IN VIVO
Newsletter of the University of Tennessee Division of Biology
VOLUME 2, NUMBER 4 OCTOBER - NOVEMBER 2002

From the Director

by Dr. Otto J. Schwarz

Dear Alumni and Friends:

The August –September issue of *In Vivo* marked a change in the Director's chair from the quiet, well-mannered academic persona of Dr. John Koontz to the gentleman you see pictured above. I accepted the opportunity to serve as Interim Director of the Division of Biology during mid-summer, at a time of budgetary turmoil and uncertainty.

My first day on the job, July 1, 2002, saw the unprecedented shut-down of most of the operations of all of the state supported campuses of higher learning in Tennessee. Those of you that follow such events know that the budgetary problems were addressed by the Tennessee State Legislature to the extent of allowing higher education to proceed across our state and on this campus.

I have spent a good deal of time and effort of my first month in the Director's chair exploring the academic and financial nooks and crannies of the Division. As you might suspect, the job provides for seemingly endless opportunities for problem solving and perhaps more importantly opportunities for encouragement and praise of the students, faculty and staff.

As a member of the Botany Depart-

See DIRECTOR, page 6

Table of Contents

Promising research on fat cells

There is a link between fat cells and diseases such as obesity, diabetes and hypertension. **Dr. Naïma Moustaïd-Moussa**, Associate Professor of Nutrition and member of the faculty of the Graduate School of Genome Science and Technology (GST) program, is trying to determine how fat cells contribute to the development of these diseases. She uses a variety of approaches and models in her search for answers.

One of her models is the human fat cell, which is investigated by only a few researchers because of the difficulties in obtaining and processing fat tissues. Her lab receives fat tissue from surgery centers including those at UT Medical Center. The tissue is digested with enzymes then filtered to separate and isolate fat cells, which can then be maintained metabolically active in defined conditions.

She is interested in how these cells respond to nutrients such as carbohydrates and fatty acids as well as hormones such as insulin, angiotensins and steroids. She wants to see how cells from lean versus obese or diabetic patients respond to nutritional and hormonal changes. Such studies will allow identification of genes that are activated or inhibited by specific nutrients, hormones or diseases. One of her post-doctoral students, **Dr. Sumithra Urs** has established in the laboratory methodologies for large scale analysis of genes called micro array techniques. The purpose of these studies is to identify genes that are activated during the development of fat cells and those that are differentially expressed in lean versus obese or diabetic patients. These studies are performed in part in the EM Facility and in part by the Genome Explorations Affymetrix Services at the University Health Sciences Center in Memphis.

As a form of control, she uses commercially available mouse 3T3-L1 cell lines that can be converted into fat cells under defined conditions. This model is well established and is routinely used to optimize experimental conditions before using human fat cells. Dr. Moustaïd-Moussa uses this model to understand the process of fat cell formation as well as fat cell metabolism and function and also to compare the results to her human and mouse studies.

The mouse provides another crucial model for Dr. Moustaïd-Moussa's research. "Studies performed in cells must be confirmed in the whole animal," she said. For this

See MOUSTAID, page 3

From the GST Director

by Dr. Jeff Becker

It is indeed a very exciting time for the ORNL-UT School of Genome Science and Technology (GST). We have been successful in recruiting a robust class of new students for the Class of 2002, and the program continues to evolve and grow. Below are featured two important enhancements of GST that occurred in the summer of 2002: the funding of a large *Genomes to Life* project at the Oak Ridge National Laboratory (ORNL) and innovative curricular changes.

Genomes to Life

ORNL was named on three of five Department of Energy awards for the *Genomes to Life* program and will receive about $21.7 million as part of a massive effort that promises payoffs for energy and environmental applications. GST's **Michelle Buchanan**, who will be the director of the new Genomes to Life Center for Molecular and Cellular Systems, noted that the project is an ambitious one. GST faculty members involved in these projects include **Mitch Doktycz**, **Loren Hauser**, **Bob Hettich**, **Greg Hurst**, **Steve Kennel**, **Frank Larimer**, **Mike Ramsey**, **Ying Xu**, **Dong Xu**, and **Jizhong Zhou**.

Researchers hope to identify and characterize protein complexes, the molecular machines of life. A better understanding of protein complexes and their regulation in microbial organisms could lead to advances in a number of areas, including improving our ability to clean up metals in contaminated soil. Through this effort, researchers also expect to learn more about the earth's carbon cycle and ways to produce clean energy sources.

