FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

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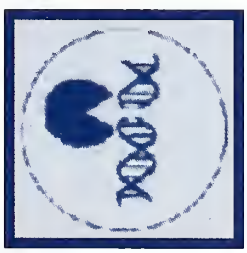
Research Festivities

CELL SOLILOQUY: TO DIE OR NOT TO DIE

by Cynthia Delgado

When confronted by damaged DNA, cells can either set about repairing themselves or commit suicide through programmed cell death. How the cell "decides" which path it will follow was explored during one of the NIH Research Festival's mini-symposiums—"Survival or Death: DNA Repair or Apoptosis." Chaired by Vilhelm Bohr, chief of the NIA Laboratory of Molecular Genetics, and Curtis Harris, chief of the NCI Laboratory of Human Carcinogenesis, the symposium focused on the pathways and players in these life-and-death cellular processes, as elucidated in ongoing studies in NIH labs.

One of the key players in DNA repair is DNA polymerase beta (β pol), which is instrumental in each of two basic excision repair (BER) pathways—single nucleotide and long-patch—and whose absence leads to "drastic" consequences, according to Rob Sobol, a research fellow in the NIEHS Laboratory of Structural Biology. Sobol presented findings from β pol knockout studies, conducted with NIEHS Deputy Director Samuel Wilson, chief of the DNA Repair and Nucleic Acid Enzymology Workgroup, that showed that "β pol is required for protection against the induction of cytotoxic hypersensitivity, apoptosis,



A view of cell death from the Apoptosis Interest Group web site

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Shalala Launches Search for Successor

VARMUS TO DEPART NIH AT YEAR'S END; COLLEAGUES LAUD LEGACY OF RESEARCH EXCELLENCE

by Celia Hooper

Late in 1996—in what he defined as a midterm interview with *The NIH Catalyst*—NIH Director Harold Varmus foreshadowed his exit from NIH with the frank declaration that he thought six years was the optimal term for an NIH director. On October 7 this year, Varmus turned the theoretical pronouncement into actuality, announcing that he would leave NIH by the end of December to head the Memorial Sloan-Kettering Cancer Center in New York.

Ernie Branson

In Good Spirits: *Minutes before the town meeting here October 12, HHS Secretary Donna Shalala (left) spoke with institute and scientific directors about the pending departure of Harold Varmus (middle) from the NIH directorship and the appointment of Deputy Director Ruth Kirschstein (right) as acting director during the search for a successor.*

Three for the Road

There were three rationales behind Varmus' late 1996 statement to *The Catalyst* (January-February 1997, page 1). His main concern was the politicization of the selection of NIH directors. "I see myself as unlinked to the electoral process," he said. "I don't believe the NIH directorship should be as politicized as it's been the past eight years or so. I didn't come in with the election . . . and my expectation is that I'll probably leave the position before the second administration is over, which would give the president a chance to name someone else who'd also span the electoral events and would disconnect the NIH nomination process from the electoral process."

Visiting the NIH campus five days after Varmus' announcement, his boss, Donna Shalala, secretary of Health and Human Services, made it clear she was sympathetic with Varmus' view. At a town meeting in Masur Auditorium, the secretary started out by saying that she believed the appointment of Varmus to lead NIH during this outstanding era of progress in biomedical research would prove to be a great legacy of the Clinton administration. "NIH ought to be seen as an extraordinary institution and, thus, politics should be left out of the process" of selecting a new director, Shalala said.

"Whether or not that is possible de-

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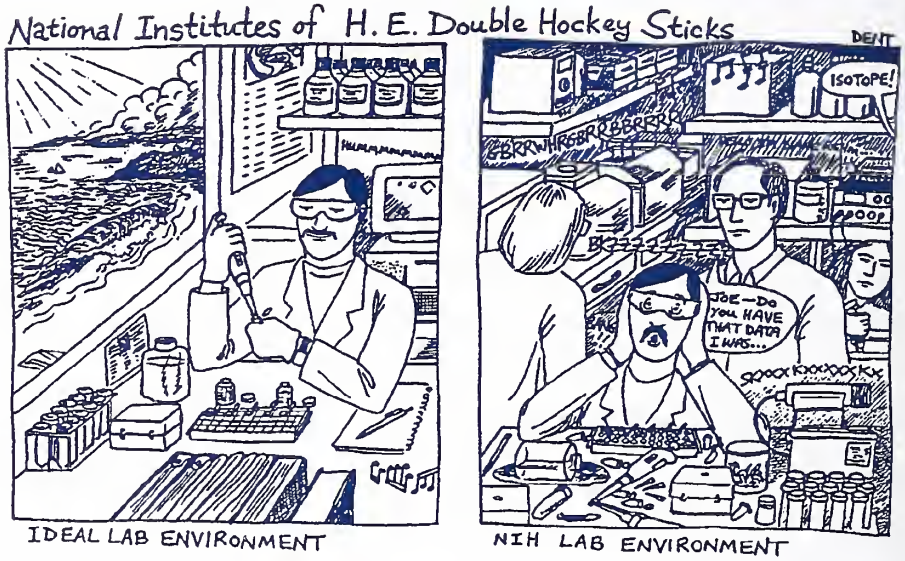
SPACE: THE FINAL FRONTIER

Michael Gottesman

Space is always on everyone's mind at the NIH. Lab directors want more space so they can expand their research programs. Postdocs want more space just so they can stretch out their arms and legs and not knock something over. We need safer, more efficiently designed space to maximize our productivity as scientists. My modest proposal in this essay is that as we develop new space at NIH we use it in equal measure to increase the space per working scientist and to provide space—at a reduced density—to support important new scientific programs.



In the next few years, the NIH Master Plan includes construction of a new Vaccine Research Center (VRC), with completion expected next summer; a new consolidated laboratory building (Building 50, the Louis Stokes Laboratories), slated for completion at the end of 2000; and a new Clinical Research Center (CRC), with anticipated lab occupancy in December 2002. And there are other—albeit not quite so dramatic—enhancements scheduled.

The intent of the CRC and Building 50 is to replace existing, outmoded laboratories and clinics with newly designed space that is safer, more esthetically pleasing, and easier to maintain and provides more elbow room per person than currently exists. The VRC will house a new program—HIV vaccine development—that is central to the NIH mission to improve the public health. As we assign people to these spaces, we will decompress existing programs by approximately 30 percent, giving each person more room. Altered laboratory design will make the corridors wider, the desks bigger, and lab bench footage longer. The net result is a safer, more user-friendly environment.

The temptation will always be there to bring in "just one more" person or large piece of equipment, but should each lab succumb to this temptation, we would, inevitably, find our collective selves again in too tight a spot. It is sheer myth that a critical mass of people in a lab leads to explosive results; the reality is that overcrowded labs frequently are not fully functional. We pay the price of crowding with irritable, less efficient, and less creative scientists and an increased risk of accidents. Former NIH postdoc Alex Dent made the point most eloquently in two *Catalyst* cartoons (one in 1995, the other in 1996), here reprised.

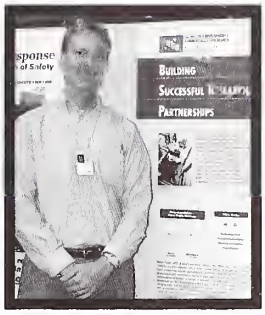
As an institution, let's resolve to be less shortsighted . . . . and less dense!

As usual, I welcome your comments.

—Michael Gottesman

TDCB: Linking NIH and Industry Labs

Collaboration between NIH scientists and non-NIH laboratories and organizations—to conduct research together or share research materials—often involves complex intellectual property issues and invariably requires negotiating agreements best handled by a separate, specially constituted body. At NIH, the Technology Development and Commercialization Branch (TDCB) is such an entity.



Fran Pollner

Tom Stackhouse, TDCB tech development and patent specialist

Housed within NCI but designated as a Competitive Service Center, the TDCB provides such technology transfer services for NCI and nine other NIH institutes (see box). Its work is called "technology transfer" because it aids the transfer to private industry of technologies or discoveries made by federal scientists that are ripe for further development, commercialization, and, ultimately, distribution to the public.

Collaborative research, involving the exchange of information and resources between NIH and industry, is often part of the technology transfer process and is the type of activity TDCB supports. Collaborative research between the federal government and industry is effected by any of several technology transfer agreements—such as a **Cooperative Research and Development Agreement (CRADA)**—and often leads to inventions that are patented and licensed. The actual patenting and licensing of NIH inventions are managed by NIH's Office of Technology Transfer (OTT).

Benefits of CRADA participation for NIH investigators include access to additional laboratory resources and the opportunity to work with industry scientists and to influence the direction of research that is heading toward commercialization.

An example of a successful CRADA is the collaboration between NCI and Bristol-Myers Squibb in the development of Taxol (paclitaxel), one the most active anticancer agents discovered in the past two decades. By the CRADA's end, three inventions had been patented and licensed—and more than 29,000 patients had been treated. Taxol has been approved for five indications, including refractory ovarian and breast cancers.

The CRADA is but one type of agreement. Others used to facilitate collaborative research include:

—Material Transfer Agreement (MTA): to obtain (or provide) research materials, such as cell lines, cDNAs, and new pharmaceutical compositions, from outside organizations

—Clinical Trial Agreement (CTA): to transfer of research materials from outside organizations for use in NCI-sponsored clinical trials

—Confidential Disclosure Agreement (CDA): to effect the free exchange of confidential research information with an outside party while ensuring control over public disclosure

In addition to negotiating various types of collaborative agreements, TDCB is using market research, the World Wide Web, publications, and exhibiting at conferences and professional meetings to inform potential research partners in the pharmaceutical and biotechnology industries of NIH scientists' discoveries and opportunities for joint research projects.

Closer to home, TDCB also reviews employee invention reports and makes recommendations to OTT concerning filing of domestic and foreign patent applications.

