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OREGON STATE UNIVERSITY

The Linus Pauling Institute

RESEARCH NEWSLETTER

From the Director

Balz Frei, Ph.D.
Professor of Biochemistry and Biophysics
LPI Director and Endowed Chair

As you look at this issue of the Linus Pauling Institute newsletter, you may notice that we changed the name from "Research Report" to "Research Newsletter." The reason for this change is that we think Research Newsletter more accurately reflects its content — articles on recent research progress in the Linus Pauling Institute written primarily for our donors and other interested, health-conscious members of the public. After changing the name from "LPI Newsletter" to "LPI Research Report" and now "LPI Research Newsletter," we intend to stick with this new name!

This year marks the 10th anniversary of LPI at Oregon State University. In the summer of 1996, the Institute, then called the Linus Pauling Institute of Science and Medicine, moved from Palo Alto, California, to Corvallis, Oregon. I wasn't part of the Institute back then and only joined as director a year later, but LPI members Stephen Lawson and Distinguished Professor George Bailey, among other researchers and administrators at OSU, vividly remember the Herculean effort it took to move the Institute and all its assets from the San Francisco Bay Area to OSU. The Institute has blossomed in the last ten years. With strong support from the University and our donors, we have grown from one laboratory and Principal Investigator with about $300,000 in annual extramural research funding to ten laboratories and Principal Investigators with about $3 million in research funding, primarily from the National Institutes of Health. The annual number of LPI scientific publications also has increased substantially, from 7 in 1997 to 47 in 2005. With this growth, we greatly expanded our research scope, which now includes heart disease, cancer, neurodegenerative diseases, inflammation, and aging, while remaining faithful to our mission to elucidate the function of micronutrients and other dietary and lifestyle factors in health promotion and disease prevention.

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Neurodegeneration

An interview with Joseph Beckman, Ph.D.
Professor of Biochemistry and Biophysics
Ava Helen Pauling Chair, LPI

Q. ***How did you get interested in ALS?***

A. It began when I watched the national news one night in 1993, when it was reported that mutations to an antioxidant gene cause ALS. The mutations affected a protein that I had been using to protect the brain against stroke and head trauma. My original research was in treating stroke, which started when I was in the army. We modified an antioxidant enzyme called superoxide dismutase, the same enzyme linked with ALS, by chemically coating it with polyethylene glycol to keep the kidneys from filtering the enzyme from blood too quickly. We found that the modified protein protected animals from stroke. This was surprising because we injected superoxide dismutase into the bloodstream and it shouldn't have crossed the blood brain barrier. These results led to clinical trials on head trauma. In the early stage of phase 2 trials, the treatment looked very promising. I was told that some people admitted to the hospital with fixed and dilated pupils recovered from severe head trauma and their intracranial pressure appeared to be decreased. However, with slightly larger doses, the effect went away. In animals, slightly higher dosages of superoxide dismutase were also not protective — the brain actually swelled more. The paradox was that the protein was not toxic even at much higher dosages in animals. I was puzzled about how a good protein could go bad.

Q. ***How did you make a connection between superoxide dismutase and ALS?***

A. In 1989, I had discovered a damaging reaction that was catalyzed by superoxide dismutase. I thought that there must be a disease in which this adverse reaction would occur. Listening to the news that evening in 1993 about a gene implicated in ALS, my interest peaked when Tom Brokow said, "and this protein is an antioxidant enzyme, raising hopes that the disease may be treated with antioxidants." I then thought that I knew exactly what the enzyme was

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Designated by the National Institutes of Health as a
Center of Excellence for Research on Complementary and Alternative Medicine

Continued from cover — From the Director

Coincidentally with LPI's 10th anniversary at OSU, this year we also mark the 20th anniversary of the publication of Linus Pauling's most popular — and arguably most influential — book, *How to Live Longer and Feel Better*. To celebrate this occasion, OSU Press will publish a 20th anniversary edition of the book in May. Although the science has progressed and new relevant findings have emerged over the last 20 years, we decided to leave the text of the book unchanged, as it represents the ideas and opinions of one of the greatest scientists of the 20th century. Not surprisingly, many of Pauling's ideas were well ahead of their time and remain topical to this day. In addition to a new introduction, the book features an afterword and some annotations. In the afterword, we refer to the Institute's extensive online Micronutrient Information Center (http://lpi.oregonstate.edu/infocenter), which provides updated information on the biological functions and health benefits of micronutrients, phytochemicals, and other constituents of the diet.

Finally, I am pleased to report to you that LPI scientists have been successful in securing four major research grants earlier this year. Professor Joseph Beckman, who is the Ava Helen Pauling Chair, together with OSU Chemistry Professors Max Deinzer and Douglas Barofsky, obtained a grant from the W. M. Keck Foundation. Their goal is to develop a new "electron capture tandem mass spectrometer" that will allow them to investigate the role of specific proteins in cancer and Lou Gehrig's disease. LPI Principal Investigator Fred Stevens secured an individual research grant from the National Institutes of Health to investigate the role of vitamin C in neutralizing lipid oxidation products that have been implicated in heart disease and cancer. Dr. Stevens also was awarded a second grant from NIH to acquire another mass spectrometer, called a "hybrid triple quadrupole linear ion trap mass spectrometer," to enhance the Institute's research programs in age-related inflammatory diseases. Additionally, LPI Principal Investigator Maret Traber obtained a grant from NIH to investigate the regulation of biochemical pathways in the liver involved in the metabolism of vitamin E. Two critical questions that Professor Traber will address are how these pathways affect the metabolism of pharmaceutical drugs and how vitamin E supplements can be used with optimal benefits for human health without lowering the effectiveness of conventional drug treatments.

Congratulations to Drs. Traber, Beckman, and Stevens for being awarded these large research grants, which, together with the support from our generous donors, are critical in sustaining and expanding the Institute's research efforts.

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and what bad thing it was doing to cause ALS. Sure enough, the mutations were affecting superoxide dismutase. So I wrote up my short hypothesis paper on ALS, superoxide dismutase, peroxynitrite, and protein nitration and published it in *Nature* in August 1993. I have been testing that hypothesis in ALS for the past 13 years. My initial ideas were too simple, but overall the concept is holding up.

Q. ***How does the body get rid of superoxide?***

A. Superoxide is removed by superoxide dismutase. There are three different forms of the enzyme — one inside the cytosol of the cell, one in the mitochondria, and one outside of cells. Detoxifying superoxide is clearly important — one-half percent of the soluble protein in your body is made up of this enzyme. My own research focused on understanding how this enzyme works to protect brain tissues. It scavenges superoxide, but it wasn't clear that superoxide was particularly toxic. The existence of superoxide was predicted and named by Linus Pauling in the early 1930s. He was thinking about reactions between potassium and oxygen and realized that a very unusual compound would be formed in that the oxygen would have a three-electron bond. He knew that potassium superoxide was unstable and even dangerous in the laboratory and called this new form of oxygen superoxide. But when you dissolve superoxide in water under conditions that occur inside the body, superoxide actually is a weak reducing agent. In the body, it is not the "super"oxidizing agent promised by its name.

Q. ***What does superoxide do in the body?***

A. It is produced in large amounts by inflammatory cells, where it helps kill pathogens. But it has a more subtle role that affects most disease processes because of its rapid reaction with nitric oxide. In 1988, I read that the endothelium-derived relaxing factor (EDRF) had been identified as nitric oxide. I had been following this story because EDRF was an unknown and mysterious factor produced by the cells that line all blood vessels. It turned out to be an inorganic molecule that's a common air pollutant. In an old chemistry textbook I had bought from a used book store while in high school, I found a reaction showing that nitric oxide reacts with superoxide to form peroxynitrite and that the peroxynitrite molecule breaks down to form two damaging free radicals — hydroxyl radical and nitrogen dioxide. In other words, superoxide — not a particularly dangerous chemical *in vivo* — could make very damaging oxidants by reacting with nitric oxide. We characterized these reactions and showed that this actually does happen in many different human diseases. By accident in the lab one night, I added peroxynitrite to bovine superoxide dismutase, which is isolated from cows. The bovine enzyme turned bright yellow and stayed yellow for years. Eventually we crystallized it and determined its molecular structure. We found that a nitro group was added to the sole tyrosine, an amino acid, on superoxide dismutase. We were fortunate to have used bovine superoxide dismutase instead of the human form because the human superoxide dismutase has no tyrosines. We discovered the process of tyrosine

nitration, which has been implicated in cardiovascular disease, arthritis, lung injury, cancer, organ rejection, Parkinson's disease, Alzheimer's disease, and ALS.