A key partner for the three-year project is Pacific Northwest National Laboratory (PNNL). Together, ORNL and PNNL have the most comprehensive collection of analytical tools within the DOE laboratory system. These tools are necessary to better understand microbes on a molecular level. Much of the challenge in this project lies in isolating proteins from a single cell, which can be an expensive and time-consuming process. PNNL and ORNL are devising new approaches to isolate these complexes in a robust, high-throughput fashion. In addition, powerful mass spectrometry-based techniques will provide an unparalleled ability to identify and characterize these protein complexes.

In the second of the three DOE awards, ORNL is a partner with Sandia National Laboratories to develop advanced computational methods and computational infrastructure to support the biological analysis and simulation of protein complexes, their regulation and collective behavior.

In the third DOE project, ORNL is a partner with Lawrence Berkeley National Laboratory. The goal is to develop computational models to describe and predict the behavior of gene regulatory networks in microbes in response to environmental conditions found in sites contaminated with metals and radionuclides.

Curriculum Changes

One of the most exciting aspects of participating in the growth of our developing GST program is the constant change and refinement necessary to insure that the students are provided with essential knowledge and expertise to be successful upon completion of their degree. To this end, for the past three years, we have been evaluating the current curriculum and based on input from students and faculty, the curriculum committee has implemented a number of changes to the GST core curriculum. These modifications are designed to enhance student's understanding of essential subject material.

See GST DIRECTOR, page 6

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MOUSTAID, *From page 1*

purpose she uses mice engineered to overexpress genes that affect obesity and hypertension in specific tissues or mice where these genes are inactivated. Through collaboration with GST faculty member **Dr. Dabney Johnson** at ORNL, she screens the mice generated as part of the Tennessee Mouse Genome Consortium. She hopes that these unique models for human diseases will provide useful information to understand, prevent and/or treat human obesity, hypertension, metabolic disorders and aging.

Her work has primarily focused on genes until now. She wants to go beyond genomics to proteomics, studying the proteins that are secreted by fat cells. In many cases, these proteins reach the bloodstream and distal organs and modulate disease states and are therefore potential therapeutic targets in fighting these disorders. She recently initiated collaborations with GST faculty member **Dr. Robert Hettich** (see page 5) to look for specific proteins that fat cells secrete. Funding to initiate this work is provided in part by the Center for Genomics and Bioinformatics Center of Excellence at the University of Tennessee, through a collaborative program project with **Dr. Cynthia Peterson** of BCMB.

One of the hormones secreted by the fat cells, angiotensin II, has been linked to hypertension because it increases blood pressure and controls fluid and electrolyte balance. So far Dr. Moustaïd-Moussa has found an additional role for this hormone in fat cells as it binds receptors in the cell membrane of fat cells to allow the excess storage of fat. This increase of adiposity eventually leads to higher blood pressure in the mouse or human body. Her focus now is to determine if it is possible to pharmacologically block this accumulation or if change of diet will have the desired impact. She is working now to test her theory in the whole mouse before applications to humans are pursued.

As part of this research on angiotensin, she is in collaboration with **Dr. Brynn Jones** of the Functional Genomics Group at ORNL and GST Adjunct Faculty, and other colleagues in Nice and Paris. This work involves using specific mice that were engineered to lack angiotensins or to produce angiotensins only in the fat tissue. Her laboratory currently uses micro array techniques to determine changes in kidney genes caused by angiotensin production by fat cells.

She receives funding from the American Heart Association for hypertension research, the U.S. Department of Agriculture for dietary regulation of fat cell genes and proteins, and the American Diabetes Association for insulin regulation of fat cell genes, obesity and type 2 diabetes. Her work has also been supported by the UT Agriculture Experimental Station and the Physicians Medical Education and Research Foundation at the UT Medical Center in Knoxville.

On a personal note

Dr. Moustaïd-Moussa is originally from Casablanca, Morocco, but moved to France for her advanced education. She received her master's in Cell Biology and Animal Physiology and her Ph.D. in Endocrinology from the University of Paris. While in the U.S. for a conference, she made the decision to move to the States and pursue her post-doctoral training in Molecular Nutrition at Harvard School of Public Health in Boston. There she met her husband, **Dr. Hanna Moussa**, who graduated from the UT Nuclear Engineering/Health Physics Program and is currently the Director of the Radiation Safety Department at UT.

