is accomplished by using a dye in a chemical test to react with MDA in such a way as to produce a compound that could be observed with visible light and quantitated. This type of test reveals, for example, the urine lipid peroxide value, or the plasma lipid peroxide value. Peroxides are thiobarbituric acid-reactive materials. Thiobarbituric acid (TBA) is the dye or the chemical reactant used to evaluate malondialdehyde-active substances, or MDA. Therefore, when we measure lipid peroxides, we are measuring the debris that comes from the breakdown of polyunsaturated fatty acids by oxidative injury, particularly omega 3 and omega 6 types of debris.

The MDA fragment, when it combines with TBA to form the colored compound, can be quantitated.

However, one needs to be cautious about what one is looking at. If you look at urine or plasma lipid peroxides as a whole-body gross factor, you have to ask what happens with kidney transport. How much urine concentration is there? Are there reactive compounds that might alter that particular test to produce variable results? We should not consider the urine peroxide test the definitive marker for individual tissue pathology, but as a gross marker for potential oxidative injury that occurs in the whole body. This is the debris, so to speak, from oxidants that are not being properly met with antioxidant protection. The TBARS test, or the urine lipid peroxide test, is a fairly good secondary biomarker for oxidative injury, but I would not consider it a primary biomarker.[4]

The 8-Hydroxydeoxyguanosine Test

Another secondary biomarker that has received quite a bit of attention recently is the 8-hydroxydeoxyguanosine test (8OHdG) test. Guanosine is one of the nucleic acids; it is the G in the AT and GC pairs of nucleic acids found in DNA. It is very susceptible to peroxidative injury. As a consequence of oxidative injury to nucleic acids, the damaged nucleic acid material in the cells is ultimately broken down and can be analyzed using sophisticated chromatography technology. Hydroxylated guanosine can be measured as 8OHdG. A number of papers have been published over the past few years showing the clinical utility of the levels of 8OHdG in a variety of oxidative disorders. This includes kidney disorders, nervous system disorders, post-myocardial infarction patients, and inflammatory bowel disease—in all of these, increased levels of 8OHdG are seen.

Finding the Right Level and Balance of Antioxidants

It would be a mistake to conclude that because we can see increased evidence of oxidative injury in individuals who have certain types of neurological disorders, taking more antioxidants by mouth will reduce 8OHdG levels. Very few clinical studies have indicated that lowering of 8OHdG levels is possible by increasing the level of dietary antioxidants.

There are many reasons why this is true, and we could spend quite a bit of time talking about it in detail, but let me summarize by saying I think it is a consequence of two general factors. First, what is the right level and balance of antioxidants that would produce a reduced oxidant injury in the tissue of concern? Can we get it across the blood brain barrier? Can it find its way to the site of oxidative stress, which is generally at the mitochondrial site? Often, it is difficult to get a nutrient transported from the blood to the mitochondrion so it can have its maximum effect in reducing oxidative injury at the portion of the cell where most of the oxygen processing is occurring.

Genetic Factors in Antioxidant Usage

Second, what is the genetic uniqueness of that individual related to his oxidant stress? Different individuals may have different sensitivities to specific antioxidant nutrients based on the genes that are being influenced by the oxidative chemistry. This field is just beginning to open up as we find that no one antioxidant fits all needs. It is the relationship of multiple antioxidants we have been consuming in our diet on a polygenic organism that gives rise to proper redox balance.

This is an area of future investigation that I think will result in a big clinical pay-off as we better understand how to assess individual need for antioxidants and range and combination of antioxidants. This is not the antioxidant-of-the-month club in which we can take vitamin C one month and vitamin E or carotenoids the next. On the contrary, the body has been consuming and is dependent on a complex array of antioxidants from foods that are high in natural colors. It is the natural carotenoids, flavonoids, and all

the materials we might consider flotsam and jetsam in foods in their natural form that create antioxidant balance.

Examination of Oxidized Lipids
A third, less-utilized test that may have strong clinical correlations is direct examination of oxidized lipids. This test is generally done by high-pressure liquid chromatography. We look at the fragments of the debris of oxidized lipids in the body, the nonenal compounds, the cholesterol oxide compounds that result from membranous oxidative injury. A number of individuals are doing that kind of work. These are the kinds of tests that are used as biomarkers to evaluate the injury to various tissues. These would be biomarkers of oxidative stress status.