Q. *What's special about tyrosine?*

A. Tyrosine is an aromatic amino acid that's important in allowing proteins to make contact with other proteins. It is also important for regulating many processes that control the major functions in cells, such as whether to divide, to differentiate, or to die. It's one of the more uncommon amino acids, occurring in only 3-5% of proteins. There are many proteins that have no tyrosines at all, so human superoxide dismutase is not an exception.

Q. *Are other antioxidants like uric acid or coenzyme Q10 useful in ALS?*

A. Uric acid may work because the pathologic process we've implicated in ALS can be inhibited by uric acid. Uric acid is being used as a promising treatment for multiple sclerosis in an animal model. The problem is that it is very difficult to get uric acid into the brain. In our cell culture models, uric acid is protective, and Kristine Robinson in my lab is testing it *in vivo* right now in animal models of ALS. In some transgenic animal models of ALS, coenzyme Q10 is protective, but only weakly. Most patients are taking large amounts of it. There are studies showing that massive amounts of coenzyme Q10 are therapeutic in Parkinson's disease and possibly in Huntington's disease.

Q. *How is ALS related to other neurodegenerative diseases like Alzheimer's or Parkinson's? Is there a common etiology?*

A. I think so. Much can be learned from what is common among the three diseases, and all three can affect some people. On the island of Guam, Parkinson's, Alzheimer's, and ALS can all develop in the same individual with high frequency. All of these neurodegenerative diseases affect motor systems. I believe they are manifestations of a similar disease process but affect different regions of the brain. The underlying mechanisms that we've been studying apply to all three. A particularly promising approach to this has come from my close colleague, Luis Barbeito, in Montevideo, Uruguay, who is interested in pathological changes initiated by astrocytes.

Q. *What are astrocytes?*

A. Astrocytes are support cells for neurons. In the brain, there are about ten times more astrocytes than neurons. They're important for providing the proper nutrition to neurons and maintaining the proper ion balances of neurons. Astrocytes are also important for defending against infections, when they can become reactive. We're finding that reactive astrocytes may have a major role in ALS, Parkinson's, and Alzheimer's. When astrocytes become reactive, they develop a star-shaped structure,

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Vitamin C and the Common Cold

The debate about the value of vitamin C in preventing and treating the common cold has raged for over three decades following the publication in 1970 of *Vitamin C and the Common Cold* by Linus Pauling. After Pauling was alerted by Irwin Stone in 1965 to the possible value of high-dose vitamin C in preventing colds, he and his wife began to routinely take several grams per day. Pauling had been troubled for many years by severe colds that interfered with his work, and he was pleased to find that the vitamin C supplements greatly decreased the number of colds he caught. He reviewed the clinical literature, then consisting of about four trials that used at least one gram of vitamin C per day, and published his book, expecting that the news that a safe and cheap substance could provide a simple way of preventing and ameliorating colds would be embraced by the medical community and the public. The medical community was generally skeptical or disdainful, but the public responded. Interest in the use of vitamin C for colds expanded, and 21 clinical studies using at least one gram of vitamin C per day were published between 1971 and 1994. These studies were reviewed by Dr. Harri Hemila in 1994. He found that vitamin C supplements reduced the duration of colds by about 23% and ameliorated symptoms, although there was no consistent effect on incidence.

In 2004, Dr. Hemila and colleagues again reviewed the relevant studies for *The Cochrane Database of Systematic Reviews*. To study the prevention of colds by vitamin C, they pooled and analyzed the results of 29 trials involving more than 11,000 subjects. With the exception of six trials with over 600 runners, skiers, or soldiers, no preventive effect was found for vitamin C. In those six subgroups, the incidence of colds was halved. Thirty studies were examined that addressed the effect of vitamin C on the duration of colds. In these studies, there was a consistent benefit, with a reducton in duration of 8% to 14%. Fifteen trials assessed the effect of vitamin C on symptoms, which were significantly ameliorated by vitamin C.

A Japanese study on vitamin C and colds was published in 2006 in the *European Journal of Clinical Nutrition*. This study was a five-year, randomized, double-blind (but not placebo-controlled) trial designed to evaluate the effect of a daily dose of 50 mg or 500 mg of vitamin C on the development of gastric cancer among 244 subjects. (The 50-mg dose of vitamin C served as a quasi-placebo.) The researchers evaluated the effects of the vitamin C supplements on the common cold at the completion of the study. The risk of contracting three or more colds in the five-year period was decreased by 66% by the daily intake of the 500-mg vitamin C supplement. There was little difference in severity or duration of colds between subjects in the low-dose or high-dose groups. This study deserves special mention because it was much longer (five years) than the trials reported by Hemila and covered many cold seasons in which subjects were probably exposed repeatedly to many cold viruses.


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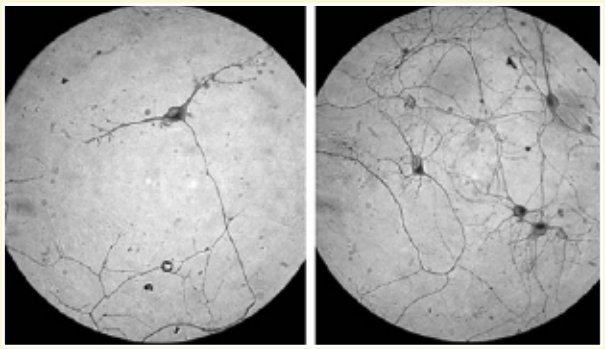
and that's why they're called astrocytes — star cells. We've worked out much of the pathway that makes astrocytes potentially damaging to neurons. This pathway involves the generation of the oxidant peroxynitrite that may be initiated by the mutant form of superoxide dismutase. Astrocytes respond differently to peroxynitrite than do neurons, which are far more likely to die. Depending upon the brain region, neurons respond differently in ALS, multiple sclerosis, or Alzheimer's disease.

Q. ***What part of the nervous system is affected by ALS?***

A. The brain contains many billions of neurons responsible for thoughts, memories, emotions, and all of our actions, directly controlling who we are, what we perceive, and how we think. These neural networks are organized to pick up sensory information, such as information from the eye, and then process the information in the outer layers of the brain. Deeper in the brain are structures that are involved in more elaborate activity, such as controlling emotional responses. But the ability to move is determined by a remarkably small number of highly specialized neurons in the spinal cord. There, only 500,000 motor neurons control the movement of every muscle in your body. These cannot be replaced. If those motor neurons die, as happens in ALS, you will be unable to move a muscle or breathe. In the final stages of the disease, patients are even unable to blink. Yet the rest of the brain is fully functional. That's what makes this disease so horrible.



Courtesy of Luis Barbeito

Motor neurons dying from exposure to peroxynitrite

Motor neurons protected from peroxynitrite

Q. ***How do you study these effects?***

A. We study the chemistry of superoxide dismutase, culture astrocytes and motor neurons, and study animal models of the disease. We also work on autopsy samples from human patients.

Q. ***Do animal models of ALS or other neurodegenerative diseases accurately represent human symptomology and brain pathology?***

A. Not exactly. A post-doc in my lab once told a famous neuroscientist, "Well, maybe humans are a poor model of the mouse disease!" But they're the most accepted model available and can teach us many things.

Q. ***Are there other substances that may be important in preventing the development of these diseases?***

A. We know from epidemiological studies that non-steroidal anti-inflammatory drugs may be protective in some forms of neurodegeneration. For example, people who have arthritis and take an NSAID or other anti-inflammatory drug have a much lower risk of Alzheimer's disease. Paired-twin studies yielded similar results. People with elevated uric acid, such as those with gout, have a much lower incidence of multiple sclerosis, and scientists have found similar correlations for Parkinson's disease.

Q. ***If some of these substances reduce the risk for these diseases, does that suggest that they also may be useful in slowing the progression or even reversing the disease?***

A. That's been studied intensely. ALS patients typically take massive amounts of vitamin E, coenzyme Q10, and vitamin C, but the effects are small once the disease has started. There are conflicting studies on vitamin E and Alzheimer's disease. The overall effect seems to be a small improvement in quality of life, but no effect in lengthening survival. But there are some important factors that have not been considered in the human trials. Maret Traber of LPI has shown that vitamin E is only absorbed if it's taken with food. In many studies no one tells the patients that, so they take vitamin E in the morning with coffee and absorb very little. That's one of the frustrating things that leads to so many conflicting and confusing results in the news.

