November 2000 Issue | Bruce Ames, PhD

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Detection of "Preclinical" Early Alzheimer's Disease—Patterns of Brain Activation—Nutrients and Brain Function

Alzheimer's disease is another common condition of aging. The authors of a report in the *New England Journal of Medicine,* titled "Patterns of Brain Activation in People at Risk for Alzheimer's Disease," discussed various genotypes related to Alzheimer's risk. In previous issues of *FMU* we have talked about the apolipoprotein E family, suggesting that the apo E4, either single or double allele, is associated with increased risk of vascular disease and dementia of Alzheimer's. The authors of this study were looking for observable functional decrements in central nervous system ability to perform that would enable them to predict the onset of early Alzheimer's. They were looking at patterns of brain activation in neurologically normal subjects. They observed changes in brain activity mirrored the known genetic risk factor apo E4. These are functional medicine questions that relate not to the singular diagnosis and the establishment of a diagnostic code, but rather to the evaluation of function.

An editorial following this paper in the same issue of the *New England Journal of Medicine*was titled "Detection of Preclinical Alzheimer's Disease." "Preclinical" in this case means functional criteria for establishing the potential for Alzheimer's disease. Early detection is necessary, if intervention is to be successful

Does this mean Alzheimer's is hard-wired in our genes? That is the question of our age. We are quick to make a gene connection to a condition, assuming flawed genes or genetic polymorphism cause the condition. We should not jump to conclusions of that type. We can modify the influence of many genetic characteristics—our sensitivities—on the basis of how we treat those genes. We really should be asking, therefore, if we can modify the environment so the apo E4 genotype does not exhibit undue influence on the physiological state that results in early neuronal death in the hippocampus and cortex. That is a very different question in medicine, a different hypothesis, and a different approach toward medical evaluation and intervention from any we have previously asked when our focus has been on diagnosis and treatment.

Environment and Nutrition Effects on Alzheimer's Disease

In Alzheimer's disease research, increasing information suggests accumulation of b amyloid plaque is related to pathophysiological mechanisms that may, in part, be modifiable by environmental and nutritional agents. A toxic mechanism may be related to the deposition of these b amyloid plaques. These neuritic plaques are associated with the loss of neuronal function we later call Alzheimer's disease.

This potential mechanism is reviewed in a recent issue of *Nature Neuroscience.* The authors discuss b amyloid deposition in the brain, which we know is a hallmark of Alzheimer's disease. They discovered b amyloid toxicity may be mediated by the interaction of the fibrillar Ab lipoprotein (amyloid protein) with neuronal membrane proteins, including amyloid precursor protein (APP). This particular process of association and its ultimate effects on apoptotic cell death, they speculate, may be mediated through a series of environmental factors that contribute to premature cell death. It is not just the genes that cause the disease; it is the gene activity as modified by interaction with environmental factors.

Nutrients and Brain Function

We might express that in another way in relation to dementia. Can nutrients modify brain function? That question was the title of a recent paper in the *American Journal of Clinical Nutrition*. This paper emerged from the laboratory and pen of Dr. John Fernstrom. He and Richard Wurtman are principal investigators at MIT who have worked on the nutrient/brain function connection and helped us understand that connection over the last 20 years.

In this paper, Dr. Fernstrom points out that over the last 40 years, many lines of investigation have shown the chemistry and function of both the developing and the mature brain are influenced by diet and dietary constituents. Examples include folate deficiency, vitamin B12 deficiency, and the effects of tryptophan and tyrosine intake on production of brain neurotransmitters, including serotonin and dopamine, respectively. We know about the role of the nutrient choline in the production of acetylcholine, one of the important neurotransmitters. We know about various kinds of toxins that might be delivered through the diet and the role they can play on neuronal function through neuronal excitotoxicity and activation of various receptor sites for neuronal excitotoxic response like the NMDA receptor site.

According to Dr. Fernstrom, nutrients can certainly influence brain function, and nutrient supplements may play a very important role in protecting the brain against functional loss in the course of aging. Asking questions about the function of the body is very different from the questions that have been asked historically by other cultures. We now expect our bodies to endure for 8, 9, or 10 decades. We want to be free of disease to the extent possible. This entirely new expectation of health requires new models for its evaluation. Dr. Fernstrom talks about the potential role of nutrient supplementation in matching an individual's genes to his or her nutritional needs to improve health span.

As Dr. John Bell explained in a recent editorial in the *British Journal of Medicine,* we are moving to a new genetics in clinical practice. It is a genetics that is a less deterministic and hard-wired than what we may have learned in an introductory genetics class. When we learned about Gregor Mendel and his laws of dominant and recessive genes, we naturally assumed everything was locked immutably into our genes and could not be changed. We believed we were hard-wired for the diseases we would get.

In contrast, the new genetics discusses modification of expression, the existence of polymorphism in the population, and the pleotropic effects of individual proteins that are locked into our genetic code. We can modify the biological activity of these molecules epigenetically with certain coenzymes and cofactors that are often nutrient-derived. These small molecules affect the function of these macromolecules, giving rise ultimately to cellular, tissue, and organ system function.

We are now looking at early assessment, stratifying patients into individual groups based on these

polymorphisms, and trying to determine how to direct specific environmental modifications toward those genotypic uniquenesses to improve the phenotypic outcome.

Medicine for Individual Genomic Characteristics

Another paper in an ongoing series in the *British Medical Journal* on this topic is titled "Are We Moving toward Rational or Rationed Medicine? The Promise of Genetics for Improved Clinical Practice." The authors, Robin Fears, Derek Roberts, and George Poste, describe how genomics research and the Human Genome Project are creating a new understanding of personalized medicine. Medicine for the average, they say, will no longer be acceptable. Medicine must be tailored to individual genetic characteristics.

This prediction is already being applied in the area of pharmacogenetics. Drug companies are being forced to understand how their drugs are metabolized or detoxified, and how those detoxification pathways can vary at the genomic level through polymorphism. There is polymorphism of cytochrome P450—the 1A1 or the 1A2 families, the 2A1 family, the 1E1 family, and the 2D6 family. All of these isoforms of cytochrome P450 monooxygenase detoxifying enzymes that appear in the liver and other tissues have genetic polymorphisms and thus functional variety in different individuals.

Individualized Drug Metabolism

The way a drug is metabolized by a specific enzyme in the average individual may be very different from the way it is metabolized by a specific individual with a unique polymorphism of that enzyme. Some people are rapid detoxifiers, for example, and others are slow detoxifiers. For the rapid detoxifier, the drug may not have its therapeutic benefit because it is so quickly metabolized and eliminated from the body. For a slow detoxifier; the standard dose may produce a neurotoxic or immunotoxic effect and result in what we used to call "atypical adverse side effects." Now we know these effects are adverse, but not atypical. They are reproducible in that individual if you ask a different question. What is the relative uniqueness of his or her ability to detoxify that molecule as a consequence of genetic metabolism uniqueness for detoxification?

Pharmacogenetics will play a role in future medical prescription patterns and medical/legal liability. In the future, a doctor who does not evaluate a patient’s detoxification profile and administers a drug to which that patient has an adverse side effect may be medically/legally liable for any adverse side effect. That is the direction in which this field is moving. It will change the questions we ask before administering certain medications. This topic is discussed in an article titled "Science, Medicine, and the Future. Pharmacogenetics," in the *British Medical Journal*.

In a nutritional example of that discussion, it is known that certain diets have traditionally been associated with lowered risk of particular diseases. Cancer incidence, for example, is lower in cultures that consume more cruciferous vegetables, including broccoli, cauliflower, cabbage, and Brussels sprouts. These vegetables contain unique phytochemicals or phytonutrients called glucosinolates. The glucosinolates are hydrolyzed by an enzyme, myrosinase, which is released from plant cells by maceration, crushing the cells of the plant by processing or chewing. Myrosinase works on the substrate, the glycosinolates, to produce a variety of secondary biochemicals that are produced in the GI system that may be absorbed and gain entry to the systemic circulation.

