FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ VOLUME 3, ISSUE 5 ■ SEPTEMBER-OCTOBER 1995

TENURE PANEL GETS FIRST-YEAR REPORT CARD

by Celia Hooper

NIH's new Central Tenure Committee recently completed its first year of life—and the first data on tenure decisions are in, along with mixed reactions from the scientists that the group evaluates and serves.

"We haven't lived with the new tenuring system long enough to see if it produces a better product" than the old system, says Mark Boguski, a NLM investigator recently tenured by the

Central Tenure Committee (CTC). "The real test will be not just whether the system is fair at the front end but whether at the back end it produces a scientist with a better post-tenure track record than before."

Actually, the idea for the committee was hatched for rather different reasons in the 1980s, under NIH Director James Wyngaarden, says CTC's executive secretary, Richard G. Wyatt. The goal of forming the new tenure committee, according to Wyatt, was to bring the expertise of senior scientists into major decisions at NIH. As is the case at universities, tenure at NIH accords a scientist permanence and independent responsibility for labora-

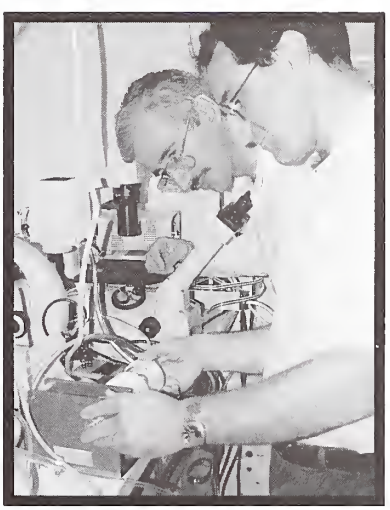
continued on page 15.

INTERSECTING ORBITS: NIH AND NASA'S JOINT EXPLORATIONS

by Rebecca Kolberg

Outer space may remain far beyond NIH's scientific reach for quite some time, but some intramural researchers are taking advantage of the next best thing—a chance to explore the earth-bound applications of technology designed for the final frontier.

"The nice thing about working with NIH is that the rest of the scientific community looks to NIH for guidance, and if useful results are reported by NIH researchers, the technology starts to spread," says Stephen Davison, a biotech program manager with NASA's Microgravity Science and Applications Division in Washington.



Lorna Hearfley

NICHD's Joshua Zimmerberg, foreground, and Leonid Margolis assemble a NASA bioreactor.

NIDCD Director James B. Snow Jr., who was appointed NIH's representative to NASA's Life Sciences and Microgravity Advisory Committee last spring, agrees that NIH and NASA make compatible partners in many areas of biomedical research. "Research on Earth could benefit from the application or transfer of technologies specifically developed for space-related purposes, and research in space or space-like environments could improve knowledge of the normal function of human biologic systems on Earth," Snow told this year's American Institute of Aeronautics and Astronautics' Life Sciences and Space Medicine Conference in Houston.

Currently, two NIH labs—one at NICHD and one at NEI—are weighing the potential applications of NASA-developed technologies in basic biomedical research. Not only do the two projects tackle quite different scientific problems, but they also diverge in the nature of the devices being tested and in the formality of their arrangements with the federal space agency. NICHD's evaluation of NASA's bioreactor for three-dimensional tissue culture is a five-year, $4.8 million interagency agreement. On the other hand, NEI and NASA scientists' fledgling collaboration to ex-

continued on page 18.

CONTENTS

Safety and Security at NIH

Michael Gottesman

The recent contamination of a water cooler in Building 37 with phosphorus-32 has raised important issues about how we protect NIH staff. Although we are a diverse community, spanning multiple campuses and a wide range of professions, we share a desire to minimize threats to our safety and security. The tough question is, how can we create a safe, secure work environment without destroying the open intellectual atmosphere essential to biomedical research?

In my view, there are two general categories of safety and security risks. First, there are risks such as fires and chemical spills that pose an immediate danger to the health and well-being of NIH staff. There is little controversy about the importance of minimizing or eliminating such risks. The second set of risks such as recombinant DNA activities and exposure to low-level radioactivity—risks that do not constitute an immediate threat to health or safety—is more problematic.

In the first category, the danger of exposures to fire, toxic chemicals, pathogenic organisms, and high-voltage equipment is a fact of life in the modern laboratory. Fortunately, scrupulous use of appropriate safety equipment and precautions, proper training, and maintenance of clear corridors can greatly reduce the chance of lab accidents and facilitate emergency response when accidents do occur.

A different sort of concern in the first category is the threat of criminal acts such as theft, personal assaults, and violent action by malicious individuals or groups. Although rare, such events do occur at NIH. To guard against crime, NIH police patrol the campus and we all take precautions such as locking unoccupied labs, limiting access to NIH buildings after normal working hours, and controlling access to buildings that house nonhuman primates. Importantly, any steps to tighten security are taken only after the risks are weighed against the possibility that tougher security measures will interfere with normal research activities. Currently, there is no plan to lock all NIH buildings during working hours because the need for such action has not been shown to outweigh the high cost of hiring enough security guards to provide "true" security and because it would significantly interfere with the normal flow of people and research materials. Our best defense against crime is for everyone to be vigilant, for example, by questioning strangers about the nature of their business in NIH buildings and reporting suspicious or criminal activity to police immediately.

The second category of potential risks at NIH includes factors that do not appear to pose an immediate danger but that, over a period of time, may result in a statistically detectable hazard. In some cases, the long-term health risks are unknown or indeterminable, but a reasonable person might perceive such a risk, or there might be public concern about the possibility of such risk. Activities that fall into this category are experiments involving recombinant DNA research on nonpathogenic organisms and gene products and the use of low-level radioisotopes. Many researchers question rules and regulations in these risk areas that they think serve no useful purpose.

However, recent events underscore the importance of observing *all* guidelines and regulations for this risk category. You may ask, what does it matter to an individual scientist if a few thousand counts of hydrogen-3, phosphorus-32 (P-32), or carbon-14 are left contaminating a lab bench, or if a researcher wants to mouth-pipet *Escherichia coli* carrying a recombinant plasmid encoding human cDNA sequences? First, such activities represent bad laboratory practice, which could lead to sloppy handling of more hazardous materials or organisms. Second, perception and acceptance of risk is very personal. Because we work in a crowded environment, one person's carelessness invariably results in the exposure of others, and it is inappropriate for one researcher to decide whether others should be exposed to questionable material—no matter how small the risk. Finally, deliberate or careless violation of rules and regulations regarding "low-level" risk subjects all of NIH to the possibility of public censure and harsh regulatory sanctions that could make it far more difficult to conduct our daily work.

The recent, apparently deliberate, P-32 contamination of a water cooler and perhaps of a scientist's food or drink in Building 37 illustrates some of these points. Although the exposures should not pose a health risk to any of the staff involved, the attendant negative publicity led to questions about our security and handling of radioisotopes and demonstrates the potential price of problems in this arena. I cannot overemphasize the emotional distress experienced by the affected individuals, the exacerbation of mistrust within our local community, and the intensity of demands that NIH "do something" to prevent such an event from recurring.

Last year, NIH suggested to the Nuclear Regulatory Commission (NRC) that security governing the storage and use of certain radioisotopes, including P-32, be relaxed to facilitate their use within labs. When the P-32 contamination occurred on June 28, NIH was undergoing an NRC inspection to determine, at least in part, the effectiveness of our security arrangements for radioisotopes and whether our request for less stringent restrictions should be granted. Although our request was based in good faith on known risks, it did not take into account the dramatic nature of any contamination with radioactive materials and the emotional reaction to such contamination. I believe that our decision to withdraw that request, to strictly enforce current security regulations, and to search scrupulously for other possible contamination—with only negative results so far—has been a reasonable response to the P-32 case. In fact, the NRC recently gave us high marks for the overall quality of our radiation-safety program.

Although we may wish to govern ourselves by safe and appropriate research practices, the reality is that NIH is governed by oversight bodies such as the NRC, the Occupational Safety and Health Administration, and the Environmental Protection Agency. One of my responsibilities is to enforce safety regulations, but another is to explain to regulators the special circumstances that affect NIH research activities. The good working relationship that we have with regulatory agencies today is due in no small part to the reputation that NIH has developed as an institution that is responsive to public concerns. I hope that everyone will work with me to maintain and foster this good reputation.

Michael Gottesman
Deputy Director for Intramural Research

CATALYTIC REACTIONS

Below are comments we received for topics raised in the July–August issue, along with some general reactions.

On ethics column on "Authorship and Ownership"

Thank you for writing a clear article on authorship and ownership. It was the first time that I can recall hearing about the *Guidelines for the Conduct of Research in the Intramural Research Program*. Is this available in the NIH Library, or do I need to look elsewhere?

—Bill Bennett, NCI

I'll be happy to send you a copy of the research conduct guidelines. Other intramural researchers who are interested in obtaining a copy of the guidelines should contact the Office of Intramural Affairs (phone: 496-3561).

—Joan P. Schwartz, NINDS

On a "burning issue" at NIH

Federal regulations forbid smoking inside NIH buildings. Smoking is, however, permitted on the NIH campus, and employees taking a "smoke break" tend to cluster around the entrances of the buildings, thereby exposing everyone who enters and leaves the building to second-hand smoke. Smoking around the Clinical Center entrances was prohibited as of July 1, and there is further discussion about whether the no-smoking zones should be extended to include all entrances to all buildings on campus. The direct benefit to all NIH employees and visitors is reduced exposure to second-hand smoke. The cost for smokers is some inconvenience, personal responsibility to minimize littering, and exposure to the elements in inclement weather.

Discussion of further smoking restrictions on the NIH campus leads to a more general policy question. Should NIH, a renowned center for health-related medical research, be a permissive partner with smokers? Or should NIH enforce a strict interpretation of the federal policy to provide a smoke-free, drug-free work environment and seek to reduce and eventually eliminate smoking on campus? The state of Maryland and the American Medical Association have taken activist roles to reduce smoking, particularly in public places, and FDA is currently examining the classification of tobacco as a drug-delivery system. NIH could also establish an active leadership role by reducing exposure of employees to toxic materials (e.g., tobacco smoke) and by helping its employees eliminate habits, such as smoking, that are linked to debilitating disease. NIH could achieve these goals in a manner that does not infringe on personal choice or privacy, perhaps, for example, by establishing programs to help interested people break these habits, and also by offering reduced health- and life-insurance rates for nonsmokers.

—Gerry Dienel, NIMH

On the National Institutes of Dent

Dent is a genius. The toiling, the drudgery, the cynicism—our world, captured in detail. Dare I say that his cartoon strip is the best thing in *The Catalyst?* ■

—Daniel Fierer, NIAID

Calendar Convenience

It's happened again. You've misplaced your Yellow Sheet, and that interesting seminar you planned on attending this afternoon turns out to have taken place yesterday. Maybe it's finally time to enter the computer age and sign up for an electronic subscription to NIH's weekly Calendar of Events. To receive the calendar via e-mail, send an e-mail message to listserv@list.nih.gov with the message "SUBSCRIBE CALENDAR Your Name". ■

Safety and Security at a Glance

If you have questions about course work on laboratory safety or other concerns about the safe use of chemicals or biological materials in the lab, contact Deborah Wilson at the Occupational Safety and Health Branch (phone: 496-2960). For nonemergency questions about fire hazards or regulations, contact the NIH Fire Department (phone: 496-2372) or John McCabe with Fire Prevention (phone: 496-0487). For nonemergency questions about crime risks or other security issues, contact Patrick Coajou with the NIH Police (phone: 496-5685). ■

Catalyst *Mailing List*

We are working toward improving our distribution system for *The NIH Catalyst*. Over the past year, many labs and offices have moved to other locations at NIH, and we are doing our best to keep the mailing list up-to-date. If you have recently moved and want to remain on our mailing list, let us know. Intramural researchers arriving at or leaving NIH should also contact us to be added to or deleted from the mailing list (phone: 496-0450; fax: 402-4303; e-mail: catalyst@od1em1.od.nih.gov). ■

Correction

The Interinstitute Interest Group Directory, pages 12–13 of the July–August issue, contained an incorrect e-mail address for Janet Yancey-Wrona, who is the contact for the Nucleic Acid Biochemistry Interest Group. Yancey-Wrona's correct e-mail address is janety@bdg10.niddk.nih.gov ■

New Ethics Panel Seeks Scientists' Input

by Joan P. Schwartz, Ph.D., NINDS

In an effort to encourage NIH researchers to adhere to high ethical standards and to ensure that allegations of scientific misconduct are handled impartially and expeditiously, the Office of Intramural Research (OIR) has established an NIH Committee on Scientific Conduct and Ethics, which is composed of scientists from most of the institutes, centers, and divisions. The OIR feels strongly that this committee can make an important contribution to the NIH scientific community.

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As chairman of this committee, I welcome all suggestions from NIH staff for topics and issues you would like to see addressed by our panel. The committee, which held its first meeting on Sept. 14, has three basic charges:

- to develop and refine guidelines for the conduct of research, including procedures to protect both whistle-blowers and scientists accused of scientific misconduct, to develop a model for binding arbitration, and to determine areas in which additional guidelines may be needed (e.g., mentorship),
- to develop effective mechanisms for ethics training in the NIH scientific community—including this ethics column, and
- to develop mechanisms to deal rapidly and fairly with allegations of scientific misconduct and with disputes related to authorship, sharing of data and reagents, mentoring, supervision, and other conflicts in the scientific workplace.

