



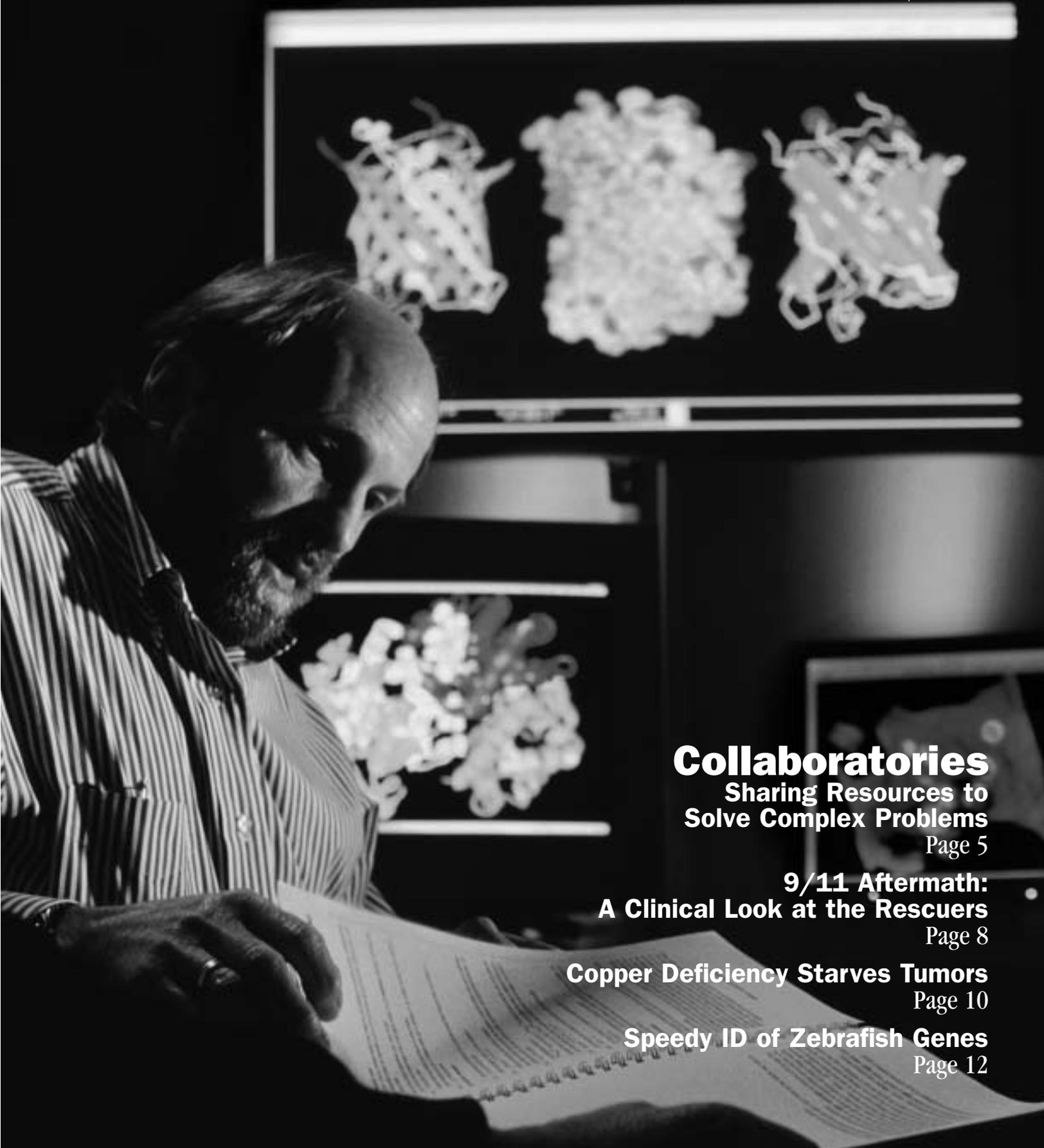
NCCR: Catalyst for Discovery

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National Center for Research Resources

NCCR Reporter

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From the Director



Forecasting Resource Needs

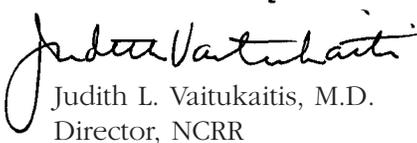
Because scientific discovery is ever changing and unpredictable, one of NCRR's greatest challenges is to forecast the future resource needs of biomedical investigators. While NCRR has long supported and will continue to support traditional infrastructure needs such as research facilities, in some instances, this forecasting requires us to consider alternative approaches to foster research. Such is the case with the virtual research environments described in "Collaboratories: Sharing Resources To Solve Complex Problems." (See page 5.)

A laboratory, a term coined in the late 1980s by combining "collaborate" and "laboratory," has been defined as a center without walls in which researchers can perform their experiments, analyze data, and interact with colleagues without regard to geographical location...in other words "working together apart." Four years ago, NCRR established several collaboratories at existing Biomedical Technology Resource Centers, primarily to provide remote access to the instrumentation and resources located at these centers. This investment has paid off—enabling research to move forward at a faster pace due to enhanced access to collaborators' expertise and state-of-the-art technologies. For instance, together with advances in automation, the collaboratory approach at synchrotrons has enabled a tenfold increase in throughput and the capability to conduct experiments remotely over the Internet.

Collaboratory support will be just one of the many resource issues NCRR will explore this year as we update our strategic plan. (See "News from NCRR," page 14.) Every five years, NCRR prepares a plan to set priorities for developing and supporting resources. To formulate the strategic plan for 2004-2008, NCRR is again soliciting input from the biomedical research community via a public notice in the *Federal Register* as well as receiving input on the NCRR web site.

Please share with us your views on those areas that will drive biomedical research in the next five years. Are there barriers to research that technology development can overcome? What resources and technologies will be critical in addressing these trends? Your comments and suggestions will be considered at an open, interactive proceeding on September 10 and 11, 2003, by an expert panel, which will include the National Advisory Research Resources Council, as well as others who are leaders in scientific disciplines related to NCRR's activities. From these proceedings, NCRR will generate an updated plan that will guide our funding priorities for the next five years.