These potential anticancer substances include indole-3-carbinol, phenyl-isothiocyanate, 2-hydroxy-3-butane, sulforaphane, and other molecules. These substance, in turn, influence on gene expression. That is the knowledge that is emerging. Some of them have specific influence on the expression of genes and the activity of the phase I and phase II detoxification enzymes.

Varying Effects of Isothiocyanates in Various Individuals

A *Lancet* article, titled "Isothiocyanates, Glutathione S-Transferase M1 and T1 Polymorphisms, and Lung Cancer Risk: a Prospective Study of Men in Shanghai, China," may not seem unusual to long-time *FMU* subscribers. Glutathione S-transferase is a phase II detoxifying enzyme that involves the ability to connect a glutathione molecule to a biotransformed intermediate to produce a nontoxic, excretable material. Isothiocyanates are the secondary byproducts of glucosinolates from crucifers of these "anticancer substances." This article points out that people with different polymorphisms of glutathione S-transferases, M1 and T1, have different sensitivities to the isothiocyanates in their diet. Not all people are affected identically. Some people might have a much more significant impact from eating crucifers in their diet and reduction of risk to cancer than others, based upon how these phytonutrients in foods interact with their genotype. Isothiocyanates appear to reduce lung cancer risk in Chinese men. Reduction in risk was strongest among those who were genetically deficient in enzymes that rapidly eliminate these compounds, meaning they have a higher residence in the body and more therapeutic effects.

We are getting back to a molecular medicine-based approach to biochemical individuality, or the genetotrophic theory of disease that Dr. Roger Williams described 60 years ago. This powerful tool is integrating genetics, metabolism, pharmacology, nutrition, and medicine to provide functional improvement for patients.

The same approach applies to medications as well as to diet. A paper titled "Association of Polymorphisms in the Cytochrome P450 CYP2C9 with Warfarin Dose Requirement and Risk of Bleeding Complications," appeared in the *Lancet.* Warfarin is a common medication used for blood thinning and anticoagulant effects. People with specific CYP2C9 polymorphisms may exhibit patterns of warfarin detoxification, which puts them at increased risk of bleeding from doses of warfarin that would be therapeutic for others. The compound may reside in the body for longer periods of time if the individual is a slow detoxifier.

There is a strong association between these variant alleles and low warfarin dose requirement. An individual who has not been properly monitored can get into trouble in this regard. It is not a matter of simply matching a standard dose to one’s body surface area; the dose should be based upon the personalized metabolism of the individual. This is a powerful example of the concept of pharmacogenetics.

N-acetylcysteine and Renal Function

Another interesting example is a *New England Journal of Medicine* paper titled "Acetylcysteine and Nephrotoxic Effects of Radiographic Contrast Agents—A New Use for An Old Drug." The radio contrast agents employed in pyelograms can induce acute renal failure, even when measures are taken to reduce the toxic effects. The adverse effects prolong hospital stays, add to the cost of medical care, and, in the

worst case, can be fatal. The incidence of radiographic contrast agent-induced acute renal failure is currently estimated to be as high as 50 percent among patients with diabetes and preexisting renal disease who receive contrast agents. That rate is likely to remain high even as the use of invasive radiologic procedures to diagnose and treat complex disease continues to grow.

In another *New England Journal of Medicine* paper, Tepel et. al. showed that just 1200 mg of N-acetylcysteine per day, given orally in divided doses on the day before and the day of the administration of the radiocontrast agent, prevented the expected decline in renal function in all patients with moderate renal insufficiency and reduce the risk of renal failure in whose who were undergoing computer tomography. N-acetylcysteine is known in pharmacology as Mucomyst or Mucosil. The mechanism by which it works has not been completely demonstrated. The proposal has been made that it works through N-acetylcysteine's antioxidant effects by soaking up free radicals that are induced as a consequence of the administration of these radio contrast agents. A second suggested possibility is that it works by its ability to improve detoxification through conjugation in the liver with glutathione.

N-acetylcysteine stimulates hepatic glutathione synthesis and thus improves phase II glutathione conjugation. Administration of a substance tailored to the need of an individual based on specific environmental circumstances—exposure to radio contrast agents—changes their need for a pronutrient. N-acetylcysteine is a precursor of glutathione, or part of the essential amino acid family. Cysteine is a sulfur amino acid.

The understanding of disease mechanisms at the molecular level and their relationship to gene expression is rapidly increasing. One area of increased knowledge is autoimmune disease, in which the body becomes allergic to itself. In the disease systemic lupus erythematosis (SLE), the body loses kidney function. We now understand that the body processes the molecular debris as a consequence of the clearance of apoptotic and necrotic cells. These dying cells release substances that serve as foreign molecules to which the body responds immunologically. The resultant antibodies can cross-react with specific tissues in organs like the kidneys.

Apoptotic and necrotic cells have become strong candidates as sources of the autoantigens that drive the autoantibody response in SLE and possibly other autoimmune disorders. Those defects in physiologic mechanisms may be much more widespread than we previously thought when we believed the immune system just went awry and began to attack the body. The physiological mechanisms for the clearance of dying cells or increased death of cells result from many different events, some of which are environmentally induced. These processes may promote disease susceptibility in genetically sensitive individuals who are susceptible to SLE. I refer to an article that appeared in *Nature Genetics*.

Using that model, we might consider reducing the risk of SLE in a genetically susceptible individual by lowering the load of substances that increase apoptotic cell death and load the system with autoantigens. To accomplish that risk reduction, one might work to lower the stress factors, antinutrients, and prooxidants in induced oxidative stress, all of which induce apoptotic cellular changes and preprogrammed cell death.
The Paradigm Shift—Evolution or Revolution?

We are moving toward molecular epidemiology. Dr. James Goodwin, a presenter at the Seventh International Symposium on Functional Medicine, made it clear that theories are nice, but the proof in

clinical practice is in how things really work. Through this presentation we got a sense that empiricism through clinical study may be preeminent over theoretic work at the fundamental science level. In talking with Dr. Goodwin after his presentation, however, I learned he recognizes that visions of new models that emerge from basic research and biomedical evaluations drive our opportunities to study clinical variations in treatment. It is somehow a hybrid of the two. Pure science drives clinical experimentation; clinical observation then drives more pure science and biomedical research. These work synergistically to produce a model to improved patient health and outcome.

Molecular epidemiology is consistent with personalized preventive medicine. I am now referring to a Journal of the National Cancer Institute article titled "Molecular Epidemiology: On the Path to Prevention?" An incentive should be provided, according to this article, to invest in areas which reduce the incidence of disease by the analysis and understanding of genotype and modifying the environment appropriately in susceptible individuals. This progress comes not from social epidemiology, but from molecular epidemiological work, according to this article.

A Paradigm Shift in Medicine

A recent paper in a British medical journal discussed moving beyond the curative model to the individualized prevention model. According to this article we are seeing a paradigm shift in thinking. The authors propose that by 2005 the principles underlying an expanded view of health based on genomics will become general knowledge. It will start to integrate more effectively into clinical practice. The traditional practice of medicine will integrate with the principles of functional and molecular medicine.

The year 2010, these authors propose, will mark the beginning of major government initiative by legislators who finally understand the interconnectedness of socioeconomic status, the public’s health, and these genomic-related issues. We will not consider genes as some kind of hard-wired legacy from our parents, but rather as plastic, variably expressed elements that can give rise to a multitude of outcomes, depending upon the way the genes are treated.