—Beth Andrews and Joanne Muskett
TDCB, NCI

TDCB Roster

The TDCB is a designated Competitive Service Center (CSC) for technology transfer and offers full technology transfer services ranging from consultation to managing all IC technology development projects. Currently, the TDCB serves NCI and nine other client institutes:

—NEI
—NIA
—NIAAA
—NICHD
—NIDA
—NIDCR
—NIH CC
—NLM
—CIT

For ICs not listed above, TDCB can direct interested parties to the appropriate technology development coordinator. For more information about technology transfer or TDCB's services, call 496- 0477, or visit **<http://tdcb.nci.nih.gov>**.

Spiegel Takes Over As NIDDK Director

Allen Spiegel

Allen Spiegel ascended to the NIDDK directorship November 15, succeeding outgoing director Philip Gorden. Spiegel has been NIDDK scientific director for the past nine years and chief of the Metabolic Diseases Branch. ■

Hispanic Scientists Directory

The Hispanic/Latino Scientists Directory —including senior scientists, postdoctoral and clinical fellows, pre-IRTAS, and other interested personnel—is now available through the FELCOM website at **<ftp://helix.nih.gov/felcom/index.html>**.

Organized by the NIH Fellows Committee with the support of the Office of Education, the directory is designed to facilitate interaction among Hispanic/Latino Scientists at NIH and FDA-CBER.

To be included in this directory and for further information, contact Nancy Vázquez-Maldonado at **<vazquez@cber.fda.gov>**.

Free Counseling

NIH's Work and Family Life Center offers free dependent-care counseling services —focusing on childcare and elder care—to NIH employees. Call the Center at 301-435-1619 between 9:00 am and 5:00 pm Monday through Friday, or leave a message at other times. ■

Varmus To Depart NIH
continued from page 1

pends on whether there is the political will in Congress to do that and on the ability to streamline the selection process," she observed. She said that she had already initiated conversations about the search with political leaders, was ready to put together a search committee, and would take an active role in the recruitment herself. "I feel very strongly that there should be a seamlessness in the NIH leadership." Asked if she felt that the needed political will was indeed present in Congress, Shalala told *The Catalyst*, "I have some indication that the political will exists" to find a replacement for Varmus before the election in November 2000.

Ernie Branson

HHS Secretary Donna Shalala at NIH town meeting

Ernie Branson

NIH Director Harold Varmus says it's so

Roses

Arguably one of the most widely respected directors in NIH history, Varmus is not perceived as having grown stale—the second reason he cited in 1996 for a limited stand by an NIH director: "You can do a lot of things in six years, but beyond that you probably start to get stale."

"Au contraire," in the case of Varmus, say NIH leaders. NIMH director Steven Hyman says "Harold Varmus has been a spectacular leader because he has invariably put the needs of science ahead of politics and has expressed his values with great clarity and force." Hyman sees Varmus' prime achievement as creating "a superb and collegial atmosphere in which to work. There were no hidden agendas—the only agenda was to make it possible to perform and to fund the most worthy science."

NIAID Director Tony Fauci concurs. "Harold Varmus has raised the bar of excellence among scientific leaders. He clearly has been an absolutely outstanding NIH director. His policies and actions are driven by a passion for science superimposed on a prodigious intellect."

In addition to expressing his enjoyment in working with Varmus, Fauci lauded Varmus' cultivation of "an atmosphere in which nothing short of the highest level of scholarship is acceptable."

Varmus' third rationale for a six-year term limit for NIH directors was the potential for faux pas and accumulation of enemies over a longer haul. Some years of experience give a director time to win supporters and forge alliances, he observed in late '96. "On the other hand, the longer you're here, the more likely you are to have some major screw-up. I've been lucky so far to have avoided major potholes, and I think my credibility is pretty good."

Thorns

If Varmus lived in fear of potholes in the second half of his tenure at NIH, it didn't show. When he perceived science to be at stake, he stood his ground in its defense. One example was sticking his neck out for universal online access to the scientific literature, despite heavy opposition from some scientific publishers (see *The NIH Catalyst*, July-August 1999, page 1).

In January 2000, a revised version of the E-biomed concept will go online as "PubMed Central" and will begin receiving, storing, and distributing content—including peer-reviewed articles, preprints, and other screened reports from existing journals, new journals, and reputable scientific organizations that have agreed to participate.

In another skirmish—the quest for degree-granting authority for an NIH graduate program—Varmus opted to retrench when faced with opposition from a few members of his Advisory Committee. A revised plan calls instead for improving and expanding opportunities for graduate students to be trained at NIH and receive diplomas from partner institutions.

Another difficult issue—human embryonic stem cell research—was still looming as *The Catalyst* went to press. Earlier this year, Varmus said that research using human pluripotent stem cells was not illegal, according to an opinion by HHS attorneys. Given its potential, the work should be supported by federal funds and in accordance with federal guidelines, he said. Such work now remains verboten pending finalization of the guidelines and oversight procedures.

'In the six years that I have been privileged to serve . . .'

Among many other firsts, Harold Varmus will enter the history books as the first NIH Director ever to send a personal thank-you note via e-mail to all NIHers to tell them of his departure. Here is the text of his message:

October 7, 1999
To members of the NIH staff:

Today I have written to President Clinton to inform him that I will be leaving NIH at the end of this year to head the Memorial Sloan-Kettering Cancer Center in New York City.

In the six years that I have been privileged to serve as Director of the NIH, I have had many pleasures, and most of them have depended on the extraordinary qualities of the people who work here. Naturally, I feel a special debt to the people I have worked with most closely—the Directors of the Institutes and Centers, the members of my senior staff in the Office of the Director, and the intramural scientists who have interacted with my laboratory group. But the spirit of commitment to the goals and standards of this remarkable agency is everywhere apparent and has given me great gratification throughout my time here.

Secretary Shalala and I are working to insure an orderly transition in the weeks ahead and will keep you informed of plans as they develop.

With sincere thanks for your help during the past six years,

Harold Varmus

The Road Ahead

For many of Varmus' admirers, it is exactly such quagmires that now make him almost indispensable. Contrary to Varmus' midterm prediction, his six years are not seen by others as a time of accumulating mistakes but of increasingly assured defense of NIH's core values. Says NHGRI director Francis Collins, Varmus "has made good science the essential currency for all discussions about NIH's present and future. He has inspired an unprecedented level of trust and respect from the leadership of the Congress and the Administration."

Looking back at some of the matters Varmus tackled earlier in his tenure, Collins says the NIH director "has been brilliant in his methods of addressing difficult problems—from mouse genomics to research tools and intellectual property—by convincing the best and brightest of this generation of scientists and policy experts to devote their most determined energies to helping solve the problems at hand. . . . He has never sacrificed ideals and truth for expediency, even if it involved bucking the tide," Collins says.

Offering promise for the future, Collins says, is that Varmus "has recruited a superb cadre of NIH scientific leadership (I can say that because I'm not one of them—I was already here!) who will continue his legacy for a long time to come."

NICHD director Duane Alexander says that the combination of gifts Varmus has brought to NIH over his short tenure here were responsible for one of the most conspicuous and enviable advances NIH has made in the past couple of years—its budget increases. "Dr. Varmus has had an enormous impact on NIH in a relatively short time. He has markedly increased the cooperation and collaboration among institutes, to the benefit of everyone," says Alexander.

"His presence here and the atmosphere he has created have made it easier for all of us to recruit top-notch scientists to NIH, both intramurally and extramurally. His personal interactions with the Congress and the sense of confidence in this institution he has inspired have been major factors in the remarkable increases in funding that have come to NIH."

Filling Varmus' shoes is just the beginning for Shalala's search committee. Finding someone whose appointment at the end of a Democratic administration could be confirmed by a Republican Congress will surely be tougher. And persuading someone to accept the position under these tenuous conditions may be the ultimate challenge. In the meantime, Shalala has named NIH Deputy Director Ruth Kirschstein to be acting director until a permanent replacement is found [see box]. "We will give Ruth all the support she needs," Shalala promised the crowd at the town meeting. "This [NIH] is the crown jewel of my empire." ■

In Good Hands

Ruth Kirschstein

Until NIH has a new permanent director, it will be back in the familiar hands of Ruth Kirschstein—one of NIH's most experienced leaders. She has served as deputy director since 1993 and was also acting director of NIH for five months before Varmus arrived in 1993.

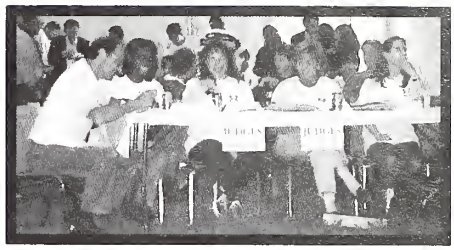
Kirschstein holds an M.D. from Tulane University School of Medicine (New Orleans). She first came to NIH in 1956; from 1957 to 1972, she did vaccine safety research at the Division of Biologics Standards (now the Center for Biologics Evaluation and Research) of the FDA. She headed NIGMS from 1974 to 1993 and served as the acting associate director of the Office of Research on Women's Health when it was first established.

Kirschstein is the author of more than 70 scientific publications and is a member of the Institute of Medicine and the American Academy of Arts and Sciences. In 1985 and 1995, she received the highest honor for career civil servants: the Presidential Rank Award for Distinguished Executives.

—*CH*

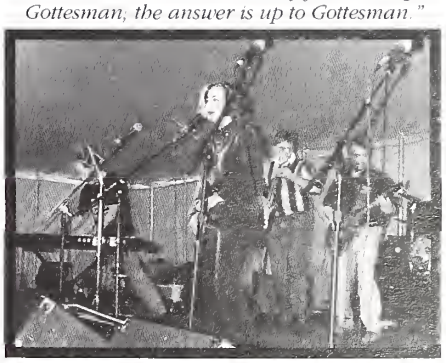
NOTE: The January-February 2000 issue of *The NIH Catalyst* will feature a farewell interview with Harold Varmus.