NIH Committee on Scientific Conduct and Ethics

Chair
Joan Schwartz, NINDS

Members
Richard Asofsky, NIAID
Bruce Baum, NIDR
Peter Blumberg, NCI
Jane Cheng, NCI
Sue Cheng, NINDS
Ted Colburn, NIAAA
Robert Desimone, NIMH
Andrew Dwyer, CC
Victor Ferrans, NHLBI
James Fozard, NIA
David Gorelick, NIDA
Christine Grady, NINR
Betty Graham, NCHGR
Victoria Hampshire, NCRR
Christy Ludlow, NIDCD
Ron Mason, NIEHS
Ralph Nossal, DCRT
John O'Shea, NIAMS
Alan Schechter, NIDDK
John Wilbur, NLM
Peggy Zelenka, NEI ■

Wednesday Afternoon Lectures: Back by Popular Demand

The popular Wednesday Afternoon Lecture Series returned for a second season on Sept. 13 with a presentation by famed epidemiologist Charles Hennekens of Harvard Medical School in Boston. And the rest of the 1995–96 lineup promises to rival the inaugural series, which attracted some of the world's most fascinating researchers to the Bethesda campus.

The series was started last year in an effort to improve attendance at NIH's top lectures by scheduling the talks in an easily accessible location and in a standard time block that scientists can set aside on their calendars. As in the 1994–95 series, most of this season's lectures will again be held on Wednesdays from 3 to 4 p.m. in Masur Auditorium, Bldg. 10. The Office of Education grants continuing medical education (CME) credits to lecture attendees. For more information on the lecture series, contact Hilda Madine of the Clinical Center's Office of Special Events (phone: 594-5595).

In a new development, all lecture hosts will try to set aside some of the speakers' time to meet with interested students, fellows, and postdocs. Young scientists who want to participate in these meetings, please contact the head of the host group as soon as possible to reserve a spot. Interest-group contacts are listed in the July–August issue of *The NIH Catalyst*. ■

Oct. 18 **Peter Kim**
Whitehead Institute for Biomedical Research, Cambridge, Mass.
"Design of Proteins and Drugs"
Host: NIGMS

Oct. 25 **Carlos Bustamante**
University of Oregon, Eugene
"Imaging Protein-Nucleic Acid Complexes with the Scanning Force Microscope"
Host: Structural Biology Interest Group

Nov. 1 **Thomas Kunkel**
NIEHS
"DNA Replication Fidelity, Mismatch Repair, and Genome Stability"
NIH Mider Lecture (OD)

Nov. 8. **Christopher Walsh**
Dana Farber Cancer Institute, Boston
"Molecular Mechanisms for Bacterial Resistance to the Antibiotic Vancomycin"
Host: Molecular Biology Interest Group

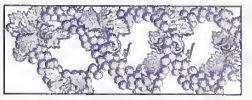
Nov. 15 **Elaine Fuchs**
University of Chicago
"Of Mice and Men: Cytoskeleton and Disease"
Host: Cell Biology Interest Group

Nov. 20 **Phillip Sharp**
Massachusetts Institue of Technology, Cambridge
"RNA Splicing and Biology"
Hosts: NIAID and the NIH Fellows Committee

Nov. 22 **John Robbins**
NICHD
"Something Old and Something New; Something Borrowed and Some Things Yet to Do"
NIH Dyer Lecture (OD)

Nov. 29 **Richard Anderson**
University of Texas Southwestern Medical Center, Dallas
"Compartmentalization of Signal Transduction in Caveolae"
Hosts: Cell Biology and Signal Transduction Interest Groups

RESEARCH GRAPEVINE



We welcome your contributions to this new feature, which is intended to provide the intramural research community with the latest news from scientific meetings in a wide range of fields. For information on submitting a brief update on a meeting that you have attended, contact The Catalyst *(phone: 402-1449; fax: 402-4303; e-mail: catalyst@od1em1.od.nih.gov).*

American Association for Clinical Chemistry

Molecular diagnostics, automation, and point-of-care testing were the themes of the American Association for Clinical Chemistry's annual meeting July 16–20 in Anaheim, Calif. In a fitting tribute to the theme of molecular diagnostics, NCHGR Director Francis Collins received the AACC's National Lectureship Award and delivered a superb talk entitled "The Human Genome Project and the Future of Medicine."

On the clinical front, James Cook of the University of Kansas Medical Center in Kansas City discussed the application of the transferrin-receptor assay for assessing a patient's iron status. Traditionally, the serum-transferrin-receptor assay has been used to gauge erythropoiesis. But Cook reported that this test may also be used to distinguish iron-deficiency anemia from the anemia produced by chronic disease. The assay found that iron-deficiency-anemia patients had serum transferrin-receptor levels that were three times higher than normal, while patients with chronic-disease anemia had normal levels of transferrin receptors. These results indicate that the serum transferrin-receptor assay may be a valuable substitute for bone-marrow examination, which has been the standard method of distinguishing between these two types of anemia.

The role of homocysteine in coronary artery disease was the focus of a presentation by Robert Jacob of USDA's Western Human Nutrition Research Center in San Francisco. Jacob provided an update on recent studies linking high concentrations of the amino acid homocysteine in the blood to an elevated risk of coronary artery disease by increasing thrombogenic tendencies. He noted that the prevalence of artery narrowing among patients in the Framingham Heart Study was found to correlate directly with serum homocysteine levels, with patients who had the highest homocysteine levels being twice as likely to have advanced arteriosclerosis as those with average levels. Other studies indicate that excessive levels of homocysteine may disrupt the anti-coagulation process, thereby predisposing individuals to thrombotic heart disease. In addition, Jacob says there's experimental evidence that B vitamins, particularly folic acid, may help to lower homocysteine levels. In addition to their implications for the treatment and prevention of thrombotic heart disease, the homocysteine findings help to underscore the often overlooked fact that coronary artery disease is a multifactorial disorder and is not solely determined by cholesterol concentrations. ■

–Ronald Elin, CC

THE INTEREST GROUP GAZETTE

NIH's family of interinstitute interest groups has grown considerably over the past year, and more new members continue to be added to the fold. Here are some details about four of the most recent arrivals, three of which are in the early, planning stages that rely heavily on the involvement of rank-and-file scientists.

Since it was established at the beginning of this year, the **Pigment Cell Research Interest Group** has attracted about 50 active members. The group, which serves as a forum for scientists from a wide variety of disciplines interested in the study of pigment cells, holds informal and interactive seminars on the third Monday of each month from 3:00 to 4:30 p.m. in Bldg. 37, Rm. 6B23. Interests include the development, growth, differentiation, and function of melanocytes, as well as what causes some melanocytes to be transformed and grow into primary malignant melanoma tumors and, eventually, to metastasize. Typically, group members make the presentations, but occasionally, outside speakers are invited to participate. The goal of the group is to foster synergistic interactions among researchers who have complementary interests and/or expertise. Members are informed by fax and/or e-mail of all official group activities and other useful news. For more information, contact Vincent Hearing (phone: 496-1564; fax: 402-8787; e-mail: hearingv@dc37a.nci.nih.gov).

The **Lymphoma and Leukemia Interest Group**, organized by Ivan Horak of NCI's Metabolism Branch, held its first meeting in September and plans to meet from 2 to 3 p.m. on the second Monday of each month in the Bunim Conference Room, 9S-235, Bldg. 10. This group is devoted to the biology and therapy of lymphoma and leukemia. The Nov. 13 meeting will feature a presentation by NCI's Jonathan Ashwell on the regulation of normal and pathological apoptosis of T cells. Organizers plan to notify members of group activities via the NIH Calendar of Events and e-mail. For more information, contact Horak (phone: 594-1127; fax: 402-3647; e-mail: idhorak@helix.nih.gov). Also in the formative stage, the **Carcinogenesis Interest Group** intends to hold its first informal meeting in November. The group's purpose is to discuss and understand the process of carcinogenesis, as well as its causes and mechanisms, its clinical and epidemiological manifestations, and its prevention. Umberto Saffiotti, the group's organizer from NCI's Laboratory of Experimental Pathology, plans to have three to four meetings per year at NIH's Bethesda campus and, possibly, some at NIEHS at Research Triangle Park, N.C. For more information, contact Saffiotti (phone: 496-2818; fax: 402-1829; e-mail: saffiotu@dce41.nci.nih.gov).

The first organizational meeting of the **Virology Research Interest Group** will be held on Nov. 9 from 2 to 3 p.m. in Bldg. 4, Rm. 433/437. All NIH scientists in the field of virology are invited to attend. The planning of future meetings and seminars will take place during the first meeting, and there will also be a discussion of additional activities aimed at enhancing the scientific and social interactions among virologists within and around NIH. Researchers who are unable to attend but would like to convey their interests and ideas should contact Bernie Moss of NIAID's Laboratory of Viral Diseases (fax: 480-1147; e-mail: bernard_moss@nih.gov). ■

–Lorna Heartley

Rousing the Sleeping Leviathan, NCI's New Leader Gets Down to Business

What's huge, innately magnificent, and could use a strong shove to get back on course? The man on the street might answer, "A beached whale." Around NIH, the response might very well be, "NCI." In an interview with *The NIH Catalyst* shortly after being sworn in as NCI's new director, Richard Klausner made it clear that his leadership team will waste no time in flexing its collective muscle to push NCI's once proud intramural research program off the shoals and into exciting new waters of scientific discovery. For starters, the 43-year-old cell biologist, who has spent most of his past 16 years at NICHD, has already streamlined NCI's division structure and established an advisory board of intramural scientists. He's also injecting some new blood into the scientific community with the recruitment of a noted molecular epidemiologist, Alfred Knudson of the Fox Chase Cancer Center in Philadelphia, and two world-class yeast geneticists from Seattle, Leland Hartwell of the University of Washington and Steve Friend of the Fred Hutchinson Cancer Research Center.

What was your perception of NCI before you came here?

Klausner: I saw it as a somewhat troubled institute that had a very top-down leadership style. It tended to be an institution that ran by fiat and fear—fear of problems, fear of crises. It's no secret that it's not been a place where people have uniformly loved to work … . That does not mean that there haven't been programs and individuals who have thrived and done well. My own feeling is that it is an intramural program that does not have the feel of the type of the intramural program that I want to be associated with—but I think that it will.

Given your accomplishments as a bench researcher and the current administrative turmoil at NCI, why did you decide to take the job as institute director?

Klausner: I really decided to do it both out of a sense of the challenge that it represented and, frankly, out of a sense of responsibility as a member of the community … . I felt if asked, I would be willing to serve. I think Harold [Varmus] has provided a fantastic model to scientists for the importance of service and the essential perspective that scientists bring to scientific leadership.

What do you think is the toughest task currently confronting NIH?

Klausner: There's no question that the toughest task is the challenge of these incredible diseases [cancers] that present such a daunting problem. Cancer will be the number one killer of Americans by the end of this century. … We need to find ways [both to] maintain the spectacular progress in basic science [but], as importantly, to somehow bridge the huge gap between this spectacular progress and the very poor progress that we have made in the cure and prevention of most cancers over the past 25 to 30 years.

What do you see as the greatest strengths and weaknesses of NCI's intramural program?

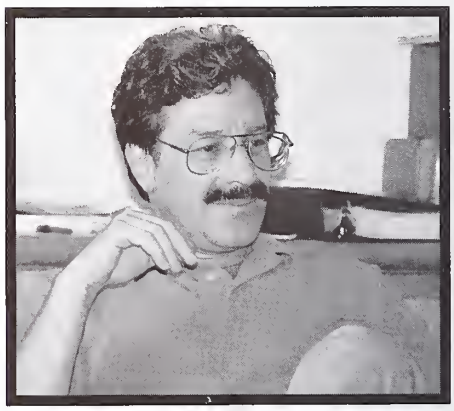
Klausner: I think the greatest strength of NCI's intramural program is the greatest strength of the NIH intramural program, that is, the available resources and the institutional opportunities to be a real community of scholars, the freedom to conceptualize research programs with very few constraints … . Such freedom places upon us a compelling demand to respond by making sure … that this is not only a great place to be, but that this is a place where great science is done.

I think it is an institution that needs a variety of both structural and cultural changes so that it functions as a true meritocracy and that it has real mechanisms [in place] to select for and reward excellence. It needs a culture in which the independent development of the careers of people can thrive. And it must become an institution where all aspects of its administrative function are structured to serve the scientist and not the other way around.

What do you see as the most fruitful and interesting avenues for basic and clinical cancer research at the institute right now?

Klausner: The major areas that I think the NCI needs to think about strengthening relate to cancer genetics and to the many fundamental aspects of the biology of the cell that we now know are directly related to cancer, including genetic and genomic instability, the relationship between genetic instability and the cell cycle, and the relationship between genetic instability and the fundamental decision between life and death that cells are capable of making. … I expect that we will be developing a very vigorous and active cancer genetics program in terms of basic, clinical, and epidemiological studies. I think the possibility of really integrating clinical, basic and population-based studies here provides many exciting opportunities. And this is reflected in the new division structure, which basically divides the intramural program into three areas based upon the three fundamental mechanisms of approaching the acquisition of knowledge and information—basic, clinical, and population-based.

I also see great opportunities in immunology. That's one of the areas in which NCI has always been strong. I want to continue to see that supported and enhanced. … I hope we can help stimulate a real renaissance in the development of a



modern tumor immunology in much the same way that there's been a renaissance in the immunology of autoimmune disease.

What steps do you plan to take to enhance the interactions between basic and clinical science?

Klausner: It is abundantly clear from the exciting advances in human cancer biology over the past few years that many of them have come out of the ability of the basic science to inform us about the disease, *and,* importantly, of the disease to inform the basic science. … For example, we are going to want to develop molecular pathology, molecular diagnostics, and cancer genetics and integrate such development among these three divisions—basic, clinical and population-based. The

by Celia Hooper and Rebecca Kolberg

three division directors, George Vande Woude, Philip Pizzo, and Joseph Fraumeni, are deep in conversation about how we actually set up working structures so that the level of communication within this institute skyrockets. And there's room for skyrocketing.

The Bishop-Calabresi report made dozens of recommendations on ways of improving NCI's intramural program, and we'd like to get your reactions on a few of them. How do you feel about the recommendation to reduce the current percentage of the budget devoted to intramural research?