Forecasting is a complex process. The health-related advances of tomorrow will depend on the availability of essential, shared research resources including nonhuman models, advanced technologies, and tools for exploring new diagnostics, therapies, and preventive strategies. However, NCRR has a long history of developing resources, and with input from the biomedical community, we will be positioned to provide the research tools and resources to attain better health for the nation's citizens.


Judith L. Vaitukaitis, M.D.
Director, NCRR

NCRR Reporter

This quarterly publication of the National Center for Research Resources fosters communication, collaboration, and resource sharing in areas of current interest to scientists and the public.

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Cover: At the University of
California, San Francisco,
Dr. Thomas Ferrin inspects
sophisticated 3-D representations
of protein and drug molecules that
can be viewed and interactively
manipulated from remote sites.
The project is one of seven NCRR-
funded "collaboratories." (Photo
by David Powers Photography)

Dermatitis Patients Lack Good Defense

Patients with atopic dermatitis, an inflammatory skin disease, often develop skin infections. Using the resources of the General Clinical Research Center satellite location at the National Jewish Medical and Research Center in Denver, scientists have discovered that this high infection rate may be attributable to abnormally low levels of two antimicrobial peptides in the skin. Normally produced in response to injury or inflammation, these two peptides help to fight bacterial, viral, and fungal infections.

The investigators compared levels of the two skin peptides in patients with atopic dermatitis, about 30 percent of whom have skin infections, and in patients with psoriasis, another inflammatory skin disease but one not usually associated with skin infections. Peptide levels were elevated in patients with psoriasis, reflecting the body's normal response to inflammation, while the levels were abnormally low in patients with atopic dermatitis. The scientists speculate that the low peptide levels might have resulted from altered immune mechanisms.

—*New England Journal of Medicine*
347:1151-1160, 2002.

Gene Therapy Success in Dogs

Gene therapy has been used previously in dogs to correct disorders that affect single bodily functions, such as vision or blood clotting. Now scientists at the University of Pennsylvania School of Veterinary Medicine in Philadelphia have devel-

oped a treatment that amends multiple malfunctions in dogs with an inherited metabolic disorder, marking the first successful multi-organ gene therapy in a large animal. The condition, known as Sly syndrome, is a lysosomal storage disease marked by a life-threatening buildup of carbohydrates in organs throughout the body. The underlying cause is a gene that produces a defective version of an enzyme—beta-glucuronidase—that normally helps to break down carbohydrates. The dog model for Sly syndrome, which also affects humans, was first identified at the university's National Referral



Center for Animal Models of Human Genetic Disease, funded by NCRR's Division of Comparative Medicine.

To prevent the disease in dogs with the mutant beta-glucuronidase gene, the researchers injected newborn pups with a genetically engineered virus carrying the normal gene. The virus transported the gene to the growing liver, where it helped to produce beta-glucuronidase. The normal enzyme was then secreted into the circulation and distributed throughout the body, thereby impeding

carbohydrate buildup in key organs and tissues.

—*Proceedings of the National Academy of Sciences USA* 99:13102-13107, 2002.

MRI vs. Histology in Mouse Disease

To monitor disease progression in an animal model, scientists traditionally have obtained tissue samples at different disease stages and performed histological analyses, thereby obtaining "snapshots" of the disease at various time points. A preferable approach might involve noninvasive technologies that permit monitoring of animals over an extended period.

Using magnetic resonance imaging (MRI) equipment purchased with an NCRR Shared Instrumentation Grant, scientists at Brigham and Women's Hospital and Beth Israel Deaconess Medical Center in Boston evaluated the suitability of MRI for monitoring juvenile polycystic kidney disease (PKD) in a mouse model. In PKD, multiple cysts form throughout both kidneys, increasing kidney size about fourfold but reducing kidney function. The researchers obtained two- and three-dimensional MRI images that showed marked differences between the kidneys of PKD mice and those of normal mice. In the 2-D images, tubule patterns could be seen in PKD kidneys that corresponded to the patterns observed in histological slices. The 3-D images provided greatly improved analysis of kidney volume compared to earlier 2-D methods. The investigators concluded that MRI is an efficient method for monitoring disease progress in PKD mice and is also suitable for other animal disease models.

—*Comparative Medicine* 52:433-438, 2002.

S.S.



Collaboratories

Sharing Resources to Solve Complex Problems

by Tina Adler

The aquaporin family of proteins, responsible for selective transport of life's key ingredient—water—throughout organs and tissues, was a stranger to scientists only 10 years ago. But today aquaporins are recognized as steadfast molecular sentinels, forming pore-like passages that permit rapid flow of water—and only water—across cellular membranes. Aquaporin channels are responsible for moving up to 400 pints of water a day through the human kidneys and for maintaining fluid balance in the brain, eye, and red blood cells. As selective gatekeepers, aquaporins also help to maintain the proper electrical balance on each side of the cell membrane by blocking passage of positively charged protons, which are even tinier than water

Scientists are embracing the concept of sharing data, resources, and expertise across geographic distances. The new BioCoRE software allows Robert Brunner and Dr. Gila Budescu (seated) to view and discuss a module of the muscle protein titin with collaborators at distant locations, while Dr. Klaus Schulten (standing) joins in via a workstation-driven projection system. (Photo by John Stone, University of Illinois)

molecules. Since 2000, researchers have understood how aquaporins work but not how the proteins could effectively prevent protons from slipping through the channel.

To tackle the problem, Dr. Klaus Schulten at the University of Illinois in Urbana-Champaign and his colleagues built a computer model of the complex protein in its natural setting, a cell membrane and a surrounding pool of water. Then six colleagues from across the country viewed the model remotely and analyzed molecular movements, atom-by-atom, using an NCCR-funded virtual laboratory, or “collaboratory.” The model revealed that each water molecule enters the aquaporin channel with its oxygen atom in the lead, but then the entire molecule does a 180° flip midway through the passage, allowing hydrogen to take the helm upon exit. These molecular gymnastics are performed as water molecules flow single-file through the channel, effectively blocking the passage of protons. Such detailed understanding of aquaporin function may eventually contribute to improved treatment of kidney disease and other disorders.