Grand Finale



Day of Judgment: *At his last NIH Research Festival attended as NIH director—and rendering possibly his heaviest decision—Harold Varmus teamed up with four other music masters to adjudicate the "battle of the bands." The historic return match pitting NIH's own "The Directors" against Johns Hopkins' (mostly) "Wild Type" was the bands' first joint appearance since the legendary play-off at the National Academy of Sciences in December 1997, when the people's award went to "Wild Type." Demanding a rematch in which they would be judged by NIH partisans, "The Directors" roared into second place—and a good time was had by all.—FP*

The Directors *(left to right): NIAMS' Steve Katz, NCI's Rick Klausner, NHGRI's Francis Collins, and NIAMS researcher John O'Shea gave a new twist to "Blowin' in the Wind," in which they capped the question of "how many years will it take . . . ?" with "the answer, my friend, is up to Gottesman; the answer is up to Gottesman."*



Wild Type: *Johns Hopkins cancer geneticist Bert Vogelstein (left) gets up to get down on keyboard, while Ellie Carson-Walter, vocalist and postdoc in his lab, belts out a tune and Hopkins postdoc Chris Torrance and Pat Morin (formerly Hopkins, now NIA) let loose on their strings.*

The Chemistry Of Bernhard Witkop

by Fran Pollner

How are organic synthesis, X-ray analysis, photo-excitation, alkaloids, catecholamines, frog venoms, protein cleavage, DNA-breakdown, metabolic enzymes, receptors, ion channels, and the Black Forest near Freiburg related? The answer is through Dr. Bernhard Witkop . . . creative and insightful investigator and mentor who has greatly extended the scope of organic chemical approaches to the fascinating frontier of biological and medical sciences."

—Yuichi Kanaoka*, October 1998

In a small back room at the end of a hallway on the second floor of Building 8, there sits a man with a typewriter. He is surrounded by floor-to-ceiling archives documenting his scientific journey over six decades of research and mentoring and his place in the evolution of his field. The art on the walls substitutes for windows.

A person might feel crowded in this room, but Bernhard Witkop feels at home. Scientist emeritus and NIH scholar since he stepped away from his post as chief of the NIDDK Laboratory of Chemistry—his niche here from 1957 to 1987—Witkop continues chronicling the discoveries and characters of his role models, colleagues, and students—the chemistry, if you will, of twentieth-century science.

A homemade poster spans most of one wall in his office. It's a branching tree of the "Roots of Biochemistry in Chemistry," on which he occupies a line in a list of names he has connected to Heinrich Wieland (1877–1957). Wieland was his mentor at the University of Munich when Witkop was a student and the person he credits with having generated his interest in oxidative mechanisms, natural products, and highly active toxins. Wieland also shielded Witkop from the Nazis, an aspect of the unfolding of the field of chemistry not visible in the poster. That Wieland captured a Nobel Prize, however, is recorded—he is one of many winners of that prize with more than a passing connection to Bernhard Witkop.

Witkop has traveled in the rarefied circles of scientists whose works, like his own, define or redefine a discipline. As often as he has been the recipient of scientific honors, he has also been called upon to memorialize the lives of scientists—a task to which he is well suited by dint of his facility with language (he knows Latin, French, Italian, Japanese, German, English, and the Swiss-German Allemanic dialect), appreciation of the accomplishments and intellect of his peers, and sense of history. Only last spring, he was elected into the rather exclusive American Philosophical Society (APS), the Biological Sciences cohort of which contains perhaps 50 individuals, compared with the 3,000 on the prestigious National Academy of Sciences roster, to which he was elected in 1969. His writings regularly appear in *APS Proceedings,* and the December 1999 issue features his paeon to "Paul Ehrlich and His Magic Bullets—Revisited."

Fran Pollner

Socratic Sandals and Winning Ways: *Witkop calls his election this year to the American Philosophical Society a "rare honor."*

Self-Appraisal

Witkop counts among his most worthy deeds his initiation of a program over 40 years ago that opened the doors of NIH to visiting scientists and paved the way for the ensuing thousands of visiting and Fogarty Scholars in Residence—an achievement recognized October 10 at a 50th anniversary celebration of Israel's Weizmann Institute of Science. The Weizmann Institute honored Witkop for having "urge(d) the authorities" at NIH in the late 1950s to launch a program that trained many of Israel's outstanding scientists, including a former president of the Weizmann Institute. It was DeWitt Stetten (then scientific director of the arthritis institute) and himself, Witkop recalls, who "had to work very hard to convince the then-director of intramural research at NIH to introduce something that would go beyond the rigidity of the Civil Service System and allow us the freedom to hire foreign scientists. That was a tremendous advance," Witkop says, noting that the greatest number of visiting scientists he personally mentored came from Japan. Many of these individuals rose to the highest levels of scientific prominence in academic and industrial spheres in Japan. In 1975, the emperor conferred upon Witkop the Kun-Ni-To, or Order of the Sacred Treasure, honoring the exchange of science and scientists between Japan and NIH.

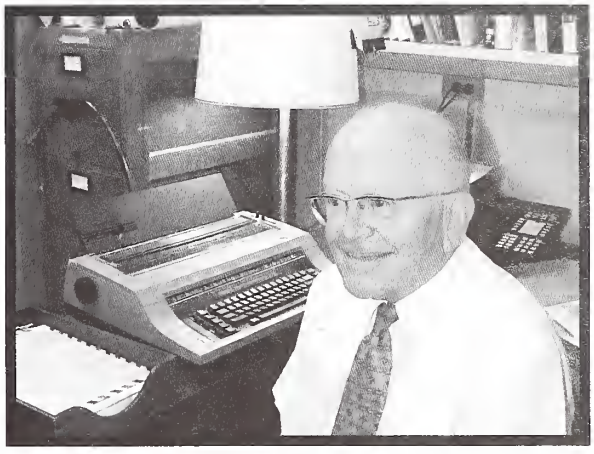
Mastering the Japanese language sufficiently to talk highly technical shop with his Japanese students and colleagues and to deliver lectures in Japan in the native tongue of his audience is another achievement of which Witkop is most proud. It was the arrival of his first Japanese postdoctoral fellow in 1957 that prompted him "at an age over 40 when, I thought, your gray matter is no longer able to handle a new difficult language" to learn Japanese.

*Yuichi Kanaoka was one of Bernhard Witkop's first students from Japan under an NIH visiting scientist program Witkop was instrumental in launching. Kanaoka went on to become president of the Japanese Pharmaceutical Society and in 1998 wrote these words in the "Preface" to the 1998 volume of *Heterocycles*, an international journal for reviews and communications in heterocyclic chemistry, published by the Japan Institute of Heterocyclic Chemistry. With contributions from nearly 300 scientists the world over, the volume was dedicated to Witkop on the occasion of his 80th birthday. John Daly, his "long-time colleague" and successor to his lab chiefdom, wrote the introductory chapter.

And it was 1961 when he delivered his first lecture in classical Japanese at meetings in Tokyo and other cities in Japan. The lecture was entitled "Protein Accountants and Protein Auditors" and elucidated the method by which Witkop and his collaborator Erhard Gross corrected a mistake in the sequence of ribonuclease that had just been established by a triumvirate of scientists who later received the Nobel Prize for that work. One of the three, Christian Anfinsen, Witkop recalls, "was at NIH when he did (the ribonuclease-sequencing work), and he got the Nobel Prize for it, together with Stanford Moore and William Stein."

The findings of Anfinsen and the correction of the sequence by Witkop and Gross ("Nonenzymatic Cleavage of Peptide Bonds: The Methionine Residues in Bovine Pancreatic Ribonuclease") were published simultaneously and "amicably" in the June 1962 issue of the *Journal of Biological Chemistry*, Witkop recounts. Witkop considers a review paper on this subject—"Chemical Cleavage of Proteins" (*Science* **162**: 318-326, 1968)—as his "star paper," for which he has received well over 1,000 requests.

Around the time Anfinsen received the Nobel, Witkop received the Paul Karrer Medal (named for the 1937 chemistry laureate) in 1971 for the discovery and development of the cyanogen bromide method to cleave large proteins that had enabled him to "audit" the ribonuclease sequencing and that has been applied to the genetic synthesis of hormones, allowing, for instance, the synthesis of the genes for the A and B chains of human insulin.



Fran Pollner

No Electronics, No Fluorescence: *"The computer is a mixed blessing. I use a typewriter, and I write my manuscripts in longhand, striking out and replacing. I lose touch with my words on a computer," Witkop says, sitting at his scholar's desk at NIH.*

Pre-NIH History

Bernhard Witkop was born in 1917 in Freiburg, Germany, near the foothills of the Black Forest. His father was a professor of German literature, who counted Thomas Mann, a Nobelist in literature, among his friends. He died in 1942 of natural causes, Witkop says. Witkop's mother, however, who was Jewish, had to flee to Holland and his siblings to Switzerland and Venezuela. Witkop recounts his own experiences as a student during the Nazi era in a chapter devoted to "personal recollections" in the mammoth multivolumed *A History of Biochemistry* (Volume 38, Chapter 3: "Stepping Stones—Building Bridges," published in 1995 by Elsevier).

Fran Pollner

A Wide Embrace: *On the wall behind him are pieces of the cultures and honors that have become a part of Witkop over the years. The hanging to the left is a water color by Munio Kotake, a leading organic chemist in Japan, whose life and contributions Witkop has memorialized; in the center is a framed award from the American Chemical Society; and at right is a gift from a Taiwanese student, a hanging in which the yin-yang symbol is encircled by the 64 hexagrams of the* I Ching, *or* Book of Changes, *the ancient Chinese system of divination. Witkop finds it "amazing" not only that the 64 hexagrams exist at all but also that they are the same number as the 64 triplets of the genetic code. Among his many explorations these days, Witkop is comparing and correlating explanations of the hexagrams with the stop codons of the genetic code.*

In the fall of 1935, Witkop wrote, he started his first semester at the University of Munich, where on September 30, 1938, he watched a motorcade including British Prime Minister Neville Chamberlain roll through the streets of the city that would become a "symbol of appeasement and betrayal."

