Linus Pauling and the Advent of Orthomolecular Medicine

Stephen Lawson[1]

Orthomolecular Psychiatry

The journal *Science* published a revelatory article in its April 19th, 1968, issue.[1] The author, Linus Pauling, was not a stranger to the pages of *Science,* but his article, "Orthomolecular Psychiatry," heralded a dramatically new direction in his thinking and research. Pauling had enjoyed widespread fame as the world's greatest chemist and tireless peace advocate for many decades, but his venture into the field of nutrition, especially concerning micronutrients and their role in maintaining mental and physical health, attracted new attention and ignited controversy. In a letter published in the June 14th issue of *Science,* Donald Oken, a psychiatrist in the National Institutes of Health, wrote:

"The article, "Orthomolecular Psychiatry" (19 April, p. 265), illustrates elegantly the pitfalls which occur when an expert in one field enters another area. With his characteristic brilliance, Linus Pauling describes a biochemical mechanism which *could* be responsible for some forms of mental illness (or, indeed, for illness of many other types). Remote plausibility, however, no matter how intriguing and creative its nature, should not be confused with evidence. Unfortunately for Pauling's thesis, there is no adequate evidence to back up his view."[2]

In response, Pauling noted that he had been working for 12 years on the molecular basis of mental illness with his research supported by the NIH, the Ford Foundation, and private donors, implying that he was not a newcomer to the field of brain chemistry. Indeed, he had published his theory on the molecular mechanism of anesthetic agents, particularly the inert gases, in 1959 and had begun working on biological molecules in the late 1930s. Those efforts culminated in the discovery of the structural themes of proteins, including the alpha-helix and pleated sheet, and the cause of sickle-cell anemia–the first disease to be characterized as a molecular disease–and established Pauling as the major founder of molecular biology. Pauling also remarked that psychiatrists had a duty, in his view, to employ the techniques of orthomolecular psychiatry in addition to the standard therapies.[3]

Oken was certainly justified in his praise of Pauling's brilliance but missed entirely the point of his genius: the ability to span diverse scientific and medical fields and synthesize original, compelling perspectives into perplexing issues. Pauling, the only person to have won two unshared Nobel Prizes, was one of history's greatest embodiments of the interdisciplinary approach, decades before it became considered essential. In the "Millennium Essay" published in *Nature* in 2000, Gautam Desiraju characterized Pauling as "one of the great thinkers and visionaries of the millennium", ranking him alongside Galileo, Da Vinci, Faraday, Newton, and Einstein. Desiraju noted that "Pauling's ingenuity and awesome intuition permeated quantum mechanics, crystallography, biology, medicine and, above all, structural chemistry" and that modern chemistry, unlike biology or physics, is utterly dependent on the work of a single scientist–Linus Pauling.[4]

What, then, was Pauling's paper, "Orthomolecular Psychiatry," about, and why did it generate such criticism? Written while Pauling was a professor in the chemistry department in the University of

1. Linus Pauling Institute, Oregon State University, 571 Weniger Hall, Corvallis, Oregon 97331-6512

California at San Diego, "Orthomolecular Psychiatry" established the theoretical basis for treating cerebral avitaminosis by "the provision of the optimum molecular environment for the mind, especially the optimum concentrations of substances normally present in the human body." Somewhat later, Pauling broadly defined orthomolecular medicine as preserving good health and treating disease by "varying the concentrations in the human body of substances that are normally present in the body and are required for health."[5] Drawing on evidence from microbial genetics and molecular reaction rates, Pauling suggested that the brain's sensitivity to its biochemistry affects the mind. While this concept seems as intuitive and obvious as some of Pauling's other discoveries like biological specificity or the molecular clock, it was very controversial when first introduced. Many nutritionists and psychiatrists like Oken felt that Pauling was trespassing on their domains and adopted an almost reactionary stance. Pauling's encyclopedic knowledge and awesome memory, as well as his great personal charm, served him extremely well in debates with his detractors over the next decades.

In 1945 Pauling had postulated the cause of sickle-cell anemia as an abnormal hemoglobin that combines with itself in deoxygenated blood, forming long rods that distort the shape of red blood cells into the characteristic sickle shape observed in the disease. Four years later, he and colleagues published a paper in *Science* that confirmed this mechanism and heralded the new era of molecular medicine.[6] Pauling returned to the concept of molecular disease in "Orthomolecular Psychiatry", noting that phenylketonuria is a molecular disease in which phenylalanine accumulates in the tissues of afflicted children because of a genetic defect in the enzyme that catalyzes the conversion of phenylalanine to tyrosine. The resultant pathology includes mental manifestations and physical problems, such as severe eczema, but can be attenuated by replacing a normal diet with one that is limited in phenylalanine–an example of orthomolecular medicine.

In "Orthomolecular Psychiatry", Pauling noted the mental manifestations of the B vitamin deficiency diseases that produce physical pathology, supporting his thesis that these vitamins play crucial roles in mental health. He explained that evolution may favor the loss of certain functions, such as the synthesis of vitamin C, if the environment supplies sufficient amounts of the critical substance. A mutant that synthesizes an adequate but suboptimum amount of a vital substance may also outcompete the wild-type organism if the energy saved by diminished synthesis can be applied advantageously elsewhere. To support this point, Pauling discussed the *Neurospora* work of his friends, Beadle and Tatum. They showed that the growth rate of a pyridoxine-requiring mutant strain, produced by irradiation, actually increased to about seven percent greater than the parental strain when large amounts of pyridoxine were supplied in the medium. Similar results were obtained for a *p*-aminobenzoic-acid-requiring strain. Citing the work of Zamenhof and Eichhorn on *Bacillus subtilis,* Pauling elaborated on observations that when nutrilite-requiring mutants were grown together with the parental strain in a medium containing the nutrilite, the mutants exhibited a selective advantage, outcompeting and overpopulating the parental strain, owing to gene deletion rather than point mutations, since the intermediate steps, including the synthesis of mRNA, would be lost.