The aquaporin study is one of many successful projects to emerge

from NCCR's recent initiative to develop collaborative laboratories, or collaboratories, that provide a virtual link between biomedical researchers and essential resources. Dr. Schulten's Biological Collaborative Research Environment (BioCoRE) for Structural Biology is one of seven NCCR-funded collaboratories. (See “Collaboratories: At Your Service,” page 7.)

“Team science is emerging as a key contributor to biomedical discovery. But such studies often demand robust infrastructure to support rapid communication and sharing of data,” says Dr. Michael Marron, director of the NCCR Division of Biomedical Technology. “The NCCR-supported collaboratories fill this critical need. By allowing researchers to cross disciplinary and geographic boundaries, the collaboratories enable and promote collaborative science.”

NCCR established the collaboratory program in 1998, in response to the growing complexity of scientific questions facing biomedical researchers, adds NCCR Program Officer Dr. Greg Farber, who oversees the collaboratory program and is a health scientist administrator in the Division of Biomedical Technology. “The goal for the program was to

develop a set of tools that would enhance the effectiveness and efficiency of research collaborations,” says Dr. Farber.

Funded as supplemental grants to existing NCCR-supported Biomedical Technology Resource Centers, the collaboratories fall into two categories: shared instruments and shared data systems. The first provides researchers with remote access to scarce, expensive, and state-of-the-art instrumentation, like the high-voltage electron microscope at the NCCR-supported National Center for Microscopy and Imaging Research at the University of California, San Diego, or the extremely bright X-rays and crystallography tools at the Stanford Synchrotron Radiation Laboratory in California. Scientists can use these and other instruments from their home offices, without the time and expense of traveling to distant sites. “These kinds of collaboratories dramatically expand scientific access to the powerful instrumentation available at NCCR-supported resource centers,” says Dr. Farber.

The second type of collaboratory, of which BioCoRE is a prime example, provides shared data systems that enable researchers to access computational resources with greater ease than was possible previously. “BioCoRE is the America Online for biomedical researchers and trainees,” says Dr. Schulten.

BioCoRE is actually software that creates a web-based workplace for researchers, with the added bonus of allowing long-distance macromolecular modeling. BioCoRE users with supercomputer accounts can have immediate access to the National Science Foundation computing centers. The computational muscle behind the aquaporin study, for example, was provided by the Pittsburgh Supercomputing Center’s terascale computing system, the

nation’s most powerful system committed to unclassified research.

Available through BioCoRE, for example, is a visualization program, VMD, and a molecular dynamics simulation program, NAMD, which won a 2002 Gordon Bell Award for outstanding performance in parallel computing. These two software packages, developed by Dr. Schulten and colleagues, produce 3-D, animated, color-coded representations of macromolecules, which can then be

• **“Collaboratories dramatically**
• **expand scientific access to**
• **powerful instrumentation.”**
•

annotated by researchers. The programs allow scientists to simultaneously watch molecules and monitor how different forces shape and alter their function.

About one-third of the functions that users perform with BioCoRE involve VMD and NAMD, but Dr. Schulten encourages scientists to take advantage of BioCoRE’s many additional functions. BioCoRE’s other offerings for collaborators include instant messaging and an online lab book for colleagues to record their research progress. Entries are automatically sorted by user and can be viewed and searched by all project members. The program also lets users archive messages, capture output of computer programs, and create a shared file system for documents, including PDF files. The system alerts users when new views are captured, entries are added to the notebook, or collaborators log in and out. Users can access only the material for their specific project.

BioCoRE users have included more than 500 researchers at uni-

versities, research institutions, and private industry across the United States and around the world.

Collaborative projects have compared computer models of neural maps, described protein mechanisms in muscle, and examined the molecular basis of lipid metabolism in humans. Dr. Rob Phillips, a professor of applied physics and mechanical engineering at the California Institute of Technology, has used BioCoRE for his molecular biology research

since October 2000 and relies on its ability to share data and other resources with colleagues. “I think it’s fantastic,” he says. He particularly appreciates that BioCoRE keeps permanent records of correspondence with collaborators.

Although commercial software comparable to BioCoRE exists, it is not designed with biomedical researchers in mind, nor does it provide easy access to supercomputers, says Dr. Schulten. BioCoRE serves as a training tool as well. “We have given many workshops to researchers who want to learn biomolecular modeling,” says Dr. Schulten. “We are often asked to give hands-on training workshops all over the world, and BioCoRE helps us tremendously.”

As BioCoRE continues to grow, Dr. Schulten’s team made the BioCoRE server available free-of-charge to researchers via their web site (www.ks.uiuc.edu/Research/biocore/localServer). Scientists who use their own BioCoRE servers can gain faster access, and those skilled in JavaScript programming can modify and add

features to BioCoRE. In the future, Dr. Schulten and his colleagues hope to build added support for analysis methods, ensure wide deployment of BioCoRE, and even create entire BioCoRE communities.

NCRR has planned some improvements too. It is developing a second funding round for the laboratory program, and an announcement requesting proposals should be issued by spring of 2003, Dr. Farber says. The new effort will focus on providing computational support to research teams, similar to BioCoRE's offerings. The revamped program also will examine some of the social issues related to collaborations, such as who should have the first right to the data, who should get credit for the findings, how should the data be shared, and what data need to be shared. "The laboratories are a work in progress," adds Dr. Farber. "It's an exciting new frontier to explore."

BioCoRE and six additional laboratories are supported by the Division of Biomedical Technology of the National Center for Research Resources.

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Collaboratories: At Your Service

The seven NCRR-supported collaboratories offer a variety of resources and services to the biomedical community, as outlined below.

Collaborative nanoManipulator, University of North Carolina at Chapel Hill, www.cs.unc.edu/Research/nano/index.html. The nanoManipulator is a virtual reality interface to an atomic force microscope that enables remote manipulation of materials. Scientists from diverse fields, including biology, materials science, and electrical engineering, have used the nanoManipulator in their research.