In this chapter, too, are accounts of Witkop's contact with chemistry Nobelist Richard Wilstätter, who was a friend of Witkop's mother's family and who resigned his University of Munich post in protest over budding anti-Semitism, and of the flight of Paul Ehrlich's widow, Hedwig, the morning after Kristallnacht in November 1938.

Witkop remained at the University of Munich, working toward his degree in chemistry in the sheltered laboratory of Wieland, one of Europe's outstanding chemists and a winner of the 1927 Nobel Prize for his work on the structure of bile acids. Wieland managed to ferry his ship through the rough waters of World War II.

Wieland, Witkop says of his mentor, "was one of the great pioneers in organic chemistry and a cofounder of modern biochemistry. And he was an upright man, fighting for his convictions, a true professor—professing his repugnance of the Nazis. Under his tutelage, the quality of science, in spite of the tyranny of the Nazis, did not suffer too much."

In 1940, Witkop completed his Ph.D. thesis on *Amanita phalloides*, the most poisonous mushroom in Europe. It was his dream, then, to go to Harvard, a dream that had to be deferred until 1947. "You could not leave the country during the war, but I did a lot of science. I worked on alkaloids and a very important reaction—a one-step synthesis of a degradation product, a metabolite of tryptophan called kynurenine," he recalls. (Tryptophan was to be a continuing research theme; see "Forty Years of Trypto-fun," *Heterocycles* **20**:2059-2075, 1983). After securing his Harvard fellowship, he struck up what was to become a lasting collegial friendship with future

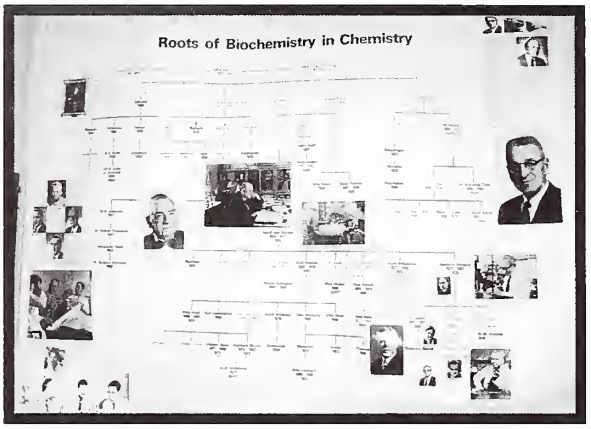
Nobelist Robert Burns Woodward. After three years at Harvard, Witkop received an invitation to work at the newly established National Heart Institute from its newly installed research director—James Shannon. He weighed his offers from industry against the NIH offer and chose the "basic research institute." Shannon recruited Witkop along with Julius Axelrod, Anfinsen, Earl Stadtman, and other luminaries.

"Shannon was marvelous," Witkop recalls. "He didn't go by mission; he went by talents. He wanted people with curiosity to look behind the secrets of nature. For instance, when we discovered cyanogen bromide degradation, we didn't know that later the approach would be extended to the chemical synthesis of human insulin at the Eli Lilly plant. How can you plan such a thing? We were looking at chemical methods to cleave large proteins; we weren't looking to advance an NIH mission in insulin research."

What's in a Name?

The insertion of politics into NIH research is something for which Witkop has little sympathy, and it is with droll solemnity that he traces the evolution of the names of institutes and buildings on the NIH campus. When Witkop arrived at the NIH campus in 1950, the institute at which he would later become chief of the Laboratory of Chemistry was still known by its original name—the Institute of Experimental Biology. But it was soon to become the National Institute of Arthritis and Metabolic Diseases. Why the name change? Witkop asks—and answers: "Because no one in Congress suffered from 'experimental biology.' " By the time Witkop left his post in 1987, the institute had gone through three additional name changes to settle upon the current NIDDK, the last in a series of permutations that left arthritis and diabetes in separate domains.

As with the names of institutes, so the names of buildings. Witkop notes that the man who hired him—Shannon—is the only NIH scientist to have a building named after him. "And I'm one of the godfathers of Building 1 who insisted that it honor the name of James Augustine Shannon," he says. All the other named buildings, he observes, are named for politicians.



On the Shoulders of Giants: *With meticulous reverence, Witkop has charted the names and dates that make up the long chain of his chemistry heritage—and has covered the wall opposite his desk with this graphic reminder of his place in the scheme. Of the Laboratory of Chemistry, NIH's oldest lab, Witkop notes that every chief—from his predecessors William Mansfield Clark, Claude Hudson, and Lyndon Small to himself and his successor John Daly—has been elected to the National Academy of Sciences.*

Chemistry at NIH

Witkop wrote the chapter on "Organic Chemistry in a Biomedical Research Organization" in the internally written NIH retrospective, *NIH: An Account of Research in Its Laboratories and Clinics* (edited by DeWitt Stetten and W. T. Carrigan, Academic Press, 1984). In both this chapter and his conversation, Witkop rejoices in the strong and enduring friendships and the decades of significant scientific collaborations formed at NIH.

With Kenner Rice, Axelrod, Sidney Udenfriend, Gordon Guroff, John Daly, Paul Torrence, and many others, Witkop "shared many research problems." These, variously, revolved around 5-hydroxytryptophan metabolism and serotinin biosynthesis; synthetic opium alkaloids, including the metabolic inactivation of lysergic acid diethylamide; catecholamine methylation (cited in Axelrod's Nobel award); hydroxylation-induced intramolecular migrations, or the "NIH Shift," "accidentally discovered," Witkop notes in his historic recap, in the course of developing a phenylketonuria assay; amphibian venoms, such as batrachotoxins and other congeners and their neurologic targets; and interferon mechanisms of action.

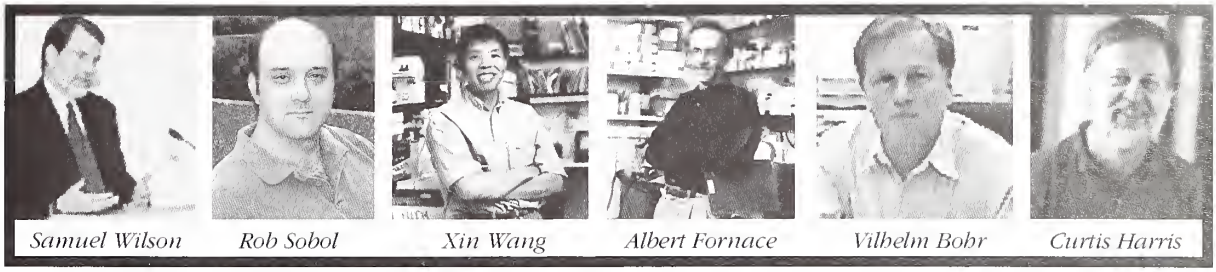
Perspective

Witkop sees his own history intertwined with the history of NIH and the field of chemistry, reflected quite tangibly in the bestowal of Nobel prizes upon so many individuals with whom he "shared research problems." Asked if he wishes he had one of those prizes for himself, he smiles and replies, "No. I think the Nobel Prize can be a nuisance. I recall what Mrs. Axelrod said: 'What a nuisance. Now I have to get a new dress.' "

Nuisance or not, he greeted the news of his friend Günter Blobel's capture of the 1999 prize with glee. He and his wife had been guests of the Blobels at their family restaurant in New York in October the night before Witkop attended the Weizmann Institute celebration. Two days later, Blobel learned of his award.

Marking a Life: *Created in 1989, this German 200-mark bill shows Paul Ehrlich and the basic skeleton of Salvarsan (arsphenamine), the first effective antisyphilis agent, on its face (and a picture of mast cells and Ehrlich's microscope on the back). Witkop, together with Ehrlich's grandson, helped design the banknote, which, Witkop observes, will be phased out with the ascendancy of the Euro. Witkop is a member of the Paul Ehrlich Foundation and Committee and has written and lectured extensively on Ehrlich's life and science—including an invited lecture at the Nobel Foundation symposium in 1981. When he left his NIDDK lab in 1987 and became an NIH scholar, Witkop inaugurated the Paul Ehrlich lecture series, which hosted some of the world's most outstanding chemists, including, in 1993, this year's Nobel laureate in chemistry and another of Witkop's friends, Günter Blobel.*

Awards are one thing, organic chemistry another. To Bernhard Witkop, organic chemistry, like beauty, is its own excuse for being. In fact, it *is* being. While other disciplines now introduce themselves as "molecular," as in "molecular genetics," organic chemistry has no such need. "That would be a pleonasm," Witkop observes. "The closer we come to the mystery of life," he says, "the more we can explain it in one language only, and that is the language of organic chemistry." ■



To Die or Not To Die
continued from page 1

and chromosomal breakage caused by exposure to methylating agents." Sobol also reviewed the findings of studies conducted by Wilson in collaboration with NIA's Bohr, in which β pol was shown to be the DNA polymerase of choice involved in the long-patch BER pathway in mammalian cells (1–3). Moreover, one of β pol's major enzymatic roles, the removal of the dRP group, was found to be the overall rate-limiting step of BER (4). He and other investigators, Sobol added, have recently demonstrated that mouse fibroblasts from β pol knockouts are highly sensitive to induction of apoptosis and chromosomal breakage by methylating agents, further emphasizing β pol's protective role (5).