Pauling then discussed the dependence of reaction rates on molecular concentrations. Echoing his interest in the 1950s on the potential role of abnormal

enzyme function in mental illness, Pauling noted that enzyme-catalyzed reaction rates are proportional to the concentration of the reactant, assuming that there are no enzyme inhibitors present. The rate decreases as the enzyme becomes saturated. If the enzyme is defective, as may be the case with those involved in abnormal brain function, the saturating concentration is larger because the enzyme has less affinity for its substrate. However, the rate may be normalized by increasing the concentration of the substrate. This provides the rationale for supplying high-dose vitamins to treat biogenic mental illness. Building on this concept, in a paper published in the *American Journal of Clinical Nutrition* in 2002, Bruce Ames discussed the remediation of about 50 human genetic diseases caused by defective enzymes with high-dose B vitamins and other micronutrients.[7]

To illustrate his hypothesis, Pauling focused on vitamin B_{12}, niacin, vitamin C, and glutamic acid. He cited a Norwegian study that found abnormally low levels of B_{12} in the blood of about 15% of patients admitted to a mental hospital, compared to values observed in the general population. He then recounted the successful application of niacin in the southeastern United States that alleviated psychosis in thousands of pellagra patients. Citing the work of Sydenstricker and Cleckley and the work of Hoffer and Osmond, he discussed the use of high-dose niacin and, in the case of Hoffer and Osmond, the combination of high-dose niacin and vitamin C, to treat schizophrenia without the side effects typically seen with drugs. Vitamin C deficiency in schizophrenics has often been reported and is also associated with depression in patients with scurvy. Pauling briefly noted that Stone estimated the optimum intake of vitamin C at 3 to 15 grams per day, based on cross-species comparisons and other arguments. A few years later, the first paper from the newly founded Linus Pauling Institute of Science and Medicine, "Results of a Loading Test of Ascorbic Acid, Niacinamide, and Pyridoxine in Schizophrenic Subjects and Controls", reported that almost all of the schizophrenic patients examined excreted abnormally low amounts of one or more of the orally administered vitamins given in doses over one gram each, compared to controls.[8] Pauling explained that several investigators in the 1940s reported that large doses of L-glutamic acid had beneficial effects in subjects with convulsive disorders or mental retardation. The effective dosage was found to be 10 to 20 grams per day, higher than the estimated intake from food of about 5 to 10 grams per day.

In the penultimate section of "Orthomolecular Psychiatry", *Localized Cerebral Deficiency Diseases*, Pauling argued that a simple model of fluid dynamics in the body leads to calculations demonstrating that localized deficiencies of vital substances could occur in specific reservoirs. In his model, such substances are used up at characteristic rates in various reservoirs, such as blood and cerebrospinal fluid; the rate of absorption from the gastrointestinal tract is constant; and the diffusion across the blood-brain barrier is a function of permeability, area of the barrier, and the difference in concentration of the substance in blood and cerebrospinal fluid. Given these parameters, the steady-state concentration of a vital substance in the brain could be much less than its concentration in blood. In schizophrenia the situation would be aggravated by genes affecting the regulation of vitamin metabolism or other critical functions so that massive doses of certain vitamins may be required to normalize cerebral concentrations and, therefore, mental function.

Pauling elaborated on and extended the concept of orthomolecular psychiatry and medicine in many publications over the next decades. In "Some Aspects of

Orthomolecular Medicine," published in 1974, he introduced new examples of orthomolecular medicine, such as the treatment of diabetes with injected insulin, the use of iodine to prevent goiter, and methylmalonicaciduria, which is treated by supplying large amounts of vitamin B_{12} (1,000 times the normal concentration) to normalize the conversion of methylmalonic acid to succinic acid.[9] *In How to Live Longer and Feel Better,* published a dozen years later, he added another example: the treatment of galactosemia–a genetic disease characterized by a deficiency of an enzyme that metabolizes galactose in lactose–by the provision of a diet free of milk sugar.[10] Pauling stressed that he used the adjective *orthomolecular* "to express the idea of the right molecules in the right concentrations" and contrasted orthomolecular medicine with the use of potentially dangerous drugs used in conventional allopathic medicine. Pauling believed that the biological plausibility of his arguments was evident and that the available evidence was supportive. Of course, as is the case with many revolutionary ideas, "Orthomolecular Psychiatry" was not greeted with universal acclaim. The American Psychiatric Association, in particular, was skeptical and dismissive.

In the Fall of 1974, Pauling contributed an article, "On the Orthomolecular Environment of the Mind: Orthomolecular Therapy", to the *American Journal of Psychiatry,* which provided an opportunity to comment on the American Psychiatric Association's *Task Force Report: Megavitamin and Orthomolecular Therapy in Psychiatry,* issued in 1973.[11] He was clearly dismayed with the negative reaction of conventional psychiatry to his ideas and the scientific evidence and criticized what he considered to be specious arguments and fallacies in the report. After discussing the probability that abnormal enzyme function may cause mental illness and listing examples of successful orthomolecular treatment with vitamins, some of which effectively shift the equilibrium rate for the formation of an active enzyme from the apoenzyme and coenzyme, Pauling faulted the APA report for ignoring evidence on vitamin C and pyridoxine; misunderstanding simple biochemistry, including the nature of vitamins and how a population of molecules can easily serve several functions—they don't all have to be committed to one reaction as implied by the task force; and intentional or unintentional bias, resulting in "a sort of professional inertia that hinders progress."

Setting the Stage

Several childhood experiences and later episodes set the stage for Pauling's codification of orthomolecular medicine and his fascination with vitamin C. His father was a druggist and, in the era before the Food and Drug Administration, concocted many medicines in his store, where Linus was exposed to this medicinal chemistry as a youngster. Later, he set up a laboratory in his basement where he carried out exciting chemical reactions. He was deeply impressed by the transformation of substances during reactions, and those early experiments stimulated an intense desire to learn more about chemistry, which was fulfilled as an undergraduate in Oregon Agricultural College (now Oregon State University) and in graduate work in the California Institute of Technology (Caltech). When Pauling and his wife were in Europe on a Guggenheim Fellowship in 1926, after earning his doctorate in chemistry and mathematical physics, his mother, Belle, died from pernicious anemia in a hospital for the insane in Salem, Oregon. Pernicious anemia, caused by a deficiency of vitamin B_{12}, is characterized by neurological problems and loss of normal mental function, resulting in delusions known as

"megaloblastic madness" and, ultimately, death. In the year that Belle Pauling died, Minot and Murphy discovered that eating raw liver reversed pernicious anemia. In 1934, they won the Nobel Prize in Medicine or Physiology for their work, and 14 years later, vitamin B_{12} was isolated independently by Pauling's friends, Karl Folkers and Alexander Todd. Another of Pauling's friends, Dorothy Hodgkin, won the Nobel Prize in Chemistry in 1964 for elucidating the molecular structure of B_{12} by X-ray crystallography in 1956.