Klausner: I don't know what the exact percentage is. It's actually not a number that's easy to get at ... . While I don't know exactly the right number, I do actually think that it's probably too high ... and we will be developing a plan that, over time, brings that percentage down.

What do you think about the suggestion to establish an open grants competition?

Klausner: Well, in the "Klausner" report [issued in 1992 by the Task Force on the Intramural Research Program, which was headed by Klausner], we talked about the NIH creating special fellowships that people would compete for. I really like that idea.

What about appointing lab and branch chiefs and scientific directors for renewable five-year terms?

Klausner: I see pros and cons of that. I'm perfectly happy if in a lab there's a group of people who would like to rotate being lab chief. But the reality is that we have certain expectations of lab chiefs, and those expectations will be reviewed. And if a person is doing spectacularly as a lab chief and wants to remain as lab chief, I don't really see how enforcing retirement helps anyone.

What does the future hold for NCI's Frederick Cancer Research Center?

Klausner: We are actively discussing Frederick. What I can say is that although there have been concerns about closing Frederick down, I see the Frederick campus as a fantastic resource for the NIH and the NCI. I think it would be very short-sighted to close it down, so we will not be doing that.

Will NCI be doing less AIDS research?

Klausner: I have no commitment to doing less AIDS [research], but I think we will be calling less things AIDS. ... We are looking at the programs very carefully to

Lorna Heartley

make sure that we are accurately describing the research we do ... . But as to whether the cancer institute does AIDS research [or other] noncancer research, one of the lessons of the past 20 years is the hubris of deciding what exactly is cancer research and what isn't.

What about drug-development activities at NCI?

Klausner: We will be reviewing the Developmental Therapeutics Program. I want the NCI to remain committed to developmental therapeutics, but I think it's a good time to look at that program. However, I'm not singling out that program. Under this new administration, we are going to have real reviews—conceptual reviews, not critique reviews pointing out what's wrong—of all of the NCI's programs, not just developmental therapeutics.

How can intramural researchers help you shape the "new" NCI?

Klausner: Part of the new governance structure is something called an IAB, an intramural advisory board that Claude Klee will chair. This will have about 15 members, intramural scientists from all the intramural divisions and people at all different levels from tenure track to lab chief. This body will be very, very important in reviewing the functioning of the intramural program on an ongoing basis. The first thing they are going to need to do is review the rules, the regulations, the administrative processes, and the communications pathways. This body will provide a filter mechanism so that decisions about the functions of the intramural program will be discussed by active intramural scientists. ... At the same time this committee will set its own agenda to interact with NCI leadership and those responsible for the administrative structures in order to constantly look at how we can improve things and to address the ongoing and changing needs of the scientists. ... This will be a very well-publicized, very accessible group. It will serve as a line of communication between all intramural scientists and the division and institute leadership, without worrying about going through chains of command.

What impact will the changes in NCI's intramural program have on intramural research at other institutes?

Klausner: We are very interested in developing things that I believe will be of real interest to all intramural programs, such as a good, user-friendly information-management system, so that we can, to the fullest extent possible, delegate authorities to the laboratories and out of separate administrative branches.

Now that we've spent a lot of time discussing NCI's future, what about the future of your own scientific career?

Klausner: I'm going to keep my lab. I love being an intramural scientist at NICHD ... and I hope they will continue to love having me there. I'm absolutely committed to maintaining my lab and maintaining myself as an active scientist. ■

Scientists, Start Your Engines! Finding Research Information on the Internet

Among the reasons that the research community has so enthusiastically embraced the Internet is the access it provides to vast repositories of scientific information and to a wealth of databases for scientific analysis. That's all fine and good, but how, in the Net's ocean of information, can an individual scientist quickly locate those sites that will be of greatest use in his or her own research?

One way to find fruitful sites is to wander around the Internet, simply using your mouse or keyboard to roam through "tunnels" on Gopher servers or to surf though the "links" between sites on the World Wide Web (WWW). Although serendipitous searching may uncover some wonderful resources, most scientists prefer a more efficient mode of exploration. "Search engines"—computer resources that can be accessed free of charge through any WWW browsing program [see box, page 9]—are what you need if you really want to soup up your research performance. These engines enable you to search for any word or combination of words in the text of a wide range of Internet sites. After the words selected for a search are entered, a list of sites will appear. Some picking and choosing might be necessary at this point. As in any computer search, if the terms are too broad, you may get a huge—and thus probably useless—list of sites. Alternatively, if you make your terms too specific, you might wind up with a "list" with nothing on it. Try to use only a couple of relatively distinctive, but not too arcane, terms to design a search of appropriate scope.

You may find one engine superior to another for your purposes.

You may also find one engine superior to another for your purposes. For example, two major search engines, InfoSeek and Lycos, return some information about the site other than its name, while another, WebCrawler, just returns a list of names. Also, the criteria resulting in rankings may vary from search engine to search engine. Finally, some Internet sites may be part of one engine's index and not another's.

As a recent "Hot Methods Clinic" helps to illustrate, the ability to smoothly navigate the World Wide Web is among the most useful computing skills that a scientist can have [see March-April issue, page 12]. To provide an idea of how search results vary depending upon the engine chosen, I conducted a simple "experiment." Using the term "PCR," I performed a search on each of the three of the most-used search engines, InfoSeek, Lycos, and WebCrawler. The results follow. Note the difference in the amount of detail each engine provides about each site, as well as the fact that although they were all given the same search word, the engines ranked some of the sites in different order. In addition, some engines returned more "hits" than others, reflecting both the incidence of such sites in the engine's index and the method used to determine what information is present at a particular site.

InfoSeek™

A list of 10 sites was returned, and the top five are listed below.

1) PCR Primer: A Laboratory Manual
Edited by Carl Dieffenbach, National Institute of Allergy and Infectious Diseases, Gabriela Dveksler, Uniformed Services University of the Health Sciences. From its first-published account in 1985, the polymerase chain reaction has become a ... http://www.cshl.org/books/pcr_primer.html (3K)

2) PCR Methods & Applications
A New Interdisciplinary Journal of Research, Methods, Reviews, and Comment. Scientists have seized vigorously on the power and flexibility of the polymerase chain reaction (PCR), and this enthusiasm is generating a host of PCR-based and other ... http://www.cshl.org/journals/pcr/ (9K)

3) PCR Reference Information
PCR (Polymerase Chain Reaction) Reference Information. What this is: This reference information is intended to provide the reader with general information regarding the process known as PCR, or the Polymerase Chain Reaction, and ... http://www.promega.com/pcrref/pcrref.html (3K)

4) A Decade of PCR
Cold Spring Harbor Laboratory and The Perkin-Elmer Corporation celebrate 10 years of amplification with a videotape library in which Nobel prize winners Kary Mullis and James Watson and 19 other distinguished scientists review the applications ... http://www.cshl.org/books/decade.html (3K)

5) MGD: PCR Primers Query Form
[MGI | User Support | Documentation | MGD | Citations | Markers | Probes |PCR | Homology | Mapping | Mapping Tools | Other Resources]. PCR Primers Query Form. Search PCR Primer Data Using the No Forms Interface. Pre-generated lists ... http://www.informatics.jax.org/pcr.html (3K)

Lycos™

The first 10 of 1,523 documents that contained the word "PCR" were printed, and the first three of those 10 are listed below.

1) http://www.panvera.com/catalog/pcrkits.html
last fetched: 02-Jul-95
bytes: 11933
links: 10
title: **PanVera Catalog, PCR Kits and Primer Sets**
outline: PCR Kits and Primer Sets LA PCR Kit Version 1*, 50 reactions Product Number: TAK RR011 PCR in vitro Single Site Amplification and Cloning (SSAC) Kit*, 20 reactions Product Number: TAK R015
excerpt: PanVera Catalog, PCR Kits and Primer Sets PCR Kits and Primer Sets LA PCR Kit Version 1*, 50 reactions Product Number: TAK RR011 Application Amplification of large DNA templates (up to 40 kb) Amplification of cloned inserts

by Dale Graham, Ph.D., DCRT
(e-mail: degraham@helix.nih.gov)

and genomic DNA Description PCR technology has been widely used in molecular genetics research, especially for genome analysis and sequencing studies. However, efficient amplification of DNA fragments greater than 5 kb has been problematic. The Takara LA PCR Kit is designed to overcome this limitation. The LA PCR Kit includes all the reagents necessary for amplification of large DNA templates; routine extension to 20 kb, with ...

2) http://twod.med.harvard.edu/labgc/estep/longPCR_protocol.html
last fetched: 19-Jul-95
file date: 02-Jun-95
bytes: 6270
links: 5
title: **Long PCR Protocol**
outline: Long PCR Reagents and Guidelines General Guidelines for Long PCR Conditions and Enzyme Mixtures Efficient Long PCR results from the use of two polymerases: a non-proofreading polymerase is the main polymerase.
excerpt: Long PCR Protocol Long PCR Reagents and Guidelines (Modified from Cheng et al. (1)) General Guidelines for Long PCR Conditions and Enzyme Mixtures Efficient Long PCR results from the use of two polym ...

3) gopher://bioinformatics.weizmann.ac.il:70/11s/bioguide
last fetched: 31-Jul-95
bytes: 1567
links: 7
excerpt: Select one of: * **What is PCR?** * What are some good reference books for PCR? * How should I select a set of primers to use for PCR? * Programs for designing PCR primers? * What is "Hot-start" PCR? * What is AP-PCR or RAPD PCR? * What is "Touchdown" PCR? * Is there

The query "pcr" found 200 documents and returned 25. The first 12 are shown below. Uniform Resource Locators (URLs), which normally are not included in WebCrawler results, are included here. When used online, WebCrawler returns a list with the site name as a live link that enables you to access the site simply by clicking on highlighted text.

1) BioGuide, http://bioinformatics.weizmann.ac.il:70/1s/bioguide
2) PanVera Catalog, TaKaRa PCR Products and Molecular Biology Kits, http://www.panvera.com/catalog/pcrmb.html
3) MGD: PCR Primers Query Form, http://www.informatics.jax.org/pcr.html
4) Long PCR Protocol, http://twod.med.harvard.edu/labgc/estep/longPCR_protocol.html
5) RegForm: PCR,http://www.vnu.co.uk/eol/pcr/PCreg.htm
6) College Nobel Laureate Lecture, http://www.physics.csulb.edu/WWWpages/nobel.html
7)http://bio-stockroom1.tamu.edu/catalog/enzym.txt, http://bio-stockroom1.tamu.edu/catalog/enzym.txt
8) PanVera Catalog Product Index, http://www.panvera.com/catalog/index.html
9) Cookie, http://wsinti05.win.tue.nl:4243/4
10) MGD Home Page, http://www.informatics.jax.org/mgd.html
11) Implications for Molecular Biology in Hypertension Research, http://www.pitt.edu/~racst12/thesis.html
12) List of Journals from CSHL Press, http://www.cshl.org/journals/

The information in this article deals only with searching WWW, or Hypertext Transfer Protocol (HTTP), sites and not with other useful Internet sites such as Gopher or File Transfer Protocol (FTP) servers. For information on locating search engines for other kinds of Internet sites, use your WWW browser to access DCRT's Information Sheet on Internet Resources. The address, or URL, for the Information Sheet is http://www.nih.gov/dcrt/expo/infos/resources.html ■

In Search of Search Engines

To reach a search engine program, fire up a WWW browser program, such as Netscape or Mosaic. If you're using Netscape, clicking on the Net Search button will take you to a page with search engine sites. Another option is to select the Open Location in Netscape or the Open URL command in Mosaic and other browsers, and then type in the Uniform Resource Locator (URL) of the search engine you want to use. Bear in mind that URLs never contain returns, tabs, or spaces. Also, remember that capital and lower case letters usually must be copied exactly.

Search Engine URLs

InfoSeek Search, http://www.infoseek.com
The Lycos Home Page: Hunting WWW Information, http://lycos.cs.cmu.edu
Webcrawler Searching, http://webcrawler.com/

Sites with Lists of Search Engines

W3 Search Engines
http://cuiwww.unige.ch/meta-index.html
This site is provided through the University of Geneva, and the search engine sites found here range from greatly useful to helpful only for searches of niche items, such as fonts.

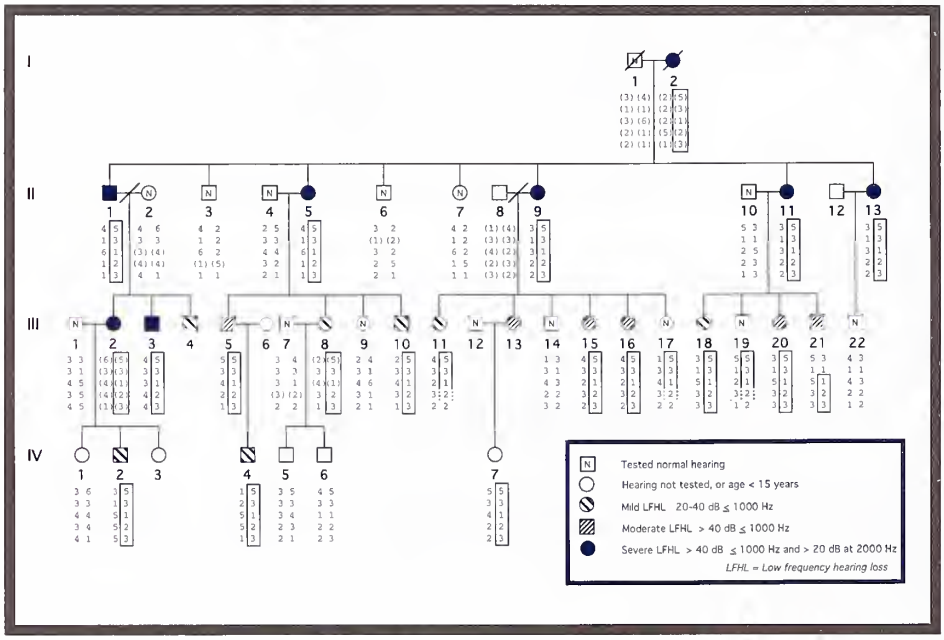
CUSI (Configurable Unified Search Interface)
http://Web.nexor.co.uk/susi/cusi.html
This site is maintained by Nexor UK. By filling out a single form, you can search several WWW engines.