Bohr elaborated on the use of Cockayne syndrome (CS)—a premature aging syndrome characterized by cell sensitivity to ultraviolet (UV) light, a deficiency or delay in DNA and RNA synthesis after irradiation, and a defect in repair of active genes—as a model to better understand DNA repair dynamics, such as transcription-coupled DNA repair (6). He also recapped mounting evidence that CS involves not only a repair defect but also a transcription defect (7).

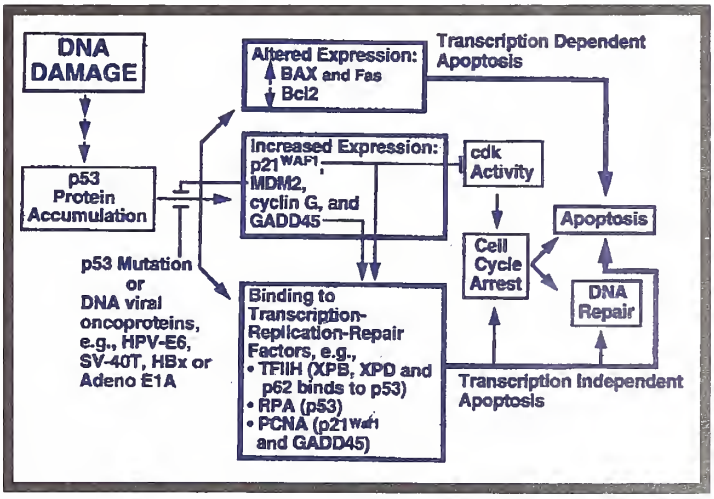
Another major player in DNA repair is the well-known tumor suppressor *p53*. While noting *p53*'s more popularly studied activities in cell-cycle delay and apoptosis, Albert Fornace, of NCI's Division of Basic Science, emphasized its equally valid "protective role in modulating cellular levels of DNA repair."

"The bottom line," Fornace said, "is that transcription-coupled repair does not appear to be perturbed appreciably by the loss of *p53*, one of the major mediators of cytotoxicity, after UV radiation." He added, however, that "global repair is attenuated" in cells lacking *p53*. His lab recently published in *Nature Genetics* the "striking find" that disruption of the *p53*-effector gene, *GADD45a*, results in genomic instability in a manner reminiscent of that seen in *p53*-null mice. Observations included centrosome amplification, chromosome aberrations, aneuploidy, mitosis abnormalities, and increased radiation-induced carcinogenesis in *GADD45a*-null cells (8). Interestingly, he said, disruption of *GADD45a* also resulted in reduced DNA-repair capacity. Compared with wild-type cells, *Gadd45a*-null cells demonstrated a significant reduction in both unscheduled DNA synthesis and global excision repair. As in *p53*-null cells, transcription-coupled repair was not reduced, whereas repair of UV-type damage to the nontranscribed strand or to bulk DNA (global repair) was reduced. These results, Fornace said, indicate that some of the features of *p53* that contribute to genomic stability and efficient DNA repair are mediated by the product of the *GADD45a* gene.

p53, said Xin Wang, of the NCI Laboratory of Human Carcinogenesis, stands "at the crossroads of life and death," a pivotal player in crucial biochemical pathways such as transcription, DNA repair, cell-cycle control, and apoptosis. Wang pointed out that many human malignancies have a *p53* mutation and that other investigators have shown that the loss of *p53* can result in genomic instability, increased chromosomal rearrangements, and mutation. He and his coworkers, therefore, reason they may find "clues to molecular carcinogenesis" by analyzing the interactive roles of *p53*.

Human diseases that have a genomic instability or cancer predisposition—such as xeroderma pigmentosum, ataxia telangectasia, Werner's syndrome, and Bloom's syndrome—may assist them in unraveling the complex network of cross-talks mediated through *p53*, he said. For example, Wang speculates that in the *p53*-mediated apoptotic pathway, *p53* may induce apoptosis by binding to and inhibiting the helicase activity of certain transcription factors.

This helicase-binding motif is also found on the DNA helicase BLM, the gene product of the Bloom's syndrome gene, *BS*. Wang said he expects these studies to enhance understanding of human cancer and ultimately to facilitate the development of effective new anticancer strategies. ■



A simplistic model of *p53*'s place on a DNA damage-response pathway (absent a cellular context and the influence of the cellular microenvironment. From X. Wang and C. Harris. "p53 tumor-suppressor gene: clues to molecular carcinogenesis." *J. Cell. Physiol.* 173: 247–255 (1997).

References

1. G.L. Dianov, R. Prasad, S.H. Wilson, and V.A. Bohr. "Role of DNA polymerase β in the excision step of long patch mammalian base excision repair." *J. Biol. Chem.* **274**: 13741–13743 (1999).

2. R. Prasad, G.L. Dianov, V.A. Bohr, and S.H. Wilson. "FEN1 Stimulation of DNA polymerase β mediates an excision step in mammalian long patch base excision repair." *J. Biol. Chem.* (in press).

3. J.K. Horton, R. Prasad, E.W. Hou, and S.H. Wilson. "Protection against methylation-induced cytotoxicity by DNA polymerase β-dependent long patch base excision repair." *J. Biol. Chem.* (in press).

4. D.K.Srivastaua, B.J. Vande Berg, R. Prasad, J.T. Molina, W.A.Beard, A.E. Tomkinson, and S.H. Wilson. "Mammalian abasic site base excision repair." *J. Biol. Chem.* **273**: 21203–21209 (1998).

5. K. Ochs, R.W. Sobol, S.H. Wilson, and B. Kaina. "Cells deficient in DNA polymerase beta are hypersensitive to alkylating agent-induced apoptosis and chromosomal breakage." *Cancer Res.* **59**: 1544–1551 (1999).

6. R.M. Brosh, A.S. Balajee, R. Selzer, M. Sunesen, L.P. De Santis, and V.A. Bohr. "The ATPase domain, but not the acidic region of Cockayne syndrome group B gene product is essential for DNA repair." *Mol. Biol. Cell* (in press).

7. M.C. Hollander, M.S. Sheikh, D.V. Bulavin, K. Lundgren, L. Augeri-Henmueller, R. Shehee, T.A.Molinaro, K.E. Kim, E. Tolosa, J.D. Ashwell, M.P Rosenberg, Q. Zhan, P.M. Fernandes-Salguero, W.F. Morgan, C.X. Deng, A.J. Fornace, Jr. "Genomic instability in *GADD45a*-deficient mice." *Nat. Genet.* **23**: 176–184 (1999).

8. E.S. Spillare, A. Roble, X.W. Wang, J. Shen, C. Yu, G.D. Schellenberg, and C.C. Harris, "p53-mediated apoptosis is attenuated in Werner syndrome cells." *Genes Dev.* **13:** 1355–1360 (1999).

ORS: Minding the Store —And the Byproducts, the Workers, the Atmosphere . . . .

Office of Research Services

by Fran Pollner

In the life of every scientist, a little rain must fall. Sometimes it's a radioactive or chemical spill or a computer crash; sometimes it's a cash shortfall for needed equipment; and sometimes it's physical fallout from ergonomically unsound lab techniques or clutter. But if the scientist happens to work at NIH, he or she is at a distinct advantage because there's an umbrella organization on campus that exists to shield researchers from such inclemencies—the NIH Office of Research Services (ORS).

ORS was out in force at this year's Research Festival, with six exhibits, 19 posters, and even a table in the vendors' tent to display the myriad ways NIH supports its intramural scientists.

SEIB: Up-Front

"For years, we weren't allowed to advertise; we couldn't participate in a show like this one and appear to be competing with the private sector," said Annie Burke, who manages the sales and rental program of the Scientific Equipment and Instrumentation Branch (SEIB) of the ORS Division of Intramural Research Services. As a result, very few scientists on campus knew what was available to them, literally steps from their door (in Building 13).

Fran Pollner

Annie Burke (left), with Stacy Jackson (middle) and Anne Treanor

That's not the case now. SEIB happily publicizes its services every chance it gets. Situating itself in the vendors' tent during the Research Festival, instead of in the Natcher Conference Center along with other ORS displays, SEIB tried to let NIH scientists know they could arrange to secure through SEIB equipment on exhibit at other vendor tables.

"We buy equipment from the vendors here and then rent it out—for a month, a year, four years—at much lower rates than you can get in the private sector. Delivery is free, maintenance is included, and you don't have to go through the procurement process—we handle the CAN," Burke said, referring to the governmental CAN number that identifies service and supply transactions.

For new labs, it's generally cheaper to set up by renting from SEIB with an option to buy; if a lab needs a high-ticket item, like a gene sequencer, it's often feasible only through a rental arrangement, Burke observed. Private sector leasing companies will charge about $200 to $300 a month for a popular item like a freezer, with an additional $150 delivery fee; SEIB charges $90 a month and throws in delivery and maintenance, she said. A sequencer that costs $130,000 (government rate) is usually beyond the means of a lab, but a $2,500 a month rental fee is often not, she added.

Other components of SEIB include a lab instrumentation repair shop and computer repair (the Center for Information Technology takes care of computer software problems, but SEIB handles hardware crashes, Burke explained) and instrument design and fabrication services. The latter, she said, produce models that rival the works of medieval artisans. "A researcher will have an idea and describe what's needed, and they'll design and fabricate it," Burke said, citing as an example a model of the uterine vascular system made of blown glass, created for a researcher who was studying drug metabolism in uterine cancer.