Examination of LDL Oxidation
Another test that is being done in some laboratories is the LDL oxidation test. This test relates to the potential risk for atherosclerosis. The oxidative modification of LDL is thought to enhance atherogenesis.[5] The resistance of LDL to induced oxidative stress *ex vivo* has been used as a possible biomarker of oxidative defense, at least the LDL particle itself. Lipid-soluble antioxidants, such as vitamin E and beta-carotene, are carried in the LDL particle. The concept, therefore, is that LDL resistance to oxidation should reflect the antioxidant defense system, particularly as it relates to lipid substrates and lipid-soluble antioxidant compounds. Unlike some assays used to measure oxidative stress, however, this assay involves challenge with exogenous oxidants in the test tube. For example, copper is commonly used to induce oxidation, followed by measurements of lag time before oxidation *in vitro.*

A variety of nutritional antioxidants, such as vitamin E and carotene, appear to affect the resistance of LDL to oxidation.[6] Vitamin E is much better than carotene in that capacity. A variety of phenolic compounds, including resveratrol, appear to help reduce LDL oxidation.[7]The more we examine the phytochemicals or phytonutrients found in various foods, the more we find they are complex food sources. Spices, as well as plant foods, have significant impact on reducing the potential for LDL oxidation.

Examination of Isoprostanes
One other family that has been the subject of recent investigation for evaluating oxidative stress is the isoprostanes. Isoprostanes are breakdown products of arachidonic acid. When arachidonic acid is oxidized through general free radical mechanisms, it is chemically converted in small levels to a new family of prostaglandin-like compounds called isoprostanes. Isoprostanes are not formed by the normal enzymatic process, like cyclooxygenase and lipoxygenase, which work on arachidonic acid in normal metabolism. These are oxidative random-hit processes occurring on arachidonic acid that chemically modify it to form a new class of compounds called isoprostanes.

One of the most abundant of these substances is F2 isoprostane, which is similar in structure to the prostaglandin F2a. It has an interesting relationship to conditions such as cardiovascular disease, risk to various types of malignancies that have been associated with increased isoprostane levels in animals. You can also measure isoprostanes, 8OHdG, urinary peroxides, and LDL oxidation.[8] These are all biomarkers.

Oxygen-Reducing Absorbance Capacity Test
On the other side of the fence, we can look at relative resistance to oxidation. What is the antioxidant

reserve? This is where we use the oxygen-reducing absorbance capacity (ORAC) test. This test combines blood with oxidants in a test tube under a specific type of assay. The oxidants cause injury to the blood, and the injury depends on the amount of antioxidants present in the blood. If the antioxidant potential is low, the oxidant, when added to the test tube, will very rapidly cause injury to the blood. If there is a high amount of antioxidant potential or antioxidant reserve when you add the same level of oxidant to the blood, it will be quenched or soaked up and will not cause injury. That is the basis of the oxygen-reducing absorbance capacity, or ORAC test.

We are seeing greater use of this test as a way of assessing the capacity of various antioxidants to prevent oxidative stress or oxidative injury. That is the other side of the coin. Rather than looking at the debris that comes from oxidative injury, we are looking at the ability to help prevent oxidative injury. If you were to assess oxidative chemistry in a person, you would probably want both sides of the equation. You would want to look at oxidative reserve as well as the oxidative injury occurring in the individual.

Other Measures of Antioxidant Potential

I believe these are very good tests to help us better understand the individual's balance of oxidation and reduction. What about measuring the levels of carotene, or vitamin C, or vitamin E in the blood? Couldn't one just measure the plasma level of these nutrients? The answer is yes, but these are probably not the best methods for evaluating intracellular capabilities of the substances for maintaining proper redox balance. They are correlated at a loose level and not at the tissue-specific level we might like to have.

What about looking at enzymes like glutathione peroxidase levels and activity in red cells? That will reveal something about selenium status and glutathione peroxidase. That is a pretty good test if you are looking for selenium deficiency, but if you are looking at levels of optimal selenium intake, it probably is not very sensitive. Glutathione peroxidase may be saturated before you can obtain optimal levels of antioxidant status.

Variations on the ORAC—TRAP, FRAP

At this point, although there are some crude ways of evaluating oxidative injury or oxidative resistance, a definitive test is not yet available. I have seen some variations on the ORAC test recently. One is the TRAP test, which measures the total peroxyl radical trapping antioxidant potential, a variation on the theme. Another is the FRAP test, which measures iron-related free radical absorbance capacity.[9] These tests are modifications of the means of measuring antioxidant reserve capacity. FRAP, TRAP, and ORAC are all part of that particular family of tests.