An article by Anna MacIntosh, PhD, ND and Karen Ball, ND, takes this discussion to the level of clinical application. The article, titled "The Effects of a Short Program of Detoxification in Disease-Free Individuals," appeared in *Alternative Therapies.*" We sometimes think the constructs we have described apply only to individuals with seriously impaired function. The authors of this paper studied a group of individuals with reasonably high function. The subjects were medical students in the first few years of their curriculum. (One might wonder how highly these students could be functioning, given the demands on a medical student’s time, nutritional status, and stress factors.) Notwithstanding our disclaimer, the authors studied these medical students before and after a lifestyle and nutrition intervention program designed to improve hepatic detoxification.

Following the seven-day metabolic detoxification program, subjects were evaluated by a variety of instruments and biochemistries for 1) clinical improvement in function; and 2) improvement in relative detoxification patterns. According to the investigators, this simple seven-day detoxification program resulted in a significant reduction in participant symptomatology and a significant increase in overall functional status. The tendency toward improvement in liver detoxification measures, as evaluated through cytochrome P450 and phase II conjugation reactions, seemed to be consistent with the improvement in their overall sense of well being. This pilot study focused on patient outcome. It was a

non-controlled clinical intervention in disease-free participants, using a variety of instruments for assessing health, including the Metabolic Screening Questionnaire. This study indicates that even people who feel they are not sick may have latitude for improved gene expression and improved function by intervening and practicing the right program.

We are discussing regulation of programmed cell death, basic approaches toward sending the right messages, the right signals, in order to insure a functional cell cycle. That seems to be the direction biomedical research is taking us. We work to allow cells to achieve the right balance of function, regeneration, and recycling; not accelerating their death and not causing them to live too long, but allowing them to be renewed at the appropriate rate and under normal control. This process is described in *Molecular Medicine Today* in an article titled "Programmed Cell-Death Regulation: Basic Mechanisms and Therapeutic Opportunities."

Antioxidants and other nutrients play a role in apoptotic cell changes in cell cycling and cell signaling messages. First, at a gross level, we can look at data on plasma antioxidant levels and longevity. A recent paper in *Free Radical Biology and Medicine* examined the level of plasma antioxidants in centenarians and found their levels were higher than in unhealthy younger-age individuals. The healthy older people had higher levels of vitamin E and carotenoids than the younger individuals. They also had higher activity of plasma superoxide dismutase and glutathione peroxidase, indicating increased antioxidant enzyme activity that depends on minerals like zinc, copper, manganese, and selenium. Therefore, it appeared that their nutritional status was improved. This does not prove causality, but the association was very strong.

The Role of Antioxidants

In the last 10 years, we have learned that antioxidants are much more than just substances that soak up oxidant garbage. They are also involved in regulation of redox potential within cells. That redox signaling, the balance between reduction and oxidation, the electromotive force of the cell, is like a storage battery that has a certain EMF, a certain voltage, a certain charge gap across it, that controls the energy and potential power of that cell. Antioxidants serve as redox-active signaling agents. This activity is described in a series of papers in *Antioxidants and Redox Signaling*. One of those papers is an editorial titled "Biomedical Aspects of Plasma Membrane Redox." The author, Dr. Aubrey De Grey from the Department of Genetics, University of Cambridge, England, stresses the importance of cellular redox regulation in controlling cell cycling, cell signaling, intercellular communication, and the balance between cell longevity, cell death, and cell regeneration.

Antioxidants, therefore, do more than just soaking up free radical substances. They help in buffering the redox potential of cells. We have proteins that buffer the pH of our plasma and ionic buffers in our osmolarity systems within cellular salt systems. We have the buffering of glucose with glycogen, another critical cellular element. And we also have intracellular redox buffering with antioxidants. The plasma membrane redox system helps control a buffer against stress-induced premature apoptotic cell death. That process is discussed in a review by Jose Villalba and Placido Navas from the Department of Cellular and Biological Energy, University of Cordoba in Spain.

Mitochondria and Redox Activities

The complex array of phytonutrients with redox potential that we consume involve more than

antioxidants. They are part of a buffering system to control cell signals through the electromotive force at the energy powerhouse of the cell, the mitochondrion. Impairment of various antioxidants leads to the loss of this redox control and an increased shift toward oxidant species.

The mitochondrion is the organelle where most oxygen is consumed in the cell, where numerous oxidation/reduction reactions take place, and the potential source of most of our oxidants. If the mitochondrial transmembrane potential and oxidative damage is not properly controlled, the loss of mitochondrial function is accelerated. The mitochondrion has its own DNA, its own genetic material that can be damaged. In cases of poor redox buffering, i.e., antioxidant insufficiencies, the mitochondria may be at increased risk of genetic damage. I refer to an article in *Free Radical Biology and Medicine.*

Years ago, Dr. Stadtman told us that when the mitochondria are uncoupled, they can produce increased production of oxidants, which results in the oxidation of proteins in the cell, and produce what he called protein carbonyls. The carbonyl proteins put a load on the detoxification and immune systems of the body. These are foreign proteins, and the body recognizes them as unwanted, as garbage, and tries to get rid of them. Therefore, there is potential for detoxification upregulation, and higher levels of certain nutrients are required to support that process. If a person is nutrient-deprived, then the detoxification process is compromised. Adverse effects of the damaged proteins can be observed on the nervous, immune, or endocrine systems, making a molecular contribution to aging, to gerontology.

Measuring the Presence of Reactive Oxygen Species

A variety of techniques have been employed to quantify the presence of reactive oxygen species and oxidant stress factors that may have been produced at the mitochondria. We can try to measure the level of antioxidants in the plasma or biological fluid. We can try to measure total antioxidant capacity, the so-called oxygen-reducing absorbence capacity. Or we can look at the presence of oxidized debris like protein carbonyls or damaged DNA. The quantitation of 8-hydroxy-deoxyguanosine is commonly used to see if DNA has been damaged. A number of methods are being explored for the functional assessment of redox balance and imbalance that occurs in redox associated with rapid molecular aging. A recent review in *Free Radical and Biology and Medicine* discusses one of these methods, cyclic voltammetry, as a means of easily monitoring redox balance.

Another method is to look at the urine, catching damaged DNA on the way out. This method was described in a recent review in *Clinical Chemistry*. Urine DNA debris can be used for evaluating certain aspects of oxidative stress. This test looks for different DNA variants that may reflect different aspects of gene expression or oxidative stress. The analysis of plasma and urine for evidence of DNA damage is becoming more routine in the laboratory.

Neopterin is another molecule in plasma that can be studied as an indicator of oxidative stress. This molecule is part of the pterin molecules of folate. It is also part of the tetrahydrobiopterin molecule, an essential cofactor of nitric oxide synthase and thus required for nitric oxide production. Release of neopterin indicates a more rapid turnover of tetrahydrobiopterin, implying more inducible nitric oxide is being produced. When nitric oxide is produced in excess quantities it can react with superoxide (which results as a consequence of uncoupled mitochondrial function) to form peroxynitrite, a new, more caustic molecule produced at rate-limiting diffusion kinetics. Peroxynitrite can nitrosate proteins that can uncouple mitochondrial oxidative phosphorylation at several steps along the path. It is a dangerous

molecule because it also has a fairly long lifetime within cells.