In 1938 Pauling gave a speech at the dedication of the Crellin laboratory in Caltech in which he said:

"There is, however, a related field of knowledge of transcendent significance to mankind which has barely begun its development. This field deals with the correlation between chemical structure and physiological activity of those substances, manufactured in the body or ingested in foodstuffs, which are essential for orderly growth and the maintenance of life, as well as of the many substances which are useful in the treatment of disease."[12]

Pauling remarked on the structural complexity of many vitamins and predicted that, given the rapid progress in the synthesis of vitamins in the preceding decade, "success will soon reward the men who are now carrying on the attack on vitamin E". Clearly, in the heyday of vitaminology, Pauling was thinking about the virtues of these vital substances. Indeed, the early part of the twentieth century, especially the 1930s, was the prime time for the discovery of vitamins and their use to correct and prevent associated deficiency diseases. For example, vitamin A was identified as a vitamin in 1914 and structurally characterized in 1930. Vitamins D_2 and D_3 were chemically characterized in 1932 and 1936, respectively. Vitamin E was discovered in 1922 but not isolated until 1936. Vitamin K was discovered in the early 1930s and identified in 1939. Pauling' s friend Albert Szent-Gyorgyi first isolated vitamin C in 1928. Thiamin was isolated in 1911 by Casimir Funk, who coined the term 'vitamine', and structurally characterized by R.R. Williams in 1936. Williams's brother, Roger J. Williams, first identified the structure of pantothenic acid in 1940 and later proposed important concepts about biochemical individuality that greatly influenced Pauling. In his classic book, *Biochemical Individuality*, Roger Williams described significant anatomical and biochemical variations due to genetic polymorphisms among humans and postulated, "practically every human being is a deviate in some respects."[13] He noted that if 95% of the population is normal with respect to one measured value, only 0.59% of the population would be normal with respect to 100 uncorrelated measured values. In December 2007, the journal *Science* heralded human genetic variation (polymorphisms) and its implication for disease risk and personal traits as the "Breakthrough of the Year."[14] Riboflavin, the first vitamin to be recognized as a co-enzyme, was isolated in 1933. Vitamin B_6 (pyridoxine and related forms) was isolated in 1938, and its structure was determined a year later. Niacin was isolated in 1867 but not identified as the anti-pellegra factor until 1937. As mentioned previously, vitamin B_{12} was not isolated until 1948, five years after another group of pharmaceutical scientists isolated folic acid. Biotin was first isolated in 1936, and its structure was elucidated in 1942. Many early vitamin pioneers won accolades for their work that spared millions of people from the ravages of debilitating and fatal deficiency diseases. From the 1920s until the mid-1960s, 16 Nobel Prizes were awarded to scientists who discovered, isolated, synthesized, or structurally characterized vitamins.

While Pauling was well aware of these developments in biochemistry and nutrition in the 1930s, his only relevant

research in that era concerned the molecular structures of some carotenoids and the flavonoid anthocyanidin. In 1939, the year in which he published papers on hemoglobin, the structures of benzene and proteins, and *The Nature of the Chemical Bond*–the most cited scientific book of the twentieth century and work for which he was awarded the 1954 Nobel Prize in Chemistry–Pauling published a quantum-mechanical explanation of the intense colors in flavonoids, carotenoids, and dyes,[15] as well as a discussion of the use of resonance theory to understand anthocyanidin and carotenoid structures.[16] "A Theory of the Formation of Antibodies" followed in 1940, after which Pauling published papers with Zechmeister on the structure of prolycopene, an isomer of lycopene obtained from the tangerine tomato, with comments on the structural characteristics of lutein, zeaxanthin, and the carotenes, among other isomers.[17,18] However, Pauling's interest in these carotenoids and flavonoids was confined to their chemical structures and the influence of structure on optical properties; he did not address their health functions.

In 1941 Pauling was diagnosed with Bright's disease, or glomerulonephritis, which was at the time an often-fatal kidney disorder. On the advice of physicians at the Rockefeller Institute, he went to San Francisco for treatment by Thomas Addis, an innovative Stanford nephrologist. Addis prescribed a diet low in salt and protein, plenty of water, and supplementary vitamins and minerals that Pauling followed for nearly 14 years and completely recovered. This was dramatic first-hand experience of the therapeutic value of the diet.

Revelations

When Pauling cast about for a new research direction in the 1950s, he realized that mental illness was a significant public health problem that had not been sufficiently addressed by scientists. Perhaps his mother's megaloblastic madness and premature death caused by B_{12} deficiency underlay this interest. At about this time, Pauling's eldest son, Linus Jr., began a residency in psychiatry, which undoubtedly prompted Pauling to consider the nature of mental illness. Thanks to funding from the Ford Foundation, Pauling investigated the role of enzymes in brain function but made little progress. When he came across a copy of *Niacin Therapy in Psychiatry* by Abram Hoffer in 1965, Pauling was astonished to learn that simple substances needed in minute amounts to prevent deficiency diseases could have therapeutic application in unrelated diseases when given in very large amounts. This serendipitous and key event was critically responsible for Pauling's seminal paper in this emergent medical field. Later, Pauling was especially excited by Hoffer's observations on the survival of patients with advanced cancer who responded well to his micronutrient and dietary regimen, originally formulated to help schizophrenics manage their illness.[19,20] The regimen includes large doses of B vitamins, vitamin C, vitamin E, beta-carotene, selenium, zinc, and other micronutrients. About 40% of patients treated adjunctively with Hoffer's regimen lived, on average, five or more years, and about 60% survived four times longer than controls. These results were even better than those achieved by Ewan Cameron, Pauling's close clinical collaborator, in Scotland.

After a long and extremely productive career in Caltech, Pauling left under political pressure in late 1963 after winning the Nobel Peace Prize for his efforts to ban the atmospheric testing of nuclear weapons. Following a short tenure in the Center for the Study of Democratic Institutions in Santa Barbara, California, Pauling became professor of chemistry in the University of California at San Diego in 1967. Two years later, he accepted an

appointment as professor of chemistry in Stanford University in Palo Alto, California, where he remained through 1973. His ideas about orthomolecular medicine had been incubated at a number of institutions over the course of over 15 years, but it wasn't until he and two colleagues founded the independent Institute of Orthomolecular Medicine, shortly renamed the Linus Pauling Institute of Science and Medicine, in 1973 that they began to flourish. Stanford had provided an academic base while Pauling continued to develop his arguments for supplemental vitamin C, culminating in an important paper, "Evolution and the Need for Ascorbic Acid", published in the *Proceedings of the National Academy of Sciences USA* in 1970 and a book, *Vitamin C and the Common Cold,* also published in 1970, that won the Phi Beta Kappa Award as the best science book of the year and sold well. Lack of adequate laboratory space in Stanford prompted Pauling to establish the Institute, which was financed by donations and the transfer of federal grants on metabolic profiling from Stanford. The Institute remained his base until his death in 1994.