Experimental Meta-Index
http://www.ncsa.uiuc.edu/SDG/Software/Mosaic/Demo/medtaindex.html
This site not only provides access to some WWW search engines, but enables you to search Gopher servers, Wide Area Information Servers (WAIS), and other useful sites. ■

A Locus for Dominant Nonsyndromic Hearing Impairment Maps Near the Huntington's Disease Gene

Investigators in the Section on Linkage Studies and Molecular Cloning, Laboratory of Molecular Genetics, NIDCD—and our collaborators at the Department of Otolaryngology at Vanderbilt University in Nashville—have discovered a novel locus for nonsyndromic hereditary hearing impairment on chromosome 4p in the region of the Huntington's disease gene. This locus causes a dominant, progressive low-frequency hearing loss (LFHL) in a large U.S. family. The region of linkage spans a distance of approximately 1.7 megabases (Mb), and we are hoping to narrow this region as additional polymorphic markers are examined. There is a possibility that mutations in known sequences, mapped during the efforts to clone the Huntington's disease locus, are responsible for this phenotype.

Genetic factors account for most cases of hearing impairment in young children. Among individuals with genetic hearing loss, approximately 74% have an autosomal-recessive mode of inheritance, about 25% have an autosomal-dominant type, and the remaining 1% having X-linked or mitochondrial types of hereditary hearing impairment. One-third of individuals with hereditary hearing impairment have other associated symptoms recognizable as a syndrome. The other two-thirds have no known associated findings and are classified as having "nonsyndromic" hereditary hearing impairment (NSHHI) (1,2). In many types of inherited hearing loss, morphologic or neuroepithelial defects are observed in inner ear structures (3). At the present time, however, little is known about the molecular mechanisms involved in the development and homeostasis of the inner ear.



Haplotype analysis of family with nonsyndromic hereditary hearing impairment. *Parentheses indicate inferred genotype. Question marks indicate unknown genotype. Markers genotyped are displayed vertically, from top to bottom: D4S43, D4S127, D4S412, D4S126, and D4S432. Boxes indicate inheritance of the chromosome linked to the disease. Dashed lines indicate that the affected parent is uninformative for that marker.*

Linkage studies have identified seven dominant gene loci and seven recessive loci for autosomal NSHHI. One of these loci is also the cause of Usher syndrome type 1B and is linked to chromosome 11q13.5 (4,5). Mutations in this gene, a novel myosin VIIA gene (6), are apparently able to cause Usher's syndrome in some families and recessive NSHHI with vestibular malfunction in others.

Two NSHHI loci map to the X chromosome (7,8). Mutations in the Pou3F4 gene have been shown to cause X-linked fixation of the stapes with perilymphatic gusher (9), a rare congenital defect leading to mixed hearing loss. Despite this progress in our understanding of the genetic causes of hearing loss, many families are plagued by inherited hearing loss in which the gene responsible is still unknown.

Mouse Models

Mouse strains that exhibit the NSHHI phenotype may help in identifying novel genes important in human NSHHI. The deafness, or dn, mouse is among the most intriguing of a couple dozen such models. The responsible dn gene maps to mouse chromosome 19 (D19MIT14, 60, and 41), which is syntenic with human chromosome 9q21 and therefore represents an additional potential location for a human NSHHI-related gene (10). Tilted mice, named for their characteristic tilted heads and discovered at Jackson Laboratory in Bar Harbor, Maine, are another possible model. The defective gene in tilted mice maps to the syntenic mouse chromosome 5 and may be the mouse version of the human dominant NSHHI gene that our lab recently localized to a region on chromosome 4p that is bounded by the Huntington's disease locus. The tilted-mouse mutant has a recessive cochleovestibular loss recognizable by the mouse's tilted head and inability to swim (11). Although in mice it is not easy to detect loss of low-frequency hearing that may be associated with the tilted mutant gene, this promises to be a valuable model.

Mutations in genes that are homologous in mice and humans sometimes share a similar but not identical phenotype. This point is well illustrated by the relationship between the shaker-1 mouse–in which mutations in the myosin VIIA gene result in hearing loss and a vestibular abnormality—and human Usher syndrome type 1B—in which mutations in the same gene result in retinal degeneration, in addition to hearing loss and vestibular abnormality. Several other mouse strains could serve as potential models for NSHHI, even though these mice also have altered vestibular function in addition to hearing loss (12–15).

Human Studies

In our current study of an extended family in the United States with more than 100 members, the majority of patients have a bilateral and symmetric hearing loss involving frequencies of 250, 500, and 1000 Hz at the onset of symptoms. The progression of the hearing loss follows one of three patterns: 1) confined to the low

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frequencies, 2) involving all frequencies and producing a flat audiogram, or 3) involving low and high frequencies while sparing the middle frequencies (2000 Hz). As a result of this comparatively mild pattern of hearing loss, few of the family members use hearing aids, and none have cochlear implants. The age of onset is generally in the second decade of life; no family members showed hearing loss before age 5, but all affected members had developed hearing loss by age 15 (16).

The family was genotyped for D4S432, D4S412, D4S127, D4S43, and D4S126 markers on human chromosome 4p. These five markers span a genetic distance of approximately 5 centimorgans (17). The region has a much higher recombination rate than would be expected for its physical size (less than 3 Mb) (18).

Four recombinants were identified by haplotype analysis (see figure). Individual 111-21 has a recombination between D4S127 and D4S412. Unaffected individuals 111-17 and 111-19 have recombinations between D4S126 and D4S432. Since 111-11 is an affected individual with the same recombinant haplotype, we postulate that the breakpoints for 111-11 vs. 111-17 and 111-19 lie on opposite sides of the gene for hearing loss. D4S126 is not a fully informative marker; the affected parents of the recombinants are homozygous at this locus, preventing detection of recombinants in their children. Thus the most likely location for the gene is between D4S412 and D4S432, a distance of 1.7 Mb (19). The maximum LOD score was 5.05 at q = 0.05 for D4S412. Given the marker order of D4S412 - D4S126 - D4S432, the multipoint mapping yielded a maximum LOD score of 6.5 when the disease gene was placed in the interval between D4S126 and D4S432.

TO OUR KNOWLEDGE, THERE HAS BEEN NO STUDY THAT SPECIFICALLY SCREENS FOR MORE SUBTLE FORMS OF HEARING LOSS IN PATIENTS WITH HUNTINGTON'S DISEASE.

This region on chromosome 4 has been well mapped. More than 20 genes were identified in the course of the lengthy search for the Huntington's disease gene, HD or IT15 (20), which was finally located at 4p16.3 in 1993 by the Huntington's Disease Collaborative Research Group. Expression of IT15 has been detected in all areas of the cerebral cortex, predominantly in neurons (21). Hearing loss has not been described as a clinical feature of Huntington's disease; however, to our knowledge, there has been no study that specifically screens for more subtle forms of hearing loss in patients with Huntington's disease. The multipoint data for our NSSHI locus suggest a location proximal to IT15 and D4S126, a region that includes the gene for the α-2C-adrenergic receptor (ADRA2C) (22). Alpha-2-adrenergic receptors are widely expressed in the brain, especially in regions with high dopamine content. Another candidate gene in this region is the α-2-macroglobulin receptor-associated protein (A2MRAP) also known as the low-density-lipoprotein receptor-related, protein-associated protein (LRPAP1) (23,24). Although the function of this protein is unknown, its corresponding receptor is important in proteinase inhibition and lipoprotein metabolism.

Now that we have documented these tantalizing potential connections to the Huntington's disease gene (25) and the tilted-mouse gene, our laboratory would like to screen any and all the candidate genes that have been mapped to the human 4p16.3 region. We are planning to breed the tilted mice to test their hearing. If these mice are good candidate models, we would expect to find close syntenic relationships in the mapping of mouse chromosome 5 and the human 4p16.3 region. ■

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Flow Cytometry: More Than Just Cell Sorting

There are two camps when it comes to flow cytometry: the believers, who appreciate the technique as a fast lane to tomorrow's research questions, and the uninitiated, who haven't heard all the things that are possible through contemporary applications of the technique. After getting its start with the crude cell counters of the 1930s and '40s, contemporary flow cytometry is no longer just for counting. New-age flow cytometry—which includes but is not limited to fluorescence-activated cell sorting (FACS)—couples a highly sensitive, automated fluorescence-detection device with sophisticated computerized analysis of data gleaned at lightning speed for measurements of numerous interesting properties of large populations of cells. Cell size, viability—including the presence of apoptotic cells, cell-cycle dynamics, kinetics, and the presence of multiple intracellular and surface proteins can be determined for each cell in a sample.

The high sensitivity of this technique, coupled with the ability to rapidly analyze multiple parameters in samples containing 5,000 to a million cells, allows the detection and definition of unique subpopulations within a sample. The ability to detect subpopulations with an abnormal pattern of protein expression is useful in diagnosing and subclassifying leukemias and lymphomas. And because flow cytometry can routinely pick out one neoplastic cell per 1,000 normal cells—and specialized techniques can improve this sensitivity to find one abnormal cell in a million normal cells—the technique is useful in hematology and hematopathology for detecting minimal residual disease.

By using appropriate standards and controls, flow cytometry can be exquisitely quantitative, allowing researchers to determine the exact number of molecules of fluorescent antibodies—and thus the molecules of antigen—bound to a cell or within a cell. This makes flow cytometry broadly useful for precise measurement of the expression of oncogenes, activation markers, adhesion receptors, and other proteins and, depending on the method of staining, allows localization of these proteins to the cell surface or cell interior.

Flow cytometry is becoming important for exploring some of the cellular activities that are under intense research scrutiny these days. Cell-cycle data can be obtained by DNA-content analysis or by detection of bromodeoxyuridine (BrdU) incorporation or cell-cycle specific proteins. Flow cytometry is, arguably, the most sensitive and easiest method for detecting apoptosis. The TUNEL method—in which the terminal deoxynucleotidyl transferase (TdT) end- labels double-stranded DNA breaks, which are generated during apoptosis—can be applied to large populations of cells by the use of flow cytometry. Another method of flow-cytometric apoptosis detection—based on light-scatter characteristics that change when the nucleus condenses during apoptosis—requires no manipulations other than preparation of a cell suspension.

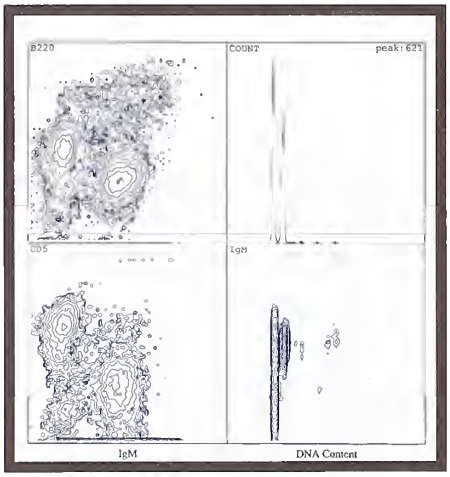


Figure 1. Flow cytometric analysis of a murine marginal zone lymphoma. *Left panels show that the majority of the cells are positive for fluorescein isothiocyanate (FITC)-staining of IgM, positive for phycoerythrin staining of CD5, and positive for dim tricolor B220, which, in this case, is indicative of marginal zone lymphoma. The right upper panel shows DNA content (x-axis) verses cell number (y-axis). The right lower panel shows a two-parametric contour plot of FITC IgM versus DNA content. Two G1/G0 populations can be seen; one is diploid and the other is aneuploid. Both are IgM positive.*

Reduced DNA content, or hypoploidy, as indicated by propidium iodide (PI) staining, is another easy procedure for detecting apoptosis. Even oxidative state and the flux of ions, such as calcium, into cells can be measured by flow cytometry. The power of flow cytometry in cell biology lies not only in its sensitivity, but also in its ability to measure multiple characteristics simultaneously on each cell in a population of 100,000 to a million cells. Therefore, for example, the nonapoptotic vs. apoptotic cells in a heterogeneous sample can be rapidly compared for levels of BCL-2 and p53 protein expression, presence of a lineage-specific surface markers, such as T-cell specific antigen, and cell-cycle phase (G1/G0, S, or G2+M).

The Method and How It Works

In flow-cytometric analysis, cells in a single cell suspension are stained with multiple fluorescent markers and transported rapidly—routinely 18,000–30,000 cells/min—to intersect a finely focused monochromatic beam of light of an appropriate frequency. A stream of fluid containing the sample cells is ejected at steady pressure and rate into a flowing, high-pressure "sheath" fluid. The convergence of the sample stream with the sheath fluid allows for the precise intersection of the sample stream that contains the single cell suspension with the laser beam. This is referred to as hydrodynamic focusing. The fluorochromes attached to the cells absorb light and emit energy at a longer wavelength that is specific for the fluorochrome. For example, fluorescein isothiocyanate (FITC) emits light at a different wave length than phycoerythrin (PE) or peridin-chlorophyll-a-protein (PerCP), allowing all three indicators to be detected simultaneously in a cell. In addition, light is scattered in proportion to the size of the cell in the forward direction, much like a shadow. Light is also scattered or reflected to the side by the intracellular granules; thus, cells with complex cytoplasm, such as the granular neutrophils, have a much higher side scatter than do less complex cells, such as lymphocytes.