Excessive quantities of neopterin can be an indirect indication of increased immunological upregulation with uncoupling of mitochondrial function and increased oxidant stress. A number of papers confirm this fact. An article in *Anticancer Research* describes the increased neopterin concentrations in plasma in patients with cancer and may suggest this is an indicator of oxidative stress induced by immune upregulation. Similarly, consistent with the role of neuronal oxidative stress in Alzheimer's patients, you see increased serum neopterin concentrations. This observation, discussed in a paper in *Clinical Chemistry and Laboratory Medicine*, again demonstrates that increased TNFa , the proinflammatory cytokine production associated with inducible nitric oxide induction, is seen as elevated neopterin concentrations in Alzheimer's patients and is associated with neuronal oxidative stress.

Finally, coronary artery disease, the atherogenic process of which is also associated with the oxidative processes and free radical damage, is associated with serum neopterin. This association, discussed in a paper in the journal *Heart*, argues that increased upregulation of the inducible form of nitric oxide synthase driven by proinflammatory cytokines, increases nitric oxide, uncouples mitochondria, and can induce oxidative stress. Neopterin may represent an up-and-coming analyte for evaluating the connections among immune upregulation, inflammatory potential, and oxidative stress across a wide range of clinical problems associated with age-related disease. A review of neopterin that appeared in *Experimental Dermatology* provides an overview of neopterin's potential assessment role in these biomedical markers.

Mitochondrial DNA Function and Age-Related Diseases

We have been talking about mitochondrial DNA, mitochondrial function, and their relationship to molecular aging. What is the relevance of this relationship to cancer, heart disease, diabetes, arthritis, inflammatory bowel disease, and Crohn's disease? In every case, mitochondrial DNA function and mutations play a role, or at least is a very strong associated process, with the onset of those age-related diseases. A recent Lancet paper, titled "Relevance of Mitochondrial DNA in Cancer," describes this association."

Modification of Mitochondrial Function with Coenzyme Q10

A variety of redox-active substances, such as antioxidants like coenzyme Q10, modify mitochondrial function. A paper in the Lancet describes coenzyme Q10 deficiency and its relationship to respiratory-chain dysfunction in individuals who have problems with cardiopulmonary function and cardiac function. Coenzyme Q10 is another "conditionally essential" nutrient.

INTERVIEW TRANSCRIPT

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JB: This month as our Clinician/Researcher of the Month we are privileged to have Dr. Bruce Ames. Dr. Ames has been a guide and mentor, influencing the way I think about the impact of science on medicine.

Dr. Ames, a professor of biochemistry and molecular biology, is director of the National Institute of Environmental Health Sciences Center at the University of California at Berkeley. In the Science Citation Index, Dr. Ames is among the few hundred most frequently cited scientists in all fields, with more than 400 publications in a number of areas. He developed the Ames Assay, the *Salmonella typhimurium* mutant assay for mutagenicity and carcinogenicity.

Biochemistry and Physiology

Dr. Ames, perhaps you could start by telling us what you've observed regarding the evolution of biochemistry and its relationship to physiology.

BA: I was always half a biochemist and half a geneticist, so I was always interested in both fields. That is good, because I kept seeing problems that geneticists turned up and didn't know how to handle. The solutions were biochemical, but the biochemists didn't know the problems existed. Insomuch as I've made a mark, it's because I like to work between two fields.

Biochemistry has been going forward like a rocket for 30 years. Now we really understand how mitochondria work and how general metabolism works. But it is so complicated, obviously, that it will take years to understand things completely. Genetics has moved forward like a rocket, too. I think people are getting a pretty good outline of metabolism—how perturbations affect health.

Testing for Mutagens

A thread through most of my own research has been DNA damage—what's causing it and how you prevent it. That's how I got into the mutagenicity tests. Some years ago, during a time when we were mutating bacteria all the time to change regulatory circuits, I read the label on a package of potato chips and saw all these food additives. It occurred to me that nobody had really been testing these things for mutagenicity. I thought it would be useful to develop a simple test for detecting mutagens, and we did that.

The first test was developed at NIH in the mid-1960s and then at Berkeley in the early 1970s. I guess it's still being used. I thought the test would be outmoded in five years or so. In fact, it seems so long ago, but that test is still being used and was a thread through my research.

Testing Chemicals

Originally, I was interested in things like food additives, but then I got interested in synthetic chemicals. Then it became apparent that most of the chemicals we ingest are natural chemicals. There's no particular reason to think synthetic chemicals are any worse than natural chemicals and 99.99 percent of the pesticides you eat, for example, are natural chemicals in plants. So my interest switched more to natural chemicals. In recent years, I think the gold is in nutrition; that is, tuning up metabolism. So that's the thread that runs through my research.

Mutagens seemed plausible as carcinogens. When we first developed the test for mutagenicity, a number of chemicals known to cause cancer in animals showed up in the test. Then, because I am partly a geneticist, I got interested in the relationship between mutagens and carcinogens. That seemed fairly

obvious to me, but at that time, people hadn't really been convinced that mutagens and carcinogens had much to do with each other. We made the argument that it was important, and metabolism is one of the reasons.

The Ames Test

No one had shown that beta-naphthyl amine, an aromatic amine used in the aniline dye industry known to cause bladder cancer in people, was really a mutagen. Then people like the Millers and Boyland and some pioneering researchers showed that the true carcinogens were metabolites of these compounds. Cytochrome P450 converted these compounds to more water-soluble compounds, and some of those were mutagens. We showed the active forms were mutagens.

Then we came up with the idea of putting some ground up rat liver on a Petri plate along with the bacteria. That way, we could get mammalian metabolism in there and mutate a defective bacteria to one that now worked. We had a mutation in one of the genes for histine biosynthesis in these bacteria so they couldn't grow in a minimal medium, but could grow if we gave them histidine. If you mutated from this mutant gene back to the normal gene, you get a colony from each mutated bacterium, so it was easy to count colonies. We put an extract of rat liver on the plate and that converted the chemical to whatever rat liver converts it to, and if any of those forms were mutagens, then they showed up. That was the so-called Ames Test.

A New Weblike Model of Medicine

JB: You opened a new, weblike, unified way of thinking. about biochemistry and physiology. Some epidemiological papers have recently confirmed this view. One, from Scandinavia, is a very large twin study published in the *New England Journal of Medicine*. It looked at identical twins and the prevalence of cancer to determine if cancer is genetic or environmental. The investigators concluded that 25 to 30 percent of cancers are traced right back to the genes, and the rest depend on what we do to the genes through the environment. The other paper, also in the *New England Journal of Medicine*, is on breast cancer and heart disease in postmenopausal women. It showed that diet and lifestyle probably play a more significant role than genes in those diseases. This is the model you pioneered in your work on dietary carcinogens and anticarcinogens back in the early 1980s. Do you feel that people are accepting this model and that science is evolving away from the deterministic perception of medicine?

BA: Yes, I think so, but there's some confusion. When Higginson first coined the word "environmental" as distinct from genetic, he was mainly thinking of lifestyle factors—diet and smoking. Somehow, then the word "environmental" turned into little traces of synthetic chemicals, which I think are mostly a distraction from the important things. Somehow, when you mention "environmental" to the public, or to a lot of scientists, they think little traces of synthetic chemicals are giving us cancer. We've been inundated with thousands of minor hypothetical risks, and the big ones got lost in the process.

The big, important risks are from smoking and from eating bad diets, and then to some extent, from chronic infection which releases all those oxygen radicals. Your phagocytic cells release all these oxygen radicals, and that leads to mutation and cancer, and then, some cancer from hormones. Those are the big guys. Little traces of pollution are just a red herring.

Mitochondrial Decay

JB: Let's move to another area of this evolving story. That is the role of mitochondria as the energy-processing powerhouse of the cell, with effects on cytochrome-mediated monooxygenase functions. Can you tell us how this area evolved, looking at the mitochondrial DNA hits and mutations, and how you became the pioneer in that area?