I am convinced that many more clinically useful evaluative tools will become available for assessing oxidative status, the redox balance. This does not mean the present tests are of no value. It would be useful for any patient who is undergoing a rapid degenerative condition to have some indications of oxidative chemistry. This might be done by looking at peroxidative status with lipid peroxides, examining 8OHdG levels, looking at LDL oxidation if there is a cardiovascular risk concern, and looking at ORAC, FRAP, or TRAP to examine reductions in potential for oxidative reserve.

All of these tests point us in an appropriate direction. But how do we apply them clinically to a specific condition? Our Clinician of the Month, Dr. Woody McGinnis, will help us answer that question.

INTERVIEW TRANSCRIPT

Clinician of the Month
Woody R. McGinnis, MD
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JB: It is time for our Clinician/Researcher of the Month interview. In 1967, Linus Pauling wrote a pivotal article that appeared in Science magazine. That article, titled “Orthomolecular Psychiatry,” in effect drew a line in the sand between a medicine that was and a medicine that would be. Years earlier, in the 1950s, Dr. Abram Hoffer and his colleague, Dr. Humphrey Osmond, had been pursuing the relationship between nutrients and brain function. They had developed some extraordinary concepts related to schizophrenia as an endogenous, hallucinogenic type of disorder associated with brain chemicals that could alter mood, mind, memory, and behavior.

In the early 1970s, Dr. Carl Pfeiffer wrote a tour de force book titled “Mental and Elemental Nutrients.” I had the privilege of knowing all three of these gentlemen in the course of my career. I worked with Dr. Pauling at the Pauling Institute and lectured with Dr. Pfeiffer in the 1970s. I have known and derived significant benefit in the last 30 years from Dr. Hoffer and his contributions. We presented the Linus Pauling Functional Medicine Award to Dr. Hoffer at our Symposium in May 2002.

Introducing Dr. McGinnis
That leads us to this month’s Clinician of the Month. We have the pleasure of introducing Dr. Woody McGinnis, a medical doctor educated at Dartmouth College and Colorado Medical School. He was in Arizona for many years and is actively involved in the area we have outlined through the Pauling/Hoffer/Pfeiffer connection—orthomolecular psychiatry related to the environment and nutrition and how they impact brain function. Dr. McGinnis has risen in national prominence through his ability to construct information and to network. He has an ability to create connections among people who are making observations and clinical connections among the environment, nutrition, behavior, and brain function. I am pleased to welcome you, Woody, to the audience of Functional Medicine Update.

WM: Greetings from balmy Ashland, Oregon, Jeff. This is my new and perhaps permanent base now; it has been over the last year or so.

Energy Centers
JB: I have always admired how you have chosen the energy centers to do a lot of your work. I know you often spend summers on Salt Spring Island in the Gulf Islands of British Columbia, and in Tucson and Ashland. Those sound like pretty good energy centers. We will drive some of that energy for this interview today.

WM: I wanted to mention that one of my very early and strong impressions was from a medical conference I attended in Palm Springs about eight or nine years ago where I had the pleasure of seeing you and David Horrobin on the same venue. Ever since that early formative impression, I have been viewing illness in the context of oxidative stress and membrane function. I still consider your 1995 review article, “Oxidants and Antioxidants in Clinical Medicine” one of the key entries in the literature.10

Endogenous Hallucinogens

JB: Thank you. Let's begin today with an area in which you have invested an extraordinary amount of time. Let's review a brilliant concept that has lain somewhat dormant over the last few years, not to the benefit of medicine. That is the concept of these endogenous hallucinogens, these kryptopyrrole compounds, or urinary pyrroles, Mauve Factors, and the discoveries Hoffer made in the 1950s. Could you give us an update regarding the status of our understanding of some disorders of brain function?

WM: The overall theme of our conversation today is probably pointing toward insights on oxidative stress in the behavioral disorders, specifically the Mauve Factor. It's a pyrrole, and was discovered by Hoffer in 1961. It's found in the urine of about 60 percent of schizophrenics. It showed a purplish lilac-colored band on the paper chromatography at the time and was designated "Mauve." It's been found in about 70 percent of Down's syndrome patients (that's the highest penetrance); 50 percent in autistic children; about 40 percent in alcoholism; and about 30 percent in ADHD (attention-deficit hyperactivity disorder). There are subgroups of mental retardation/ depression/delinquency that have it. Some folks without behavioral diagnoses are high- Mauve and feel better with treatment. There is a very strong familial tendency. Hoffer found that if he treated the high-Mauve schizophrenics with high doses of niacinamide, later adding vitamin C, that their measurable urine levels dropped and their behavior improved. The ones whose urine cleared are those who got out of the hospital.