Q. ***Has exposure to any environmental toxin been associated with the development of ALS or other neurodegenerative diseases?***

A. The general consensus is that there's not a strong association between any particular environmental chemical and ALS. There have been lots of studies that find suggestive clues. But ALS is rare, and it is extremely hard to do good epidemiology. The exception is the Island of Guam, where, after WWII, the incidence of ALS was enormous. It's been studied extensively, but no one has figured out the cause. We're collaborating with an investigator, Chris Shay of the University of British Columbia, who has isolated some toxic chemicals from cycad nuts that may play a role. ALS patients have very slightly elevated levels of lead. We tested this with transgenic animals and got the paradoxical result that lead was slightly protective, so we've been investigating that further. Of course, this does not imply that ALS patients should take lead, but it shows us some of the difficulties in studying this disease.

Q. ***What's wrong with the abnormal superoxide dismutase implicated in some cases of ALS?***

A. The superoxide dismutase loses its affinity for zinc, becoming toxic to motor neurons and contributing to development of peroxynitrite.

Q. ***Have you tried to restore normal superoxide dismutase in animals by supplementing them with zinc?***

A. We've approached it from two different angles. If we deplete the animals of zinc and they carry a mutant superoxide dismutase, the animals develop the disease faster. If we supplement with zinc, the animals still develop the disease, but are slightly protected. However, large amounts

of zinc caused the animals to die when they were very young. We have shown the deaths resulted from the large doses of zinc that impaired copper absorption. If copper isn't absorbed, iron cannot be inserted into hemoglobin. We have to be careful about taking zinc supplements because of this anemia. Another problem is getting zinc into the motor neuron, which is inside of the spinal cord, protected by the blood brain barrier. Zinc itself is rather toxic to neurons, so a sudden influx of zinc kills neurons in a culture dish.

Q. *Can motor neurons be regenerated?*

A. Unfortunately, the answer is still no. Adjacent motor neurons will sprout and take over some of the functions of dead motor neurons. We hope to stop the progressive nature of the death of one set of motor neurons after another, which may provide an effective treatment for the disease.

Q. *Do you think that stem cell therapy holds promise for ALS patients?*

A. Yes, but it's a long way off. I am concerned because some implanted stem cells can develop into tumors. It's still a process that needs to be studied carefully for a more complete understanding. We need to have better access to more stem cell lines to really unravel this mystery. I think our work with micronutrients will be of greater benefit much sooner.

Q. *Is there something people can do to reduce the risk of developing ALS, aside from taking vitamin E?*

A. Vitamin E is not proven to help, but it is the only compound that has ever been identified that might reduce the risk of developing ALS. I think maintaining adequate zinc levels might help, but there is no evidence for this. It is based only on our animal experiments. Only 2% of patients who develop ALS have mutations to superoxide dismutase, but members of their families who have not developed ALS may carry superoxide dismutase mutations. Low-dose zinc supplementation in those family members may be helpful. Zinc deficiency is also a chronic problem in the U.S. population — after magnesium, it's the second greatest mineral deficiency.

Q. *Are there geographic concentrations of ALS?*

A. Other than Guam and a couple of places in southern Japan, the incidence is remarkably constant around the world.

Q. *Are there specific gene mutations that have been identified in Alzheimer's or Parkinson's?*

A. A number of genes have been identified in Alzheimer's and Parkinson's. Some of them cause what is called early-onset familial autosomal dominance Alzheimer's disease. Mutations to genes called presenilin 1 and presenilin 2 account for 60% of patients with a familial origin of the disease. A lipid-carrying protein called ApoE4 predisposes people to develop Alzheimer's disease. But all these mutations account for only 5-10% of the total number of Alzheimer's patients.

Q. *Are motor neurons attacked in Parkinson's disease?*

A. No. In Parkinson's, the striatal cells in the substantia nigra are affected. Some of the neurons turn black from oxidation of the neurotransmitter, dopa, which is indicative of oxidative stress.

Q. *Is oxidative stress a common denominator in many of these pathologies?*

A. Oxidative stress plays a role. So does brain inflammation, as illustrated by the activation of astrocytes. But are these events early causal agents or tombstones — something that's happened after the die has been cast and the cells are already dead?

Q. *Have you had to develop special analytical tools to study peroxynitrite, motor neuron death, and astrocyte activation?*

A. Absolutely. Probably 95% of our effort goes into developing analytical methods. But once working, the experiment generally moves along quickly. You have to be suspicious when you get the answer that you want and perform control after control to make sure you haven't deceived yourself. I am particularly excited by the use of mass spectrometry to isolate superoxide dismutase directly from tissues. This method allows us to determine precisely whether it has copper, zinc, both, or neither bound to it. No one's been able to do that until now. We now have a

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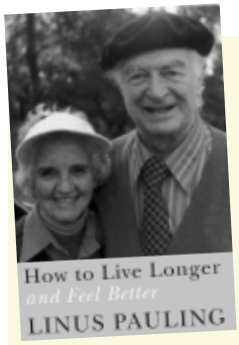

20th Anniversary Edition of *How to Live Longer and Feel Better*

How to Live Longer and Feel Better by Linus Pauling was published in 1986 and quickly became a *New York Times* bestseller. The book presented Pauling's ideas on the value of micronutrients and vitamins, especially vitamin C, in preventing and treating disease and reviewed the older, relevant clinical literature. In preparing *How to Live Longer and Feel Better*, Pauling drew from his three previous popular books for the public, *Vitamin C and the Common Cold*; *Vitamin C, the Common Cold, and the Flu*; and (with Ewan Cameron) *Cancer and Vitamin C*, and added new information and insights. *How to Live Longer and Feel Better* has been unavailable for several years.

The 20th anniversary edition features a new introduction that traces Pauling's work on hemoglobin and in molecular medicine that led to his development of the theoretical basis of orthomolecular medicine, a term he coined to refer to varying the concentration of molecules normally present in the body to attain optimum health and to prevent and treat disease. *How to Live Longer and Feel Better* also features a new afterword and annotations, as well as name and subject indices. The 360-page book will be published in May by Oregon State University Press as an oversized paperback (6 by 9 inches), available for $19.95.

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method to measure directly superoxide produced in tissues, which is exciting because it gives us insights into what's happening inside motor neurons and astrocytes. We can also use those analytical tools to understand why vitamin E works. That's not been proven yet, but the epidemiological evidence indicates that it may have important effects in the brain.

There is strong evidence that the brain is susceptible to vitamin E deficiency. For instance, horses that are deficient in vitamin E develop an ALS-like syndrome. Humans, on the other hand, develop a sensory neuropathy not usually associated with a primary motor neuropathy defect. The tools for genomics, the advances in mass spectrometry, and the technology to study how every gene in a cell is turned on or off were unimaginable 15 years ago. Now you can really investigate these processes at a level that is just truly astonishing. And yet it still comes back to a lot of the science that Linus Pauling invented when he applied quantum mechanics to chemistry. When we study the structure of superoxide dismutase and visualize it in color — all 1,200 atoms — we use the coloring scheme called CPK that Corey, Pauling, and Karplus devised many decades ago. Much of what we study was anticipated or predicted by Pauling in the 1930s and 1950s. I heard a story about a young student in a lab in Albany, New York, that was famous for developing methods using gas chromatography. The student was working at night, and there was an old man working in the corner of the lab, injecting things into the gas chromatograph. The student was really impressed with how friendly and knowledgeable the guy was. The next day he came into the lab and asked, "Who's that old guy down at the end of the bench?" "Oh, that's Linus Pauling." He had come to the lab in his 70s to learn the latest gas chromatography technology so that he could analyze metabolites in urine and plasma, a method he called orthomolecular diagnosis.

Q. What other micronutrients may be helpful in ALS?

A. We have become very interested in Tory Hagen's research on lipoic acid and acetyl-L-carnitine, two "age-essential" substances that induce genes that help you resist stress. We have seen benefits with acetyl-L-carnitine and lipoic acid in our transgenic animals. Nobody's tested these particular substances in ALS patients. Based on the evidence that Tory's gathered, we have a plausible mechanism for how they might work. Tory has observed mitochondrial dysfunction in old rats, and it's also a problem in ALS. Energy is produced in the cell's mitochondria, and Tory refers to them as the "Achilles' heel" of aging. They are fascinating organelles that also play an important role in nerve degeneration.

Q. Does peroxynitrite attack mitochondria?

A. Absolutely. Mitochondria are a key site for the generation of superoxide. Nitric oxide will diffuse into the mitochondria and combine with superoxide to form peroxynitrite, which then attacks the superoxide dismutase in the mitochondria. We're collaborating with Tory's group on this new probe that can measure superoxide. In fact, we've used it to measure superoxide in heart mitochondria from old rats. Kristine Robinson in my lab is doing most of this.

Q. Does peroxynitrite have some beneficial effects?

A. It kills pathogens and is critical for defense in the immune system. If you knock out the ability to make both superoxide and nitric oxide in a mouse, it's nearly impossible for it to survive to adulthood without dying from massive infections. However, immune cells that produce peroxynitrite almost always die as a consequence.