BA: My two main interests are mitochondrial decay and aging, and tuning up people's metabolism through diet and nutraceuticals. I'll talk first about the mitochondrial decay. I was always interested in oxidation, because we're doing that in our normal metabolism. You are also oxidizing DNA when you smoke, because you're breathing in all these nitrogen oxides. When you have a chronic inflammation, your phagocytic cells are pouring out oxidants. So oxidants seemed like a major contributor to DNA damage from just living, from smoking, and from chronic inflammation.

That area interested me early on, and we tried to measure how much oxidized DNA was made every day in a rat. We did this by looking at oxidized bases that were repaired out of DNA that went into the urine. We looked at several different oxidized bases. Radiation is an oxidative mutagen, and the radiation biologists have done all the chemistry. The number we came up with was something like 100,000 oxidative hits per cell per day. That amount of oxidized bases was repaired out and went into the urine.

Oxidant Leakage

That number shook us up because it seemed awfully high. We did a lot of controls and still came up with that number. It's a fairly soft number in the sense that it's hard to control for cell turnover. We don't really know how to factor in mitochondrial turnover so some of it might come from the mitochondria. Anyway, there's all this oxidation going on. Where is oxidant leakage coming from? It's from the mitochondria mostly, because to generate energy, you burn fat or carbohydrate, which means pulling electrons from them and adding the electrons to oxygen.

When you add four electrons to oxygen, you're home safe to water, but if you add them one at a time, you make superoxide, hydrogen peroxide, and hydroxyl radical which are the very substances you get from radiation. We measured the steady state level of oxidative damage in tissues, and again that number seemed pretty high. It was something like 26,000 oxidative lesions per cell in a young rat, and in an old rat it was close to 70,000. That number has gone up and down. People in the field were getting widely different numbers. If you grind up a cell you release iron. Iron can oxidize the DNA, and you're measuring one oxidized space per million non-oxidized spaces, so it's easy to fool yourself.

Oxidizing DNA

Those numbers have gotten better now. Most people think the numbers I'm giving you are about the right ones. You're oxidizing your DNA all the time; you're repairing it, and the steady-state level is slowly increasing. Our defense systems include good DNA repair. Then we have all the enzymes—superoxide dismutase, catalase and glutathione peroxidase—that protect us against oxidative damage. But it never pays nature to be 100 percent perfect.

With age, things slowly degenerate. We have shown that the mitochondria are degenerating. The

mitochondria are the most complicated organelle in the cell, and they're getting hit by oxidants all the time. One defense they have is that the lysosome eats up 10 percent of the mitochondria every day, even in tissues like the brain, where the cells aren't dividing. Mitochondria are turning over, and they have their own DNA. They're always making new mitochondria, and a thousand proteins are made in the nucleus and shipped into the mitochondria.

Lysosomal System

One way the cell defends itself is this lysosomal system eating up mitchondria and taking out the bad ones. Nobody has exactly proved that, but that's what everybody thinks. With age, the lysosomal system gets clogged up. Brunk in Sweden has looked at that. You're not taking out the bad mitochondria, junk slowly accumulates, and the mitochondria degenerate. The cell has other defenses. If there's too much damage to mitochondria, you release cytochrome C and the cell undergoes apoptosis and is killed.

We have lots of defenses, but they don't quite keep up. We have shown that by the time a rat is old, the membrane potential in the mitochondria is down quite a bit. The mitochondria are very heterogenous. The cardiolipin, a key lipid in the mitochondrial membrane, is down, and the oxidant leakage is up. We've figured out how to reverse a lot of that by adding high levels of normal mitochondrial metabolites. I think the trick there is as you oxidize protein in the mitochondria, you're altering the structure of these proteins and cleaning those up, too. But nothing is quite 100 percent cleaned up.

Minimizing the Accumulation of Rancid Lipids

As Earl Stadtman showed, you accumulate oxidized protein, and we showed you accumulate rancid lipids that stick to protein, and it gets worse with age. Your mitochondrial proteins start losing activity because they're being oxidized. But you can get some of that activity back because it's an affinity (Km) problem with the substrate.

We found that if we add the normal mitochondrial metabolites—carnitine (we use acetyl carnitine) and lipoic acid, both of which are involved in mitochondrial metabolism—then these old rats look very lively, and their mitochondria look more like young mitochondria. We're busily working on that and trying to move it to people. I'm excited about that. I think we're going to be tuning up metabolism.

Antioxidants or Redox-Active Substances

JB: When we talk about these oxidants in mitochondrial function and the role of substances like acetyl carnitine and lipoic acid, people often conclude those are antioxidants. One might also look at this as balancing the reduction/oxidation potential of the cell. Is there anything more than a philosophical difference between the concept of an antioxidant and a redox-active substance in terms of how this research is unfolding?

BA: I don't think it's just antioxidants. The main damage to the mitochondria seems to be oxidative leaking of electrons, and lipoic acid is certainly an antioxidant. Lester Packer has been arguing for years that lipoic acid is an important antioxidant for mitochondria. It's the coenzyme for pyruvate dehydrogenase to get carbohydrate energy into the mitochondria.

Carnitine really isn't an antioxidant. It's a transporter for getting fatty acids into the mitochondria. It's just that the enzyme activity goes down with age, but you can restore it somewhat by adding more of the substrate, the acetyl carnitine. You can buy these compounds in the health food stores. We're interested in how to extend this to people.

Increasing Need for Redox-Active Substances with Age

JB: Is there a functional difference in the need for these substances as the animal ages? That's one of the questions molecular gerontology has considered for some time—whether the functional need for these substances changes as we get more into these oxidative-base reactions.

BA: Normally, you don't think of these compounds as vitamins because you make them in your own body, and there are little traces in food. In fact, however, when you're older, you may need more to get the enzymes to work well. They may be called conditional micronutrients—those you require when you're older. I think there are going to be lots more of these and the amounts may be different. I'm interested in tuning up metabolism, getting everything in the mitochondria working smoothly in an old rat so I can extend it to people.

In a fit of enthusiasm, I called up my son in New York, who is in computers, and told him one of my students seems to be changing old rats into young rats. There was silence, and then my son said: "Well, that's all very well and good, but let me know when you do the next step and change old people into young rats." Your children don't let you get away with anything.

Nutrition in Cancer Prevention

Let me add a footnote to the previous section. The reason I think nutrition is really important in cancer prevention. Epidemiological studies show that the quarter of the population eating the fewest fruits and vegetables has double the cancer rate of the quarter eating the most fruits and vegetables for practically every type of cancer. There are 200 studies showing that. Top epidemiologists have reviewed this field, and everybody is starting to agree that somehow fruits and vegetables protect you against cancer. I've been very interested in why. One of the reasons, I think, is micronutrients.

We require about 40 micronutrients in a normal diet—vitamins, minerals, the essential amino acids, and essential fatty acids. We were interested in vitamin C and vitamin E, but we got excited through our work on folic acid.

Folic Acid

Folic acid is a "hot" vitamin now. Folia is the Latin word for leaf or foliage, and you get folic acid from things like spinach and greens. Folic acid moves one carbon unit around in metabolism. Methyltetrahydrofolate methylates homocysteine to make methionine with the help of vitamin B12. Then, methylene tetrahydrofolate, that is a different pool, methylates the base uracil to make the base thiamin for DNA. So dUMP gets methylated to dTMP.

A fellow named Jim MacGregor, a cytogeneticist, had been developing methods for measuring DNA chromosome breaks in mice, showed that if you irradiate the mice, you get more chromosome breaks. He

stumbled on the fact that when you made the mice folic acid-deficient, you get chromosome breaks just like radiation. He spent a sabbatical in my lab. A graduate student worked on the problem and worked out the mechanism. It turns out that when you don't methylate uracil to make thiamin, you start putting uracil in your DNA.