Understanding the Mauve Factor

JB: In the early stages of understanding the chemistry of thiscompound, we thought it might have been a kryptopyrrole. Later, with improved resolution of high-pressure liquid chromatography, researchers found different chemical compounds. Do we now have a better understanding of the chemical nature of this compound and its metabolic origin?

WM: That's correct. It was initially identified as krytopyrrole, but that was erroneous. The krytopyrrole is very close to what we think the true Mauve Factor is. Some labs are still using the kryptopyrrole standard and it appears that the clinical information we get is just as valid as the use of the beta lactam, actually the hydroxylactam of hemopyrrole, so-called HPL, which from the work with GLC in the 1970s, appears to be the moiety in question. What we've found is that it's a very unstable compound in the urine and that from a practical point of view, it has to be protected very carefully from light on collection, placed in ascorbate preservative, and shipped overnight for valid results on the testing.

In the 1970s, Carl Pfeiffer started using vitamin C, vitamin E, zinc, and particularly high doses of B6 for a wide range of behavioral diagnoses with high urinary pyrrole. He also found that the pyrrole level dropped and symptoms improved, often markedly. Kurt Vonnegut's son, Mark Vonnegut, was one of his early and successful patients and is now a happy, productive pediatrician in Massachusetts. Vitamin B6 and zinc deficiency are key aspects of the high-Mauve patient. The pyrrole implies a much higher zinc requirement. Some of our children with high pyrrole require as much as 150 mg of zinc picolinate daily. It also implies a really high need for B6.

My current idea about the origin of this Mauve Factor makes both the zinc and the B6 requirements more understandable, since both can play strong anti-oxidant roles, and zinc is known to block the formation of pyrrolic tissue adducts. I usually give the activated B6, called P5P, and patients sometimes benefit from as much as 200 mg of P5P daily in divided doses.

Some of the signs and symptoms associated with Mauve Factor are pretty clearly related to the need for zinc and/or B6. Patients often have spots on the nails, or stretch marks consistent with very low zinc, or poor dream-recall, which is B6. The skin is often pale; it may have a so-called “China doll”appearance. I started looking at the inability to tan, and at vitiligo, which is being understood now in the context of excessive local levels of hydrogen peroxide, reversible in tissue culture with the addition of catalase, as a possible model for what we’re seeing in these patients with elevated Mauve. They tend to be really emotionally labile. There’s at least one mass murderer who was high-Mauve who became a very docile, agreeable guy with treatment. I’ve been studying this for over six years, particularly for the last six months as a primary focus. I think I probably have the comprehensive bibliography on Mauve Factor. (Available upon request from mcginnis@mind.net.)

Autism and Mauve Factor

JB: When we look at your interesting observation of the B6 and zinc connection, it sounds reminiscent of what Dr. Rimland has observed with certain types of autism, knowing that 50 percent of individuals with autism have high Mauve Factor. Do you think that’s part of the correlation?

WM: I do, though we haven’t studied this in detail to see if the approximately 50 percent of autistic children who respond to high-dose B6 are the ones who are the high Mauves. I think they likely are. There are other factors at work in autism which could be inhibiting B6 function, including yeast overgrowth, but I think there’s a likely strong overlap with high-Mauve. We have some good data in submission generated by Tapan Audhya on the levels of various nutrients in autism against a matched control group. We find that on functional tests, B6, B12, folate, and biotin are deficient, below-normal levels, in about 50 percent of the children. All of them can have significant antioxidant roles. Serum vitamin A, which I think of as an antioxidant factor, is low in nearly 70 percent of autistic children; red blood cell zinc in nearly 50 percent; selenium in 65 percent. The nutritional status of these children sets them up for oxidative stress.

Mauve Factor and Reaction to Stress

One of the most salient features, clinically, of these high-Mauve patients, is that they are exceedingly intolerant of stress, any kind of stress. This is a really obvious clinical fact that emerged and about which all the clinicians agree. These patients, irrespective of their behavioral diagnosis, have a spike in their urinary pyrrole measurement and a decline in their behavior if they lose sleep, if they travel, if there is an infection, or even an emotional upset. Everyone is in agreement about this and I consider it a bedrock basis on which to analyze the significance of Mauve Factor. To me, these forms of stress imply increased oxidative stress.

Oxidative Stress Connection

I started to think about the possibility that the Mauve Factor is caused by excess oxidative stress. I took a two or three-month trip into heme biosynthesis, which is full of pyrroles. I spent many hours looking and perhaps came up with a plausible way to get the Mauve Factor enzymatically from porphobilinogen and also relate the phenomenon to oxidative stress. It was a little tooconvoluted and didn’t really fit so I started looking for other things that impinge on the body exogenously which cause the creation of pyrrolic compounds. I found a really large universe of things from the outside that create pyrrolic compounds. The first one was hexane.