Pauling's fascination with his favorite molecule–vitamin C–led to numerous papers and was the focus of hundreds of his speeches from the 1960s until his death. Pauling was stimulated to think deeply about vitamin C after being contacted by Irwin Stone in 1966. Stone had been in the audience in 1966 when Pauling gave a talk at the reception for his acceptance of the Carl Neuberg Society for International Scientific Relations Medal in New York City.[21] In his speech, Pauling remarked that he hoped and expected to live a long time. Stone wrote to Pauling about hypoascorbemia (a genetic disease affecting all humans and caused by the inability to synthesize vitamin C) and suggested that he might well live for a long time, perhaps enjoying another fifty years of good health, by taking supplemental vitamin C. In his reply to Stone, Pauling cited his 1962 paper with Zuckerkandl on molecular diseases in which they argued that the loss of the endogenous synthesis of a vitamin can be considered to be a molecular disease, corrected by a palliative diet.[22] Pauling reviewed the evidence supplied by Stone and decided to take three grams of vitamin C per day, partly for optimum health and partly to prevent the serious colds that had afflicted him for many years, seriously interfering with his work. His wife, Ava Helen, also began to take supplemental vitamin C, and both reported better health and a greatly reduced incidence of colds, in accord with the scant clinical literature. Pauling was so impressed that he decided to write a book on the use of vitamin C to prevent and treat the common cold. The book was also a response to a letter from a critic, Victor Herbert, who complained about "vitamin hucksters" and challenged Pauling on statements he made in a talk at the dedication of the Mt. Sinai Medical School in 1968 on the efficacy of vitamin C in preventing and ameliorating colds.[23] Herbert asked for evidence from properly controlled trials, and Pauling discussed evidence from four such trials in his book. In a new edition of that book, *Vitamin C, the Common Cold, and the Flu,* published in 1976, Pauling added material on influenza, especially concerning the work of Jungeblut and Murata on the inactivation of viruses by vitamin C and the work of Klenner, Morishige, Murata, and others on the prophylactic and therapeutic effect of vitamin C in viral diseases.[24] Pauling noted that in 1935 Jungeblut was the first to report that high-dose vitamin C inactivates poliomyelitis virus, and he was intrigued by Klenner's use of very high-dose vitamin C, usually given intravenously, to treat viral diseases like hepatitis, poliomyelitis, and pneumonia, and toxicological conditions like venomous snake bites. Klenner

had published his work in regional medical journals since 1948. In the late 1980s, Pauling's attention returned to infectious disease and vitamin C. On the basis of *in vitro* and clinical evidence, he and his associates argued that vitamin C should be used in conjunction with newly introduced antiviral drugs like AZT, which prevents the *de novo* infection of cells, to inhibit replication of HIV and prevent the formation of abnormal giant T lymphocytes called syncytia, which are markers of viral infectivity and cytopathology.[25] Pauling and Cameron completed a draft of a new book, never published, on vitamin C and AIDS.

Szent-Gyorgyi and Pauling shared the opinion that the optimum intake of vitamin C is much larger than the RDA, the amount set by the Food and Nutrition Board to prevent scurvy. Pauling wrote to Szent-Gyorgyi in 1970, asking about Stone's ideas. Szent-Gyorgyi replied:

"As to ascorbic acid, right from the beginning I felt that the medical profession misled the public. If you don't take ascorbic acid with your food you get scurvy, so the medical profession said that if you don't get scurvy you are all right. I think that this is a very grave error. Scurvy is not the first sign of the deficiency but a premortal syndrome, and for full health you need much more, very much more....there is an enormous scattering in the need of vitamins and it is quite easily believable that many diseases which have not been connected til now with vitamins are really expressions of a lack of vitamins."[26]

Robert Cathcart, an orthopedic surgeon in California, read Pauling's book on vitamin C and the common cold in 1971 and began taking large doses of vitamin C to prevent colds from developing. Based on his success, he treated patients with high-dose oral vitamin C and observed the "bowel tolerance" threshold effect, which refers to a laxative function of high-dose vitamin C that depends on the health status of the subject.[27] Cathcart used this observation to titrate the therapeutic dose of vitamin C. A recent study suggested that vitamin C, by stimulating the cystic fibrosis transmembrane conductance regulator (CFTR), increases fluid secretion in epithelial cells, such as those found in the lung and intestine.[28] This may account for the observed laxative effect and could be variable depending on the individual's health status.

Pauling's public celebrity became increasingly associated with the advocacy of high-dose vitamin C to prevent and treat infectious diseases, even though he continued to work productively for the rest of his life on theoretical problems in chemistry and physics, notably his closed-pack spheron theory of atomic nuclei, as well as on solving chemical structures of organic and inorganic substances. Of course, he also continued to honor a commitment to his wife and himself to advocate for peace among nations at every opportunity. A collaboration with the Scottish surgeon Ewan Cameron on the adjunctive use of high-dose oral and intravenous vitamin C in advanced cancer that began in 1971 continued until Cameron's death in 1991. Cameron had written a book, *Hyaluronidase and Cancer,* in 1966 about the quest for a physiological hyaluronidase inhibitor (PHI) that would interfere with the action of the enzyme hyaluronidase in attacking hyaluronic acid in the ground substance that permits the growth of tumors.[29] Such a strategy might enhance "host resistance" to cancer and slow the growth of solid tumors, making cancer a manageable disease. Cameron read about Pauling's statements on the putative value of vitamin C in controlling cancer and wrote to him in 1971.[30] Cameron began to give his patients hospitalized with advanced cancer about 10 grams of vitamin C per day for about 10 days or longer, typically by slow-drip intravenous administration

followed by oral dosage. Pauling and Cameron argued that vitamin C benefits cancer patients by stimulating the synthesis of a PHI or by being incorporated into one, augmenting the immune system, and optimizing collagen synthesis, thus encapsulating tumors and enhancing tissue integrity.[31] Pauling also thought about the cytotoxicity of vitamin C, involving copper and redox chemistry, as early as 1975[32] and in 1983 published a paper with Japanese colleagues implicating hydrogen peroxide as the cytotoxic molecular species, based on *in vitro* and animal studies.[33] Despite repeated denials of federal grant requests over eight years, Pauling and his colleagues managed to publish scores of papers on vitamin C and cancer encompassing in vitro research, animal experiments, and clinical work. In 1979, he and Cameron published *Cancer and Vitamin C,* which remains in print in an expanded and updated edition.