MAPB: They've Got the Look

Continuous video demonstrations attracted a lot of passers-by to the ORS Medical Arts & Photography Branch (MAPB) exhibit, where Ken Ryland, chief of the video section, boasted that not only were the videos high quality they were also 10–25 percent less ex-

New DIRS Director Aims To Please

Shirl Eller, the first permanent director for the reconstituted ORS Division of Intramural Research Services, has a few questions for NIH intramural scientists. What services are most appreciated? What services should be improved? What services are needed that are not yet available? Formerly the chief of financial management at the Army Research Lab in Adelphi, Maryland, Eller arrived here in May with visions of a "business model" for DIRS, namely, that the division be run along the lines of the private marketplace, with customer satisfaction the driving force. She'll be conducting customer surveys in each of the four DIRS components: the Scientific Equipment and Instrumentation Branch, the Medical Arts & Photography Branch, the NIH Library, and the Veterinary Resources Program. Right now, each service is undergoing a one-by-one review, but the future, Eller said, will bring a more comprehensive strategy for the ORS overall. ■

Fran Pollner

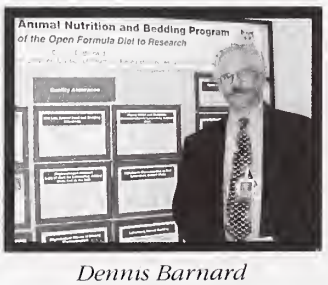
Shirl Eller

—FP

Animal TLC

Two million pounds of feed and 1.5 million pounds of bedding yearly go into the tending of laboratory animals at NIH. They arrive under the auspices of the laboratory animal nutrition quality assurance program provided by the DIRS Veterinary Resources Program (VRP).

Unlike commercially available animal diets, which have proprietary quantitative formulations, the open-formula diets developed by the VRP Nutrition Office provide standard reference diets with known quantitative formulations. The open-formula diets help to eliminate unknown variables from research; the VRP quality assurance program ensures the nutritional quality and safety of the feed and bedding. Because research projects involving laboratory animals amount to $427 million a year, this is no small matter, noted laboratory nutritionist Dennis Barnard. For nutrition consultation, diet formulation, and quality assurance, and problem solving, call Barnard at 402-7255. ■



Dennis Barnard

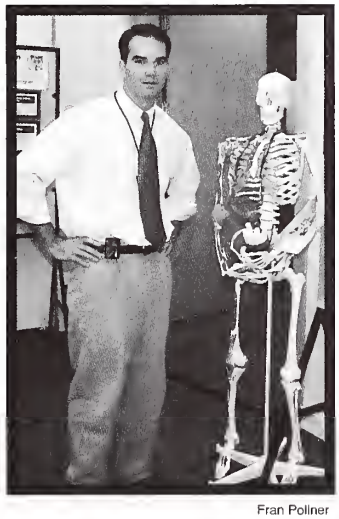
pensive than what one could expect to pay in the outside marketplace. According to administrator Nancy Guerin, MAPB handles 50,000 jobs a year—serving all the institutes and producing anything from slides to exhibits to full video production and distribution.

Fran Pollner

Ken Ryland (middle), with MAPB branch chief Lem Canady and design chief Linda Brown

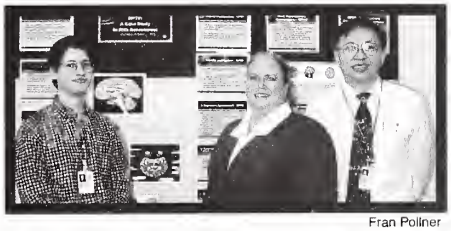
Safety First

Attached to the tools of the trade are those who use them—sometimes to their detriment, especially if pipetting or keyboarding are major components of one's day. With the aid of a skeleton to illustrate every bone in one's body that could be laid waste by repetitive or improper technique, the ORS Division of Safety dedicated two of its multiple posters to the subject of laboratory ergonomics. There's nary an object in the laboratory—table, chair, microscope, cabinet, computer, microtome, pipette, cryostat, flow cytometer, centrifuge rotor, glovebox—that cannot be a vector for injury, according to industrial hygienist Jason Barr, who reviewed all NIH laboratory injury and illness reports filed from 1991 to 1997. Between 1991 and 1995, he found, there were 59 cases of repetitive strain injuries (RSIs), representing 2 percent of all reports; in the following two years, there were 29, or 4.5 percent of all case reports—a reflection, Barr thinks, of increased awareness and reporting. There was a higher proportion of RSIs among younger (40 and under) than older workers and more RSI case reports from women, inspiring the speculation that women might perform more repetitive activities.



Fran Pollner

Jason Barr and Bony Friend



Fran Pollner

Albert Lock (right), with Mike Pallay, occupational safety specialist, and Roz Rutledge-Burns, chief of the Safety Operations Section

The upper body—shoulders, wrists, and thumbs—is most vulnerable. For example, turning a microtome to cut paraffin for histology specimens affects the wrist; removing the ovaries of a fruit fly with small tweezers affects the thumb, Barr noted. Using cushioned forceps would help, he said, as would alternating the fingers used to hold them. "We ask manufacturers to redesign products, and until that happens we improvise—like gluing foam around the forceps."

The division routinely sends questionnaires to labs, interviews personnel, checks out worksites, and consults with manufacturers—no fee involved.

For pithy, ergonomically sound, illustrated advice related to typical lab environments, click onto **<http://odp.od.nih.gov/whpp/ergonomics/>**.

MPTP: A Case Study

Albert Lock, toxicologist-pharmacologist in the Occupational Safety and Health Branch (OSHB), is in the business of assessing the risks of adverse health effects run by scientists who work with hazardous substances. MPTP, a chemical used to model the effects of Parkinson's disease in the brain, is a risk-analysis case in point. "This is a chemical that can cause permanent brain damage. We've been evaluating it since the mid-'80s, updating our literature searches, monitoring its use, and doing routine lab checks," Lock said. The OSHB has 14 specialists, assigned by institute, whose work includes attending animal care and use meetings and signing off on protocols involving chemicals, toxins, or recombinant DNA.

Fran Pollner

Beth Reed

Radiation Rigors

NIH labs are surveyed monthly and inspected two to six times a year to keep tabs on another potential hazard—radiation. Beth Reed, health physicist in the ORS Division of Safety's Radiation Safety Branch, was pleased to report that although her branch responded to 129 potential problem calls between 1989 and 1999, there were no radiation injuries. "There have been no adverse effects, not even from the notorious case" (involving a pregnant scientist who ingested radioactive material).

"Accidents happen. It's what you do afterwards that counts," Reed said. Most of the 129 incidents involved unforeseeable spills in restricted areas. The most typical scenario is that someone drops a container of radioactive phosphorus 32, a common material used to label cells. Should such an accident occur, the key action, Reed said, is to take action right away by calling the Radiation Safety Branch, based in Building 21 (496-5774), and clean up the spill immediately. Radiation Safety Branch staff clean up spills that occur in common areas; lab staff usually clean up spills within their labs. Generally, cleaners found in labs are effective—Windex, 1 percent acetic acid, or Radiacwash.

That such accidental spills have not had any biomedical effects on exposed personnel can be attributed largely to maintaining the low radiation limits set for both restricted (2,200 dpm/100 cm^2) and unrestricted areas, Reed observed. Aside from possibly jangled nerves, the worst consequence for personnel may be having contaminated articles placed in storage—they are held to allow for radioactive decay, a period determined by the half-life of the contaminant. An article contaminated with P^{32}, with a half-life of 14 days, for instance, would be held for 20 weeks. ■

Hot Mouse Tips: a Three-Part Series

Part 3. The Routes To Right Dosing

by Tory Hampshire, DVM, NINDS, and Judy Davis, DVM, NINDS

Parts 1 and 2 "inside the mouse hospital" addressed old and new ideas for surgery and perioperative support of the mouse and rat. Part 3 presents some available products and techniques that may make medication of the mouse less labor-intensive and more efficient.

Some General Tips

Dosing and achieving steady-state blood concentrations are challenging in a tiny patient with a high metabolic rate whose surface-to-volume ratio is roughly ten times that of humans.

When an oral route of administration is selected, it's a good idea to measure pooled blood samples via retro-orbital bleeds (under anesthesia) several times over the course of 24 hours to make certain that steady-state blood concentrations are achieved. Often, this step is left out of study design. A volume per mouse of 100 µL is acceptable. Alternatively, mice can be terminally bled, serially, to construct a pharmokinetic curve.

A common myth is that mice and rats are resistant to infection, but in reality even oral dosing entails a risk of infection in genetically or pharmacologically immunosuppressed mice. Always pay attention to osmolality, pH, and sterility of preparation in dosing immune-compromised hosts like SCID or nude mice. And remember that although a healthy immune-competent mouse may have an LD50 for infection somewhat higher than that of a person, a dog, or a cat, it *does* have an LD50. For intravenous, intramuscular (IM), or intraperitoneal injections, continue to observe precautions in preparing drug solutions or fluids.

Oral Administration

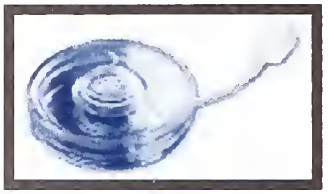
Most scientists use gavage needles to deliver substances via the oral route. Gavage needles are readily available from almost any scientific supply source. Stomach capacity is generally 5–10 mL/kg or about 0.2 mL at a time for a mouse. Some mice will willingly drink off the end of a gavage needle if the substance is highly palatable. Gavage needles should be checked for rough edges as the esophagus in mice is very delicate.

The Jell-O recipe mentioned in our first article (*The NIH Catalyst*, May-June 1999, page 10) is also highly palatable and may also serve for noncritical dosing regimes. Keeping in mind that mice typically revisit food throughout the day, it might be wisest when using Jell-O as a medication vehicle to place the total desired daily dose in one Jell-O cube of known volume. The same considerations should guide the process of adding medications to water and pelleted feed, a popular way to administer drugs orally. Again, measure pooled aliquots of serum before arriving at conclusions about drug efficacy. We have had good luck with a source called National Medical Services (Willow Grove, Penn., at 215-657-4900 or e-mail at <**nms@nmslab.com**>), which runs a large number of bioanalyses for a broad range of compounds and will also consult on special problems.