Hexane, by itself, is considered relatively non-toxic, but it is metabolically converted to a g-diketone

called 2-5-HD. This is a really nasty neurotoxin that has been recognized for the last 10 years. This 2-5-HD is a linear compound with twocarbonyl groups which form a cyclical pyrrolic adduct to tissue proteins. It especially binds the e-amino group of lysine. That is where the P5P forms its Schiff's base. The pyrrolic adducts from 2-5-HD crosslink and you can see resultant degeneration of neurofilaments. In fact, there seems to be a particular selectivity for neurofilament damage in 2-5-HD neuropathy. This is the accepted pathophysiology for chronic hexane axonopathy and was my first model for what I think is going on with the Mauve Factor.

Pyrrolizidine Alkaloid Compounds

The next thing I looked at was the pyrrolizidine alkaloid compounds. These are from various plants, including comfrey, which has been used, at least in the past, for herbal teas. Parenthetically, I think we should warn people about the danger of oral comfrey. It really shouldn't be consumed. There are quite a few flowers, including the common groundsel and senecio species, which have these alkaloids, and their toxicity is based on formation of a pyrrolic tissue adduct at that e -amino group of lysine and subsequent protein crosslinking. From these 2-5-HD type compounds—and there are others besides hexane—and the pyrrolizidine alkaloids, you get measurable pyrrole excretion in the urine. The University of Lisbon in Portugal has been trying to identify these pyrroles in the urine. They found that administering zinc to animals prior to hexane or 2-5-HD exposure would decrease pyrrolic urine excretion, which is of course what we see with Mauve Factor.

Oxygenation of Unsaturated Fats

Then I discovered a very substantial source of pyrroles in the body—free-radical oxidation of unsaturated fats. That's where I think the Mauve Factor derives. In most tissues this would mean especially oxidation of the arachidonic acid in the second position of the membrane phospholipid. In the brain and retina where it is really dense, it would be oxidative damage to DHA. Over the past decade, a huge literature has evolved in this area.11,12 We're beginning to understand how free-radical peroxidation of membrane lipids produces significant quantities of pyrrolic tissue adducts, which have that particularly high affinity for the
e-amino group of lysine. Lipid peroxidation and its sequelae are part and parcel of the pathogenesis of many illnesses. This includes the strictly "somatic" illnesses, such as atherosclerosis or complications of diabetes, and is now arching over to the neurodegenerative illnesses—Down's syndrome, Parkinson's, Alzheimer's, and so on.

Lipid Urine Peroxide Measures

Functionally, this oxidative stress can be measured as lipid peroxide in urine or blood, as apoptosis, or as pyrrolic tissue adducts. The pyrrolic tissue adducts form from arachidonate-derived oxidation products called levuglandins, and also from secondary oxidation products such as hydroxynonenal (4-HNE) and malondialdehyde (MDA), which are well-studied. Ethane or butane in the expired breath also reflect free-radical oxidation of unsaturated fats, ethane from the peroxidation and secondary oxidation of omega-3 polyunsaturates and butane probably from protein oxidation. Oxidized nucleic acids, or even nitrates and nitrites from the peroxynitrite radical, are other measures in use. And we are finding abnormalities in these measurements in the neurodegenerative diseases. I just finished a 70-article synopsis on this for my collaborator, Robert Solomon of Case Western Reserve University, yesterday on all of these measurements of oxidative damage in the neurodegenerative diseases, ranging from Down's syndrome to Alzheimer's. (If interested, contact Dr. McGinnis at mcginnis@mind.net)

The levuglandins are particularly reactive. They were discovered by Robert Solomon. He is helping us understand how these levuglandins are key to pyrrole formation and crosslinkage in a number of neurodegenerative diseases.

An Organic Chemistry Explanation of Oxidative Stress Processes

JB: You have given us some brilliant information. I applaud your detective work. For the sake of the listeners, let's go back a step to help them understand where you have taken us. From what I understand, the metabolic oxidative pathway, let's call it the detoxification pathways for what had been considered benign hydrocarbons, like hexane, end up going through oxidative steps that produce diketones. There are other substances, like polyunsaturated fatty acids, which, when they undergo oxidative chemistry, will also create along their unsaturated linkages, aldehydes, or ketones, principally aldehydes. Hydroxynonenal is one breakdown product. Another is malondialdehyde (MDA). These compounds have very high reactivity toward amino groups.