Q. Does peroxynitrite figure in autoimmune diseases or immunological problems?

A. It has a huge role in autoimmune diseases. In fact, immunologists have recently begun to pay attention to tyrosine nitration because if you put a nitro group on a protein, it makes it antigenic. That explains how

Recent Pilot Project Awards

The LPI Pilot Project Awards are designed to stimulate innovative research projects by Oregon State University scientists that are relevant to the mission of the Institute. These $20,000, one-year awards, made possible by financial support from LPI donors, enable investigators to obtain preliminary results that can be used to support research grant applications to federal funding agencies for more extensive projects.

Four awards were made in 2005. **Dr. Theresa Filtz**, Assistant Professor of Pharmaceutical Sciences, is studying the "Effects of hawthorn (*Crataegus*) plant extracts on cultured cardiomyocytes and endothelial cells." **Dr. Emily Ho**, LPI Principal Investigator and Assistant Professor of Nutrition and Exercise Sciences, is investigating "Zinc status and age-related macular degeneration (AMD)." **Dr. Chrissa Kioussi**, Assistant Professor of Biochemistry and Biophysics, is determining the "Roles of selenoprotein W during mammalian development and growth." **Dr. Fred Stevens**, LPI Principal Investigator and Assistant Professor of Medicinal Chemistry, conducts research on the "Enzymatic ascorbylation of lipid peroxidation products."

Three awards were made in April 2006. **Dr. Christopher Draheim**, Assistant Professor of Nutrition and Exercise Sciences, will investigate "Atherosclerosis and inflammation response in a Down syndrome mouse model." **Dr. Gayle Orner**, LPI Research Assistant Professor, will conduct studies on the "Prevention of aflatoxin-induced carcinogenesis by combinations of chlorophyllin and NovaSil." **Dr. Debbie Mustacich**, LPI Research Associate, will study "Cisplatin-induced neuropathy and vitamin E deficiency."

Please look for reports by these investigators in future LPI Research Newsletters.

antibodies can be developed against endogenous proteins that are then recognized by the immune system. I read a paper today showing that a huge number of antibodies to nitrotyrosine are found in the synovium of arthritis patients.

Q. *Does uric acid scavenge peroxynitrite?*

A. Technically, uric acid is not a scavenger of peroxynitrite. It competes for tyrosine nitration, which sounds like a subtle difference, but is biologically very important. Uric acid as an experimental tool is useful to study tyrosine nitration and to distinguish that from other actions of peroxynitrite. Uric acid seems to afford some protection in a wide range of different diseases, but the levels that you need for protection are about three- to four-fold higher than what are normally present in your plasma. In multiple sclerosis, immune cells cross the blood brain barrier, and uric acid is very effective in preventing that. But ALS involves the motor neurons and probably wouldn't be affected.

Q. *What about clinical trials with micronutrients?*

A. We're quite excited that these can be tested in patients. When we've tried to develop drugs, there's an eight- to ten-year process trying to get a drug company interested. Because of potential side effects and toxicity with drugs, the protocol development is slow and painstaking. With micronutrients, you can proceed quite rapidly into clinical trials because of their excellent safety profiles.

Q. *Do you have collaborators with whom you work closely?*

A. Yes, I work with a couple of excellent scientists in South America, particularly from Uruguay, who've been very instrumental in all of our work on ALS. They have provided most of the ideas that have driven this project forward. And they are the ones who are developing the connections between ALS, Alzheimer's, and Parkinson's disease. We also work with scientists in Zurich and all over the world. With the Internet, it's easy to share data and even look through a microscope 12,000 miles away. The world has become much smaller. LPI

Cruciferous Vegetables and Cancer Risk

Jane Higdon, Ph.D.
LPI Research Associate

What are cruciferous vegetables?

Cruciferous or *Brassica* vegetables come from plants in the family known to botanists as Cruciferae or alternately, Brassicaceae. Plants in the Cruciferae family have flowers with four equal-sized petals in the shape of a cross. "Brassica" is the latin term for cabbage. Commonly consumed cruciferous vegetables include broccoli, Brussels sprouts, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard, rutabaga, turnips, bok choy, and Chinese cabbage. Arugula, horseradish, radish, wasabi, and watercress are also cruciferous vegetables.

What's so special about cruciferous vegetables?

Like other dark green vegetables, many cruciferous vegetables are rich in folate and chlorophyll. One of the unique things about cruciferous vegetables is that they are rich sources of glucosinolates, sulfur-containing compounds that give them their pungent aromas and spicy (some say bitter) taste. Chopping or chewing cruciferous vegetables releases myrosinase, an enzyme that breaks down glucosinolates into biologically active compounds, such as indoles and isothiocyanates (see figure at right).

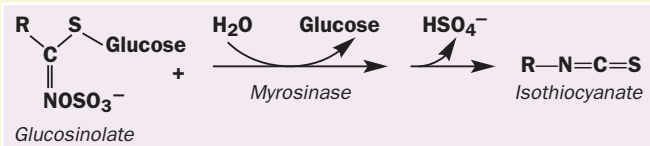
How might compounds in cruciferous vegetables help protect against cancer?

Folate plays an important role in DNA repair and the methylation of DNA, which affects the expression of cancer-related genes. Chlorophyll can form tight complexes with chemicals from tobacco smoke or charred meat (cooked-meat mutagens) that are known or suspected to cause cancer. The tight binding of chlorophyll to these potential carcinogens may interfere with their absorption and reduce the amount that reaches susceptible tissues. Glucosinolate breakdown products, which are unique to cruciferous vegetables, could help prevent cancer in several ways. Isothiocyanates and indoles may increase the activity of enzymes that play an important role in the detoxification and elimination of carcinogens. Indoles may alter the metabolism and activity of estrogens in the body, which could play a role in hormone-associated cancers, such as breast or prostate cancer. Some isothiocyanates, such as sulforaphane, can act as histone deacetylase inhibitors, which have the potential to suppress the development of cancer by turning on tumor suppressor genes in precancerous cells.

Is there any evidence that high intakes of cruciferous vegetables prevent cancer?

Cell culture and small animal studies have found some anticancer effects of substances isolated or derived from cruciferous vegetables. However, some potential risks of supplementation with these substances, such as indole-3-carbinol, have also been identified (see the article by Susan Tilton on page 12).

continued on page 8



Myrosinase released by chopping or chewing cruciferous vegetables breaks down glucosinolates to isothiocyanates, releasing glucose and sulfate in the process. "R" designates side chains of other elements in the molecule.

C=carbon, S=sulfur, N=nitrogen

Continued from page 7 — Cruciferous Vegetables and Cancer Risk

An extensive review of epidemiologic studies published prior to 1996 reported that the majority (67%) of 87 case-control studies found an inverse association between some type of cruciferous vegetable intake and cancer risk. At that time, the inverse association appeared to be most consistent for cancers of the lung and digestive tract. The results of such retrospective case-control studies are more likely to be distorted by bias in the selection of participants and dietary recall than prospective cohort studies, which collect dietary information from people years before they are diagnosed with cancer. In the past decade, results of prospective cohort studies and studies taking into account individual genetic variation suggest that the relationship between cruciferous vegetable intake and the risk of several types of cancer is more complex than previously thought.

• Breast Cancer

The results of epidemiologic studies of cruciferous vegetable intake and breast cancer risk are inconsistent. Several recent case-control studies in the U.S., Sweden, and China found that measures of cruciferous vegetable intake were significantly lower in women diagnosed with breast cancer than in cancer-free control groups, but cruciferous vegetable intake was not associated with breast cancer risk in a pooled analysis of seven large prospective cohort studies that included more than 350,000 women.

• Prostate Cancer

Four out of eight case-control studies published since 1990 found that cruciferous vegetable intake was significantly lower in men diagnosed with prostate cancer than in men in a cancer-free control group. Of the four prospective cohort studies that have examined associations between cruciferous vegetable intake and the risk of prostate cancer, none found statistically significant inverse associations overall. However, the prospective study that included the longest follow-up period and the most cases of prostate cancer found a significant inverse association between cruciferous vegetable intake and the risk of prostate cancer when the analysis was limited to men who had a prostate specific antigen (PSA) test. Since men who have PSA screening are more likely to be diagnosed with early prostate cancer, limiting the analysis in this way reduces detection bias. Presently, epidemiologic studies provide only modest support for the hypothesis that high intakes of cruciferous vegetables reduce prostate cancer risk.