Light detectors collecting the forward and side scatter and fluorescence emissions thus rapidly gather information for each cell on its size, cytoplasmic complexity, and fluorescent markers. Three different fluorochromes emitting light at three different frequencies are routinely used in clinical laboratories, whereas research facilities may use five or more fluorochromes. Fluorochromes may be bound to antibodies for the detection of specific proteins or of BrdU incorporation in studies of cell-cycle kinetics. Alternatively, the fluorochromes may be used for direct staining of cellular elements. An example of this would be PI intercalation into DNA.

Flow cytometry measures all parameters for all cells passing through the focused beam, and then the cells can be classified into categories based on any combination of the detected parameters, including presence of cell-surface lineage-specific markers or activation antigens, oncogene expression, or DNA content. Complex computer programs then quantify the numbers of cells within each defined category and record the fluorescent intensity, indicating levels of protein expression or quantities of DNA, for example. The computer then displays the data in two- or three-dimensional plots of parameters and calcu-

by Maryalice Stetler-Stevenson, M.D., Ph.D., NCI, and Gerald E. Marti, M.D., Ph.D., FDA

lates the percentage of cells falling into any category specified by the investigator.

Protocols

Mention of a specific product in the following description does not constitute an endorsement.

Protocols differ depending on the information a researcher is seeking—cell-cycle analysis or cell-surface antigen, for example—and the type of specimen—cell lines in media, whole blood, or bone marrow, for example. The two most widely used protocols are whole-blood lysis (WBL), for surface immunophenotyping of blood or bone marrow, and PI staining of isolated cells, for analyzing the cell-cycle (to detect S phase cells) and for detecting aneuploidy.

In WBL, a 10- to 100-mL sample of whole blood or bone marrow is stained with two to five conjugated monoclonal reagents. After the staining is complete, the red cells are lysed using either dilute HCl, hypotonic solution, detergents, ammonium chloride, or proprietary preparations. The lysed cells are washed and fixed for analysis. Compared with mononuclear cell isolation followed by antibody staining, WBL is more convenient, quicker, and less likely to cause selective cell loss, which could bias the data. Fixation stabilizes cell membranes, crosslinks the antibody to its antigen, and reduces the risk that researchers will be contaminated with infectious material. Fixed cells are also stable for longer periods than are fresh cells.

To stain intracellular antigens, the cells are permeabilized and stained with conjugated monoclonal antibodies. Red cells are lysed. If the permeabilization method does not fix the cells, they must be fixed after lysis of the red cells. For simple cell-cycle analysis, cells are permeabilized and stained with a DNA dye such as PI. Combined antigen-expression and DNA-content analysis usually consists of staining with an FITC-labeled antibody, followed by gentle fixation, permeabilization, and PI staining. Both FITC- and PE-labeled reagents can be used together with 7-amino-actinomycin D (7AAD) instead of PI, which emits light in the same wavelength region as PE. Describing the actual acquisition and computer analysis of the data on the flow cytometer is beyond the scope of this article. In our opinion, acquisition and analysis of flow-cytometry data is best done by—or under the supervision of—someone experienced in the field. Labs with substantial flow-cytometry experience and facilities are listed in the "contacts" section of this article.

Trouble-Shooting Tips

Attention to several technical details helps to prevent problems and to identify the source of problems that do occur. We wash all cells that are derived from living animals, including humans, to remove adherent proteins that may bind labeled antibodies or dyes in a specific or nonspecific manner. Using too high a concentration of antibody promotes nonspecific binding. The appropriate concentrations

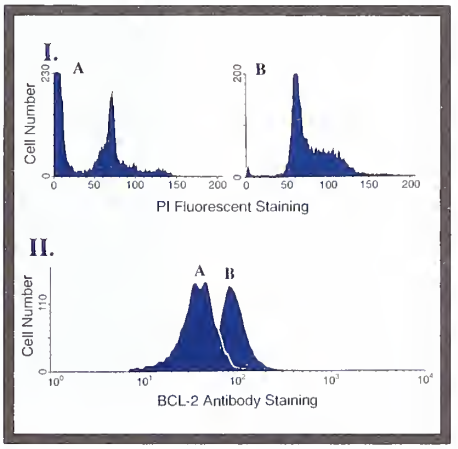


Figure 2. I. DNA content analysis to detect apoptosis in cells after cold shock. *Cell line A is prone to apoptosis in response to cold shock. Sub-diploid apoptotic peak is shown. Cell line B is resistant to cold shock-induced apoptosis and shows no sub-diploid apoptotic peak. II. BCL-2 expression in cell lines. Apoptosis-resistant cell line B has higher level of BCL-2 protein expression than apoptosis-prone cell line A.*

for antibodies are often specified by manufacturer or can be empirically determined by staining control cells with serial dilutions. When working with antibodies, we use isotypic controls against nonmammalian proteins to detect nonspecific binding of antibodies. Negative and positive controls for all biological processes that are being characterized in a flow-cytometry study should be run to ensure sensitivity and specificity. Protocols of preparations for flow-cytometric analysis of cell-surface, intracellular, and intranuclear antigens and DNA content are given below.

Cell Washing

1. Add 20 mL or less of peripheral blood, bone marrow, or cell suspension to a 50-mL centrifuge tube. With peripheral blood or bone marrow, mark the level.
2. Add room temperature phosphate-buffered saline (PBS) to bring the volume to 45 mL. Invert to mix and centrifuge at 1200 rpm in swinging-bucket rotor centrifuge at room temperature for 10 min.
3. Aspirate the supernatant and repeat step 2. above two times.
4. Restore to original volume with PBS. Dilute to 2 x 10^6 cells/mL.

Cell-Surface-Antigen Staining in Whole Blood or Bone Marrow

1. Add appropriate amount of antibodies to labeled tubes (usually 5–20 µL). Three antibodies, each complexed with a different color fluorochrome (e.g. FITC, PE, and PerCP) can be placed in the same tube for FacScan analysis. For five-color analysis or UV excitation, more complex flow cytometers are required. Add 150 µL PBA (PBS with 0.1% bovine serum albumin and 0.1% NaN3) or PBS with 10% fetal calf serum (FCS) to each tube.
2. Add 100 µL of washed whole blood or bone marrow to each of the prepared reagent tubes and incubate in the dark for 30 min at room temperature. Because light bleaches fluorochromes, tubes should be kept covered with aluminum foil.
3. Wash cells by adding 4 mL PBA and centrifuging at 1200 rpm for 8 min in a swinging-bucket rotor centrifuge at 4–6 °C. Aspirate the supernatant. Approximately 100 µL PBA and cells will remain.
4. Lyse the specimens using a proprietary lysing kit (e.g., Immunolyse, Q-prep, and Facslyse). We find that manually lysing is quickest and provides an excellent specimen in the hands of an experienced technician. The manual lysing methods vary from product to product but usually entail 1) adding a lysing reagent, 2) incubating for a precise period of time, 3) adding a fixative that stops the lysing and 4) washing the cells to remove the lysing reagent. Timing, in manual lysing, is critical. We recommend using an electronic timer and restricting the number of tubes to be lysed to a number that can be handled promptly. Experienced technicians in our laboratory do not lyse more than 20 tubes in one batch. Machines that perform the entire lysing procedure (e.g., the Coulter Q-prep) are available but can lyse just one tube at a time. The main benefit of the Q-prep is that it requires no experience to produce an excellent specimen. Its main drawback is that it is not as fast as manual lysing in the hands of an experienced technician.
5. After washing the cells with PBA, resuspend each specimen while vortexing lightly in 500 µL of 1% paraformaldehyde in the isotonic solution that the cells will be analyzed in. Cover and keep at 4–6 °C in the dark for at least 1 h but, preferably, overnight.

continued on page 22.

LAB BEHIND THE LEADER
NEW NIAID SCIENTIFIC DIRECTOR

In its quest for a first-rate scientific director, NIAID didn't have to go far afield—it found just what it needed in its own back yard: noted immunologist Thomas Kindt. Recognized for his contributions to the understanding of human T-cell leukemia virus-1 (HTLV-1), Kindt joined NIAID in 1977 and went on to become the chief of the Laboratory of Immunogenetics. Before arriving at NIH, Kindt, who received his Ph.D. from the University of Illinois at Urbana-Champaign in 1967, held academic appointments at The Rockefeller University and Cornell University Medical College in New York. He has received numerous scientific awards, including the Elliot Osserman Award from the Israel Cancer Research Fund and has advised the Howard Hughes Research Scholars Program. Kindt offers this description of his research.

Our laboratory studies the human retrovirus HTLV-1. Although most of the 10 to 20 million people infected with HTLV-1 worldwide suffer no overt disease, about 5% are afflicted with an acute and often fatal leukemia, a debilitating neurologic disease, or one of a variety of chronic conditions ranging from arthritis to uveitis. In certain areas of the world, such as southern Japan, the prevalence of HTLV-1 infection may be as high as 20%. This problem of endemic HTLV-1 infection, along with the recent increase in the infection rate in the United States, mainly among intravenous drug users, makes HTLV-1 a significant health threat.

We would like to understand why HTLV-1 infection produces such variable responses in different individuals. What events or factors dictate the difference between asymptomatic infection and fatal or chronic disease? Possible candidates include the nature of the cell infected; mutations in the virus genome, which spans approximately 9 kilobases (kb); variations in host-virus interactions; the genetic background of the infected subject; or some combination of these parameters.

We have developed in vitro and in vivo systems to learn more about how HTLV-1 exerts its highly variable effects on the host. The laboratory rabbit, which is highly susceptible to HTLV-1 infection, is an animal model that mimics human infection in several ways. As its name implies, HTLV-1 infects T cells, and rabbit T-cell lines infected with HTLV-1 survive in culture indefinitely. We have administered HTLV-1–infected T-cell lines to rabbits and monitored the animals to see whether they develop disease or asymptomatic infections. In an effort to determine what is responsible for the divergent outcomes, we then studied the characteristics of the various HTLV-1–infected cell lines, along with the structure of the integrated virus, or provirus, and the functions of the provirus' component genes.

Lorna Heartley

Thomas Kindt

To date, we have identified HTLV-1 cell lines that cause acute disease in rabbits that is similar to human leukemia and, in other cases, chronic cutaneous lymphoma. We are now examining these lines in detail to ascertain how they differ from lines that give rise to asymptomatic infection. We have observed certain small differences in gene expression between the so-called lethal and nonlethal cell lines, but none of those differences absolutely correlates with the disease-causing potential of the infected cell. We have cloned proviruses from such lethal and nonlethal lines, and, through direct injection, have used those DNA clones to infect normal human and rabbit cells in vitro and rabbits in vivo. In a further effort to pinpoint the genes that control HTLV-1's pathogenicity, we are now exchanging different component genes among the viruses from the lethal and nonlethal cell lines to create chimeric HTLV-1 strains.

> **WE WOULD LIKE TO UNDERSTAND WHY HTLV-1 INFECTION PRODUCES SUCH VARIABLE RESPONSES IN DIFFERENT INDIVIDUALS. WHAT EVENTS OR FACTORS DICTATE THE DIFFERENCE BETWEEN ASYMPTOMATIC INFECTION AND FATAL OR CHRONIC DISEASE?**

Our current data suggest that a major factor in pathogenicity is the expression of certain, as-yet-unidentified cellular genes—expression that is very likely induced by infection with the lethal virus. Our in vitro studies show that expression of such genes predisposes the cell to overcoming host resistance. One route by which this occurs is by the apoptosis, or programmed death, of host T cells, which are presumably the cells that keep the infected cells in check.

In the future, our lab, which includes R. Mark Simpson, Tongmao Zhao, Mary Ann Robinson, Michel Leno, and Florence Bowers, will concentrate on exploring the effects of genes from lethal and nonlethal viruses on the expression of host cellular genes. First, both viral and cellular genes that exert either a positive or negative effect on pathogenesis will be identified by screening HTLV-1–infected cell lines by in vitro molecular and cellular methods. When candidate genes are found, nonlethal HTLV-1–infected cell lines transfected with these pathogenesis-promoting genes and lethal HTLV-1–infected cell lines transfected with pathogenesis-suppressing genes can be tested for in vivo effects. ■

TENURE TRACK
continued from page 1.

tory research decisions, staff, and resources. But NIH tenure goes further, actually providing scientists with the resources to conduct the research without obliging them to apply for grants, as their extramural colleagues must do.

Under the old system, intramural staff researchers were nominated for tenure by lab and branch chiefs, then reviewed by their institute, center, or division's (ICD's) promotion and tenure committee. If their scientific director (SD) selected them as tenure candidates, their packets of credentials were presented to the Board of Scientific Directors—a group composed of the SDs for all institutes that meets biweekly with the deputy director for intramural research (DDIR). The SDs discussed each candidate's merits and voted for or against recommending that the DDIR grant tenure to the candidate. The DDIR almost invariably approved the candidates that the SDs recommended. About 95% of the candidates brought to the SDs were recommended.

Data from CTC's first 14 months support the proposition that the new committee is no tea party. Of the 28 scientists whose cases were reviewed, 21—75%—were recommended for tenure. Although the percentage of canidates tenured by the CTC has gone up slightly in the past few months, the new system appears to be tougher. Wyatt says the reasons may in part relate to lack of familiarity with the new system and a new emphasis on distinguishing between independent scientists, who may be granted tenure, and staff or collaborative scientists, who may be granted permanence but not tenure.

The most conspicuous change in the system is that the final packets of credentials no longer go to the SDs, but instead to a 15-member panel of scientists selected to serve on the CTC by the DDIR. Many of the other changes in the new system are not universal or substantive, but are refinements intended to make tenure policies more uniform among the various institutes and understandable to everyone. "The members of the lab I work in have less anxiety than before with respect to the tenuring system," says Pat Becerra, who is at the start of NEI's tenure track. "Before, the tenuring system was an unknown. ... Now we all know where we stand."

To make sure the new system is widely understood, DDIR Michael Gottesman has met with groups of postdocs and junior scientists to explain the new system and answer questions. And at each step in the new system—starting with intramural postdocs just entering NIH for training—there is an emphasis on letting everyone know their career status and what NIH offers them and expects from them. For example, all scientists placed on the tenure track now receive letters congratulating them on their new status and describing the resources that will be at their disposal during the six years that they have to establish themselves as independent scientists on the tenure track.