Uracil

Uracil is normally in RNA, not in DNA. Every time you put uracil in DNA, when you remove it by repair enzymes, you make a break in one of the strands. So, it's a nick in the DNA. If you have one break across from another, then the whole chromosome falls apart. We found 4,000,000 uracils per human cell when somebody was folate-deficient, along with a lot of chromosome breaks. If you give these people folate, both the uracil and chromosome breaks go away. When I looked up what percent of the population was at a level of folate intake where they were breaking their chromosomes, it was 10 percent, and a much higher percentage of the poor. The poor are the ones who are not eating their fruits and vegetables. They're eating terrible diets. That got me all excited.

Since then, we've shown that vitamin B6 deficiency does the same thing, because that's how you provide the methylene group in the methylene tetrahydrofolate from serine via a vitamin B6-dependent enzyme. Vitamin B12 deficiency also increases uracil, so they all work in the same way. They work the same way as radiation does in breaking the chromosomes and that's the dangerous part of radiation. B6 deficiency is 10 percent of the population; folate deficiency was 10 percent of the population; B12 deficiency is 14 percent of the elderly ,and they're not the same people.

The Need for Micronutrient Supplementation

We're talking about a lot of people who are breaking their chromosomes because of bad diet. Since then, there's been a lot of work on zinc deficiency damaging DNA. iron deficiency. We've just shown iron deficiency damages DNA, and 19 percent of the menstruating women in the U.S. are almost anemic because they're losing iron all the time and not getting enough of it.

Right now, one of my passions is tuning up people's metabolism by making sure they all get their micronutrients, and it's cheap. A multivitamin pill cost less than a penny to make and you can buy them for 2 or 3 cents in stores like Costco. So, it's less than $10 a year to give somebody a multivitamin pill every day. People in northern climates aren't getting enough vitamin D because of a lack of sunshine. You can get it from a multivitamin pill that contains vitamin D, zinc, and iron. Women should take a pill with iron and men one without iron. Right now I'm trying to get multivitamin pills into the poor to tune up metabolism. I think it's going to have a huge effect on preventing cancer and heart disease, and perhaps improving cognitive function. We're also showing that sperm DNA gets damaged when you don't have enough vitamin C or folic acid.

Rethinking the RDAs

JB: The Human Genome Project has identified a number of genetic polymorphisms, such as the methylene tetrahydrofolate reductase C® T polymorphism and its relationship to folate. That gene penetration may exist in approximately 20 percent in the population. That seems to represent a strong argument for the model you're describing and against the traditional approach to establishing the RDAs.

BA: Yes. There are committees establishing the RDAs. They raised vitamin C, but I think they should have raised it higher. Mark Levine did the pharmacokinetics, and at about 100 mg of vitamin C or 110, he saturated all the tissues. The rest is excreted in urine. So instead of raising it to 90, I think they should have raised it to Mark Levine's level—110 or 120.

You could quibble about these things, but in general, the committees do their best and they're coming up with what they think is a reasonable amount. Now they're putting in an upper limit—what amount starts to be toxic. If you tell everybody selenium is good for them, you know that 5 percent of the population will go out and take too much selenium and poison themselves. Every micronutrient has a level at which it's useful and a level at which you get too much.

Multivitamins, Fruits, and Vegetables

The first piece of advice is that everybody take a multivitamin pill and try to eat five portions of fruits and vegetables a day. Just take the multivitamin as insurance; 25 percent of the population is doing that. It is primarily the rich who eat good diets, and it's the poor who need them

Polymorphisms, as you mentioned, may influence all of this. For example, somehow nature selected for an alternate form of methylene tetrahydrofolate reductase. That mutation decreases the size of the methyltetrahydrofolate pool, which increases risk of heart disease, and it increases the size of the methylenetetrahydrofolate pool, which puts you less at risk for chromosome breaks and cancer. Why was that gene selected? Heart disease and cancer come too late in life. In both rats and humans we have shown that the sperm count goes down and the quality is bad if one is folate-deficient. That's due to the methylene-THF pool; it's affecting reproduction when you're folate- or B12- or B6-deficient. That could have a strong selective advantage. We suspect it's reproduction.

Folate Deficiency and ALL

That's the selective force for this MTHFR polymorphism in the northern populations that were chronically folate deficient. They may have selected for this to protect the sperm. Anyway, those polymorphisms are turning out to be really useful for determining which cancers are associated with which deficiencies. Folic acid deficiencies have been associated with colon cancer, pancreatic cancer, and acute lymphocytic leukemia (ALL).

Martin Smith, who did the ALL work, used the polymorphism as a clue to tracking down that folate had something to do with ALL. The polymorphisms are going to be very useful in helping us get clues as to what's causing different types of cancer. I think more and more is going to come out of diet because that's where the gold is.

Measuring Oxidative Damage in the Laboratory

JB: Clinicians frequently ask what is the best laboratory marker to evaluate oxidative damage or oxidant stress. One that has been in the news recently is 8-hydroxy-deoxyguanosine, or 8OHDG.

BA: That's one we've used. Now, there's no perfect marker. There are technical problems, too. If you grind up a cell, you release iron and oxidize the DNA so you get a lot of artifacts. You have to have

chelators to tie up the iron and be careful to have the minimum number of manipulations. We keep on improving the method, and other people are improving the method, but it's tricky. You can measure malondyaldehyde and other aldehydes coming out of oxidized lipids. We developed a mass spec assay for doing that, which we think is pretty good, but again, you need to be careful. So, there's no easy perfect method, but a lot of people are working in this area. I'm sure that's going to continue to improve.

Acknowledging the Contributions

JB: Thank you for the many contributions you have made and continue to make in opening our eyes to these connections. It will be fascinating to see how our medical thoughts and technologies evolve over the next decade from these principles. We wish you the very best in your continued work, and we'll keep in touch.

BA: It's a pleasure.

Dr. Ames mentioned the studies he and his colleagues have done, looking at the effect of various redox-active, mitochondrially-active nutrients on age-related processes. One area he discussed was the impact of lipoic acid supplementation on mitochondrial redox. One of those papers appeared in the *FASEB Journal* (Federation of American Societies for Experimental Biology). It is titled "Age-Associated Decline in Ascorbic Acid Concentration, Recylcing, and Biosynthesis in Rat Hepatocytes—Reversal with

(R)-a -Lipoic Acid Supplementation." In this paper Dr. Ames and his colleagues point out that as animals age, their redox-recycling mechanisms, a kind of ping pong shuttle system that regenerates antioxidants, tend to wear out and run down.

Vitamin C is not as easily converted and recycled from dehydroascorbate and tends to lose its antioxidant buffering capacity. Dr. Ames and his colleagues found that by administering (R)-a -lipoic acid to older animals, they were able to increase the rate of ascorbate recycling (dehydroascorbate/ascorbic acid) and improve the redox buffering system.

Thermogenic Response Changes in Aging

A companion paper Dr. Ames mentioned is titled "(R)-a -Lipoic Acid-Supplemented Old Rats Have Improved Mitochondrial Function, Decreased Oxidative Damage, and Increased Metabolic Rate." One of the hallmarks of aging is the lowering of the basal metabolic rate. When this happens, individuals tend to be less thermogenically responsive. They seem to have symptoms similar to those associated with hypothyroidism, cold hands and feet, symptoms we often think of as an endocrine disturbance. In fact, it is really a reflection of a fundamental physiological process associated with lowered brown fat thermogenic response and decreased thermogenic responsiveness of other tissues as a consequence of altered mitochondrial function.