You talked about the reaction of amino groups, which we know are present in proteins, particularly the primary amino group found in the e amine of lysine, which can react to form what are called Schiff's bases, to use the organic chemical term. The nitrogen reacts with the aldehyde to give an imine. In the structure of that intermediate compound, if there is another aldehyde or ketone present, that imine can react with the other ketone to produce a cyclized product. That can be a five-membered ring, which is a pyrrole. Now we have a biosynthetic pathway from a linear organic hydrocarbon that could lead us into a pyrrole nitrogen-containing intermediate that would be endogenously synthesized on the basis of this oxidative chemistry occurring in situ. Is that another way of saying it?

WM: That's a really cogent summary. The specifics of the story on the levuglandin go this way. The bound arachidonic acid from that second position in the phospholipid can be released by phospholipase and go free and undergo enzymatic conversion to eicosanoids by cyclooxygenase or lipoxygenase. There's probably a way that can get confounded and kicked over into more reactive compounds if your glutathione peroxidase function is decreased. The primary source of levuglandin isn't through cyclooxygenase or lipoxygenase, but through free radical injury to arachidonic acid while it is still in the lipoprotein, while it's still membrane bound, or in the LDL.

The free radicals attack the unsaturated fat, the arachidonic acid or the DHA. This creates a g-ketoaldehyde, a prostanoid which has a ketone and an aldehyde group. These levuglandin prostanoids are highly reactive and want to form pyrrolic adducts about one hundred times more than hydroxynonenal. The levuglandin and the hydroxynonenal both form pyrrolic adducts, which goes on to cross-linking. Membrane damage, enzyme inhibition, and ultrastructural impairment ensue and ultimately affect the cellular calcium levels, function and viability of the cell.

Isolevuglandins

Once the free radicals have created this g-ketoaldehyde prostanoid through an endoperoxide intermediate, the oxidized ketoaldehyde may just sit there in the membrane, or it can be released. Increasingly, we find that Phospholipase A2 (PLA2) levels are really quite deranged in behavioral disorders, including autism, where it's highly elevated. Free radical and lipid peroxide levels definitely increase PLA2 activity, so elevations may reflect excess oxidative stress. On release from the membrane, then—and this is a key point—the oxidized arachidonate or DHA has to be released from the membrane before it can form the highly reactive levuglandin, or one of numerous reactive prostanoid isomers, which are called isolevuglandins. There are many of these levuglandin isomers, 64 isomers in just one levuglandin family.

These free-radical formed levuglandins are extremely reactive and they especially love to do one thing. They love to bind those lysine amino groups and, in the process, form the pyrrolic ring, that 5-member ring with the nitrogen in it—the pyrrole. This is directly analogous to what's happened with the 2-5-HD hexane poisoning and the plant alkaloid poisonings, in which measurable pyrroles are found in the urine.

These pyrrolic tissue adducts from oxidative injury to fat is measurable in blood and specific tissues such as brain. That's what they're doing at Case Western right now. They have a very sensitive and reproducible immunoassay to measure the arachidonic or DHA-derived levuglandin ketoaldehyde tissue adduct. It is also useful to understand that not all the lipid peroxides go to formation of pyrrolic tissue adducts: There is a parallel process in which some of the oxidized fat forms pentanyl rings which do not react to form pyrrolic tissue adducts. These latter compounds are called isoprostanes, which are stable and measurable in the urine. We are collaborating with Domenico Pratico at the University of Pennsylvania to measure urinary isoprostanes in autism.

Pyrrolic Compounds and the Mauve Factor

The pyrrolic compounds would be a direct indication of injury to both lipid and protein. Therefore, if the Mauve Factor is derived from these pyrrolic compounds, this would take us into deep layers of pathology. As it turns out, the levuglandin adduct has long remnant side chains from the original fatty acid, and molecular Mauve Factor, HPL, only has simple methyl and ethyl groups in these positions on the pyrrolic ring. Fatty-acid side-chains are really susceptible in vivo to myriad cleaving and shortening secondary oxidative reactions, which are exceedingly diverse. So I propose that ongoing oxidative stress shortens the side chains of these levuglandin adducts and produces the Mauve Factor molecular structure. No one has looked for urinary pyrrole excretion with levuglandins as they do find in the 2-5-HD and pyrrolizidine models. All I have to do is cleave-down the side chains on the pyrrolic adduct from the levuglandin and I get structurally precise Mauve Factor, which for levuglandin and isolevuglandin pyrrolic adducts, is always the hydroxylactam, as is Mauve Factor.