• Lung Cancer

When evaluating the effect of cruciferous vegetable consumption on lung cancer risk, it is important to remember that the benefit of increasing cruciferous vegetable intake is likely to be small compared to the benefit of smoking cessation. Although a number of case-control studies found that people diagnosed with lung cancer had significantly lower intakes of cruciferous vegetables than people in cancer-free control groups, the findings of more recent prospective cohort studies have been mixed. Prospective studies of Dutch men and women, U.S. women, and Finnish men found that higher intakes of cruciferous vegetables (more than three weekly servings) were associated with significant reductions in lung cancer risk, but prospective studies of U.S. men and European men and women found no inverse association.

• Colorectal Cancer

Although a number of case-control studies conducted prior to 1990 found that people diagnosed with colorectal cancer were more likely to have lower intakes of cruciferous vegetables than people without colorectal cancer, most prospective cohort studies have not found significant inverse associations between cruciferous vegetable intake and the risk of developing colorectal cancer over time. One exception was a prospective study of Dutch men and women, which found that those with the highest intakes of cruciferous vegetables (averaging about 1/2 cup/day) were significantly less likely to develop colon cancer than those with the lowest intakes.

Why can't epidemiologic studies give us a clear answer about the cancer-protective effects of cruciferous vegetables?

Recent research suggests that genetic factors affecting the metabolism of glucosinolate breakdown products may influence the relationship between cruciferous vegetable intake and cancer risk. Glutathione S-transferases (GSTs) are a family of enzymes that metabolize a variety of compounds, including isothiocyanates from cruciferous vegetables, in a way that promotes their elimination from the body. Genetic variations that affect the activity of GSTs have been identified in humans. Specifically, individuals who inherit two copies of the GSTM1-null or GSTT1-null gene cannot produce the corresponding GST enzyme. Lower GST activity in such individuals could result in slower elimination and longer exposure to isothiocyanates after cruciferous vegetable consumption, thus enhancing cancer prevention. In support of this idea, several epidemiologic studies have found that inverse associations between isothiocyanate intake from

cruciferous vegetables and the risk of lung cancer or colon cancer were more pronounced in GSTM1-null or GSTT1-null individuals. These findings suggest that the protective effects of high intakes of cruciferous vegetables may be enhanced in individuals who eliminate potentially protective compounds, like isothiocyanates, more slowly. However, low GST activity in smokers may also slow the elimination of tobacco carcinogens. A large case-control study in the U.S. recently found that high intakes of cruciferous vegetables were associated with reduced lung cancer risk only in GSTM1-present individuals, especially current smokers. The complex effects of gene-diet interactions on cancer risk continue to be an area of active scientific research.

Is there a recommended intake for cruciferous vegetables?

Although many organizations, including the National Cancer Institute, recommend the consumption of 5-9 servings (2 1/2-4 1/2 cups) of fruits and vegetables daily, separate recommendations for cruciferous vegetables have not been established. Much remains to be learned regarding cruciferous vegetable consumption and cancer prevention, but the results of some epidemiologic studies suggest that adults should aim for at least 5 servings (2 1/2 cups) per week of cruciferous vegetables.

Does cooking alter the cancer-protective effects of cruciferous vegetables?

Glucosinolates are water-soluble compounds that may be leached into cooking water. Boiling cruciferous vegetables for 9-15 minutes results in 20-60% decreases in the total glucosinolate content of cruciferous vegetables. Cooking methods that use less water, such as steaming or microwaving, may reduce glucosinolate losses. However, some cooking practices, including boiling, steaming, and microwaving at high power (850-900 watts) may inactivate myrosinase, the enzyme responsible for the breakdown of glucosinolates into isothiocyanates and indoles. Although human intestinal bacteria can break down glucosinolates to some degree, several studies in humans have found that heat inactivation of myrosinase in cruciferous vegetables substantially decreases the bioavailability of isothiocyanates. To maximize the bioavailability of isothiocyanates and indoles, it's probably best to eat cruciferous vegetables raw or microwaved at low power.

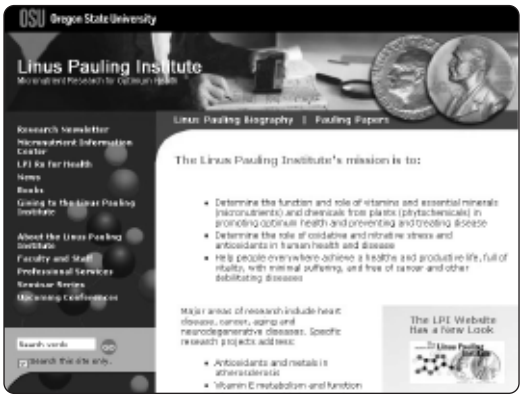
Are there cruciferous vegetables that are particularly rich sources of specific isothiocyanates or indoles?

Different cruciferous vegetables contain different profiles of glucosinolates, which result in different profiles of isothiocyanates or indoles when they are chopped or chewed (see table above). For example, broccoli is a rich source of glucoraphanin, the precursor of sulforaphane, and glucobrassicin, the precursor of indole-3-carbinol. Broccoli sprouts are concentrated sources of glucoraphanin, providing 10-100 times more by weight than mature broccoli plants. Broccoli sprouts that are certified to contain at least 73 mg of glucoraphanin (also called sulforaphane glucosinolate or SGS) per 1-oz serving are available in some health food and grocery stores. LPI

Glucosinolate (precursor)	→	Indole or Isothiocyanate	Food Sources
Glucobrassicin	→	Indole-3-carbinol	Broccoli, Brussels sprouts, cabbage, cauliflower
Glucoraphanin	→	Sulforaphane	Broccoli sprouts, broccoli, Brussels sprouts, cabbage
Gluconasturtiin	→	Phenethyl-isothiocyanate	Watercress
Glucotropaeolin	→	Benzyl-isothiocyanate	Cabbage, garden cress, Indian cress
Sinigrin	→	Allyl-isothiocyanate	Cabbage, horseradish, mustard

Some food sources of selected isothiocyanates and their glucosinolate precursors that are under investigation by cancer researchers.

New LPI Web Site Debuts



The redesigned LPI Web site was launched in April. More compelling and easier to navigate, the site has been under development for several months. Pertinent information about the LPI mission, research programs, and faculty and staff are clearly highlighted, and special sections present the Micronutrient Information Center and the LPI Research Newsletters. A biography of Linus Pauling, news releases, information on intramural seminars and the LPI biennial conference, special research services available to professionals, books for sale, and information about making gifts to LPI are also featured. The Internet address for the LPI Web site is http://lpi.oregonstate.edu.

We hope you enjoy the new site and look forward to your comments!

More News about Intravenous Vitamin C and Cancer

As reported in the Fall/Winter 2005 LPI Research Report ("Vitamin C and Cancer — Renewed Interest"), vitamin C has re-emerged as a promising substance in the adjunctive treatment of cancer, 35 years after the beginning of the productive collaboration between Drs. Ewan Cameron and Linus Pauling. Cameron gave high-dose vitamin C intravenously and orally to terminal cancer patients, many of whom reported an increased sense of well-being and lived longer than expected. Cameron and Pauling documented their observations in a series of technical papers and a book for the public, *Cancer and Vitamin C*. Recent work by Dr. Mark Levine and colleagues at the National Institutes of Health continued to demonstrate the efficacy of high concentrations of vitamin C in killing cancer cells *in vitro* and emphasized the potential value of attaining these high concentrations in the body by intravenous, rather than oral, administration.

A new study by Dr. Levine and colleagues was published in the *Canadian Medical Association Journal* in March 2006. In this study, the investigators carefully reviewed the clinical cases of three patients who responded especially favorably to high-dose intravenous vitamin C in an attempt to assess the clinical plausibility of this modality in cancer therapy. To accomplish this, the investigators followed the National Cancer Institute Best Case Series guidelines in their analysis. One case involved renal cancer with lung metastases that regressed following the intravenous administration of 65 grams of vitamin C twice per week for ten months. The second patient had invasive bladder cancer. He received 30 grams of vitamin C intravenously twice per week for three months, followed by somewhat irregular infusions for about four years. The third patient had B-cell lymphoma and received 15 grams of intravenous vitamin C twice per week for two months, followed by similar doses given less frequently. All three patients experienced complete remissions. Additionally, Dr. Drisko and colleagues at the University of Kansas reported in the *Journal of the American College of Nutrition* in 2003 two cases of women with late-stage ovarian cancer who experienced complete remissions after receiving 60 grams of intravenous vitamin C twice per week.

While not providing definitive proof that only vitamin C was responsible for these responses, the cases nonetheless suggest that the use of high-dose intravenous vitamin C should be evaluated in controlled clinical trials.