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Unraveling the secrets of misfolded proteins

It is not a perfect world, including at the molecular level. For example, after proteins are constructed at the ribosome, they have to fold into the proper shape. However, recent experiments show that up to 50 percent of these protein molecules do not fold properly and this sometimes causes problems in the body.

GST faculty member **Dr. Ronald Wetzel** spends his time trying to determine the causes and consequences of protein misfolding. His research laboratory focuses on the amyloid hypothesis of neurodegenerative diseases such as Alzheimer's and Huntington's.

Under normal circumstances, the cell responds by either tagging the defective protein for destruction or by attempting to salvage or correct these molecules. However, if the defective protein escapes the cell or misfolds after leaving the cell, it will have a tendency to aggregate further and form an amyloid fibril or other aggregate. These structures build up, causing cell death and tissue destruction. Over 30 human diseases are associated with the build-up of such aggregates.

Many proteins can make amyloid fibrils. Although these proteins differ from each other in a number of ways, the structures of their amyloid fibrils are quite similar at the electron microscopy level, and probably at the atomic level as well. Dr. Wetzel and his collaborators are working to learn what the amyloid structural motif looks like.

Not only is this of fundamental importance, it may also be a productive approach to finding cures of some of these diseases. Dr. Wetzel said, "The amyloid fibril is a rogue structure that is doing a lot of damage and we would like to think that, theoretically, there should be some generic approaches to preventing amyloid formation that might apply to many fibrils. If we can successfully target and inhibit amyloid fibril growth, this could be useful for many amyloid diseases. One therapy for many diseases - that would be the ultimate goal."

Dr. Wetzel came to Knoxville five years ago committed to continuing his Alzheimer's disease research. Since that time, he has added the study of Huntington's disease to his laboratory's portfolio. Comparative studies on both diseases in parallel may be particularly effective because of the similar protein aggregation features shared by these diseases. Both are brain diseases that are neurodegenerative and develop with age. While only five to ten percent of Alzheimer's is currently known to be primarily genetic, all Huntington's disease cases are genetic in origin. Amyloid fibrils build up outside of the neurons in Alzheimer's disease, but are found inside the neurons in Huntington's disease.

An electron micrograph of the amyloid fibrils associated with Alzheimer's Disease

Dr. Wetzel's laboratory is at the University of Tennessee Medical Center (UTMCK), neighboring the laboratories of **Dr. Alan Solomon**, another GST faculty member. Dr. Solomon works on amyloid diseases that occur predominantly outside of the nervous system, such as in the heart and kidney. Together the Wetzel and Solomon laboratories constitute one of the largest centers of amyloid disease research in the world. One of the shared interests of these laboratories is a class of antibodies, called pan-amyloid antibodies, which have the unique ability to recognize amyloid folding motifs regardless of the underlying protein. Dr. Solomon's group is working to exploit these antibodies in amyloid therapy, while Dr. Wetzel's group is focusing on how to use these antibodies to learn about amyloid structure. He is aided by his two GST students **Erica Johnson** and **Matt Sega**.

Dr. Wetzel enjoys a number of productive collaborations with other GST faculty including **Drs. Engin Serpersu, Chris Dealwis, Kelsey Cook**, **Douglass Gilman**, **Peter Zhang** , **Dong Xu,** and **Ying Xu**.

Dr. Wetzel wears many hats. Besides his work as a GST faculty member, he is also adjunct professor in both the BCMB and Chemistry Departments on the UT campus. In addition, he is on the graduate school faculty of the Comparative and Experimental Medicine Program at UTMCK, where he serves as a professor in the Department of Medicine. Dr. Wetzel also serves on the Board of Directors of the East Tennessee Chapter of the Alzheimer's Association.

His ongoing financial support is from grants from the National Institute on Aging of the NIH as well as the Hereditary Disease Foundation. Dr. Wetzel's laboratories were initially outfitted thanks to a generous gift from **Mr. Lindsay Young** of Knoxville. Dr. Wetzel will continue to follow the amyloid hypothesis in his search for cures for neurodegenerative diseases. He said, "The basic premise that protein aggregation is central to a number of disease mechanisms is sound, being supported by a wealth of research. In the end, however, perhaps the best proof of a disease mechanistic hypothesis is when an effective drug can be developed that is based on that hypothesis. If we do that, we will not only help the patients but also advance our science."