I really think that this Mauve Factor is derived from the pyrrolic levuglandin isomers which result from oxidation of lipid membrane and ensuing protein adduction. This hypothesis may or may not prove valid. Regardless, it is very important to emphasize that the Mauve Factor has very high utility clinically. And I think it's now time for us to open the best minds and the best technology to the notion of oxidative stress as etiology and basic pathogenesis in the behavioral disorders. I find it quite exciting stuff.

Oxidative Mechanisms and Disease

JB: This could be a threshold of tremendous importance. It ties together so many potential variables that we know have some relationship to altered brain chemistry and mood, mind, memory and behavior. I refer to things like heavy elements and the concepts of allergies, fungal infections, gut dysbiosis, and various things that seem like outliers. We may just have been missing the central theme that may be explained through these oxidative mechanisms.

WM: There are so many things, Jeff, that fit in autism. We've known for 25 years that they have low glutathione peroxidase function in red cells. This work was done by the French and just this last year, the Turks came up with a confirmation study also showing low superoxide dismutase activity in the children. We have a number of very low antioxidant nutrients in the children. We have high autoimmune markers in at least half of these children—increased auto-antibodies to myelin basic protein, and to a couple of very key intermediate filaments, neurofilament and glial fibrillary acidic protein. This is published data. Autoimmune disease may, in fact, stem primarily from oxidative insult to protein and nucleic acid in the cell.

Levuglandin and Isoprostane Formation

JB: Your model seems to imply some kind of relationship between levuglandin and isoprostane formation. Has that been reported?

WM: It has been, but it can be misleading. In one group of animals exposed to very high oxidative stress, they got high levuglandin tissue adducts, but no corresponding elevation of isoprostanes, so they don't always go hand in hand. They are parallel processes, but there's a complexity there that means the two can function independently, or at least are not measurable synchronously.

Pyrrolizidine, Pyrroles, and Levuglandin

JB: You mentioned that with pyrrolizidine alkaloids that there was some correlation between pyrroles and pyrrolizidine, but no correlation that you're aware of yet between pyrroles like Mauve and levuglandin.

WM: We haven't looked at that yet, though we may be stimulating some interest in it. It would be one of the first things we want to do in our research. We are jumping ahead a bit and currently are executing the Oxidative Stress Study in Autism, which is going to take a half-dozen of the best technologic measures for oxidative stress, apply them to an autistic group, and see what we come up with in terms of correlation and utility.

Heme Porphyrin and Pyrroles

JB: Are we sure there is no heme porphyrin connection?

WM: No. Not at all. I wouldn't give up on that notion one bit. The HPL is what we think is the true structure for the native Mauve Factor in urine. It's pretty close structurally to porphobilinogen, which, hypothetically, could be enzymatically converted to Mauve. It would need action by at least three enzymes to get the groups in all of the ring positions to coincide, but it's a possibility. It is also possible that oxidative stress is generating Mauve, but not the way I propose. The brain is very low on catalase, and there are variations in the gut, as well. Hydrogen peroxide itself can attack hemoglobin and release free iron, which we both know can really create havoc in terms of free radical generation. So I am very ready to agree that an alteration in the heme biosynthetic pathway could explain the Mauve Factor. I'm submitting this notion about the origin of Mauve Factor from oxidation of lipids as a hypothesis, but my mind is open to the other, for sure.

The Oral Glutathione Myth

It's a mistake for us to get locked in. We have a tendency, I think, sometimes, even in our progressive wing of medicine and applied medical science, to form our own myths. One of them that I've been trying to get over in the last couple of years has been the myth about poor utilization of oral glutathione. Oral glutathione, in fact, gets really excellent absorption by the epithelial cells in the gut. There's awfully good literature on how high doses of oral glutathione will increase circulating plasma levels by as much as five times in healthy adults, probably even greater increases in the tissue levels in patients who have high degrees of oxidative stress. There's one pharmaceutical company now which is in phase 3 on high-dose glutathione for AIDS which is showing that circulating monocytes have a doubling of their glutathione levels within 30 minutes of large oral doses.

I've been using oral glutathione in several high-Mauve autistic children with really excellent results, starting with about 10 mg per kg in divided doses and working up. They do this in Cystic Fibrosis children, who have very high oxidative stress. I digress a bit, I guess.

Oral Glutathione

JB: That's very useful information. From my experience, which is not nearly as vast as yours in this area, I would concur that oral glutathione at high dose can influence plasma levels quite significantly because it's not broken down; it's not a normal peptide linkage. Many people feel it's broken down into requisite amino acids that make up the tri-peptide of glutathione, but that's not true.