With funding from the National Cancer Institute, the Mayo Clinic conducted two randomized controlled trials of high-dose vitamin C and advanced cancer.[34,35] Both studies failed to demonstrate any benefit of supplemental vitamin C, which was given only orally and for a short period. Pauling, Cameron, and others noted serious methodological flaws in the Mayo studies, which have been amply discussed elsewhere.[36] In recent years, Mark Levine and colleagues in the NIH have studied the pharmacokinetics of vitamin C in young, healthy men and women.[37,38] Based on their results demonstrating dramatic differences in plasma concentration of vitamin C depending on the mode of administration–intravenous administration produces plasma levels of 14,000 umol/L compared to about 220 umol/L with oral dosing–Levine considered the anticancer role of intravenous vitamin C,[39] extensively used by Cameron and by Riordan and colleagues.[40] He published several papers showing that, with high concentrations attained by intravenous infusion, the ascorbate radical is formed in the extracellular milieu around cancer cells, helping to generate hydrogen peroxide that then diffuses into malignant cells, inducing apoptosis and pyknosis and disrupting mitochondrial function.[41] This activity is selective–normal cells are unaffected–and appears to be dependent on the presence of an unidentified small molecular weight protein. Other recent papers have reported that vitamin C modulates hypoxia-inducible factor-1 (HIF-1), a transcription factor induced by hypoxia in cancer cells.[42,43] Vitamin C inhibits HIF-1 induction and related gene expression, resulting in decreased growth of tumor cells. It's possible that multiple mechanisms are involved in the anticancer effect of vitamin C and, based on Cameron's results with oral vitamin C, that some types of cancer may be more therapeutically sensitive to vitamin C. Fortunately, interest in this area has been renewed, and phase 1 clinical trials have been published and are under way.[44]

In the late 1980s, Pauling's renewed friendship with a German cardiologist led to the formulation of a novel hypothesis on the possible cause of atherosclerosis: lipoprotein(a), a major constituent of atherosclerotic plaque, serves as a surrogate for vitamin C in chronic vitamin C insufficiency.[45] A number of related concepts were derived from this putative surrogacy, including the role of lysine and vitamin C in ameliorating exercise-induced severe angina pectoris in patients with advanced heart disease. Indeed, Pauling wrote three case reports in the early 1990s that discussed such relief associated with the use of 3-6 grams per day each of lysine and vitamin C.[46-48] Clinical studies in recent years have repeatedly demonstrated that high-dose vitamin C promotes relaxation of the arteries and improves blood flow–reversing endothelial dysfunction in

patients with heart disease or diabetes[49,50] probably by stabilizing or increasing tetrahydrobiopterin, a molecule involved in nitric oxide synthesis.[51] Other clinical studies have shown that high-dose vitamin C reduces systolic blood pressure in hypertensive subjects by about ten points.[52] Of course, none of these beneficial effects are directly related to the classic role of vitamin C as a vitamin in preventing scurvy by promoting collagen synthesis.

The Work Continues–A Brief Survey

We have witnessed an explosion in research in orthomolecular medicine in the last 40 years. Nutritional epidemiological studies, mainly observational studies, have reported associations between many dietary factors and the risk for disease, and these initial associations have been followed up by biochemical and molecular biological studies to determine the substances and molecular mechanisms responsible for the putative benefits. While nutritional epidemiological studies do not prove a causal relationship, they do suggest possibly fruitful areas for further research. Foremost among epidemiological studies in the U.S.A. are the Nurses' Health Study I, organized in 1976 with 122,000 women; the Nurses' Health Study II, established in 1989 with 117,000 women; and the Health Professionals' Follow-up Study, organized in 1986 with about 51,500 men. Subjects report periodically on their diet and health using food-frequency and health-status questionnaires, and some biological samples, such as toenail clippings, blood, and urine, have been collected. Compliance has been extremely good, with about a 90% response rate, and the value of the food-frequency and health questionnaires has been generally verified,[53] although many scientists remain skeptical of associations derived from observational studies. One of the outcomes of the Nurses' Health Study has been the discovery of the strong association between the intake of *trans* fat and coronary heart disease, which has led to the reduction of *trans* fat in the American diet through labeling and food industry practices. In the Netherlands, the work of Martijn Katan has been equally important in this area.[54] Other large nutritional epidemiological studies have been organized, including the Netherlands Cohort Study (121,000 men and women, begun in 1986) and the European Prospective Investigation into Cancer and Nutrition (EPIC) study (440,000 men and women, begun in 1993), which found a link between the consumption of red meat and colorectal cancer, as well as myriad other findings, such as impressive inverse relationships between plasma vitamin C and all-cause mortality[55] or risk of stroke.[56]

Reports from these large-scale studies and others have identified associations between the consumption or avoidance of certain foods and supplements with disease risk, and scientific reductionism and biological plausibility have prompted the investigation of dietary constituents putatively responsible for the observed effects. Ellagic acid and anthocyanidins in berries; flavonoids like catechins in tea and chocolate and others in fruit and vegetables; isothiocyanates, including sulforaphane, and indole-3-carbinol from cruciferous vegetables; resveratrol in wine, grapes, and peanuts; chlorophyll and its derivative, chlorophyllin; carotenoids like lutein and lycopene; allicin and its derivates from garlic; phytosterols; lignans; fiber; essential fatty acids; curcumin; and soy isoflavones are some of the dietary phytochemicals for which substantial literature has emerged in recent years. Research on the role of fish-derived omega-3 fatty acids in attenuating cardiovascular disease, inflammatory diseases, and mental illness has also been robust. The antioxidant function of flavonoids has been emphasized, but their poor absorp-

tion and rapid metabolism has led to the suggestion that fructose, not flavonoids, in ingested fruit increases the antioxidant capacity of plasma by stimulating the synthesis of uric acid, a strong physiological antioxidant, in the liver.[57] A new focus on the cell-signaling properties of flavonoids and transient antioxidants like alpha-lipoic acid has emerged.[58] The Micronutrient Information Center on the Linus Pauling Institute Web site provides a resource for updated and comprehensive information on micronutrients, phytochemicals, and other dietary substances and their roles in health and disease (http://lpi.oregonstate.edu/infocenter).