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Mass spectrometry made available to GST faculty

Dr. Robert Hettich of ORNL is part of the Bioanalytical Technologies focus area of the GST program. He is a senior research staff member in the Organic and Biological Mass Spectrometry Group of the Chemical Sciences Division. This research group develops and demonstrates technologies that are based on mass spectrometry (MS) for a wide variety of research areas, including biomolecule characterization, novel instrumentation, and Homeland Security issues, such as explosives detection and illegal drug identification.

The other staff scientists in this laboratory are group leader **Dr. Gary Van Berkel**, **Dr. Greg Hurst**, **Dr. Doug Goeringer**, and **Mr. Keiji Asano**. Dr. Hettich said, "We have a wide range of mass spectrometry equipment in our group that represents virtually all the technology available in this field." He is particularly pleased with the high-resolution, state-of-the-art Fourier Transfer Mass Spectrometer (9.4 T magnetic field) that is one of only 12 in the world. He said, "The advantages of mass spectrometry in the characterization of biomolecules like proteins and DNA is that it is an accurate, high resolution tool that provides measurements not available with conventional technology."

Dr. Hettich's current research involves two related but distinct areas of biological MS; structural characterization of isolated or purified proteins and protein identification from very complex mixtures, such as organism proteomes. For example, a research effort is underway at ORNL to characterize bacterial proteins and protein-complexes as part of the DOE *Genomes to Life* initiative. The particular bacteria being studied are *Rhodopseudomonas palustris* and *Shewanella oneidensis*.

The DOE is interested in the characterization of the complete genomes and proteomes of bacteria in the hopes of understanding the "molecular machinery of life". Such information would be useful for the fundamental understanding of how certain bacteria such as *S. oneidensis* can reduce harmful metals at toxic waste sites for bioremediation purposes.

Dr. Hettich's research interests in characterization of isolated or purified single proteins stems from a desire to develop and apply MS as a sophisticated biological tool for protein characterization at a mass accuracy and resolution level that previously has been unattainable. GST students **Joshua Sharp** and **Tomoaki Uchiki** are critical components of this research effort. Josh is developing a chemical oxidation method that, when combined with MS, affords protein surface mapping information. Tomoaki is using MS technology to study the structures and properties of an important DNA repair protein, Sml1p, in yeast.

In addition to single protein characterization, research is also underway at ORNL to develop MS approaches for identifying and characterizing the entire suite of proteins present in a bacteria cell, which is termed "proteomics." In this work he is aided by colleague Dr. Hurst and GST student **Nathan VerBerkmoes**. Dr. Hettich said that they "are using mass spectrometry as a high-throughput tool for understanding the complex mixtures of proteins and proteome in a bacteria cell." For this work, the bacteria of interest are cultured under different growth conditions, such as metal-rich versus starved, and then examined in detail to obtain a comprehensive picture of the proteins that are present in each condition. Such information provides exquisite detail about protein function.

The MS laboratory at ORNL provides this same high performance technology for collaborations with the other GST faculty in their research endeavors. **Drs. Chris Dealwis**, **Cynthia Peterson, Stephen Wilhelm, Barry Bruce, Engin Serpersu, Liz Howell,** and **Jeff Becker** all have ongoing projects where high resolution MS is key to their investigations. These collaborations are often initiated and conducted by GST students during their rotations in the MS laboratories.

Dr. Hettich did not anticipate serving as an advisor for biology graduate students. His background is in analytical chemistry, and his Ph.D. thesis work was focused on fundamental gas phase studies of metal clusters and organometallic compounds. He received his undergraduate B.S. degree at the South Dakota School of Mines and Technology and his Ph.D. from Purdue University. He went directly from graduate school to a staff position at ORNL in 1986 with the focus of bringing his knowledge of MS and its instrumentation to biological research.

Dr. Hettich is heavily involved in the GST program. Although working with the students adds extra time to his busy work week, he feels it is a good investment. He particularly enjoys the summer rotations where the GST students can put in 40 to 50 hours a week during a six-week term. This is a reasonable scenario for teaching complex instrumentation to students who may have limited or no background in MS. Many of the GST students who have rotated through the MS laboratory have chosen to incorporate MS in either a major or minor part of their thesis research project.

He said, "The GST program gives students a unique opportunity to bridge different research areas. I think that is going to make them very desirable in the workplace today. For the most part, these students are top-notch quality and have responded very favorably to the flexible design of this graduate program."