One key difference in the system is only beginning to come into play—a new emphasis on outside recruitment to the tenure track. When NIH switched from the old system to the new one, each institute was allowed to nominate intramural scientists for "grandfathering" onto the tenure track based on evidence of high-quality, independent research. Around 180 intramural scientists were placed on the tenure track under the grandfather clause. Currently, however, the only scientists being added to the tenure track are those selected as the top candidates in rigorous, nationally advertised searches for the positions.

Some fellows and trainees erroneously believe that this emphasis on outside recruitment means they do not have a crack at the tenure track after their allotted five years as postdocs in the intramural program. "What is worrisome is the fellows who have this view that there will not be a chance to make a life here at NIH if you come in as a postdoc," says NCI's Elise Kohn, recently tenured by CTC. "We are starting to see an exodus of outstanding postdocs leaving for opportunities elsewhere and grave concern among postdocs about coming here."

Although it is likely that in the future, a smaller percentage of scientists tenured at NIH will have been trained here, doing a postdoc stint at NIH is not the kiss of death for one's intramural tenure prospects. "By virtue of the size and excellence of our training programs, many of the best young investigators in this country are coming out of our labs and clinics," says DDIR Gottesman. "It would be a travesty if we didn't snap up the best of our own for the tenure-track." Gottesman gives intramural postdocs information on new tenure track positions by advertising them on his electronic bulletin board a few weeks before the ads appear in journals like Science. NHLBI's SD, Edward Korn, says his institute has capitalized on the new system to snare some excellent postdocs for tenure-track positions. "The tenure-track policy provides more opportunities for NIH postdocs than the previous policy in that NIH postdocs now can and do compete for the many positions outside their own laboratory rather than just the few or none that might arise within their own lab." Korn says that of the five nationally advertised tenure-track positions NHLBI has filled in the past year, three went to postdocs from other institutes who were unknown to NHLBI's search committee before they applied, and one went to a postdoc in another NHLBI lab who was previously unknown to the lab that selected him.

David J. Clark, who has been in his tenure-track position at NIDDK for just a couple of months, says his case proves that intramural fellows can make it to the tenure track. Clark was selected through a national search as the best candidate for a tenure-track position within the institute in which he was trained, but in a different lab. "It is now more difficult for intramural fellows to get a tenure-track job at the NIH, but I believe that the new system is fairer and will help to raise the quality of research at NIH," says Clark, who chose the NIH position over several outside offers.


Lorna Hearley

Richard G. Wyatt

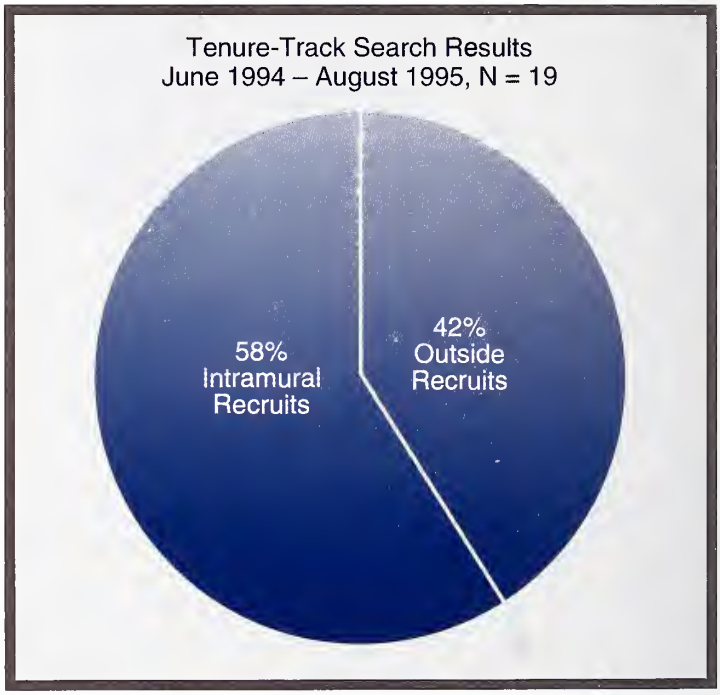
Once scientists make it to the tenure track, their work is cut out for them. In the course of the next six years, the tenure-track investigator must build up a portfolio of research that impresses a series of judges, starting with the lab or branch chief and the institute's Board of Scientific Counselors (BSC)—the panel of outside scientists that convenes at NIH

every three to four years to review all tenured and tenure-track investigators. If, based on these evaluations, the scientist is kept on the tenure track, in six—or perhaps fewer—years, his or her credentials will be passed to the institute's tenure and review committee, which will solicit letters from outside reviewers and weigh these along with the BSC review and the candidate's publications and other scientific achievements. The ICD tenure committee makes a recommendation to the institute's SD, and if the recommendation is that the candidate should be tenured, the SD and the candidate's lab or section chief send the candidate's packet to CTC. Two regular CTC members plus one ad hoc member are generally assigned to review the case. At the CTC meeting, the SD and the lab chief present the candidate's credentials to the entire CTC and then leave before the committee's discussion.

"Some people say [the CTC review] is too rigorous," says Igor Dawid, an NICHD scientist who serves on CTC. "It is definitely quite rigorous. ... Those who are assigned to the cases are preparing quite carefully and people do take [the review discussions] very seriously," Dawid says. "The process is supposed to take a half hour per case, but it almost always takes longer." CTC reviews two cases per meeting, with meetings called as often as there are candidates. In addition to the 15 regular members of CTC, numerous other tenured scientists have lent their specialized expertise as ad hoc members. Ad hoc members are invited to stay for the entire meeting and are welcome to participate in discussion of both cases under review, but they do not vote. At least eight eligible CTC members must vote on a case, and CTC members from a candidate's institute are not eligible to vote.

Now the burning question has become, What is CTC really looking for? "People don't know what it takes to make it at NIH anymore," says one recently tenured scientist. Kohn says her recipe for success would include "one part perseverance, one part persistence, one part creativity—or more." Kohn would also include "independence and productivity—but not just publications. ... You need to be able to prove that you will be able to sustain the momentum in your line of investigation with direction and focus." Thus far, teaching and patents have not been decisive factors in cases CTC has seen, Dawid says. Wyatt says that BSC reviews, SD evaluations, and outside letters are all important and that CTC's emphasis when it looks at publications is on the quality of a few key publications rather than sheer numbers. Dawid's recommendation to people on the tenure track is simple and unsurprising: "Do some good work and publish it."

One of the main reasons people have been turned down for tenure by CTC is weak evidence of scientific independence from mentors. The emphasis on this criterion is new. Under the old system, Wyatt notes, there was a fuzzy line between permanent, collaborative scientists and true tenured scientists with independent resources. The Board of SDs "did not have to resolve the issue of independence fully in every case," Wyatt says. Dawid notes that teasing out the issue of independence can be very tough in instances where the work requires extensive collaboration or where a mentor's achievements loom large. "In such cases, it is more important to bring to bear other evidence of independence and originality," such as outside letters and invitations to speak at international meetings, he says.



Boguski says he was confident as his case came before CTC, even though, as an investigator in the National Center for Biotechnology Information, his achievements were made in front of a computer screen rather than at a lab bench. "From what I hear, there was a good, in-depth discussion of what it means to be an independent scientist at NIH, and how someone like me fits in." Boguski attributes his success to traditional measures of achievement. "I know what I've done, the journals where I've published. I felt good about my record," says Boguski, who also received competing job offers from other institutions. One bonus of the new system for Boguski was that CTC reviewed his resources and made recommendations to his SD on appropriate levels of support for his research. "They did a careful job of evaluating me, and once they decided I was worthy, of seeing that there were adequate resources for me to do my job."

In the long run, says Dawid, CTC's standards for tenure will emerge from the cases it reviews and will trickle down to the local tenure-review committees, "No one knows exactly what we're trying to evolve," says Dawid, but he anticipates that "a rather high and uniform set of standards will be applied." Korn, who has not yet taken a tenure candidate to CTC, strongly supports the tenure-track concept but says the feedback of information on tenure standards may not be as good as it used to be. "This is a loss to the scientific directors," Korn says. He notes that because the entire board of SDs is only informed when a tenure applicant is successful, there is limited or no feedback to other institutes, either on exciting science being done by the successful tenure candidates or, in the case of unsuccessful candidates, on what credentials are insufficient to achieve tenure.

One scientist who has just started the

tenure track worries that, in the final analysis, the judgment of his credentials for tenure may be as political as the judgments on who would be "grandfathered" onto the tenure track. "The way things have gone as I've been through this makes me think it could still be a political process [in the tenure evaluation] at the end and may not involve totally objective criteria," the scientist says. "People have different perspectives on science. What is 'hot' changes dramatically. ... Let's say you recruit someone in to work on p53 today. That work might be relegated to specialized journals in five to 10 years," when the candidate comes up for tenure.

Although that scientist is optimistic that the new tenure system will be an improvement and that he will, ultimately, achieve tenure, he has been saddened by one change in his lab that he believes was caused by the new system. "Our lab used to fire like an engine; now you have a bunch of pistons—everyone is trying to be independent," he says. "I don't think this system fosters team work ... but rather creates a possessiveness and territoriality. People are less interactive and more guarded about what's theirs and what isn't."

Spiegel, who has had two people under him come up for tenure before the committee, is satisfied that, on the whole, the new CTC is doing a good job with "to-the-point, incisive, rigorous reviews." Korn sees the changes in the tenure system as necessary and important. Pointing to the many outstanding and productive scientists who were tenured at NIH under the old system, he says he is optimistic that the new system will be as good or better. "It's like any experiment—we have to wait and see."

Meanwhile one of NHLBI's tenure-track stars, Cynthia Dunbar, echoes her boss's guarded optimism. Dunbar's work has been going well and she hopes to be put up early for tenure. "I'm not losing sleep over it. I've got too many other things to worry about," she says of the tenure system she will be facing. "People have received more information than previously about how the system is supposed to work, and, in theory, the procedures are improved in terms of fairness and reproducibility from institute to institute, but only time will tell what the outcome will be." ■

Tenure-Track and Tenure-Appointments Process

STEP 1	The institute, center, or division (ICD) director, scientific director (SD), and lab or branch chief, in consultation with senior scientists in the ICD, determine the need for a new tenure-track position.
STEP 2	The SD establishes a search committee with concurrence of the ICD director and advertises for tenure-track candidates.
STEP 3	The search committee evaluates applications, including reference letters, invites promising candidates to campus for interviews and seminars, and recommends one to three candidates to the lab or branch chief, SD, and ICD director. The SD and the ICD director select one name and forward it to the deputy director for intramural research (DDIR) for approval. The DDIR reviews and approves the selection process and the candidate.
STEP 4	The SD and lab or branch chief, in consultation with the potential candidate, prepare and sign a Tenure Track Agreement. A copy is sent to the DDIR.
STEP 5	The candidate signs the Tenure Track Agreement and is appointed or converted to a tenure-track position, starting the tenure-track "clock."
STEP 6	Each year, the section or lab or branch chief prepares an oral and written performance evaluation of the candidate.
STEP 7	Approximately every three years, the Board of Scientific Counselors (BSC) reviews the candidate's performance and qualifications for tenure and decides whether the candidate should be continued in the tenure track, dropped from the track, or advanced for a tenure decision.
STEP 8	Before time elapses on the tenure-track clock, the SD and ICD director review the candidate and decide whether to propose the candidate for tenure, continue the candidate in tenure track, or drop him or her from the track.
STEP 9	The candidate is informed in writing of the decisions of the BSC, lab or branch chief, SD, and ICD director.
STEP 10	If the candidate is advanced for consideration, an ICD Promotion and Tenure Review Committee is formed to solicit outside letters and assemble and review credentials. This committee, in concurrence with the SD and ICD director, makes a recommendation to the NIH Central Tenure Review Committee (CTC).
STEP 11	CTC reviews the candidate's credentials and makes a recommendation to the DDIR.
STEP 12	The DDIR makes a tenure decision.
STEP 13	The DDIR informs the SD of the decision. In turn, the SD informs the candidate, in writing, of the decision.
STEP 14	If the candidate is not approved for tenure or is dropped from tenure track, he or she has one year to wrap up work and find another job. ■

NIH and NASA
continued from page 1.

plore the utility of a fiber-optic probe for cataract detection involves no exchange of funds and resembles traditional collaborations between NIH researchers and scientists in academia. What the two projects do have in common is that both arose through avenues that NIH researchers often overlook while scouting around for collaborative opportunities.

Space-Age Bioreactors

Until a friend in academia alerted him to one of NASA's bi-annual "Research Announcements," Joshua Zimmerberg, chief of NICHD's Laboratory of Theoretical and Physical Biology, didn't know that NIH scientists were even eligible for NASA grants, let alone that one of NASA's proposal requests dovetailed neatly with his lab's interests in three-dimensional tissue culture and the imaging of complex tissues [see box]. The request called for research projects that might facilitate the space-to-ground transfer of NASA's bioreactor, a fluid-filled, rotating-wall cylinder originally developed to allow cells to be cultured in liquid medium—despite the weightless conditions found in the space shuttle—and to protect the cell cultures aboard.

In the first year of the joint project, which got under way in August 1994, Zimmerberg's group, which includes three-dimensional-culturing expert Leonid Margolis, created a two-room core facility in Building 10 that is equipped and staffed to assist NIH and NASA researchers who want to use the bioreactor to address basic biomedical questions. For its part, NASA has moved one of its experienced technicians, Wendy Fitzgerald, from Houston to Bethesda to train scientists in the fine points of using the bioreactor. In addition, the collaborative agreement calls for Zimmerberg and his colleagues to use the NASA bioreactor to create tissue cultures for other NIH projects that require a higher level of cell organization than is available through standard monolayer or suspension culture. The NICHD lab is currently using the bioreactor to create a human lymphoid tissue model for AIDS research.