Presently, it is impossible to reliably predict which cancers and at what stage may be most sensitive to vitamin C or the magnitude of its benefit. Cameron and Pauling estimated that about 10% of patients with advanced cancer who take high-dose vitamin C would experience tumor regression or tumor death. Controlled clinical trials will help expand our knowledge in this area.

Clinical trials to test new drugs or therapies proceed in stages. A Phase I trial enrolls a small group of patients (typically 20-80) to evaluate safety, side effects, and dosage, and to gain insights into mechanism of action and efficacy. Results from these trials permit the appropriate design of Phase II trials, which involve more subjects (a few hundred) to further assess side effects and get preliminary information on effectiveness. If the results of this phase are promising, the final trial, Phase III, may be initiated. This trial enrolls more subjects (hundreds or thousands) to address efficacy and evaluate overall risks and benefits.

A Phase I clinical trial of intravenous vitamin C alone or in combination with chemotherapy is under way at McGill University in Montreal. Additionally, a small-scale Phase I trial was published by Dr. Hugh Riordan and colleagues in December 2005. In that trial, 24 late-stage cancer patients were given high-dose intravenous vitamin C for up to eight weeks to ascertain safety and side effects. Only minor adverse effects were observed, except for one patient with a prior history of kidney stones who developed one after 13 days of therapy, suggesting that high-dose intravenous vitamin C is generally safe unless there is a predisposition to kidney stone formation. LPI

INVITED ARTICLE

Vitamin C for the Treatment of Charcot-Marie-Tooth Disease

Davide Pareyson, M.D., National Neurological Institute "C. Besta", Milan, Italy

What is Charcot-Marie-Tooth Disease?

Charcot-Marie-Tooth disease (CMT) is the most common inherited neuromuscular disorder, with an estimated prevalence of 17-41 in 100,000 people. It is genetically heterogeneous, but 60-90% of the patients affected by the demyelinating variety CMT1 (accounting for about two-thirds of all CMT cases) carry a gene duplication on a specific chromosome (CMT1A). CMT1A, therefore, is the most frequent CMT subtype, with an estimated prevalence of 1 in 5,000. The affected chromosome encompasses the gene coding for the peripheral myelin protein 22 (PMP22), which is located in the compact myelin of peripheral nerves and plays a crucial role in the formation and maintenance of myelin, and possibly in cell growth regulation and shaping. Myelin is a mostly fatty substance (80% lipids and 20% proteins) that insulates nerves. Affected patients carry multiple copies of the PMP22 gene and overexpress the protein in peripheral nerves, leading to the pathological manifestation of the disease.

Current therapy

Currently, physiotherapy and surgery for skeletal deformities and tendon tightening are the only treatments for CMT, and there is no pharmacologic therapy. However, animal models of the human disease have been developed. Transgenic rats and mice overexpressing PMP22 are good models for studying disease mechanisms and for testing possible treatments.

Why vitamin C?

Recently, Passage and colleagues reported in *Nature Medicine* that chronic treatment with vitamin C is a very effective treatment for mice overexpressing PMP22. The researchers performed three sets of experiments involving physical performance and balance and observed that mice treated with vitamin C had less severe neuropathy and performed much better than untreated mice. For some clinical motor tests, such as the grip test, the performance of treated mice was even better than before treatment, indicating that vitamin C not only prevents disease progression, but also partially reverses the phenotype. Histological analyses revealed that the number and percentage of myelinated nerve fibers was much greater and that the myelin was thicker in treated mice than in untreated animals. Moreover, treated mice had a normal life span compared to untreated ones, which had a decreased life span. It is well known that vitamin C *in vitro* promotes normal myelination. The researchers performed more experiments that showed that vitamin C decreases the amount of the PMP22 protein, possibly through a cAMP-mediated mechanism. It is therefore possible that vitamin C attenuates neuropathy by decreasing PMP22 expression.

The study

The mouse study is very important because it suggests that vitamin C may be useful to treat this disease in humans. It is possible that what works in the mouse will not work as expected in humans, but since there is no effective therapy for CMT and vitamin C is very safe, a clinical trial is now appropriate. A randomized controlled trial (RCT) with vitamin C will be the first RCT for CMT. If we find that vitamin C is an effective therapy for CMT, it will be a fundamental step in the field of therapy of inherited degenerative disease.

How the trial is organized

The CMT-TRIAAL (CMT-TRial Italian with Ascorbic Acid Long term) has been recently funded by Telethon and started in March 2006. It is a randomized, double-blind, placebo-controlled trial that involves eight Italian centers and recruited over 200 patients, aged 18-70 years. These patients will be treated with either 1.5 grams per day of vitamin C or placebo and then followed for two years, which is a reasonable time period in which to observe a difference between treated and untreated patients if the effect of vitamin C is appreciable. We also considered that it is not easy to ask patients not to consume extra vitamin C. Therefore, we will check patient compliance by measuring plasma vitamin C levels. The primary outcome we will assess is the composite impairment scale CMT neuropathy score. We will also measure changes in electrophysiological parameters and in the following clinical scales: distal arm and leg strength (measured by maximum voluntary isometric contraction), 10-meter timed walking, 9-hole-peg test, Overall Neuropathy Limitations Scale, visual analogue scale for pain and fatigue, and health-related quality of life (assessed with the 36-item short-form questionnaire). Clinical-electrophysiological assessment will be performed at baseline and every six months thereafter. In a subset of patients skin biopsies will be performed to evaluate PMP22 expression at baseline and at the end of the study. We will also take plasma samples for the evaluation of antioxidant capacity if, indeed, the therapeutic activity of vitamin C in the transgenic mouse is linked to its antioxidant action. This last assay will be run by LPI-affiliate investigator Dr. Francesco Visioli.

Who has organized the study?

The Principal Investigator is Dr. Davide Pareyson of the National Neurological Institute "C. Besta" in Milan, Italy, and the trial coordinator is Dr. Alessandra Solari. This trial is the first and largest of a potential collaborative network of European (and one American) institutes and hospitals. There is the potential for the evaluation of roughly 500 patients worldwide, depending on funds.

The timetable

We have recruited the patients, and the trial is under way. It will last for two years, then we will perform statistical analyses on the data. Finally, if other centers start their own trials, we might be able to merge the data to increase statistical power. Altogether, we expect a final report in approximately three years. LPI

Benefits and Risks of Supplementation with Indole Phytochemicals

Susan C. Tilton, Ph.D.
LPI Graduate Fellow (2004-2005)

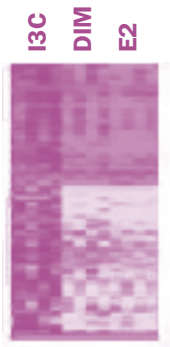
Consumption of cruciferous vegetables, such as broccoli, cauliflower, Brussels sprouts, and cabbage, has been associated with decreased risk of several types of cancer in epidemiological studies. These vegetables contain substantial amounts of sulfur-containing compounds known as glucosinolates, which break down into isothiocyanates and indoles when the vegetables are chopped or chewed. There is strong interest among researchers to evaluate the health effects of these glucosinolate-derived compounds compared to the whole vegetable. Our laboratory is particularly interested in the cancer chemoprotective effects of the indole compounds, indole-3-carbinol (I3C) and its primary acid condensation product in the stomach, 3,3'-diindolylmethane (DIM). We are also interested in determining if there are any possible toxicities associated with long-term consumption of I3C and DIM, which are both currently available over the counter as dietary supplements. Supplement companies suggest an intake of 200-1,200 mg/day, which equates to 3-17 milligrams/kilogram/day (mg/kg/day) for a 70-kg adult.

From previous studies in our lab and by other scientists, we know that I3C can inhibit the development of cancer in the trout liver and in multiple tissues of rodents, including breast, colon, stomach, lung, and liver. Protection by I3C is typically observed in animals when it is fed in the diet prior to or concurrent with exposure to the carcinogen. Thus, I3C can be considered a "blocking agent" because it blocks the initial steps required for cancer development. It likely does this by inducing enzymes responsible for the metabolism and excretion of the carcinogen. However, when I3C is fed to animals in the diet long-term *following* exposure to the carcinogen, it actually promotes or enhances the development of some cancers by an unknown mechanism. We have observed cancer promotion by I3C in the trout liver, and subsequent studies found similar promotion by I3C in rat thyroid, uterus, liver, and colon. The contradictory effects of I3C in animal studies raise questions about the safety of long-term supplementation with indole phytochemicals in humans. Therefore, we are currently investigating how I3C enhances cancer development in the rainbow trout model to better understand its potential relevance for humans.