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DIRECTOR, *from page 1*

ment for some thirty plus years I was never entirely aware of the academic diversity and talent present within the Division. As of the beginning of the Fall 2002 term we have 128 full-time graduate students, of which 28 are new this Fall, as well as, 39 support staff and some 70 full-time faculty who are distributed over our support facilities, the four departments and a Graduate Program in Genome Science and Technology (GST).

Mention of GST allows a convenient segue to the subject of this issue of *In Vivo*, the ORNL-UT School of Genome Science and Technology. **Jeff Becker**, the Interim Head of the Microbiology Department and also the Director of the joint ORNL-UT GST program, speaks to some of the exciting program advances that have become part of GST as well as some progressive tweaking of the graduate curriculum.

The accompanying articles provide only a fractional sampling of the enormous scientific talent base that has been assembled under the auspices of GST. Next month I hope to be able to report on the progress of the long needed renovation of old and new Hesler Hall as well as some other interesting undertakings of Biology here at UT.

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GST DIRECTOR, *from page 2*

The current first year students will be the first group to benefit from these changes.

In the revised curriculum, the courses and subjects covered are arranged as follows: GST I Genetics/genomics including model systems analysis; GST II & III Bioinformatics and computational biology including genome and protein analysis; GST IV Analytical technologies including spectroscopy, separation technology, x-ray crystallography, NMR, and mass spectrometry.

The curriculum committee believes the revised arrangement will allow a more efficient and in depth coverage of the areas of study.

Leaving a legacy

by Dr. John Koontz

In 1980 **Dr. W.D. "Pete" Wicks** moved from a professorship in Pharmacology at the University of Colorado Health Science Center to serve as Head of the Biochemistry Department at the University of Tennessee. He was selected not only for his strong record of scholarly activity supported by national funding agencies but also because he was recognized for his willingness to make difficult decisions. He served the department in this capacity for twelve years.

He guided the department during what was the beginning of very difficult financial times at the university. His most enduring legacy as head will be the faculty whom he recruited to the department. Virtually all of these recruits have gone on to be research and/or administrative leaders at this or other universities, a record difficult for anyone to match. And he did make the difficult decisions, denying tenure to two faculty members who were probationary at the time he was hired as head.

His service to the department was far greater than that evidenced by his role as head. He had a boundless passion for knowledge. Like most of us his office was strewn with recent scientific journals. Pete had actually read articles from most of them. He conveyed this passion to his students in all of the classes he taught. His reputation as a teacher among the students and among the rest of the faculty was matched by very few of us. He was demanding and compassionate. Numerous students have commented on the impact he had on their desire to learn and their lives.

The one thing that Pete regretted in assuming greater administrative responsibilities was that it took him away from the research bench. His passion for learning was matched by a passion for trying to figure out how living systems worked, going from the molecular to the whole organism level. Even when his own research efforts were on the wane, he carried this passion with him in serving on the thesis and dissertation committees for the students of other faculty members. His faculty colleagues knew that when they advised students to ask Pete to serve on their thesis or dissertation committees, they were preparing their students for a thoughtful, insightful and rigorous examination of their research projects.

He carried this passion into all the varied aspects and interests of his life. Pete did not invest himself half way in anything. Talk to those with whom he competed in athletic endeavors. He would never admit to being so far behind that he was out of the contest, nor could he ever get too far ahead. No golf shot was ever quite as good as it might have been. He always had perfection as his goal.

He also had a passion for music. He continued to practice and play the piano and was an avid vocalist in his church choir. He became legendary among the classical music lovers in the Knoxville area. The local public radio station (WUOT) would solicit input into the name of the composer during one of its regularly scheduled evening programs. Pete probably provided the correct answer to at least half the pieces played.

Everyone who came into contact with Pete benefited from it. Few of us knew of all his varied interests and talents. I'm sure there are many like myself who wish we had taken the time to let him know how much we liked and enjoyed him. Perhaps, we are only now realizing how much that was. Pete died on July 22, 2002. All who knew him are saddened by this loss.

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Alumni News

1983 Elaine Harris Lemings, D.D.S.
Completed her bachelor's degree in Microbiology and then went on to graduate from the UT College of Dentistry in 1987. She has her own practice in Athens, Tenn.