The unique properties of the NASA bioreactor, which costs about $4,500 and is manufactured by Synthecon Inc. of Houston, aren't immediately apparent. Outwardly, the bioreactor looks virtually the same as a conventional "roller bottle" bulk-culturing device. However, NASA bioengineers have nested a second, slightly smaller rotating cylinder within the roller bottle to form a rotating wall of medium that keeps cultured cells or tissues in constant, gentle suspension rather than allowing them to slosh roughly about as they do in a standard roller bottle. The inner wall is semipermeable, to allow the free exchange of oxygen and carbon dioxide within the bioreactor.

Although NASA scientists designed the bioreactor to protect cells during space travel, they decided to pursue on-ground applications after discovering that cells cultured in the bioreactor's low-turbu-

Histoculture: Entering the Third Dimension

by Birgit An der Lan, NICHD

To adapt John Donne's familiar phrase, no cell is an island. Although many types of cells can be grown through conventional culture techniques, such methods provide, at best, a pale imitation of the complex microenvironment that influences cell growth and activity within living tissue. Factors such as proximity to blood vessels and interactions with other types of cells don't come into play when cells are cultured under standard in vitro conditions. Recognizing a need to develop better ways of studying cells in a biomedically relevant context, Leonid Margolis' group at NICHD's Laboratory of Theoretical and Physical Biology has for the past seven years been working with methods that make it possible to culture and image three-dimensional blocks of tissue.

It has been known for decades that tiny chunks of tissue can be cultured for up to six weeks on collagen sponges floated in growth medium—a system used primarily for studying the tissue cells' invasion of the sponges. The NICHD group has gone one step further, however, by using the collagen-substrate system to study cell-cell interactions within tissues. Furthermore, Margolis, Joshua Zimmerberg, and their colleagues have taken advantage of a relatively recent technological advance in light microscopy—laser confocal fluorescence microscopy–to analyze the native architecture and dynamic processes within such tissue specimens, which are too thick for conventional microscopy. Compared with standard fluorescence microscopy, images formed using confocal fluorescence microscopy have much better spatial resolution because they have less out-of-focus light. Each plane of focus can also be conveniently stored in a computer database, thus facilitating quantitative analysis and allowing the reconstruction of three-dimensional images.

So far, Zimmerberg, Margolis, Boris Baibakov, and Svetlana Glushakova have used their three-dimensional histoculture and imaging techniques to track individual melanoma cells as they invade lung tissue. Using cubes cut from human tonsils, the researchers have also observed the fusion of healthy T cells with various types of human cells expressing the envelope glycoprotein of the human immunodeficiency virus (HIV). The lab has also used its culturing and imaging expertise to trace the innervation of the epithelial cells in rat tongue tissue by neuroblastoma and trigeminal ganglia cells and to observe differentiation of human breast tissue in vitro. ■

Additional Reading

L.B. Margolis, S.E. Glushakova, B.A. Biabakov, C. Collin, and J. Zimmerberg. "Confocal microscopy of cells implanted into tissue blocks: cell migration in long-term histocultures." *In Vitro Cell Dev. Biol.* **31**, 221–26 (1995).

L.B. Margolis, S.E. Glushakova, B.A. Baibakov, C. Collin, and J. Zimmerberg. "Syncytium formation in culture human lymphoid tissue: fusion of implanted HIV glycoprotein 120/41-expressing cells with native CD4+ cells." *AIDS Res. Hum. Retroviruses* **11**, 697–704 (1995).

L. Margolis, B. Baibakov, C. Collin, and S.A. Simon. "Dye-coupling in three-dimensional histoculture of rat lingual epithelium." *In Vitro Cell Dev. Biol.* **31**, 456–61 (1995).

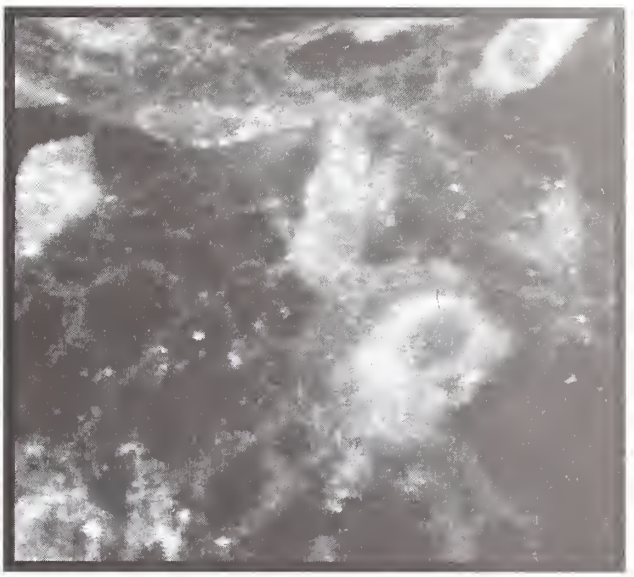
lence environment on Earth grew into three-dimensional masses that bore a greater structural and physiologic resemblance to natural tissues in vivo than did cells cultured by traditional methods.

Although the fledgling NIH-NASA bioreactor venture has yet to yield any publications, Zimmerberg says the most impressive results from the bioreactor to date fall into two general categories, engineering tissue-like structures from single cells and keeping tissue alive with its architecture intact in vitro.

The first set of achievements—making cells clump together and differentiate to form tissue-like spheroids of up to 3 mm in diameter—was accomplished by using the bioreactor exactly as NASA envisioned. "At this point in time ... if someone wants to grow spheroids for their research, this is the best environment in which to grow spheroids," Zimmerberg says. However, the NICHD team's recent success in maintaining 2- to 3-mm fragments of lymphoid tissue in the bioreactor for up to three weeks represents a more innovative application of the device. The ultimate goal of such efforts is to develop a uniform, controlled environment in which to study various aspects of human immunodeficiency virus (HIV) infection in human lymphoid tissue.

NICHD researchers have also learned a few things about the limitations of bioreactor technology. For example, Zimmerberg states that a "big drawback" of the bioreactor is its inability to run multiple controls in the same experiment. "In a 96-well plate, you can have 96 different conditions for cells growing, whereas in the bioreactor, it's just one homogeneous volume," he says. The lab is currently trying to get around that problem by wrapping different types of tissues in separate "envelopes" of agarose before they are placed in the bioreactor.

The rewards may be substantial for those NIH research teams whose research projects happen to mesh well with the bioreactor's strengths, according to Zimmerberg. For each of the three or four intramural projects deemed to be the best tests of the bioreactor's scientific potential, the NASA-NIH center will provide funding for a three-year postdoc position. That's on top of the training and support services of two technicians and a histologist funded by NASA.



Confocal micrograph of bovine endothelial cells grown in the NASA bioreactor for 11 days. Cells attach to microcarriers and, due to the low shear forces in the bioreactor, bridge them. [Courtesy Leonid Margolis, NICHD.]

To date, eight groups of intramural scientists have tried or are attempting to use the NASA bioreactor in their projects, with varying results. On the disappointing side, NHLBI's Maurice Burg says that although the bioreactor did keep renal medullary cells alive outside of the intact kidney somewhat longer than did other techniques, it did not keep those cells "lively enough long enough" for Burg's lab to perform its desired series of experiments. But Burg says his experience shouldn't dissuade others from testing the bioreactor: "They [the bioreactor center staff] were good people to work with—they work quickly and well." Like Burg, NICHD's David Klein found that the bioreactor did little to advance his research. "We failed to get encouraging results," says Klein, who had hoped the bioreactor environment would allow pineal gland cells to proliferate more freely and respond better to challenges from stimulants such as norepinephrine than they do in standard cell-culture conditions.

Others have had a bit more luck. Using a scaled-down version of the bioreactor, NIDDK's Gary and Liliane Striker report promising early results in culturing mesangial glomerular cells that more closely resemble those found within normal kidneys than cells grown by traditional techniques. "The initial bioreactor was too large," says Liliane Striker, who says the bioreactor center helped to modify the device for her group's experimental needs.

Although he only began testing the bioreactor a couple of months ago, NCI's William Stetler-Stevenson says that so far, he's seen nothing to make him stop exploring the use of the device in his studies of tumor-cell invasion. In their experiments, Stetler-Stevenson and his colleagues are attempting to grow tumor-like spheroids using a human melanoma cell line transfected with genes encoding tissue inhibitors of metalloproteinases (TIMPs), which suppress cell invasion by inhibiting matrix metalloproteinases. The researchers hope to use those spheroids to examine how varying the expression of TIMPs affects cell adhesion and migration in culture.

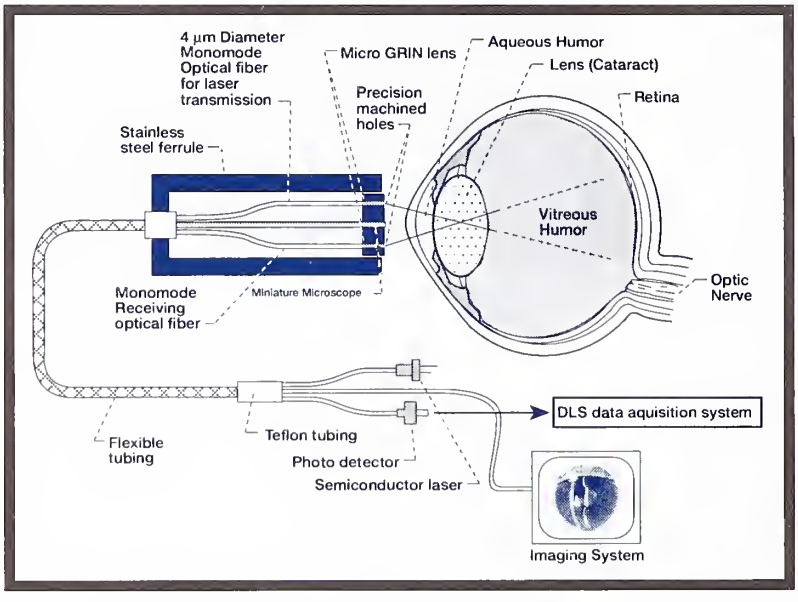
NIA's Steven Sollott is enlisting the bioreactor in his efforts to develop better in vitro models for studying cardiovascular disease. In experiments just recently started, Sollott's group is using bioreactors in attempts to culture vascular smooth muscle cells and endothelial cells in a manner that maintains the differentiation seen in the body. Under standard cell-culture techniques, such cells de-differentiate and behave differently than they do in vivo. Sollott and his colleagues are also co-culturing vascular smooth muscle cells and endothelial cells in the bioreactor in hopes that they may form structures similar to small blood vessels. "We're very excited about the potential of the bioreactor," Sollott says.

"It's hard to test out this technology simply by reading about it," says Zimmerberg, urging more intramural researchers to stop by and check out what's going on with the 18 or so NASA bioreactors the center has on hand. Although NASA naturally would like to see its brain child become a standard fixture in biomedical labs around the globe, Zimmerberg says that space agency officials have not interfered with his research and have made it clear they want the assessment of the bioreactor to be as objective as possible. "They were very excited about the idea of getting a completely unbiased evaluation," he says. "I've received nothing but support from the NASA people. ... There's no micromanagement."

Other institutions where NASA bioreactors are being or have been tested include Harvard University and the Massachusetts Institute of Technology in Cambridge, Mass., New England Deaconess Hospital in Boston, Huntington Medical Research Institutes in Pasadena, Calif., The Wistar Institute in Philadelphia, and the University of Texas Health Science Centers at San Antonio and Houston. But what NASA finds particularly appealing about the NIH center is the easy access it provides to a wide range of top-notch biomedical researchers. "What NIH brings to the table is a critical mass of scientific intellect that can be pulled together and applied to a given problem," says Neal Pellis, a project director for biotechnology at NASA's Johnson Space Center in Houston. "Within academia, you have to go to multiple universities stretched across the United States to get the same level of expertise, and you often cannot get the same level of cooperation."

Seeing Eye to Eye?

NEI's interest in another NASA device can be traced to some fortuitous page flipping. Leon Ellwein, special adviser to the NEI director, says he was thumbing through *TechReach*, a newsletter put out by the Great Lakes Industrial Technology Center in Cleveland, when an article reprinted from the *Federal Lab Consortium Newslink* grabbed his attention. The topic? A small fiber-optic probe—originally designed to measure the growth of protein crystals in experiments aboard the space shuttle—now being tested as a "compact eye diagnostics device" by Rafat Ansari at NASA's Lewis Research Center in Cleveland.

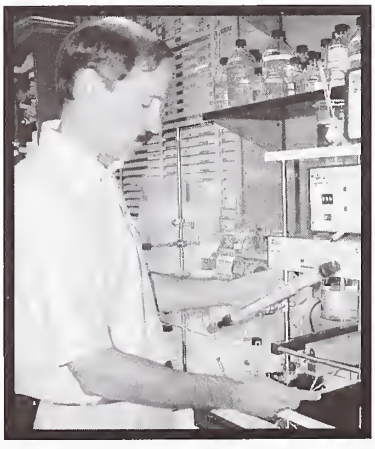


Schematic drawing of NASA's fiber-optic eye diagnostics device. [Courtesy R.R. Ansari, NASA Lewis Research Center, Cleveland.]

"I decided to call Ansari out of the blue and ask him all about it [the device], and he was reasonably aggressive on following up," says Ellwein. During his first face-to-face meeting with Ansari in Bethesda, Ellwein realized that the NASA probe might prove useful in the animal studies of potential anti-cataract drugs being conducted in NEI's Laboratory of Mechanisms of Ocular Disease. He called the head of the lab, J. Samuel Zigler Jr., over for a spur-of-the-moment discussion with the NASA scientist.

"Our realms of expertise are totally different—I know the biology and he [Ansari] knows the physics. But we may be able to find some common ground in the middle," says Zigler.