In this study with elderly rats, the investigators administered lipoic acid in supplemental doses as a conditionally essential nutrient. They were able to improve mitochondrial function and decrease oxidative damage (looking at 8-hydroxy-deoxyguanosine as a marker for damaged DNA), and increase metabolic rate, showing increased thermogenic responsiveness.

The Role of a -Lipoic Acid

As Lester Packer at the University of California at Berkeley has stated, a -lipoic acid plays an important role in a variety of functions, including liver disease. In studying the effects of amanita mushroom poisoning on liver toxicity, Dr. Bert Berkson found N-acetyl-cysteine and lipoic acid participated in protecting the liver against amanita toxins. More recently, Dr. Berkson has studied protection of the liver against hepatitis C infection and reduction of the incidence of hepatocellular damage by administering the hepatoprotective nutrients lipoate and N-acetylcysteine. Dr. Packer and his colleagues describe this same process in a review article titled "a -Lipoic Acid in Liver Metabolism and Disease."

A few years ago, an *FMU* subscriber told us about the response he had observed in a number of patients with severe liver disease following the administration of lipoate and acetylcysteine. The results were remarkable, with liver enzymes coming down, symptoms of liver dysfunction clearing, and patients at high risk even to liver failure seeming to recover. I with to reemphasize the important role of this family of nutrients in liver function.

Incidentally, the Berkson interview on *FMU* took place in 1996, so we have known for some time about changes in the area of functional medicine/molecular medicine. Liver protection and protection against other diseases associated with oxidative stress, according to Dr. Packer, may be very responsive to lipoate supplementation.

Lipoate and Insulin Sensitivity

In previous issues of *FMU* we have discussed lipoate’s role in improving insulin sensitivity in the hyperinsulinemic, insulin-resistant individual. Lipoate is a hypoglycemic agent in those cases, helping to normalize blood sugar. A number of papers have described this effect in both animals and humans. One such paper, which appeared in *Diabetologia*, discussed lipoate’s role in improving nerve function, assessed by measuring nerve conduction velocity in diabetic animals. Again, we are moving in the right direction.

One of the authors of the paper I just described is Dr. David Horrobin, who has achieved prominence in research on gamma-linolenic acid (GLA). Dr. Horrobin has been a fundamental contributor over the last several decades to the evolution of molecular medicine. He is an important figure in our field.

MTHFR Polymorphisms

Dr. Ames also talked about methylenetetrahydrofolate reductase (MTHFR) polymorphisms and explained that 20 to 30 percent of the population may have a single allele penetration of this polymorphism that makes them less responsive to folate. A review of this polymorphism family, the C® T polymorphism, the cytosine 677 T polymorphism, appeared in *Nutrition Reviews.* Titled "Methylenetetrahydrofolate Reductase Polymorphisms, Folate, and Cancer Risk: a Paradigm of Gene-Nutrient Interactions in Carcinogenesis," this article follows beautifully from Dr. Ames’ comments.

We have learned that if you have a block at that step in the pathway of the folate cycle, you can move downstream one step and use the product of that reaction, which is 5-methyl-tetrahydrofolate. By doing so, you circumvent the rate limiting step caused by a change in primary structure of MTHFR, and

improve the 5-methyl-tetrahydrofolate coenzyme catalyzed methylation of homocysteine to methionine. It is a molecular medicine approach using 5-methyl-tetrahydrofolate rather than folic acid itself, for people who may have a defect at that step in their pathways.
Eighth International Symposium on Functional Medicine

This discussion will be a feature of the Eighth International Symposium on Functional Medicine. The symposium will focus on applied endocrinology across a range of age-related dysfunctions. This polymorphic consideration of folate will be one part of a story that will develop from some world-renowned presenters at the symposium. The symposium will be held at the Westin Resort in Vancouver, B.C., in May of 2001 over the Memorial Day Weekend. This resort, which has just experienced a $30,000,000 renovation, is located along the waterfront with a beautiful view of the mountains in Vancouver. Mark that week on your calendar, and we will send more information on this symposium.

In continuing the folate discussion, let us consider anti-folates used in chemotherapy. First-generation cancer drugs like methotrexate work principally by blocking the reduction of dihydrofolate to tetrahydrofolate and then supposedly causing preferential death of the more rapidly dividing cells, which you hope are the cancer cells, rather than uncoupling other cells of the body at the time when the medication is administered.

Does that mean one should never give folate or recommend folic acid-enhanced diets to a person with cancer? Rather than answer that question, I would like to give you some interesting "food for thought." This comes from a paper in the *Lancet,* titled "Comparison of Fluorouracil with Additional Levamisole, Higher-Dose Folinic Acid, or Both as Adjuvant Chemotherapy for Colorectal Cancer: a Randomised Trial." Recalling what you heard from Dr. Ames, in colorectal cancer, part of the story may be related to altered methylation patterns of DNA associated with certain kinds of genetic alterations in the folate cycle that may respond to folic acid and B12 at higher doses.

Colorectal Cancer Study

Patients with colorectal cancer, without evident residual disease, were randomly assigned either fluorouacil or high-dose L-folinic acid. I emphasize that this is a much higher dose than that used in traditional diet supplementation. Normally, we talk about the 400-800 m g level. This is the 25 mg level of L-folinic acid, which is 25,000 m g, or nutrient pharmacology. Some of you may wonder if that might be a toxic dose of folinic acid. My response is that folic acid is a very safe nutrient in terms of toxicity, certainly in the range we are talking about.

The RDA was set at the 400 m g dose in non-pregnant women because high-dose folate can mask vitamin B12 insufficiency, which can go on to produce irreversible neurological damage. To be sure the clinical signs of B12 deficiency (hematological signs, increased MCVs, mean corpuscular volumes) were clear, therefore, folic acid was regulated at a lower dose. If you are administering folate and B12 simultaneously at levels that maintain sufficiency of both, you need not worry about that. The toxic issue is not the problem with folic acid; it is the masking of vitamin B12 insufficiency. Always make sure the patient is adequately nourished with B12. That might even require intramuscular injections of B12 to make sure his or her gastrointestinal absorption processes are not impaired by atrophic gastritis type B or poor intrinsic factor release, and that cellular B12 is adequate.

Fluorouacil or L-Folinic Acid

This paper in the *Lancet* discusses fluorouacil and L-folinic acid, either active or placebo. The fluorouracil and folinic acid could be given either as six five-day courses, with four weeks between the start of the courses, or as 30 once-weekly doses. Levamisole or placebo was given three times daily for three days. The primary endpoint was mortality from any cause. We first discussed these studies in 1995, when the first reports were coming out. This data now covers 1994 to 1997 and the 4927 patients who were enrolled. Of those, 1776 had recurrences and 1576 of the 4927 patients died. Survival was similar with high- and low-dose folinic acid, as were three-year recurrence rates. Survival was worse with levamisole than with placebo. The inclusion of levamisole in chemotherapy regimens of these nutrients does not delay recurrence or improve survival. Higher-dose folinic acid produced no extra benefit in these regimens beyond that from low-dose folinic acid. Trials of chemotherapy versus no chemotherapy will show whether these four treatments are equally effective or ineffective. Those trials are now ongoing.

One might wonder about the relative effects of folinic acid. Low-dose folinic acid clearly has a beneficial effect. That is the 25,000 m g, as contrasted to a high dose of folinic acid, which is 175,000 m g. One need not use doses as high as 175 mg. The effect is realized at 25 mg. Whether this in fact has significant clinical benefit in individuals who have not had chemotherapy remains to be seen. Whether or not part of the problem in colorectal cancer is a consequence of poor gene expression control through altered hypomethylation or methylation patterns at the genome is a question that remains for clinical study. I believe there is something important here clinically related to folic acid status, cellular regulation, genome expression, cell cycling, and oncogenesis. We should be looking at these MTHFR polymorphisms and the relationship to sufficiency of folate as needed at that level for that individual.