Rainbow trout have been used as a biomedical research model for over 40 years at Oregon State University and in the Linus Pauling Institute by multiple investigators, including Drs. George Bailey, David Williams, and Rod Dashwood. Data from studies on cancer development and progression, carcinogen sensitivity, and responsiveness to dietary supplementation in trout support the use of these animals as a model for human carcinogenesis, particularly in liver cancer. We have also examined genes important for development of trout liver tumors and found they are very similar to genes expressed in liver tumors in humans and other animals.

Recent studies indicate that comparative analyses of gene profiles across diverse species are more likely to highlight functional gene interactions important in key mechanisms of human carcinogenesis. We've applied the same comparative approach to our studies of tumor enhancement by indole phytochemicals in trout.

To examine how I3C may promote liver cancer, we first looked for proteins in the liver that were induced by I3C and could be used as markers for I3C exposure. We found that I3C induces a metabolizing enzyme, CYP1A, which was expected, since we know I3C can induce certain metabolism enzymes in liver. We also observed the unexpected induction of a protein that only responds to estrogens in trout liver, suggesting that I3C may be acting like an estrogen. We know that estrogens can enhance cancer development in the liver of trout and rodents. We also know that some inducers of the CYP1A enzyme can promote liver cancer, so we continued to investigate the possibility that I3C was acting through one of these mechanisms in its ability to promote liver cancer.



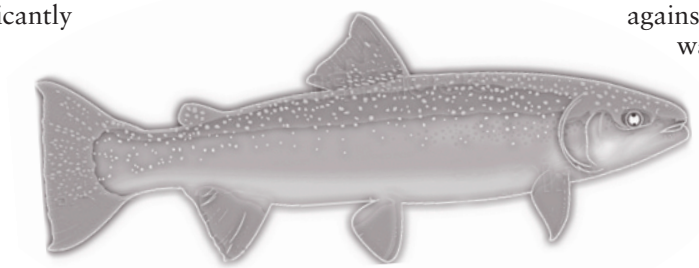
Similarity in gene expression from livers of animals fed 24 mg/kg/day I3C and DIM compared to 17β-estradiol (E2). Dark area at top represents genes up-regulated and lighter area at bottom represents genes down-regulated at least 2-fold from control animals.

Adapted from Tilton et al., Toxicol. Sci. 90:61-72 (2006)

Our next step was to take a more global approach by using microarray analysis to examine the expression of several thousand genes in the liver simultaneously after exposure to I3C rather than looking at only a few protein markers. We analyzed gene expression in trout livers after dietary exposure to I3C and DIM (24 and 78 mg/kg/day) and compared the gene expression profile to those of two known liver tumor promoters, 17β-estradiol (endogenous estrogen hormone) and β-naphthoflavone (a known inducer of CYP1A). We found that both I3C and DIM have very similar gene profiles to estradiol, suggesting that they act like estrogens in trout liver and likely promote liver cancer by this mechanism. Interestingly, DIM was a stronger estrogen than I3C. This data led us to question whether DIM would also promote liver cancer in trout similar to estradiol and I3C. Only a few studies have examined the effects of DIM on cancer development in animals, and none have examined the potential for DIM to promote cancer.

We performed a tumor study in trout to examine the effects of DIM on cancer development compared to 17β-

estradiol. Feeding the trout either DIM (24 mg/kg/day) or estradiol in the diet long-term following acute exposure to a liver carcinogen resulted in more tumors compared to control animals. Concentrations of DIM lower than 24 mg/kg/day did not significantly increase the number of tumors. We confirmed that DIM was promoting cancer by acting like an estrogen by comparing gene expression in the liver samples over the course of cancer development. At all timepoints examined, gene expression in liver samples from DIM and estradiol-treated animals were very similar, indicating they are working by the same mechanism.



Metastasis is a process in which malignant tumors spread from their primary site to other areas in the body and is associated with a decreased prognosis for treatment and higher mortality. Interestingly, the malignant tumors observed in our study may be less likely to metastasize to other organs and, therefore, may be less aggressive. Therefore, the prospect that dietary indoles may protect against metastasis is a very interesting area of future research for our laboratory. However, these potential benefits need to be weighed against the tumor enhancement that was observed in this study.

Overall, our data indicate that I3C and DIM can promote liver cancer in trout by acting as estrogens, but we don't yet know if this is relevant to other animals.

These results may help explain some of the contradictory data observed on the cancer protective and promoting effects of dietary indole phytochemicals. However, it continues to raise questions about the safety of supplementation with I3C and DIM. Research in our lab will continue to evaluate the relative risks, as well as benefits, of long-term supplementation with dietary indole phytochemicals. LPI

Oxygen Club of California Annual Meeting

The Oxygen Club of California held its annual scientific conference, co-sponsored by LPI, in Santa Barbara, California, in mid-April. *Oxidants and Antioxidants in Biology* featured 35 presentations in four sessions: "Obesity, the Metabolic Syndrome, and Oxidative Stress"; "Obesity, Uncoupling Proteins, and Micronutrient Action"; "Dietary Modulation of Cell Signaling Pathways"; and "Mitochondrial Function, Aging, and Disease."

Addressing obesity, several speakers noted that it is associated with inflammation and oxidative stress, which can be ameliorated with weight loss and decreased macronutrient intake. Additionally, fish oil (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) exerts anti-inflammatory effects that may reduce the risk of heart disease in obese individuals. Insulin resistance and inflammation are also associated with obesity in pregnant women, increasing the risk for gestational diabetes. Insulin resistance, oxidative stress, and dysfunction of the insulin-producing pancreatic beta cells may be attenuated with alpha-lipoic acid and acetyl-L-carnitine. Selenium supplementation in rats alleviated inflammation associated with liver damage by regulating manganese superoxide dismutase, an endogenous antioxidant enzyme.

Other speakers addressed how flavonoids, phytochemicals, and other dietary constituents affect cell-signaling pathways, contributing to the reduction of oxidative stress and the prevention of cancer. For example, catechins in tea may inhibit growth factors implicated in cancer. In rats, white tea and certain catechins in tea provide some protection against colon tumors induced by "cooked-meat mutagens." Resveratrol in wine and grapes and isothiocyanates in cruciferous vegetables induce apoptosis, or programmed death, in cancer cells. The omega-3 fat DHA suppressed amyloid plaque formation in the brain when fed to mice that mimic Alzheimer's disease. Selenium compounds block the cell cycle and lead to apoptosis in melanoma cells and also inhibit metastasis of injected melanoma in mice. Additionally, acetyl-L-carnitine inhibited inflammation and oxidative stress in rat astrocytes (cells in the nervous system that support neurons). Astaxanthin, an orange-colored carotenoid, ameliorates oxidative stress in the kidney cells of diabetic mice.

In the final session, scientists discussed mitochondria, the energy-producing organelles in cells that can be damaged by oxidants generated within the mitochondria themselves. Such damage can be attenuated by glutathione, an endogenous antioxidant, and glutathione peroxidase, an enzyme that inhibits lipid oxidation in mitochondria. Mitochondrial dysfunction and defects have been implicated in neurodegenerative diseases like Parkinson's. Additionally, loss of calcium regulation associated with ischemia and reperfusion results in increased oxidative stress in mitochondria, and the signaling molecule nitric oxide plays a role in mediating ischemic damage. (Ischemia refers to the partial or complete obstruction of an artery, resulting in reduced blood flow and oxygen supply to the heart and other tissues. Symptoms may include angina, pain in the arm, shortness of breath, and nausea.) LPI

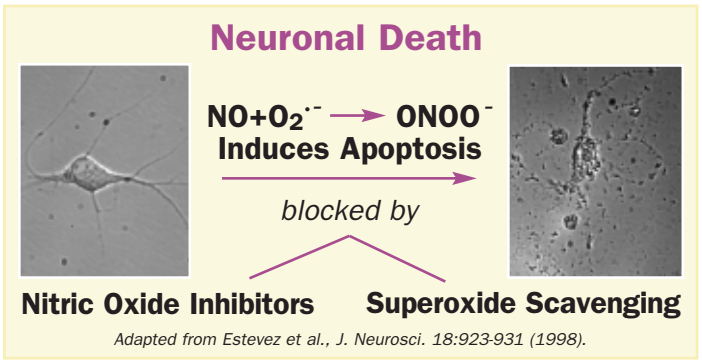
The Human Side of Research

Kristine Robinson
LPI Graduate Fellow

At the age of eighteen I began to dream of opening my own flower shop or becoming an engineer. However, the course work in business or physics required to accomplish these goals either did not interest me or was a struggle. Surprisingly, when other students groaned at their test scores in chemistry class, I sat quietly in shock when I got nearly perfect scores. I was fascinated by chemical theory and atomic structure, so I followed my aptitude and completed my bachelor's degree in chemistry. I always knew I would someday want to apply that knowledge in the medical field and, hopefully, make a significant contribution in disease research. Changing my focus from chemistry to biochemistry in graduate school was the first natural step toward accomplishing my goal. Oregon State University offered a unique opportunity to work in an atmosphere more focused on biophysics and the chemistry of biochemistry. The Department of Biochemistry is also linked to the Linus Pauling Institute, which has the attractive aspect of disease-oriented research. I was introduced to LPI Principal Investigator Dr. Joe Beckman, whose laboratory focuses on Lou Gehrig's disease. My dreams of working on disease were starting to become a reality.