Interest in improving health span by dietary strategies has also accelerated, leading to remarkable studies with acetyl-L-carnitine and lipoic acid. Supplementation with these compounds has increased ambulatory activity and cognitive performance in old rats and old dogs, suggesting that they may be useful in slowing or even reversing age-related deficits in humans.[59,60] Mitochondrial dysfunction, caused partly by oxidative damage and inflammation, has been implicated in neurodegenerative diseases, such as Parkinson's, ALS, and Alzheimer's, as well as in age-related decline, and therapeutic efficacy for coenzyme Q_{10}, lipoic acid, and other antioxidants has been suggested.[61,62]

Vitamin D is critical for cell differentiation, immune function, calcium utilization, and bone health, and it is required to prevent rickets and osteomalacia. A number of conditions, such as skin color, advanced age, fat malabsorption syndromes, obesity, and inflammatory bowel disease, are associated with increased risk for vitamin D deficiency. Concern about chronic suboptimum levels of vitamin D in northern latitudes, implicated in increased cancer risk, autoimmune disease, and osteoporosis, has prompted discussion of increasing the AI (Adequate Intake).

Clinical studies have also been conducted to determine if intervention with supplements identified by observational studies will attenuate disease risk. In 1993 two prospective studies from the aforementioned epidemiological research showed that the intake of vitamin E supplements significantly reduced the risk for coronary heart disease in men and women.[63,64] While this had been common knowledge among those familiar with the Shute's work in Canada and with Pauling's writings, the papers stimulated much clinical interest in high-dose vitamin E and heart disease, leading to several randomized controlled trials. Results have been inconsistent, possibly owing to insufficient dose and/or duration, inadequate instructions about how to take vitamin E with fat-containing food for sufficient absorption, and the polypharmacy of patients with heart disease. For example, investigators have speculated that vitamin E may induce drug-detoxifying enzymes in the liver that could interfere with the therapeutic efficacy of certain drugs.[65,66] Additionally, one recent study in hypercholesterolemic subjects found that oxidative stress, as measured by plasma F_2-isoprostanes, was significantly suppressed (by 35% and 49%) only by daily doses of *RRR*-alpha-tocopherol of 1,600 IU or 3,200 IU, respectively, for at least 16 weeks.[67] Smaller daily doses (400 IU or 800 IU) resulted in non-statistically significant reductions in plasma F_2-isoprostanes.

Recent studies have also identified *in vitro* and *in vivo* anti-inflammatory roles for gamma-tocopherol in alleviating oxidative and nitrative stress,[68,69] despite the more rapid metabolism and clearance of gamma-tocopherol compared to alpha-tocopherol,[70] for which a transport protein has been discovered. About 70% of the vitamin E intake in the U.S. is in the form of gamma-tocopherol. One recent review of the role of gamma-tocopherol in the prevention of heart disease and

cancer noted that the results of prospective studies of gamma-tocopherol and the risk for heart disease are inconsistent, but some evidence suggests that high plasma gamma-tocopherol levels are associated with a decreased risk for prostate cancer.[71] The media have compounded confusion about vitamin E and heart disease by not carefully distinguishing primary prevention trials in which subjects at baseline have not manifested heart disease from secondary prevention trials in which patients with heart disease have been supplemented with micronutrients to determine if supplementation decreases clinical events like myocardial infarction, stroke, or death.

Cellular transport mechanisms for vitamin C–the sodium vitamin C transporters (SVCT 1 and 2)–have been discovered in recent years.[72] Dehydroascorbic acid (DHA) and glucose are facilitatively transported by GLUT1, GLUT3, and GLUT4.[73,74] DHA has a half-life of only about seven minutes, and its levels in plasma are about 1000-fold less than circulating glucose, so its uptake is likely to be competitively inhibited by glucose.[75] Ascorbic acid, on the other hand, is actively transported by the SVCT proteins, and the activity of one, SCVT1, declines with age,[75] suggesting that older people need higher intakes to maintain a plasma status similar to young people at lower intakes of vitamin C.[76] New biochemical functions have been reported for vitamin C, such as the ascorbylation reaction in which vitamin C combines with reactive aldehydes, potentially protecting biomolecules from damage.[77] Mechanistic and pharmacokinetic studies on vitamin C and vitamin E and their roles in disease prevention and treatment will continue to be further explored in the near future.

Nutritionally essential minerals, such as selenium, zinc, and magnesium, have also garnered attention after inverse associations with risk for disease, especially cancer, emerged from epidemiological studies. Deficiencies of selenium or zinc impair immune function and increase susceptibility to infectious diseases, including HIV/AIDS.[78,79] A long-term intervention trial found that daily supplementation with selenium-enriched yeast was associated with about a 50% reduction in prostate cancer incidence,[80] although the risk for non-melanoma skin cancer was increased by 25%.[81] In a large-scale randomized controlled trial, daily zinc supplements alone or in combination with antioxidant vitamins significantly reduced the risk for age-related macular degeneration by about 25% or more.[82] In another large-scale, long-term study, high serum magnesium levels were associated with substantial decreases in all-cause mortality (40%), cardiovascular disease mortality (40%), and cancer mortality (50%), compared to low serum magnesium.[83] Over half of Americans appear to ingest less than the daily Estimated Average Requirement (EAR) for magnesium, and a significant percentage of pre-menopausal American women ingest less than the daily EAR for iron, which increases vulnerability for heme deficiency and anemia.[84]

From these studies we can ascertain with certainty that relationships between micronutrients are complex and, in many cases, poorly understood. It is also clear that many people do not have adequate intakes of vital micronutrients, resulting in poor health and increased risk for disease. There is much to learn about the optimum intake of specific micronutrients or combinations of micronutrients, and new emphasis on translational research and evidence-based medicine will continue to stimulate research. Uncertainties propel research forward, and the future of orthomolecular medicine is bright. On the 40th anniversary of his paradigm-shifting paper, Linus Pauling would be pleased.