Laboratories for Biomedical Research Software Development, Pittsburgh Supercomputing Center (PSC), <http://collaboratory.psc.edu>. PSC has used its collaboratory grant to make its supercomputing-class hardware, software, storage systems, and more available to researchers at remote locations. It provides video conferencing and other tools to enable remote collaborations.

Laboratory Testbed for Macromolecular Crystallography, Stanford Synchrotron Radiation Laboratory, <http://smb.slac.stanford.edu>. Stanford's synchrotron produces extremely bright X-rays that investigators can access remotely and simultaneously for analyzing everything from atomic and molecular-size objects to synthetic materials with unusual properties.

High-Resolution Biological NMR Spectroscopy, University of Wisconsin-Madison, <http://kamba.nmrfam.wisc.edu/Sesame>. Currently in great demand by the biomedical community, NMR spectroscopy provides a unique look at multiple areas of a molecule's structure. Researchers at the National Magnetic Resonance Facility at Madison codeveloped Sesame, a collaboratory that provides instrumentation and software along with training to NMR users for biological research.

Microstructure Image-Based Collaboratory, National Center for Microscopy and Imaging Research, University of California, San Diego, <http://ncmir.ucsd.edu/MIBC>. Provides remote groups of researchers with access to 3-D images of cell samples, as seen through the center's intermediate high-voltage electron microscope. Its use by researchers in Chicago in 1992, over an early version of the high-performance Internet, demonstrated for the first time the feasibility of instrument-based collaboratories.

3-D Image Visualization and Manipulation Collaboratory, University of California, San Francisco, www.cgl.ucsf.edu/Research/collaboratory. The collaboratory provides access to Chimera, an interactive molecular graphics program. Multiple users in remote locations can share a full array of data related to complex, 3-D molecular models. Using Chimera, scientists have investigated the molecular mechanisms of mutagenesis, DNA repair, and more.

BioCoRE: A Collaboratory for Structural Biology, University of Illinois, Urbana-Champaign, www.ks.uiuc.edu/Research/biocore. As described in the feature article, BioCoRE provides tools that enable multiple collaborators to share molecular visualizations over the Internet, run applications on supercomputers, coauthor papers and other documents, automatically alert team members to project changes, keep a lab book, and more.

Research Highlights

9/11 Aftermath: A Clinical Look at the Rescuers

Frightening dreams of the World Trade Center collapse awoken some New Yorkers from their sleep. But scores of city firefighters are facing their own 9/11 nightmare: a horrible cough that plagues them night and day. It came from breathing air—if you can call it that—at the site. One firefighter described it as “darker than a sealed vault and thicker than pea soup.”

The men not only inhaled it, they ingested it. Then they coughed it back up—dark sputum infiltrated with pebbles or particles, say researchers who evaluated the rescue workers. Many firefighters acquired gastrointestinal disorders along with the lung disease, a condition so pervasive and persistent that doctors dubbed it the “World Trade Center cough.” The condition first came to the attention of clinical researchers when the rescue workers sought medical care in late September 2001.

Dr. David J. Prezant, a pulmonologist with the Fire Department of New York City and the Albert Einstein College of Medicine, members of the New York University School of Medicine’s General Clinical Research Center (GCRC), and colleagues evaluated and treated the firefighters. Their study, published in the September 12, 2002, *New England Journal of Medicine*, is one of the few that describes the incidence of reactive airway disease after short-term exposure to respiratory irritants. In contrast, most occupational cases of reactive airway disease—a condition marked by constricted bronchial passages—develop only after years of long-term, low-level exposure to toxic air, as may occur in mining or construction work.



New York City firefighters breathed in a thick soup of pollutants at the World Trade Center site. Many developed a severe cough as a result. (Photo courtesy of the New York Fire Department)

Of the 10,993 New York City firefighters who survived the disaster, 10,116 were later evaluated as part of a medical monitoring program, and more than 400 underwent additional testing at the GCRC and other clinical facilities. To determine how the level of exposure to airborne debris had affected the health of rescuers, the researchers characterized firefighters as having a high level of exposure if they were at the site as the towers collapsed, a moderate level if they arrived after the collapse but within the first two days, and a low level if they arrived between the third and seventh days after the disaster.

The high-exposure group included more than 1,600 surviving firefighters. Of these, 8 percent had World Trade Center cough, defined as a respiratory condition severe enough to require medical leave for at least a month. The debilitating cough also was identified in 3 percent of the nearly 7,000 firefighters who had moderate-level exposure and in only 1 percent of the 1,320 who had low-level exposure. In sum, 332 firefighters were diagnosed with the cough six months after the collapse.

Even if they did not develop the cough, early responders were at high risk of developing another respiratory condition, known as bronchial hyperreactivity, marked by inflammation and reduced lung function. Among the cough-free cohort, nearly one-fourth of the high-exposure group suffered from the condition, as did only 8 percent of those who had moderate exposure.

“One surprising finding is that some people can develop long-term problems after only a short-term exposure to contaminants, while others seem to recover more quickly,” says Dr. Michael Weiden, the GCRC assistant program director for genomics at New York University and a Fire Department pulmonologist. Dr. Weiden and his colleagues are exploring the possibility that individuals have a genetic predisposition to lung disease. Exposure to contaminants caused inflammation either in the sinuses, leading to sinusitis, or in the lungs, causing asthma symptoms such as coughing and wheezing. The patients who developed only sinus problems were more easily treated and got back to work sooner, says Dr. Weiden. Firefighters can return to the field only when they are off their medications, which included cough suppressants with codeine, bronchodilators, decongestants, antibiotics, and anti-inflammatories.

So few firefighters used respirators in the throes of the disaster and rescue efforts, that the study could say little about their value. Less than one-fourth of the firefighters with or without the cough used the devices regularly during the first week after the collapse. The equipment the firefighters had available was sufficient, says GCRC director Dr. William N. Rom, but the condi-

tions at Ground Zero didn't lend themselves to taking protection. "The challenge is that these situations are so stressful," he says.