Free Radicals and Chronic Disease

Going from something as severe as cancer to more chronic conditions, free radicals play a role in chronic disease, such as chronic fatigue syndrome (CFS). A recent paper in *Redox Report* discusses the correlation between CFS and free radical oxidative stress. This follows from work we have published over the last nine years, and work we have also described in past editions of *FMU*. Redox-potential substances, detoxification therapy, and chronic fatigue/fibromyalgia, using coenzyme Q10, lipoic acid, N-acetyl-cysteine, vitamin E, are also very important.

You now have an overview of the functional age/molecular age construct. Dr. Ames has been one of the pioneers, a founding father of the field of molecular gerontology. We look forward to watching this field open up for new clinical opportunities as we move into 2001.

Bibliography

1. Futterman LG, Lemberg L. Fifty percent of patients with coronary artery disease do not have any of the conventional risk factors. *Am J Critical Care.* 1998;7(3):240-244.
2. Braunwald E. Shattuck lecture—cardiovascular medicine at the turn of the millennium: triumphs, concerns and opportunities. *N Engl J Med.* 1997;337(19):1360-1369.
3. Herrington DM, Reboussin DM, Brosnihan KB, et al. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. *N Engl J Med.* 2000;343(8):522-529.
4. Stampfer MJ, Hu FB, Manson JE, Rimm EB, Willett WC. Primary prevention of coronary heart

disease I women through diet and lifestyle. *N Engl J Med.*2000;343:16-22.
5. Bookheimer SY, Strojwas MH, Cohen MS, et al. Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med.* 2000;343(7):450-456.
6. Skoog I. Detection of preclinical Alzheimer's disease. *N Engl J Med.* 2000;343(7):502-503.
7. Lorenzo A, Yuan M, Zhang Z, et al. Amyloid b interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease. *Nature Neurosci.*2000;3(5):460-464.
8. Fernstrom JD. Can nutrient supplements modify brain function? *Am J Clin Nutr.*2000;71(suppl):1669S-1673S.
9. Bell J. The new genetics in clinical practice. *BMJ.* 1998;316:618-620.
10. Fears R, Roberts D, Poste G. Rational or rationed medicine? The promise of genetics for improved clinical practice. *BMJ.* 2000;230:933-935.
11. Wolf CR, Smith G, Smith RL. Science, medicine, and the future. Pharmacogenetics. *BMJ.* 2000;320:987-990.
12. London SJ, Yuan JM, Chung FL, et al. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. *Lancet.* 2000;356:724-729.
13. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet.* 1999;353:717-719.
14. Safirstein R, Andrade L, Vieira JM. Acetylcysteine and nephrotoxic effects of radiographic contrast agents—a new use for an old drug. *N Engl J Med.*2000;343(3):210-212.
15. Tepel M, van Der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. *N Engl J Med.* 2000;343:180-184.
16. Walport MJ. Lupus, DNase and defective disposal of cellular debris. *Nature Genetics.*2000;25:135-136.
17. Perera FP. Molecular epidemiology: on the path to prevention? *J Natl Cancer Inst.*2000;92(8):602-612.
18. Health and Health Care 2010. The paradigm shift: evolution or revolution? http://www.rwjf.org/iftf/chapter_12/ch12_section_9.htm
19. MacIntosh A, Ball K. The effects of a short program of detoxification in disease-free individuals. *Alt Therapies.* 2000;6(4):70-76.
20. Roy S, Nicholson DW. Programmed cell-death regulation: basic mechanisms and therapeutic opportunities. *Mol Med Today.* 2000;6:264-266.
21. Mecocci P, Polidori MC, Troiano L, et al. Plasma antioxidants and longevity: a study on healthy centenarians. *Free Rad Biol Med.* 2000;28(8):1243-1248.
22. Halliwell B. Antioxidant and redox regulation of genes. Book review. *Antioxidants Redox Signaling.* 2000;2(2):369.
23. De Grey A. Biomedical aspects of plasma membrane redox. *Antioxidants Redox Signaling.* 2000;2(2):155-156.
24. Villalba JM, Navas P. Plasma membrane redox system in the control of stress-induced apoptosis. *Antioxidants Redox Signaling.* 2000;2(2):213-230.
25. Lizard G, Miguet C, Bessede G, et al. Impairment with various antioxidants of the loss of mitochondrial transmembrane potential and of the cytosolic release of cytochrome C occurring during 7-ketocholesterol-induced apoptosis. *Free Rad Biol Med.*2000;28(6):743-753.
26. Kohen R, Vellaichamy E, Hrbac J, Gati I, Tirosh O. Quantification of the overall reactive oxygen

species scavenging capacity of biological fluids and tissues. *Free Rad Biol Med.* 2000;28(6):871-879.
27. Lo YM. Molecular testing of urine: catching DNA on the way out. *Clin Chem.*2000;46(8):1039-1040.
28. Murr C, Fuith LC, Widner B, Wirleitner B, Baier-Bitterlich G, Fuchs D. Increased neopterin concentrations in patients with cancer: indicator of oxidative stress? *Anticancer Res.* 1999;19:1721-1728
29. Leblhuber F, Walli J, Demei U, Tilz GP, Widner B, Fuchs D. Increased serum neopterin concentrations in patients with Alzheimer's disease. *Clin Chem Lab Med.*1999;37(4):429-431.
30. Garcia-Moll X, Cole D, Zouridakis E, Kaski JC. Increased serum neopterin: a marker of coronary artery disease activity in women. *Exp Dermatol.* 1999;8:167-176.
31. Hamerlinck FF. Neopterin: a review. *Exp Dermatol.* 1999;8:167-176.
32. Hochhauser D. Relevance of mitochondrial DNA in cancer. *Lancet.* 2000;356:181-182.
33. Rotig A, Appelkvist EL, Geromel V, et al. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. *Lancet.*2000;356:391-395.
34. Lichtenstein P, Holm NV, Verikasalo PK, et al. Environmental and heritable factors in the causation of cancer. *N Engl J Med.* 2000;343(2):78-85.
35. Lykkesfeldt J, Hagen TM, Vinarsky V, Ames, BN. Age-associated decline in ascorbic acid concentration, recycling, and biosynthesis in rat hepatocytes—reversal with (R)-a -lipoic acid supplementation. *FASEB J.* 1998;12:1183-1189.
36. Hagen TM, Ingersoll RT, Lykkesfeldt J, et al. (R)-a -lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *FASEB J.* 1999;13:411-418.
37. Bustamante J, Lodge JK, Marcocci L, Tritschler HJ, Packer L, Rihn BH. a -lipoic acid in liver metabolism and disease. *Free Rad Biol Med.* 1998;24(6):1023-1039.
38. Cameron NE, Cotter MA, Horrobin DH, Tritschler HJ. Effects of a -lipoic acid on neurovascular function in diabetic rats: interaction with essential fatty acids. *Diabetologia.* 1998;41:390-399.
39. Kim YI. Methylenetetrahydrofolate reductase polymorphisms, folate, and cancer risk: a paradigm of gene-nutrient interactions in carcinogenesis. *Nutr Rev.* 2000;58(7):205-217.
40. QUASAR Collaborative Group. Comparison of fluorouacil with additional levamisole, higher-dose folinic acid, or both, as adjuvant chemotherapy for colorectal cancer: a randomised trial. *Lancet.* 2000;355:1588-1596.
41. Richards RS, Roberts TK, Dunstan RH, McGregor NR, Butt HL. Free radicals in chronic fatigue syndrome: cause or effect? *Redox Rpt.* 2000;5(2/3):146-147

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