Instech Company's single-channel infusion system uses the Harvard Apparatus infusion pump, stainless steel single-channel swivel (25 gauge), 3.5" counterbalanced lever arm, covance infusion harness for mice, and an 8.5" clear animal container with feeder and water bottle.

Subcutaneous Administration

Because of the large space between skin and subcutaneous tissues, the subcutaneous route is very attractive for chronic medication of rodents. Generally, it is very easy to restrain mice and to deliver up to 5.0 mL in this space. For optimal absorption, drugs should be hypoosmolar (less than 300 milliosmoles). Necrosis at the site of entry is a major side effect with highly acid or base substances, but sterility is not as much of a problem with subcutaneous administration as it is with the intramuscular, intraperitoneal, or intravascular routes. Immunosuppressed mice, SCID mice, and nude mice should not receive anything that has not been carefully sterilized.



*The Infu.Disk by Med.e.cell (San Diego, 619-552-0781; website <**www.med-e-cell.com**>) can be mounted to a swivel arm and serves as a lower-cost alternative to the Harvard system for infusion. This company makes five different 10-mL discs delivering a range of 0.02–4.0 mL/h.*

Slow- and continuous-release options have also become available in transcutaneous and subcutaneous rodent delivery systems.

Several catheter delivery systems are also available for rodents. Alzet osmotic pump models allow continuous delivery of agents at controlled rates when placed subcutaneously or intraperitoneally. If you want targeted delivery of a drug to an area remote from the site of implantation, you can attach a catheter to the pump. Alzet pumps have been used in gene therapy experiments. The pumps come in several sizes with different volume reservoirs. For more information: <**www.alzet.com**> or 800-227-9953; e-mail: <**alzet@alza.com**>.

The ESOX implantable pump is also an option. This system, with its refillable reservoir, enables truly long-term drug delivery. (Contact Access Technologies at <**www.norfolkaccess.com**> or 847-674-7131.)

Innovative Research of America (Sarasota, FL, 800-421-8171) has come out with a time-release matrix-driven biomedical delivery system in a pelleted form that easily can be placed in a subcutaneous pocket. They list nearly all compounds of drugs and do special formulations as well.

Transcutaneous Routes

The advent of pain medications in topical gels and creams is here. Systemic absorption of active drugs after topical application of ketoprofen (a nonsteroidal anti-inflammatory), nitroglycerin, motion-sickness drugs, and the like has led to a growth industry of creating and evaluating penetrating emulsions. Organogel, a lecithin-based matrix, has been used by our consulting pharmacist (Foer's pharmacy, Bethesda, MD, 301-657-3500) to compound 40 percent (38 percent stronger) lidocaine cream for studies confounded by narcotics and nonsteroidals. We apply this along the incision line of rodent patients. It may represent another avenue for innovative stress-free application of drugs.

Intravenous Route

As we mentioned in our second article (*The NIH Catalyst*, September-October 1999, page 10), with lots of practice, it is possible to cannulate mouse jugular veins. It's definitely possible to purchase mice and rats already instrumented with intravascular access—from Taconic Farms (through the Veterinary Resources Program procurement, 301-

496-3575)—or put them in yourself. In mice, if you are successful with this route, several intravenous delivery solutions are possible. Harvard makes a terrific mouse infusion system, with a swivel that has low rotational friction. This system, with all attachments, runs around $3,500.00 and is sold through Instech (5209 Militia Hill Road, Plymouth Meeting PA, at 610-941-0132; fax: 610-941-0134).

IM and Intraperitoneal Delivery

We discourage the use of IM injections. Necrosis and pain at the injection site are not uncommon outcomes due to the small hindlimb muscle mass of rodents. If you must use the IM route, you should learn the location of the sciatic nerve and limit the volume delivered to 0.05–0.1 mL/site in mice and.0.1–0.3 mL/site in rats. The lumbar muscles are also good IM injection sites. Due to the small volume per site, one drug dose generally must be delivered in several places.

Intraperitoneal delivery is still overused in rodent studies and may carry with it numerous concerns, such as contamination, splenic trauma, serosal hemorrhage, and other untoward effects, and should be performed thoughtfully. Drug delivery by convection or viral-vector delivery to the spleen or liver is best performed under direct visualization of these structures. Drug delivery is possible but must follow general guidelines for sterility, pH, and osmotic compatability with mammalian systems.

Attention to such detail and careful practice with NIH's tiny research subjects will surely pay off with better data. ■

Innovative research products can be located on the web at ***<http://www.innovrsch.com>***

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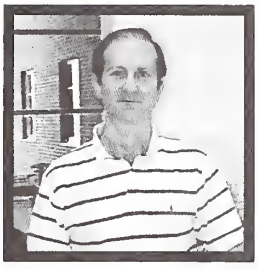
After 11 years, Tory Hampshire is leaving NIH in December to start her own business—Advanced Veterinary Applications—in Bethesda. She'll be focusing on refinements to animal care and veterinary technician team building.—Ed.

Recently Tenured

Harris Bernstein *received his Ph.D. in biology from the Massachusetts Institute of Technology in Cambridge in 1987 and did postdoctoral work at the University of California, San Francisco, before joining the Genetics and Biochemistry Branch of NIDDK in 1992. He is now a senior investigator there.*



Fran Pollner

Harris Bernstein

My laboratory studies protein translocation across and insertion into cell membranes. Most of our work has focused on the transport of proteins across the mammalian endoplasmic reticulum (ER) and the bacterial inner membrane (IM), which are evolutionarily related processes. Although the cellular factors that decode ER and IM targeting signals and the components of the conserved protein-conducting channel ("translocon") have been identified, many steps in the transport pathway are still poorly understood. How membrane proteins are incorporated into the lipid bilayer with the correct topology is particularly enigmatic.

Work initiated in the 1980s showed that in mammalian cells a ribonucleoprotein complex called the signal recognition particle (SRP) recognizes both the signal sequences of presecretory proteins and the transmembrane segments of integral membrane proteins cotranslationally and then targets nascent chain complexes to the ER. Interaction of SRP with an ER-bound receptor then catalyzes insertion of the nascent chain into the translocon. We have been particularly interested in understanding the mechanism by which the SRP 54-kD subunit (SRP54) recognizes signal sequences with a high degree of fidelity and releases them only after arriving at the ER. We have found that the three domains of SRP54—an NH_2-terminal four-helix bundle ("N domain"), a central GTPase ("G domain"), and a COOH-terminal signal peptide binding domain ("M domain")—play distinct roles in the targeting cycle. Our studies show that the N domain promotes high-affinity signal peptide binding and that the GTPase acts as a switch that promotes signal peptide release at the ER. The data suggest that the N domain serves as a "lid" for the signal peptide binding pocket that is opened by a GTP-induced conformational change. We are currently continuing to explore how the three domains work together to promote accurate protein targeting.

While several laboratories were busy characterizing the mammalian SRP pathway, several other groups showed convincingly that presecretory proteins are targeted to the *Escherichia coli* IM post-translationally by molecular chaperones. Their results predicted that SRP would be unnecessary in prokaryotes and thus found only in eukaryotic cells. The surprising discovery of SRP in bacteria through sequence gazing in 1989, however, created an apparent paradox. Our most significant recent contribution has been to resolve this long-standing puzzle. Using a combination of genetic and biochemical methods, we showed that SRP targets integral membrane proteins to the IM in *E. coli*. In addition, we have obtained evidence that the SRP-targeting pathway has been widely conserved in prokaryotes not only because it increases the efficiency of membrane protein biogenesis, but also because it prevents the toxic accumulation of mislocalized membrane proteins in the cytoplasm.

Our studies on protein transport pathways in bacteria have also led us to some new insights into the function of the translocon. We have identified a mutant form of *E. coli* SecY, the most highly conserved translocon subunit, that has a specific defect in membrane protein insertion. Studies on this mutant demonstrate that the membrane protein insertion and protein translocation functions of the translocon are at least partially separable. We are now attempting to isolate additional translocon mutants in an effort to understand how the translocon performs two related but distinct functions. Finally, we have found that the bacterial SecA protein, which was previously thought to participate only in the export of proteins targeted post-translationally, also facilitates the insertion of membrane proteins targeted by SRP. Our most recent results suggest that SecA may play a wider role in membrane protein insertion than was previously expected.

In a departure from our work on ER/

Recently Tenured

IM translocation, we have recently begun to study an unusual phenomenon known as "nonclassical secretion." More than a dozen secreted cytokines (for example, interleukin 1 and basic fibroblast growth factor) and viral proteins (for example, HIV Tat) have been described that lack typical NH_2-terminal signal sequences. The export of these proteins does not appear to involve passage through the normal ER-Golgi route. Currently, we are using genetic approaches to identify cellular factors that promote nonclassical secretion in *Saccharomyces cerevisiae.*

Charles Egwuagu *received a masters degree in public health and a Ph.D. in epidemiology and microbiology from Yale University in New Haven, Conn., 1987. He joined the NEI Laboratory of Immunology in 1987 and is currently a senior investigator and head of the Section of Molecular Immunology.*

The focus of my research is on molecular mechanisms that underlie the etiology and susceptibility to organ-specific autoimmune diseases. We emphasize a group of intraocular inflammatory diseases (uveitis) of presumed autoimmune etiology.

Fran Pollner

Charles Egwuagu

In the early part of my work, I demonstrated that T cells expressing T-cell antigen receptors (TCRs) of the Vβ8 family are amplified in the retina of uveitic rats and might therefore be responsible for the induction of experimental autoimmune uveitis, the animal model of human uveitis. A significant number of the Vβ8 TCRs contain a conserved Val-Gly motif in the third complementarity-determining region, suggesting that this TCR motif may provide an immunotherapeutic target. These results moved us to extend our studies to human diseases. We have found evidence of selective recruitment and amplification of $V\gamma2^+$ T cells in tear ducts of patients with ocular sarcoidosis and are now examining other uveitic conditions.