WM: We agree. If you poison the enzymes for synthesis and for the disassembling absorption, you still get really good increase in cellular levels. In this population of behavioral kids with autism we have very significant inflammatory changes throughout the GI tract. For such populations, applying the oral glutathione can be really efficacious. The body, at considerable metabolic expense, secretes high volumes of reduced glutathione in the bile. There's a 24-hour a day trickle down through the gut. The biliary concentration of reduced glutathione may be 20 or 30 times as much as it is in the plasma. Unfortunately, when the body function suffers from toxic exposure, one thing which happens is cessation or diminution of choleresis. Bile flow shuts off, which is just what you don't want. I look at the reduced glutathione that's trickling past the gut as the body's first good defense against all the hot electrophiles—poisons really—which are ingested. Altered foods, peroxidized foods, heavy metals, insecticides, and the rest are less damaging to the gut, and ultimately the system, if you can neutralize them with glutathione in the lumen of the gut. I think that's an area for clinicians that really has large promise.

Antioxidants and Autism

JB: We could spend hours talking about this; it's fascinating. You've talked about vitamin C, zinc, B6, and oral glutathione as some of the nutrients that help improve metabolism of these porphyrin-like compounds. Is there anything else that we should be aware of that you see on the horizon as useful, given this oxidative theory? I guess we would look at a range of antioxidants that might be important, as well.

WM: You would. You'd look at some fat solubles like CoQ10; and there may be some synthetic antioxidants and probably some current pharmaceuticals which are appropriate. Deprenyl appears to have quite an antioxidant effect. There are two studies by which I am most impressed in autism in the last 10 years. They were well-controlled studies in blue chip journals which used very good psychometrics and demonstrated excellent improvement in autistic children using single nutrients. Limitation to a single nutrient places a pretty high bar for such studies. Combinations are usually so essential. At any rate, the first study used multi-gram doses of vitamin C and saw wonderful improvement in the autistic children.13 The other, more recent study, by Michael Chez in Chicago, also very well-run, showed excellent improvement in autism with 800 mg of carnosine, which is known for its lipid antioxidant effect.14

These are some of the ideas we have. It's a wonderful field, full of creative intellect. Bill Walsh at the Pfeiffer Treatment Center is showing the way in oxidative stress in autism in his examination of metallothionein. Mary Megson has so much to offer. She presumes a defect in the G-protein switch consistent with the night blindness we're seeing in the families, and the supernumerary nipples in the children, and many other clinical clues. This ties in very closely to elevations in the intracellular calcium levels reflected by very elevated parathyroid hypertensive factor (PHF) measurements we're getting through the University of Alberta. The autistic children have the very highest PHF measurements of all the groups measured. This all makes a nice confluence with what I presume is a significant oxidative injury, as seemingly reflected by higher pyrrole measurements.

Frontiers of Research on Oxidative Stress

JB: I can't thank you enough, both for your professional dedication and for sharing this information with the FMU audience. This represents a lifetime of work for individuals to piece this puzzle together, but I think it's a direction finder. Often, we need to get on a beachhead and start on a new journey. I think your concept that we're looking at central mechanisms of oxidative injury and the secondary byproducts that are endogenously produced, which then alter neuroreceptive pathways is a fascinating frontier that will lead to opportunities for new therapies.

If you were to take where we are right now, knowing there is always a tempering of our optimism with the reality of what we don't know, what insights would you leave with our listeners?

WM: Whether or not my hypothesis about Mauve Factor is valid, we have enough clues to mandate a real focus on the broader theme of oxidative stress in the neurologic disorders, including especially the behavioral disorders. It's time for us to open our best minds and our best technology to the oxidative mechanisms for the behavioral disorders. I think in the trenches, clinicians should consider getting the various tests available to ascertain oxidative stress in the behavioral diagnoses and titrate remedies accordingly.

Lastly, I want to remind everybody to take their zinc. Zinc is the ultimate antioxidant, I think, in terms of membrane protection. I might be overstating it a bit. Then, I want to thank you, Jeff, for a fine interlude with you today, and also for your inspiration and your leadership.

Future of Autism Research

JB: Thank you, Dr. McGinnis. I think you have pointed all of us toward a new theme that can take us over a hump. Since Dr. Rimland first brought the concept of autism and B6 to the field, with significant resistance from his peers, this has been an upward battle. I think we may now be reaching the top of the hill. Thank you very much for giving us this energy and insight.

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