Ready access to GCRC resources provided critical support during the clinical investigations, Dr. Rom adds. "The GCRC supplied state-of-the-art pulmonary equipment in the form of high-resolution CT scans," he says. The scans proved surprisingly effective at revealing the inflammation and air trapping that characterize reactive airway disease.

Firefighters also underwent spirometry, which measures the volume of air exhaled as a function of time. During an asthma attack, for example, the amount of air the patient can exhale declines significantly. Firefighters with World Trade Center cough scored poorly on



Drs. William Rom (left) and Michael Weiden are studying airway hyperactivity in firefighters exposed to air pollution at the World Trade Center collapse. (Photo courtesy of New York University School of Medicine)

• *The GCRC had the infrastructure in place to respond to the immediate needs of firefighters.*

spirometry tests of forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁). Vital capacity is the maximal volume of air that a person can breathe out slowly after breathing in as much as possible. FVC is the amount of air expired with maximal force. FEV₁ is the volume of air forcefully expired during the first second after a full breath, explains Dr. Weiden.

The firefighters' FEV₁ and vital capacity were reduced in proportion to one another, which alone would have led the researchers to suspect pulmonary disorders other than reactive airway disease. But further detective work pointed them to their final diagnosis, says Dr. Weiden.

Seven months after the collapse, 48 percent of the firefighters with World Trade Center cough had returned to work, but it made a difference whether their symptoms were in the upper or lower airways. Thus 93 percent of those with predominantly upper-airway symptoms had returned to work, in contrast to only 64 percent of those with lower-airway symptoms.

The scientists are planning to perform biochemical analyses of the firefighters' serum, bronchoscopies to examine what remains in their lungs, and additional high-resolution CT scans. Another cutting-edge technology now available at the GCRC is magnetic resonance imaging that uses polarized gases as contrast agents. This technique can image a single inhalation of the gas and show its distribution in the lung, says Dr. Weiden.

GCRC researchers also are now studying residents of lower Manhattan and telephone company employees who worked in the area, to see if their exposure to the contaminated air has increased their risk of developing asthma.

Although the GCRC had the infrastructure in place to respond to the immediate needs of the firefighters, Dr. Rom says the center is working on improving its emergency readiness. Studies are under way on how to analyze blood, tissue, or lung samples "for whatever agent or condition one could envision," such as anthrax, in an emergency situation.

The evaluation and care of the firefighters who braved Ground Zero is a long-term project, Dr. Weiden notes. "This was the first of many studies of the firefighters that will utilize GCRC," he says.

—Tina Adler

This research is supported by the Division of Clinical Research of the National Center for Research Resources, the Centers for Disease Control and Prevention, the National Institute of Occupational Safety and Health, the Stony Wold-Herbert Foundation, and the American Lung Association.

For more information about the NCCR Division of Clinical Research, see www.nccr.nih.gov/clinical_rsrch.asp.

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Copper Deficiency Starves Tumors

The story of how the copper-reducing drug tetrathiomolybdate (TM) came to be considered a potential cancer treatment is a prime example of how research in one field can produce benefits in totally unrelated areas. The story begins at the turn of the 20th century, when cows and sheep grazing in pastures in New Zealand and Australia developed a strange disease, characterized by symptoms such as broken bones, diarrhea, and anemia. Eventually, researchers traced the disease to a copper

⋮ ***Combining TM with radiation therapy***
⋮ ***reduced the volume of tumors in mice***
⋮ ***more than either treatment alone.***

deficiency caused by ingestion of molybdenum, an element that was highly concentrated in the grasses in those countries. In the stomachs of the animals, molybdenum was transformed into compounds called thiomolybdates, which formed complexes with copper in the diet and prevented copper absorption from the digestive tract. The resulting copper deficiency inhibited the activity of body enzymes that depend on copper, leading to the livestock disease.

In the early 1990s, Dr. George Brewer, professor of internal medicine and human genetics at the University of Michigan in Ann Arbor, was looking for a fast-acting copper-binding compound to treat Wilson's disease. This rare genetic disorder is marked by life-threatening accumulation of copper in the body, which may lead to neurological symptoms and liver failure. He figured that TM, the most potent of the thiomolybdates, might effectively eliminate excess copper in these patients, and the NCCR-supported General Clinical Research Center (GCRC) at the university provided an ideal setting for testing this hypothesis. "I've now treated over a hundred Wilson's disease patients with TM, and it's worked beautifully," says Dr. Brewer. (For more information about Dr. Brewer's studies of Wilson's disease, see the *NCCR Reporter*, September/October 1997, pp. 8-9.)

Impressed with TM's potency and almost total lack of toxicity, Dr. Brewer began to suspect that the drug's copper-binding effects also might prove useful in the treatment of cancer. Scientists have known for more than 30 years that tumors larger than 2 millimeters need a

new blood supply to feed their rapid growth, and researchers have long sought anticancer compounds that would block blood vessel formation, or angiogenesis, around growing tumors. "The need for angiogenesis might be an Achilles' heel of cancer," says Dr. Brewer.

Dozens of angiogenesis inhibitors have been identified and more than 20 are in clinical trials. Dr. Brewer decided to add TM to the growing list because copper is a key component of many enzymes and immune system mediators that promote angiogenesis, both under normal, healthy conditions and around growing tumors. By reducing copper levels and thus the activity of angiogenic factors, TM might hinder formation of new

blood vessels, thereby choking off tumor growth.

In collaboration with oncologist Dr. Sofia Merajver and others, Dr. Brewer has administered TM to more than 40 patients with various

types of tumors that metastasize, or spread throughout the body. Patients are given six TM doses daily—three with meals and three between meals. The TM given at mealtimes prevents the absorption of copper from food, much like the thiomolybdates prevent copper absorption in livestock, while TM given between meals is absorbed into the bloodstream, where it complexes with copper



Dr. George Brewer discovered that a compound formed in the stomachs of molybdenum-poisoned livestock could shut down blood vessel formation around growing tumors. (Photo by Bob Kalmbach, University of Michigan)

and is removed from the body. Patients are monitored, in part at the University of Michigan GCRC, to ensure that the drop in copper levels does not cause anemia, the first clinical sign of copper deficiency.