References

1. Pauling L: Orthomolecular Psychiatry. *Science,* 1968; 160: 265-271.
2. Oken D: Vitamin therapy: treatment for the mentally ill. *Science,* 1968; 160: 1181.
3. Pauling L: Letter. *Science,* 1968; 160: 1181.
4. Desiraju G: The all-chemist. *Nature,* 2000; 408: 407.
5. Pauling L: *Vitamin C and the Common Cold.* San Francisco: W. H. Freeman, 1970.
6. Pauling L, Itano H, Singer S, et al: Sickle cell anemia, a molecular disease. *Science,* 1949; 110: 543-548.
7. Ames B, Elson-Schwab I, Silver E: High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased Km): relevance to genetic disease and polymorphisms. *Am J Clin Nutr,* 2002; 75: 616-658.
8. Pauling L, Robinson A, Oxley S, et al: Results of a loading test of ascorbic acid, niacinamide, and pyridoxine in schizophrenic subjects and controls. In: Hawkins D and Pauling L, eds. *Orthomolecular Psychiatry.* San Francisco: W. H. Freeman, 1973, 18-34.
9. Pauling L: Some aspects of orthomolecular medicine. *J Intern Acad Prev Med,* 1974; 1:1-30.
10. Pauling L: *How to Live Longer and Feel Better.* New York: W. H. Freeman, 1986.
11. Pauling L: On the orthomolecular environment of the mind: orthomolecular theory. *Am J Psychiatry,* 1974; 131: 1251-1257.
12. Pauling L: The future of the Crellin Laboratory. *Science,* 1938; 87: 563-566.
13. Williams R: *Biochemical Individuality.* New York: John Wiley & Sons, 1956; 3.
14. Breakthrough of the Year: Human Genetic Variation. *Science,* 2007; 318: 1842-1843.
15. Pauling L: A theory of the color of dyes. *Proc Natl Acad Sci,* 1939; 25: 577-582.
16. Pauling L: Recent work on the configuration and electronic structure of molecules; with some applications to natural products. Fortschr Chem organis *Naturstoffe,* 1939; 3: 203-235.
17. Zechmeister L, LeRosen A, Went F, et al: Prolycopene, a naturally occurring stereoisomer of lycopene. *Proc Natl Acad Sci,* 1941; 27: 468-474.
18. Zechmeister L, LeRosen A, Schroeder W, et al: Spectral characteristics and configuration of some stereoisomeric carotenoids including prolycopene and pro-gamma-carotene. *J Am Chem Soc,* 1943; 65: 1940-1951.
19. Hoffer A, Pauling L: Hardin Jones biostatistical analysis of mortality data for cohorts of cancer patients with a large fraction surviving at the termination of the study and a comparison of survival times of cancer patients receiving large regular oral doses of vitamin C and other nutrients with similar patients not receiving those doses. *J Orthomol Med,* 1990; 5: 143-154.
20. Hoffer A, Pauling L: Hardin Jones biostatistical analysis of mortality data for a second set of cohorts of cancer patients with a large fraction surviving at the termination of the study and a comparison of survival times of cancer patients receiving large regular oral doses of vitamin C and other nutrients with similar patients not receiving those doses. *J Orthomol Med,* 1993; 8: 157-167.
21. Stone I: *Letter to L. Pauling,* 4 April 1966.
22. Pauling L: *Letter to I. Stone,* July 1966.
23. Herbert V: *Letter to L. Pauling,* 11 November 1968.
24. Pauling L: *Vitamin C, the Common Cold, and the Flu.* San Francisco: W. H. Freeman, 1976
25. Harakeh S, Jariwalla R, and Pauling L: Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. *Proc Natl Acad Sci USA,* 1990; 87: 7245-7249.
26. Szent-Gyorgyi A: *Letter to L. Pauling,* 15 April 1970.
27. Cathcart R: Vitamin C, titrating to bowel tolerance, anascorbemia, and acute induced scurvy. *Med Hypotheses,* 1981; 7: 1359-1376.
28. Fischer H, Schwarzer C, and Illek B: Vitamin C controls the cystic fibrosis transmembrane conductance regulator chloride channel. *Proc Natl Acad Sci USA,* 2004; 101: 3691-3696.
29. Cameron E: *Hyaluronidase and Cancer.* Oxford: Pergamon Press, 1966.
30. Cameron E: *Letter to L. Pauling,* 30 November 1971.
31. Cameron E and Pauling L: Ascorbic acid and the glycosaminoglycans. *Oncology,* 1973; 27: 181-192.
32. Pauling L. *Letter to E. Cameron,* 19 August 1975.
33. Kimoto E, Tanaka H, Gyotoku J, et al: Enhancement of antitumor activity of ascorbate against Ehrlich ascites tumor cells by the copper:glycylglycylhistidine complex. *Cancer Res,* 1983; 43: 824-828.
34. Creagan E, Moertel C, O'Fallon J, et al: Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. *N Engl J Med,* 1979; 301: 687-690.