1993 Randolph Caldwell
Graduated from Microbiology and is now a staff scientist at Creatogen AG in Augsburg, Germany. He is currently working on his doctorate degree in biology at the University of Würzburg.

1995/1998 Gregory Anderson
Received both his bachelor's and master's degrees in Microbiology. He is now a Health Scientist in the Office of the Director for the National Centers for Infectious Diseases in Atlanta. His job involves research coupled with Congressional briefings, public relations issues during a public health crisis, i.e. West Nile Virus, and serving as a grant administrator on several grants and cooperative agreements issued by the CDC. He is currently pursuing a second master's in Public Health at Emory University.

1998 Laura Haynes
Received her bachelor's degree in Ecology and Evolutionary Biology. She initially planned to enter a career as a physician's assistant, but instead has entered the world of politics. She was recently promoted to Legislative Assistant to Congressman **Bob Clement** and now lives in Washington, D.C. She researches and advises on bills related to agriculture, environment, health and education issues. She said, "Tell **Dr. Fox** that he was one of my favorites."

Remembering Gordon Carlson

by Dr. Ben Hochman, Professor Emeritus

When I joined the faculty of UT's Department of Zoology in 1963, Gordon Carlson was its head, having served in that capacity since 1947. I expected to find a department head who was a first-class scientist and a nice person as well. Gordon more than met these expectations, and I was proud to call him a friend for the remainder of his long life.

Gordon's interest in biology began in high school and continued at the University of Pennsylvania where he obtained a bachelor's degree in 1929 along with a Phi Beta Kappa key. He did some teaching at Bryn Mawr College while working toward the Ph.D. at Penn, achieving that goal in 1935. Important also during this period were the summers that Gordon spent taking courses and doing research at the Marine Biological Laboratory at Woods Hole, Mass.

The years 1935-1946 found Gordon at the University of Alabama, Tuscaloosa. He was first an instructor with a salary of $1,800 for nine months. There his teaching load included courses in general biology, anatomy, physiology, and invertebrate zoology. Despite these academic demands on his time, Gordon found time to attend faculty dances and, at one of these, he met the lovely **Elizabeth Shirley**, an instructor in Mathematics. They were married in December 1936.

The nine-month contract at Alabama did allow Gordon to concentrate on research during the summers, and he spent them at places such as Woods Hole, Harvard University's Bussey Institute and the National Institutes of Health (NIH) in Bethesda, MD. His research began to shift from descriptive to experimental cytology, especially the effects of X-rays on chromosomes and mitosis in the grasshopper, *Chortophaga viridifasciata*.

Especially important was Gordon's association with **Dr. Alexander Hollaender** at NIH. In 1946 Hollaender became the Director of the Biology Division at ORNL and a year later, in part on Hollaender's recommendation, Gordon was offered the headship of the Department of Zoology and Entomology at UT. And the rest, as they say, is history. A fruitful relationship between several science departments and ORNL developed, UT increased student enrollment from below 10,000 to over 25,000, and Zoology's staff grew accordingly.

During his tenure, Gordon was an exemplary department head. He was calm, patient and a fair-minded administrator. Throughout this period and into his retirement, Gordon continued doing research. He published over 40 papers with the final ones coming in 1988, in collaboration with **Dr. Mary Ann Handel**, and in 1989 at age 81.

Despite all his accomplishments, when I remember Gordon, it is the intelligent and kind person I recall and not just the scientist. We had many enjoyable lunches at Regas and other restaurants in Knoxville. We talked about the possible benefits of large doses of vitamin C, and Gordon attributed his lack of colds and the elimination of various allergies to this vitamin. We both laughed when Gordon told stories of his his 97-year-old aunt working on her roof or shoveling snow. I like to think of him tooling around in his Porsche or perhaps rowing his canoe off Cape Cod or on the Tennessee River.

In Vivo
An alumni newsletter published by the Division of Biology

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IN VIVO
Newsletter of the University of Tennessee Division of Biology

VOLUME 2, NUMBER 4 OCTOBER - NOVEMBER 2002

First-year GST students display the fruits of their labor

There will be a poster presentation on the second floor of Walters Life Sciences Building on the UT campus Friday, December 6, 2002 from 12:00 pm to 2:00 pm. First-year students will display the results of research conducted during their lab rotations. It is open to the public. Please contact **Gaynelle Russell** in the GST office at (865) 974-1531 or by e-mail at **russellg@utk.edu** for more details.