Working in Dr. Beckman's lab allowed me to find a way to apply chemistry to disease by investigating the role of free radicals in neurodegeneration. Recent breakthroughs in scientific techniques have revealed the significant role free radicals play in disease progression, establishing a new frontier in free radical research — a perfect fit for my background.

The research on Lou Gehrig's disease (also called amyotrophic lateral sclerosis, or ALS) in Dr. Beckman's lab focuses on the mechanism that causes the progressive death of motor neurons. ALS is a fatal neurodegenerative disease, and the cause remains elusive, as does a cure. Dr. Beckman observed an increased production of free radicals in ALS and asked the question, "Did free radicals kill Lou Gehrig?" My research in the lab has been on two free radicals, nitric oxide (NO) and superoxide ($O_2^{\cdot -}$), and how they can combine to create a toxin, peroxynitrite ($ONOO^-$), which we believe could be responsible for killing motor neurons (see illustration below). This past year, my research has been specifically focused on detecting the peroxynitrite precursor, superoxide. Unfortunately, the most popular fluorescent moiety used to detect superoxide, dihydroethidium, is not actually specific for superoxide at all.



Adapted from Estevez et al., J. Neurosci. 18:923-931 (1998).

Publications from the mid-1980's demonstrating this lack of specificity seem to have been forgotten. I was asked to characterize the chemistry of a new derivative of dihydroethidium, which might serve as a more specific tool. To do this, I learned several techniques, such as mass spectrometry, nuclear magnetic resonance spectroscopy (NMR), culturing primary neuronal cell lines, imaging those cells by confocal microscopy, and isolating mitochondria from rat hearts to measure superoxide generation. While analyzing this new probe, I found a simple fluorometric method that provides the specific detection of superoxide. I now intend to apply this technique of superoxide detection to motor neurons in culture and test for increased superoxide generation in an ALS animal model. Moreover, I can use this method to test agents, such as metal chelators, that might inhibit the increased generation of superoxide in ALS.

These investigations have also emphasized to me the importance of collaboration in the international scientific community. I have worked with scientists from Uruguay on the isolation of motor neurons and with a scientist from England, Dr. Michael P. Murphy, on the use of electrochemical probes. Attendance at international meetings, provided for by my LPI graduate fellowship, has been a major part of facilitating these connections. Presenting research at these meetings has also helped me discover the enjoyment of communicating my research to other people. I intend to pursue a research career applying my background in chemistry to the question of free radicals in neurodegeneration. I am also excited by the prospect of teaching biochemistry.

One valuable lesson I have learned while working for Joe Beckman is the importance of research to patients. Joe does an excellent job of educating ALS patients not only by giving lectures in hospitals and elsewhere, but also by inviting patients to visit our lab. Moreover, I've watched him take time for the seemingly smaller tasks, such as making driving arrangements for patients to attend ALS meetings. Developing and maintaining contact with patients has also become a very important goal for my research career. During the past few years I was able to volunteer to help with an ALS patient. I first met "Bob" when Joe had invited him to come visit our lab. Over the period of one year, Bob's condition had deteriorated. He was able to visit our lab, but then he became immobilized at home. We volunteered to do jobs at his home that he could no longer do, like pruning trees in his orchard and hanging up a birdhouse by the bedroom window. I also remember a very sad visit with Bob's wife at his funeral — another vivid reminder of the tragedy of ALS. I was the only member of the lab at the funeral, and I felt like a representative from the group that could have potentially prevented this loss but had not yet found a way to do so.

My experience with Bob taught me that the work we do is very important to real people. Combining academic research with human affairs completes my dream of trying to make a difference by working on neurodegenerative diseases. LPI

Developments

Scott Palmer
LPI Director of Development

As I was sitting in his office listening to him speak, it occurred to me that Dr. Fred Stevens, Assistant Professor of Medicinal Chemistry at OSU and one of LPI's Principal Investigators, is a very patient man. He has to be. He was explaining how vitamin C reacts with and neutralizes the toxic byproducts of human fat metabolism and, for my benefit, he was going slowly, trying to make sure that I understood the amazingly complex chemical processes involved.

The discovery of this heretofore unknown vitamin C chemistry is a remarkable achievement by Dr. Stevens, Dr. Balz Frei, and other researchers here at the Linus Pauling Institute that sheds more light on the role that vitamin C plays in helping to prevent cancer and other chronic diseases, such as heart disease, Alzheimer's disease, and autoimmune disorders. Earlier this year Dr. Stevens received a five-year, $1.5 million grant from the National Institutes of Health (NIH), enabling him to expand his research in this area.

Despite Dr. Stevens' best efforts, I still don't completely understand the chemical reactions between vitamin C and oxidized fat. But I do know that this discovery significantly expands our understanding of the many different ways that vitamin C works in the human body.

This discovery highlights two important issues. First, there is still much we don't know about how the body uses vitamins, minerals, and other micronutrients. Second, the financial support the Institute receives from our supporters is absolutely critical in furthering our research. One year ago, Dr. Stevens received a $20,000 pilot project grant from LPI for this preliminary study.

The money for that grant was given to the Institute by people like you. The results from that preliminary study led to the $1.5 million grant from the NIH. Dr. Stevens' work was published in the *Proceedings of the National Academy of Sciences USA,* and he wrote an article for the Spring/Summer 2005 LPI Research Report.

As is evident with Dr. Stevens' work, the potential implications of the research we conduct at the Linus Pauling Institute are significant. And the research projects our Principal Investigators are working on are very exciting.

Dr. Emily Ho is studying the relationship between prostate cancer and zinc. Her studies have determined that there is a link between reduced levels of zinc and an increased risk of prostate cancer in laboratory animals. Dr. Tammy Bray, who is also the Dean of the College of Health and Human Sciences at Oregon State University, is looking at diabetes and the role that vitamin C plays in promoting wound healing. She has developed a vitamin C ointment and is using it to treat wounds in diabetic laboratory animals. Dr. David Williams is testing his hypothesis that a cause of some childhood cancers is the trans-placental transfer of certain carcinogens from the mother to her fetus and that diet and nutrition might play a role in helping to prevent the transfer of those toxins.

The scientists here at LPI are working on dozens of projects like these, and while the initial results of many of these pre-clinical research studies are promising, more work needs to be done.

That is where you come in. Unfortunately, funding from the federal government for this kind of research is becoming more restricted. The donations that we receive from you and our many supporters are critical to provide the essential resources we need to follow-up on the work mentioned above as well as to pursue research opportunities that no one else is investigating.

One of the easiest ways to provide for the Institute is to include LPI in your will or estate plan. There are numerous ways that you can help make a difference, either through a direct bequest, a charitable gift annuity, or a charitable trust. For more information about how to include LPI in your estate plan, or if you would like to know more about the research projects we are working on, please don't hesitate to contact me either by phone or email.

In the meantime, I am looking forward to my next "lesson" from Professor Stevens. Hopefully, by the time you call, I will be able to talk to you about vitamin C conjugates, oxidized lipids, and genotoxins.

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LPI

LPI is deeply grateful for the bequests we have received from the following friends since September of 2005

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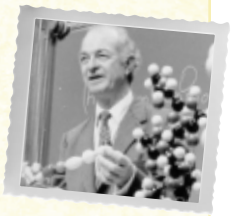
Linus Pauling Day



Ted Kulongoski, Governor of Oregon, proclaimed February 28th, Linus Pauling's birthday, "Linus Carl Pauling Day." Dr. Pauling was recognized in the Proclamation for his "efforts to bring about world peace and the banning of nuclear weapons testing" and for his "genius in chemistry [that] provided the basis for modern chemistry and set the stage for major discoveries that benefited humankind." Governor Kulongoski also noted that "Dr. Pauling was unafraid of controversy in his pursuit of the truth and devoted his entire life to the good of humanity."

Look for these informative articles inside!

Special thanks to Barbara McVicar and Tracy Oddson for editorial assistance and photographs, authors of signed articles, and Dick Willoughby for the logo photograph of Linus Pauling.

Micronutrient Research for Optimum Health

Linus Pauling Institute

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