35. Moertel C, Fleming T, Creagan E, et al: High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. *N Engl J Med,* 1985; 312: 137-141.
36. Richards E: *Vitamin C and Cancer: Medicine or Politics?* New York: St. Martin's Press, 1991.
37. Levine M, Conry-Cantilena C, Wang Y, et al: Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA,* 1996; 93: 3704-3709.
38. Levine M, Wang Y, Padayatty S, et al: A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci USA,* 2001; 98: 9842-9846.
39. Padayatty S, Sun H, Wang Y, et al: Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med,* 2004; 140: 533-537.
40. Riordan H, Hunninghake R , Riordan N, et al: Intravenous ascorbic acid: protocol for its application and use. *P R Health Sci J*, 2003; 22: 287-290.
41. Chen Q, Espey M, Sun A, et al: Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. *Proc Natl Acad Sci USA,* 2007; 104: 8749-8754.
42. Vissers M, Gunningham S, Morrison M, et al: Modulation of hypoxia-inducible factor-1 alpha in cultured primary cells by intracellular ascorbate. *Free Radic Biol Med*, 2007; 42: 765-772.
43. Gao P, Zhang H, Dinavahi R, et al: HIF-dependent antitumorigenic effect of antioxidants in vivo. *Cancer Cell,* 2007; 12: 230-238.
44. Riordan H, Casciari J, Gonzalez M, et al: A pilot clinical study of continuous intravenous ascorbate in terminal cancer patients. *P R Health Sci J,* 2005; 24: 269-276.
45. Rath M, Pauling L: Hypothesis: lipoprotein(a) is a surrogate for ascorbate. *Proc Natl Acad Sci USA,* 1990; 87: 6204-6207.
46. Pauling L: Case report: lysine/ascorbate-related amelioration of angina pectoris. *J Orthomol Med,* 1991; 6: 144-146.
47. McBeath M and Pauling L: A case history: lysine/ascorbate-related amelioration of angina pectoris. *J Orthomol Med,* 1993; 8: 77-78.
48. Pauling L: Third case report on lysine-ascorbate amelioration of angina pectoris. *J Orthomol Med,* 1993; 8: 137-138.
49. Frei B: On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction. *Proc Soc Exp Biol Med,* 1999; 222: 196-204.
50. Gokce N, Keaney J, Frei, B, et al: Long-term ascorbic acid administration reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation,* 1999; 99: 3234-3240.
51. Huang A, Vita J, Venema R, et al: Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin. *J Bio Chem,* 2000; 275: 17399-17406.
52. Duffy S, Gokce N, Holbrook E, et al: Treatment of hypertension with ascorbic acid. *Lancet,* 1999; 354: 2048-2049.
53. Willett W, Sampson L, Browne M, et al: The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol,* 1988; 127: 188-199.
54. Mensink R, Katan M: Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N Engl J Med,* 1990; 323: 439-445.
55. Khaw K, Bingham S, Welch A, et al: Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norwalk prospective study: a prospective population study. *Lancet,* 2001; 357: 657-663.
56. Myint P, Luben R, Welch A, et al: Plasma vitamin C concentrations predict risk of incident stroke over 10 y in 20,649 participants of the European Prospective Investigation into Cancer–Norfolk prospective population study. *Am J Clin Nutr,* 2008; 87: 64-69.
57. Lotito S, Frei B: The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. *Free Radic Biol Med,* 2004; 37: 251-258.
58. Suh J, Shenvi S, Dixon B, et al: Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proc Natl Acad Sci USA,* 2004; 101: 3381-3386.
59. Hagen T, Liu J, Lykkesfeldt J, et al: Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proc Natl Acad Sci USA,* 2002; 99: 1870-1875.
60. Milgram N, Araujo J, Hagen T, et al: Acetyl-L-carnitine and alpha-lipoic acid supplementation of aged beagle dogs improves learning in two landmark discrimination tests. *FASEB J,* 2007; 13: 3756-3762.
61. Lin M, Beal M: Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases.

Nature, 2006; 443: 787-795.
62. Rodriguez M, MacDonald J, Mahoney D, et al: Beneficial effects of creatine, CoQ_{10}, and lipoic acid in mitochondrial disorders. *Muscle Nerve*, 2007; 35:235-242.
63. Stampfer M, Hennekens C, Manson J, et al: Vitamin E consumption and the risk of coronary disease in women. *N Eng J Med,* 1993; 328: 1444-1449.
64. Rimm E, Stampfer M, Ascherio A, et al: Vitamin E consumption and the risk of coronary disease in men. *N Eng J Med,* 1993; 328: 1450-1456.
65. Brigelius-Flohe R: Vitamin E and drug metabolism. *Biochem Biophys Res Commun,* 2003; 305: 737-740.
66. Traber M: Vitamin E, nuclear receptors and xenobiotic metabolism. *Arch Biochem Biophys,* 2004; 423: 6-11.
67. Roberts II L, Oates J, Linton M, et al: The relationship between dose of vitamin E and suppression of oxidative stress in humans. *Free Radic Biol Med,* 2007; 43: 1388-1393.
68. Jiang Q, Elson-Schwab I, Courtemanche, et al: gamma-Tocopherol and its major metabolite, in contrast to alpha-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. *Proc Natl Acad Sci USA,* 2000; 97: 11494-11499.
69. Devaraj S, Leonard S, Traber M, et al: gamma-Tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radic Biol Med,* 2008; 44: 1203-1208.
70. Leonard S, Paterson E, Atkinson J, et al: Studies in humans using deuterium-labeled alpha- and gamma-tocopherols demonstrate faster plasma gamma-tocopherol disappearance and greater gamma-metabolite production. *Free Radic Biol Med,* 2005; 38: 857-866.
71. Dietrich M, Traber M, Jacques P, et al: Does gamma-tocopherol play a role in the primary prevention of heart disease and cancer? A review. *J Am Coll Nutr,* 2006; 25: 292-299.
72. Daruwala R, Song J, Koh W, et al: Cloning and functional characterization of the human sodium-dependent vitamin C transporters hSVCT1 and hSVCT2. *FEBS Lett,* 1999; 460: 480-484.
73. Rumsey S, Kwon O, Xu G, et al: Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. *J Biol Chem,* 1997; 272: 18982-18989.
74. Rumsey S, Daruwala R, Al-Hasani H, et al: Dehydroascorbic acid transport by GLUT4 in Xenopus oocytes and isolated rat adipocytes. *J Biol Chem,* 2000; 275: 28246-28253.
75. Michels A, Hagen T: Vitamin C status declines with age. In: Asard H, May J, and Smirnoff N, eds. *Vitamin C.* New York: Garland Science/ BIOS Scientific Publishers, 2004; 203-227.
76. Brubacher D, Moser U, Jordan P: Vitamin C concentrations in plasma as a function of intake: a meta-analysis. *Int J Vitam Nutr Res,* 2000; 70: 226-237.
77. Sowell J, Conway H, Bruno R, et al: Ascorbylated 4-hydroxy-2-nonenal as a potential biomarker of oxidative stress response. *J Chromatogr B Analyt Technol Biomed Life Sci,* 2005; 827: 139-145.
78. Shankar A, Prasad A: Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr,* 1998; 68: 447S-463S.
79. Baum M, Campa A: Role of selenium in HIV/ AIDS. In: Hatfield D, Berry M, and Gladyshev V, eds. *Selenium: Its Molecular Biology and Role in Human Health.* 2nd ed. New York: Springer, 2006; 299-310.
80. Duffield-Lillico A, Dalkin B, Reid M, et al: Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. *BJU Int,* 2003; 91: 608-612.
81. Duffield-Lillico A, Slate E, Reid M, et al: Selenium supplementation and secondary prevention of nonmelanoma skin cancer in a randomized trial. *J Natl Cancer Inst,* 2003; 95: 1477-1481.
82. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol,* 2001; 119: 1417-1436.
83. Leone L, Courbon D, Ducimetiere P, et al: Zinc, copper, and magnesium and risks for all-cause, cancer, and cardiovascular mortality. *Epidemiology,* 2006; 17: 308-314.
84. Moshfegh A, Goldman J, Cleveland L: *What We Eat in America, NHANES 2001-2002: Usual Nutrient Intakes from Food Compared to Dietary Reference Intakes.* USDA Agricultural Research Service, 2005.