Of 42 TM-treated patients, 18 have reached the point at which they can be evaluated; in these cases TM has been shown to prevent further growth of tumors for an average of 11 months. One patient with chondrosarcoma, a tumor derived from cartilage cells, had no evidence of disease progression for about four years, and a patient with breast cancer went almost three years before experiencing disease progression at one site.

Although the ongoing study does not have a control group, Dr. Brewer says that the long periods of disease stabilization observed in some TM-treated patients provide evidence of its efficacy, since extended stabilization is unusual in untreated metastatic cancer. "I expect that TM will be very effective in treating cases of metastatic cancer in which the tumors are still small," he says.

The drug may be even more effective when combined with other cancer treatments, says Dr. Brewer. In a recent study conducted by Dr. Mohamed Khan and others in Dr. Merajver's laboratory, the researchers found that combining TM with radiation therapy reduced the volume of tumors in mice more than either treatment alone. "I think that TM has great potential for use with other agents, particularly if those agents reduce tumor size," says Dr. Brewer. "Under those conditions, I think that TM will have a significant role in preventing regrowth."

TM also holds promise for preventing cancer in people who inherit genes that predispose them to the disease. In an experiment conducted by Drs. Merajver, Brewer, and their colleagues, the investigators administered either TM or water to female mice that were genetically predisposed to develop mammary gland tumors. After seven months, none of the TM-treated mice had palpable tumors, while tumors were found in half of the group of mice that had received only water. However, when TM-treated mice were taken off the therapy, measurable tumors developed within two weeks, suggesting that the mice had developed undetectable microtumors that were kept in check during TM treatment. This was confirmed when examination of mice undergoing TM therapy showed that their mammary glands contained microscopic regions of precancerous and cancerous cells that did not develop into full-blown tumors, possibly because TM restricted the growth of their blood supply.

According to Dr. Brewer, pathologic angiogenesis also appears to play a role in various noncancer diseases, such as diabetic retinopathy and rheumatoid arthritis.



Dr. Sofia Merajver and her colleagues found that tetrathiomolybdate prevented tumor growth in mice genetically engineered to develop mammary gland tumors. (Photo by D.C. Goings, BMC Media)

He has plans for clinical trials to evaluate TM treatment for these conditions, as well as certain diseases of inflammation and fibrosis, such as liver cirrhosis. Recent studies have indicated that TM can inhibit the immune system chemicals involved in these diseases as well. "It looks like TM may have many potential uses," says Dr. Brewer.

—*Steven Stocker*

This research is supported by the Division of Clinical Research of the National Center for Research Resources, the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, the Food and Drug Administration, the Department of Defense, the American Cancer Society, the Cleveland Foundation, and the Tempting Tables Organization.

For more information about the NCCR Division of Clinical Research, see www.nccr.nih.gov/clinical_rsrch.asp.

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••• Critical Resources

Speedy ID of Zebrafish Genes

Zebrafish embryos have long been a favored animal model for studying vertebrate development. The fish's fast reproduction time and transparent early-stage embryos allow scientists to quickly and easily assess details and variations in the initial phases of life. To identify the specific genes involved in development, researchers have traditionally used chemicals to induce mutations in male zebrafish and then looked for deformities in the developing offspring. But this method typically produces mutations that are only single-point changes in DNA, making it difficult and time-consuming to identify the mutated gene. Identification of just one gene can easily take one to several years.

••• *This technique has shortened the time for identifying mutated genes from years to days.*

To simplify the process, NCRR-funded researchers developed and then applied a faster method, known as insertional mutagenesis, for identifying genetic mutations in the zebrafish. This technique, which uses a virus to induce mutations in the fish embryos, has shortened the time for identifying mutated genes from years to days,

says Dr. Nancy Hopkins, Amgen professor of biology at the Massachusetts Institute of Technology in Cambridge.

Over the past decade, traditional chemical mutagenesis has allowed zebrafish researchers worldwide to isolate only about 70 of the 2,400 zebrafish genes believed to be necessary for normal embryonic development. But in just the past few years, Dr. Hopkins' lab has used insertional mutagenesis to identify and clone more than 250 of the genes. Along the way, her team may help to identify genes involved in human development and disease, since many of the zebrafish genes have human counterparts.

"Our goal is to find and clone the genes that are needed for a fertilized zebrafish egg to develop into a free-swimming embryo and larva," Dr. Hopkins says.

"This includes all the genes that are essential to make functioning vertebrate organs, including the heart, liver, kidney, and brain." Considering that there are probably at least 30,000 genes in the zebrafish genome, identifying the 2,400 that are involved in development is no small feat.

That is where insertional mutagenesis can be of benefit. The technique uses a genetically engineered mouse retrovirus to induce mutations and also serve as a molecular tag, or beacon, making the mutated gene easy to locate. "Instead of using chemicals that damage DNA, insertional mutagenesis uses small pieces of viral DNA that are readily recognizable and insert themselves



By adapting a new technique for inducing mutations, Dr. Nancy Hopkins' lab has been able to identify more than 250 zebrafish genes that are necessary for normal embryonic development.
(Photo by Donna Coveney, Massachusetts Institute of Technology)

at random into the zebrafish genome,” Dr. Hopkins explains. When the inserted pieces of DNA disrupt a developmental gene, the resulting fish embryos often are deformed. Scientists can then search the fish DNA for the viral genes and subsequently sequence the DNA on either side of these genes to obtain a partial sequence of the mutated zebrafish gene. This method of identifying mutated genes “can take as little as a few days to one or two weeks,” says Dr. Hopkins. “If the genome sequence of an organism is available, the cloning time is even shorter.”

Insertional mutagenesis had been used previously in the fruit fly *Drosophila* and in other invertebrates, so the basic groundwork already existed. But Dr. Hopkins’ team was the first to apply the technique to a vertebrate, and the method had to be specifically adapted for zebrafish. “We found that we could use mouse retroviral vectors as mutagens for this purpose,” she says. “The particular viruses we use were first developed for use in human gene therapy. I think no one could have foreseen their use in the zebrafish.”