NASA's noninvasive probe, which is about the size of a pencil, consists of two optical fibers. The first fiber transmits a safe, low-power laser beam into the eye. The second fiber detects the laser's light as it is scattered within the eye and bounces back toward the probe. The resulting dynamic light-scattering data are processed by computer to generate a three-dimensional scan showing both the size and location of protein aggregates in the eye's lens. Some aggregation of proteins in the lens is part of the normal aging process, but when the clumps of proteins grow too large, they precipitate and create opaque, light-scattering centers. Such light scattering degrades the optical transparency of the lens, causing cataracts. Currently, surgical replacement of the clouded, natural lens with a plastic lens is the only treatment for cataracts, and more Medicare dollars—$3.4 billion in 1991—are used to pay for cataract surgery than for any other single procedure. There are no drugs on the market in the United States now to prevent or slow cataract formation.


Lorna Heartley

J. Samuel Zigler Jr.

Unlike the clinical collaborations Ansari has established with the Wills Eye Hospital and Drexel University in Philadelphia, Zigler envisions using the NASA probe primarily

in mouse models to screen for drugs that may halt or slow the progression of cataract formation. Preliminary results from mouse eye lenses that Zigler sent to Ansari indicate that the fiber probe can detect protein aggregations indicative of early-stage cataract formation. It remains to be seen whether the device can provide similar data when used on living animals. Currently, researchers must rely on slit-lamp examinations, which detect dark areas or shadows in normally homogenous tissue, to make relatively subjective assessments of changes within the animals' lenses. "If the difference between groups [in an anti-cataract-drug study] is small, it is difficult to establish anything with current methods," Zigler says. "This [NASA] device would be most valuable to us if it would provide an easy and reliable way to monitor changes in the lenses of the animals over time."

In addition to in vivo drug studies, Zigler says, he may try out the probe during in vitro experiments aimed at better characterizing how a molecular chaperone called alpha-crystallin acts to prevent the aggregation of denaturing proteins in the lens of healthy eyes. Ansari suggests that other possible biomedical applications of the NASA probe include detection of cholesterolosis in the eye's aqueous humor and of disorders affecting the vitreous humor.

Snails in Space

To date, the bulk of the interactions between NIH and NASA on scientific projects has been conducted through extramural programs. NIDCD and NASA have established a ground-based center for balance and vestibular research at Northwestern University Medical School in Chicago. Through the Space Tissue Loss Program, NIAMS and NASA are funding extramural projects to conduct a series of experiments aboard the space shuttle that focus on the changes in bone and muscle cells during space flight. NINDS and NASA expect to fund up to seven extramural proposals for "Neurolab" experiments that will use the space shuttle as a unique environment in which to study neurological development and function. And the list goes on.

Among the NIH scientists who have served as consultants on extramural projects is NINDS's Daniel Alkon, who says the contacts he developed through such work paved the way for scientific exchanges between his lab and the Japanese space agency, NASDA. Alkon has provided the Japanese researchers with his simple-system model of learning and memory, the snail *Hermissenda crassicornis*. For their part, the Japanese scientists are working on developing an aquaculture environment in which the snails can be trained and maintained aboard a spacecraft.

Hermissenda crassicornis
[Courtesy Daniel Alkon and Carlos Collin, NINDS]

The researchers want to use the snail system to analyze the biophysical properties of memory and visual-vestibular associative learning under microgravity conditions. Some astronauts have experienced short-term memory deficits after space travel, but the basic mechanism underlying those deficits is unknown. Before the snails go up in space aboard a U.S. space shuttle—an event likely to occur within the next five years—Alkon says several Japanese scientists will probably come to NIH to study his lab's techniques. In exchange, Alkon hopes that he can share his Japanese colleagues' technological advances in high-resolution microscopy to obtain visual images of neuronal branches at the same time that electrophysiological recordings are being made.

In addition to the scientific incentives, the growing emphasis on efficiency in government may serve to promote greater interactions between NASA and NIH's intramural community. "Collaborations will occur whenever an opportunity to cooperate and coordinate will move the science forward faster and to a higher plane and when there will be cost savings to both agencies in accomplishing mutual goals," says Snow, adding, "Both NASA and NIH scientists possess enormous expertise and dedication." ■

Seeking Postdocs? Consider PRAT

Laboratories interested in recruiting a postdoc with pharmacological or related research skills should be aware that the deadline for NIGMS's Pharmacology Research Associate (PRAT) program is Jan. 1. Projects by PRAT fellows may be in the areas of signal transduction, drug metabolism, immunopharmacology, chemistry and drug design, structural biology, endocrinology, neuroscience, and clinical pharmacology, for example. During their two-year appointments, funded by NIGMS, fellows receive competitive salaries, supplies, and travel funds to support research in their preceptors' labs. Postdocs who hope to obtain PRAT funding should apply together with a preceptor before coming to NIH, even if they plan to come earlier through other funding arrangements. Only U.S. citizens or permanent residents are eligible. Any tenured NIH scientist may identify a PRAT candidate and apply to become a PRAT preceptor. To receive a 1995-96 PRAT Fact Sheet, contact the PRAT program assistant (phone: 594-3583; e-mail: prat@gm1.nigms.nih.gov). ■

NIH Collaborators Receive Eastern Bloc Grants

Five researchers who are collaborating with NIH intramural scientists are among the 90 scientists in Eastern Europe and the former Soviet Union chosen to receive a new type of grant from the Howard Hughes Medical Institute (HHMI), based in Bethesda. The grants range from $22,000 to $35,000 annually for five years, and 60 of the grants also provide $2,500 to $3,500 per year for collaborating scientists.

Acknowledging that the grants are small by U.S. standards, HHMI President Purnell Choppin says he thinks that the money will still go a long way toward strengthening international scientific ties and helping researchers in former Eastern Bloc nations to modernize their labs and undertake new experiments. The funds can be used to pay for salaries, travel, supplies, equipment, computer and communication services, and journals.

Selected from more than 2,000 applicants, the grant recipients with collaborative links to NIH intramural scientists are László Hunyady and András Lipták of Hungary, Mariusz Jaskólski of Poland, and Sergei Nedospasov and Dmitry Anatoly Gordenin of Russia.

Hunyady is an associate professor in the physiology department of Semmelweis University of Medicine in Budapest. In conjunction with NICHD's Kevin Catt, Hunyady is examining how the receptor for angiotensin, a polypeptide in the blood, generates signals and is regulated. Lipták, a professor of biochemistry at the L. Kossuth University in Debrecen, Hungary, is working with NICHD's Vince Pozsgay to develop a vaccine against *Shigella sonnei*, a parasite that causes dysentery. Jaskólski, who is an associate professor at the Institute of Bioorganic Chemistry in Poznan, Poland, collaborates with Alexander Wlodawer of NCI's Frederick Cancer Research and Development Center (FCRDC) on studying the structure of certain enzymes useful for treating leukemia.

Nedospasov is working with Nancy Rice, also of NCI-FCRDC, on how the transcription factor NF-κB/Rel regulates gene expression by interacting with DNA. Nedospasov is the head of the cytokine molecular biology unit at the V.A. Engelhardt Institute of Molecular Biology in Moscow. Together with NIEHS's Michael Resnick, Gordenin is exploring the genetic consequences of inverted DNA repeats, which are common in many organisms. Gordenin is a leading research fellow in the physiological genetics laboratory at St. Petersburg State University in Russia.

"We were very impressed with the quality of their [the grant recipients'] research, especially since so many of them are working under extremely difficult conditions," HHMI's Choppin says.

–Anne Blank, NICHD, and Lorna Heartley

Hot Methods
continued from page 13.

Staining Cell-Surface, Intracellular, and Intranuclear Antigens

1. Place 5–20 mL of PE-conjugated monoclonal antibody for the detection of the antigen of interest (e.g., CD3 for T cells) into labeled, flow-cytometer-compatible tubes. Add 150 mL PBA and 100 mL washed cells. Incubate for 30 min at room temperature in the dark.
2. Add 2 mL 1x Ortho PermaFix* to each tube, vortex for a second or two, and incubate for 40 min at room temperature in the dark.
3. Centrifuge for 8 min at 1400–1600 rpm in a swinging-bucket rotor centrifuge at 4–6 °C. Aspirate off the supernatant and vortex the pellet thoroughly.
4. If specimen is whole blood or bone marrow, lyse the red blood cells by adding 2 mL PBS, vortexing thoroughly, and incubating at room temperature for 10 min in the dark. Vortex again and centrifuge for 8 min at 1400–1600 rpm in a swinging-bucket rotor centrifuge at 4–6 °C. Decant supernatant and vortex gently.
5. Add 20 mL appropriately diluted antibody [e.g. TdT or myeloperoxidase (MPO)] to tubes and vortex lightly. Incubate in an ice bath for 1 h in the dark.
6. Wash cells by adding 2 mL PBA, centrifuging at 1400–1600 rpm in a swinging-bucket rotor centrifuge at 4–6 °C, and decanting supernatant.
7. Resuspend pellet in 0.5 mL 1% paraformaldehyde in isotonic saline, pH 7.4. Analyze samples on flow cytometer within 24 hours. Other permeabilization reagents can be used, but method must be optimized for each reagent.

Surface-Antigen and DNA-Content Detection

1. For whole blood and bone marrow, begin by separating mononuclear cells by density gradient (e.g., by using Ficoll Hypaque). Wash this cell suspension or others, such as cells from a cultured cell line or cells teased from lymph node, with PBS prior to staining.
2. Place 100 mL PBS in labeled, flow-cytometer-compatible tubes and add an appropriate amount of FITC-conjugated antibody for detection of the surface antigen of choice. Add one million to two million cells in 100 mL. Incubate in the dark at 4–6 °C for 30 min.
3. Wash by adding 4 mL cold PBS (4–6°C), centrifuging at 1200 rpm in swinging-bucket rotor centrifuge at 4–6 °C for 10 min, decanting supernatant, and resuspending pellet by agitating tube or light vortexing. Repeat wash.
4. Add to resuspended cells 1 mL 70% cold (4–6 °C) ethanol per 1 x 10^6 cells while vortexing gently. Incubate in the dark overnight at 4–6 °C to fix cells.
5. Wash by adding 4 mL cold PBS (4–6 °C), centrifuging at 1600 rpm in swinging-bucket rotor centrifuge at 4–6 °C for 5 min, decanting supernatant, and resuspending pellet by agitating tube or by light vortexing.
6. Add 500 mL PI/RNase solution (50 mg/mL PI, 200 units RNase/mL in PBS). Incubate 45 min to 1 h at room temperature. Analyze samples on flow cytometer within 4 h.

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A FARE Deal Fellows Research Award

Postdoctoral and clinical fellows should start fine-tuning their abstracts now for the second annual Fellows Award for Research Excellence (FARE) competition. Last year, about 450 fellows applied and 30 were chosen to receive the merit-based awards, which provide up to $1,000 toward travel expenses to a domestic scientific meeting. The deadline for submitting applications for this year's FARE awards is Dec. 15, and the winners will be announced in February. All postdoctoral and clinical fellows—including foreign and visiting fellows—are invited to apply for the awards, which are based on a peer review of submitted abstracts. Application forms and further information are available from each ICD's representative on the NIH Fellows Committee and from the Office of Science Education (phone: 496-3887). ■

Tapping the Talents of "Re-entry" Scientists

The research community is encouraged to attend a special workshop this fall showcasing the scientific achievements of participants in the Offiice of Research on Women's Health's (ORWH's) Re-entry Program. The 1 1/2-day workshop, entitled "Re-Entry Into Biomedical Research Careers," will take place Nov. 13 from 8:30 a.m to 5:00 p.m. and Nov. 14 from 8:30 a.m to 12:00 p.m. at the Natcher Building Conference Center.

The workshop's first day will feature keynote speakers, scientific presentations, and poster sessions by scientists who were awarded grants through ORWH's Re-entry Program. The second day will focus on career options, NIH resources, and mentoring and networking to facilitate productive science careers. Issues, concerns, and experiences of principal investigators and mentors will also be discussed. The workshop, sponsored by ORWH, is free and open to the public. For more information, contact Joyce Rudick (phone: 402-1770).

ORWH has established two pilot programs—intramural and extramural—to encourage fully trained women and men to resume active research careers after taking a break to meet family demands. Since 1992, the re-entry program for intramural scientists, which was developed in conjunction with the Office of Education, has supported the placement of three scientists, two at NCI and one in NINDS. The re-entry program for extramural scientists has supported the placement of 26 researchers over those same three years. ■

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The Flow Cytometry Consortium Web Page:
http://www.cber.nih.gov/welcome.html ■

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Catalytic Reactions

In this issue, we are asking for your reactions in four areas: safety and security, NCI's changes, tips for our Hot Methods Clinic, and postdoc concerns. **Send your responses on these topics or comments on other intramural research concerns to us via e-mail: catalyst@od1em1.od.nih.gov; fax: 402-4303; or mail: Building 1, Room 334.**

In Future Issues. . .

- Postdoc Life: When Dreams And Reality Collide
- Linking Scientific Devices to Computers
- Cultural Crossroads, The NIH Experience
- Chromosome Mapping: Stretching the DNA

1) What do you think poses the greatest health or safety risk to NIH staff? What specific suggestions do you have for improving safety and security at NIH?

2) What is your reaction to the changes under way in NCI's intramural program? What advice would you give the institute's new director?

3) Do you have any suggestions or comments about the flow cytometry techniques featured in this issue's Hot Methods Clinic? What methods would you like to see covered in future issues?

4) We plan to devote our next issue to postdoc concerns, so now is the time for postdocs and their mentors to fire away. What do you think is the biggest challenge facing postdocs at NIH today? What can be done to improve the postdoc experience? And, postdocs, what are your pet peeves about life at NIH?

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 334, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; e-mail: catalyst@od1em1.od.nih.gov

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