An important and unresolved problem in autoimmunity is defining risk factors for development of an organ-specific autoimmune disease. Why are some individuals resistant while others are susceptible? Recent studies in my laboratory have shed some light on this. We discovered that ocular-specific antigens that are the targets for pathogenic autoimmune processes are expressed in the thymus of some animals. Animal species that possess the thymic antigens are resistant, while those that do not are susceptible to disease induction. Furthermore, the degree of susceptibility or resistance depends on the relative amounts of the autoantigens in the thymus. These data suggest a novel explanation for differences in susceptibility to autoimmune diseases: Resistance to an organ-specific autoimmune disease may be regulated at least in part by capacity to establish central tolerance to the relevant autoantigen. We have extended these studies to humans, and preliminary results indicate that the level of thymic expression of two putative ocular autoantigens (S-Antigen and IRBP) may serve as a useful indicator of susceptibility or resistance to uveitis. The general applicability of this concept to other autoimmune diseases remains to be established.

One of the cytokines that has been implicated in the immunopathogenic mechanism of a number of organ-specific autoimmune diseases is γ-interferon (IFN-γ). However, whether IFN-γ plays a role in the induction or recovery from the disease is still a matter of debate. Recent studies in the mouse have shown that IFN-γ confers protection against experimental allergic encephalomyelitis, a model of multiple sclerosis. To explore the potential benefits of IFN-γ in the management of uveitis, we generated transgenic (TR) rats and mice with targeted ectopic expression of IFN-γ in the eye. These models enabled us to study the consequences of prolonged exposure of ocular tissues to this cytokine. The IFN-γ rat strain is the first TR rat generated at NIH.

Analysis of these rats revealed that an important consequence of prolonged exposure of ocular cells to IFN-γ—as may occur during chronic or recurrent uveitis—is the induction of choroidal inflammation, formation of retinal folds, activation of pro-inflammatory genes, and enhanced susceptibility to anterior and posterior uveitis. Thus, in contrast to the protective effect of systemic IFN-γ in the mouse, constitutive secretion of IFN-γ in the rat eye predisposes the animal to severe uveitis. The TR rats also show progressive degeneration of the neuroretina and selective apoptosis of ganglion cells. These are early signs of glaucoma and nutritional amblyopia. TR rats are clearly a unique and important animal model for studying etiologic mechanisms of glaucoma and uveitis.

During the course of studies on our IFN-γ TR mice, we discovered that several members of the interferon regulatory factors (IRFs) family of transcription factors are constitutively expressed in the lens. We have also shown that the expression of these IRFs is tightly regulated. Perturbation of the levels, spatial distribution, and subcellular localization of ICSBP, IRF-1, and IRF-2 in the developing mouse lens are strongly correlated with disruption of lens differentiation and development of lens cataracts. Constitutive expression of IRFs, including the lymphoid-specific IRFs, ICSBP, and LSIRF/Pip in the ocular lens, makes a compelling case for IRFs in transcriptional regulation of lens genes. Taken together with our previous finding that aberrant activation of the JAK/STAT signaling pathway can alter the developmental fate of ocular cells, we believe that the IFN-γ TR model provides a useful biologic system for understanding competing signaling pathways that influence the development of the vertebrate lens.

Jeffrey Rubin *received his M.D. and Ph.D. in molecular biology from Washington University (St. Louis) in 1983. Following an internal medicine residency program at The Jewish Hospital of St. Louis, he joined the Laboratory of Cellular and Molecular Biology at the NCI in 1986 as a biotechnology fellow. He is now a senior investigator in the LCMB.*

From 1986 to 1996, my research dealt primarily with the purification and biological activities of two heparin-binding mitogens—keratinocyte growth factor (KGF, also known as FGF-7) and hepatocyte growth factor/scatter factor (HGF/SF). These proteins are mediators of mesenchymal-epithelial communication that can stimulate cell migration, differentiation, proliferation, and tissue morphogenesis.

Through collaborative studies, I have explored the role of these factors in development, tissue repair, reproductive tract biology, and neoplasia. We and others have shown that KGF has remark-

able cytoprotective effects, consistent with the hypothesis that it functions as a homeostatic factor to maintain epithelial barrier function. This has led to its use in clinical trials to reduce mucositis associated with chemoradiotherapy. My colleagues and I also identified two truncated HGF/SF isoforms, designated HGF/NK1 and HGF/NK2, which bind with high affinity to Met (the HGF/SF tyrosine kinase receptor). We demonstrated that these isoforms act as partial agonists or antagonists of HGF/SF activity. We determined that the amino-terminal domain of HGF/SF retains the heparin-binding properties of the full-length protein, and we established an important role for proteoglycan in HGF/SF isoform signaling.

My ongoing KGF research is collaborative and concerns its potential effects on development and function of the immune system. A collaborative study of HGF/SF will provide a detailed map of its heparin-binding site. This could yield more potent agonists or antagonists with potential clinical uses.

Fran Pollner

Jeffrey Rubin

For the past three years, the major focus of my research has been a soluble protein we discovered that has a cysteine-rich domain homologous with the putative Wnt-binding site of Frizzleds, the cell surface Wnt receptors. We have shown that this secreted Frizzled-related protein (sFRP-1) can bind directly to Wnt and modulate its activity. Thus, we believe it regulates Wnt-dependent developmental processes. sFRP-1 might also have an effect on Wnt signaling in neoplasia.

We have generated an abundant source of recombinant sFRP-1 and are currently studying its structure and biological activity. Gene targeting and transfer projects are underway to assess sFRP-1 function in vivo. With the support of an NCI Intramural Research Award, we have begun to screen peptide phage display combinatorial libraries to identify motifs responsible for binding to sFRP-1. Such information could lead to the development of analogs that would modulate Wnt and sFRP-1 activities.

In another series of experiments, we have characterized the promoter region of the human *sfrp-1* gene and identified several potential binding sites for transcription factors, including members of the GATA family.

This work adds a new dimension to my research program, which has centered on the discovery and analysis of soluble polypeptide factors involved in the regulation of growth and differentiation. The projects have been highly interactive, involving collaborations on and off the NIH campus, and have the potential to generate reagents of therapeutic relevance. ■

Beyond 2000: NIH-Duke Training Program

Applications for the 2000–2001 NIH-Duke Training Program in Clinical Research will be available beginning **December 1, 1999,** in the NIH Office of Education, Building 10, Room 1C129.

Designed primarily for clinical fellows and other health professionals who are training for careers in clinical research, the program offers formal courses in research design, statistical analysis, health economics, research ethics, and research management. Courses for this program are offered at the Clinical Center via videoconferencing from Duke or on site by adjunct faculty.

All persons taking courses in the NIH-Duke Training Program in Clinical Research must be nominated by the NIH Admissions Committee and formally admitted by the School of Medicine at Duke University.

1999–2000 NIH-Duke Clinical Research Fellows: *(standing left to right): Chen-Sen Wu, Michael Brennan, John Gribar, Hiroyu Hatano, Stefan Weiss, Raphaela Goldbach-Mansky, Yogen Saunthararajah; (seated left to right): Giovana Thomas, Elizabeth Higgs, Marcia Slattery; (not pictured: Robin Boineau, Hani El-Gabalawy, Howard Fine, Jay Giedd, Alfred Gordon, Julie Gulya, Winnie Rossi, Robert Walsh, Mary Lynn Dell)*

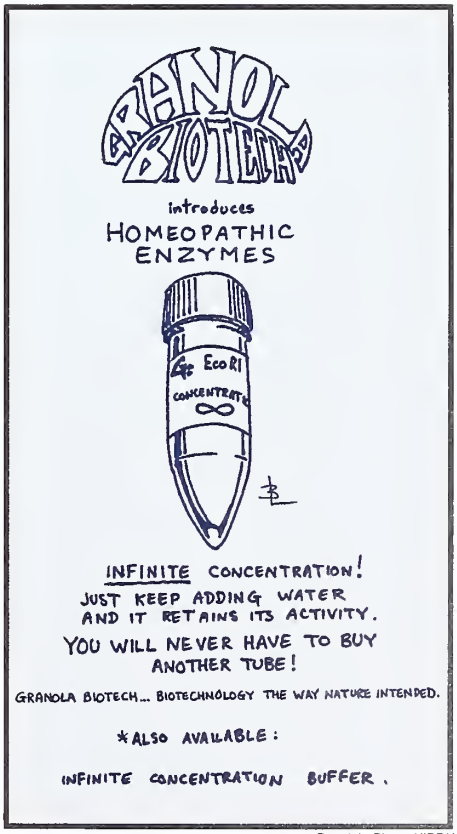
The deadline for receipt of applications is **March 13, 2000**. Applicants accepted into the program will be notified by July 1, 2000.

For more information on coursework and tuition costs for the 2000–2001 academic year, visit the program web site at

<http://tpcr.mc.duke.edu/>.

Enrollment in this program is limited. E-mail queries regarding the program may be addressed to William E. Wilkinson, Program Director, at

<tpcr@mc.duke.edu>.



Benoit LeBlanc, NIDDK

HAPPY NEW MILLENNIUM!

Call for Catalytic Reactions

In this issue, we are asking for your reactions in four areas: space at NIH, what awaits a new NIH director, the NIH Research Festival, and research ground rules for the next century.

Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: <catalyst@nih.gov>; fax:402-4303; or mail: Building 1, Room 209.

In Future Issues...

- Genomics-Based TB Drug Search
- Rethinking Rejection
- Policing the NIH Campus

1) Do you have a strategy for sensible space allocation at NIH?

2) What do you see as the biggest challenge for the next NIH director?

3) What suggestions do you have for new or improved ORS services?

4) We can't resist asking an end-of-century question, to wit: Since we'll be starting at ground zero-zero, would you establish any new rules as a basis of biomedical research for the next hundred years?

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 209, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

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