• Knowledge of zebrafish genes • may lead to insights into human • development and disease.

Dr. Hopkins credits NCRR for making the project possible. It was initially rejected for funding by other organizations several years earlier because the zebrafish was then still a relatively new animal model, and technology development is notoriously unpredictable. “It took vision and risk-taking to back this project,” says Dr. Hopkins. “Fortunately for me and others, NCRR saw early on the huge potential of the fish system and decided to support it.”

Dr. Hopkins expects that knowledge of zebrafish genes may lead to insights into human development and disease. “There is a gold mine of future discoveries here, with huge implications for understanding human health,” she says. “Every gene we have isolated has a close human homologue, since fish and humans are both vertebrates.” Dr. Hopkins notes that her research team has already isolated several genes in the fish that are also mutated in human disease. For example, researchers in her laboratory have identified mutant fish with kidney defects that result from mutations in about a dozen different genes. “Among these, two genes are already known to cause kidney defects in humans,” she says.

“This suggests that others may also prove to be the basis for human diseases for which genes have not yet been identified.”

In the future, Dr. Hopkins and her colleagues plan to use their mutant lines of zebrafish to explore the mechanisms by which genetic mutations cause developmental defects. Researchers in her laboratory are examining genes involved in the development of the kidney, brain, jaw, and liver. One investigator is looking at genes that underlie certain inherited pediatric disorders. Dr. Hopkins and her colleagues also plan to make their mutant lines of zebrafish available to other researchers, so that further phenotype defects in the mutant lines can be identified, and these valuable tools can be widely studied.

Dr. Hopkins says it is uncertain when all 2,400 development genes will finally be pinpointed and sequenced. “Many people still do not seem to understand the critical importance of many of these genes,” she says. “Some genes get labeled ‘boring,’ for example, and no one wants to work on them. Other genes become ‘stars’ and are much sought after. But as our work and that of many other laboratories is showing, there probably are very few boring genes.”

Several zebrafish laboratories around the world are working to decode the entire fish genome, and this in turn could benefit Dr. Hopkins’ project. “A crude genome sequence is now available for the zebrafish, and this has already speeded up our cloning efforts,” she says. “When it is finished, it should make the cloning about a one- to few-day-long process for most mutants.”

—Steve Mitchell

This research is supported by the Division of Comparative Medicine of the National Center for Research Resources and by Amgen.

For more information about the NCRR Division of Comparative Medicine, see www.ncrr.nih.gov/comparative_med.asp.

Additional Reading

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NCRR Seeks Input for New Strategic Plan

Every five years, NCRR develops a strategic plan to anticipate the research resource needs of the NIH-supported biomedical community. To prepare the Strategic Plan for 2004–2008, NCRR is asking scientists around the country to identify emerging scientific trends and make recommendations concerning research resources and technologies that will be needed in the future. The information provided by researchers will then serve as a framework for discussions at a scientific planning forum on September 10 and 11, 2003, in which distinguished members of the biomedical research community generate recommendations for the new strategic plan.

In addition to providing input about emerging trends and future needs, researchers are asked to recommend strategies for eliminating barriers to progress and enhancing access to research resources and technologies. Also, they may recommend individuals to serve as panel members for the September planning forum. *The Federal Register*, dated January 29, 2003, contains all of NCRR's questions related to updating the plan.

Those interested in participating may submit recommendations on or before May 15, 2003, at NCRR's web site: www.ncrr.nih.gov/sprecommend.asp. Those without web access may send recommendations to: Office of Science Policy and Public Liaison, NCRR/NIH, One Rockledge Centre, 6705 Rockledge Drive, MSC 7965, Suite 5046, Bethesda, MD 20892-7965. NCRR's strategic plan for 1998-2003 may be accessed at www.ncrr.nih.gov/about_ncrr/plan98.asp.

Greenwood Award Winners Named



An NCRR associate director and a scientist researching preterm birth were the winners of the first Greenwood Awards, which are presented biennially to a scientist in recognition of research excellence involving minority health issues and/or a research administrator for long-time meritorious service to

minority institutions. The awards are named in honor of the late Dr. Frederick C. Greenwood, who did much to increase the ranks of minority scientists and promote research on health issues that affect ethnic minorities.

The Greenwood Award for Service Excellence was presented to Dr. Sidney McNairy, Jr., associate director of NCRR's Division of Research Infrastructure (DRI). Dr. McNairy has been on the staff of NCRR since 1975. In 1985, he was named director of the RCMI Program, which was launched in that year to enhance research capacity and infrastructure at minority institutions nationwide. Dr. McNairy, associate director of DRI since 1994, gave the closing address to the more than 450 symposium participants.

The Greenwood Award for Research Excellence was presented to Dr. Greenwood's widow, Dr. Gillian Bryant-Greenwood, a molecular endocrinologist at the University of Hawaii at Manoa. Dr. Bryant-Greenwood researches hormones and other factors involved in premature birth, which disproportionately affects women in lower economic groups. (For more information on Dr. Bryant-Greenwood's research, see the *NCRR Reporter*, Winter 1999, pp. 10-11.)

The awards were presented at the Eighth Research Centers in Minority Institutions (RCMI) International Symposium on Health Disparities, held in Honolulu in December 2002.

Wisconsin Primate Research Center Opens New Wing

The Wisconsin National Primate Research Center at the University of Wisconsin-Madison has formally opened its new 43,000-square-foot research wing, which was funded in part by facilities improvement grants totaling nearly \$5 million from NCRR's Division of Research Infrastructure. Included in the new wing are housing space for 440 monkeys, surgical suites, and histology and pathology testing facilities. The wing also has centralized equipment for washing monkey cages, a new card-based security system, and a ventilation system that allows for constant monitoring of room temperature and humidity. In addition, the rooms for housing the monkeys and the AIDS laboratory are built to prevent the spread of microbes to the outside environment. The lobby is open to the public and contains a 36-foot-long mural and live marmosets in a glass enclosure. In addition, the facility offers a live videocast of marmosets, which is popular with elementary school children.

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NCRR Resources Aid New IOM Members

Among the 65 new members elected to the Institute of Medicine (IOM) in October are 15, listed below, who have depended on NCRR-supported resources for their research. New members are selected based on their outstanding contributions to health, medicine, and related fields. As part of the National Academies in Washington, D.C., the IOM provides advice concerning health and science policy to government, corporations, professional organizations, and the public.

Dr. Salvatore DiMauro, professor of neurology at Columbia University in New York City, studies a group of muscle diseases that involve abnormal mitochondria function. In this research, Dr. DiMauro depends on General Clinical Research Center (GCRC) resources and a whole-body magnetic resonance system purchased with partial funding from an NCRR Shared Instrumentation Grant (SIG).

Dr. Stanley Fahn, professor of neurology at Columbia University, investigates movement disorders, particularly Parkinson's disease and dystonic disorders. With the assistance of the Columbia University GCRC, he studies the natural history of these conditions and the effects of drugs and other treatments.

Dr. Caroline Hall, professor of pediatrics and medicine at the University of Rochester in New York, utilizes the resources of her university's GCRC in studies of infectious agents—particularly respiratory syncytial virus, human herpesvirus 6, and parainfluenza viruses—in children and adults.

Dr. Bertil Hille, professor of physiology and biophysics at the University of Washington in Seattle, investigates cellular ion channels, which influence the electrical properties of cells. With assistance from an NCRR-supported computer resource, Dr. Hille used drugs and toxins to characterize ion channel function in skeletal muscle and neuroblastoma cells.

Dr. Stanley Korsmeyer, professor of pathology at Harvard Medical School in Boston, studies the processes involved in programmed cell death in mammals. In this work, Dr. Korsmeyer uses the NCRR-supported microscopy resource at the New York State Department of Health in Albany and instruments purchased with SIG funding.

Dr. Richard Lifton, professor of genetics and medicine at Yale University in New Haven, Connecticut, researches genetic disorders that cause hypertension, osteoporosis, and other conditions. His studies have drawn on the resources of the Yale University adult and pediatric GCRCs and the GCRC at Brigham and Women's Hospital in Boston.

Dr. James Lupski, professor of molecular and human genetics and pediatrics at Baylor College of Medicine in Houston, utilizes Baylor's pediatric GCRC in his studies of disorders caused by DNA rearrangements.

Dr. Karen Matthews, professor of psychiatry, epidemiology, and psychology at the University of Pittsburgh, studies how stress and other psychological factors contribute to the development of heart disease, infertility, and other disorders. In this research, Dr. Matthews has used GCRCs at the University of Pittsburgh and the Harbor-UCLA Medical Center in Los Angeles.

Dr. Charles Nemeroff, professor of psychiatry at Emory University in Atlanta, is assisted by the Emory GCRC in his research on the neuroendocrine stress system, the relationships between increased heart disease and depression, and antidepressant treatment of cancer patients.

Dr. Erkki Ruoslahti, professor in the Cancer Center of the Burnham Institute in San Diego, investigates the role of cell adhesion in tumor metastasis. In this research, Dr. Ruoslahti has used the protein crystallography resource at the University of California, San Diego, and an electron microscope purchased with SIG funding.

Dr. Debra Schwinn, professor of anesthesiology, surgery, pharmacology, and cancer biology at Duke University, studies the molecular and cellular mechanisms that underlie heart disease and urogenital disorders. Dr. Schwinn uses the resources of the Duke University GCRC and the tissue and organ resource at the National Disease Research Interchange in Philadelphia.

Dr. Ralph Steinman, professor and senior physician at Rockefeller University in New York City, researches immune cell responses in cancer, HIV infection, and other diseases. He is currently using the resources of the Rockefeller GCRC to develop and test cancer vaccines and is studying the spread of immunodeficiency viruses in macaque immune cells provided by the Tulane National Primate Research Center.

Dr. John Trojanowski, professor of pathology and laboratory medicine at the University of Pennsylvania in Philadelphia, uses his university's GCRC in studies of biomarkers and genotypes associated with neurodegenerative diseases. Additional investigations of embryonic tissue transplants and stem cells have been assisted by the pediatric GCRC at the University of Colorado in Denver and the Wisconsin National Primate Research Center in Madison.

Dr. David Valle, professor of pediatrics at Johns Hopkins University, has long utilized the GCRC in his studies of bone development disorders, inborn errors of metabolism, and therapies for a hereditary eye disease called gyrate atrophy.

Dr. R. Sanders Williams, professor of medicine and principal investigator of the GCRC at Duke University, has studied the effects of exercise on red blood cell and heart function.

(continued from page 15)

National Primate Research Centers (NPRCs) are a network of eight specialized facilities for nonhuman primate research that are primarily funded by NCR's Division of Comparative Medicine. Besides maintaining more than 20,000 nonhuman primates, NPRCs also provide nonhuman primate cells, tissues, organs, and biological fluids for biomedical research purposes.



Synchrotron X-Ray Researcher Wins Nuclear Energy Award

Among the winners of the 2002 E. O. Lawrence Award is Dr. Keith Hodgson, professor of chemistry

at Stanford University in California and director of the Stanford Synchrotron Radiation Laboratory, which is

partially funded by NCR. Presented annually by the U.S. Department of Energy, the Lawrence Awards recognize contributions to nuclear energy research and are named in honor of Dr. Ernest Orlando Lawrence, Nobel Prize-winning physicist who invented a type of particle accelerator called the cyclotron.

Dr. Hodgson's award, in the chemistry category, cited his development of methods that use synchrotron X-rays to study the structure and function of molecules. In the 1970s, Dr. Hodgson showed that the brilliant X-rays generated at synchrotron facilities could be used to determine the structure of macromolecular crystals more rapidly than conventional X-rays. Later, he developed a technique that uses multiple wavelengths of synchrotron radiation, rather than just one wavelength, to determine the structure of a crystal. Dr. Hodgson also pioneered new methods for using synchrotron X-rays to study biomolecules in solution, rather than in a crystalline state, and for studying active sites in metalloproteins. Dr. Hodgson has played a major role in advocating and promoting the development of synchrotron radiation research for chemistry and biology in both the United States and abroad.

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