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The Rockefeller University

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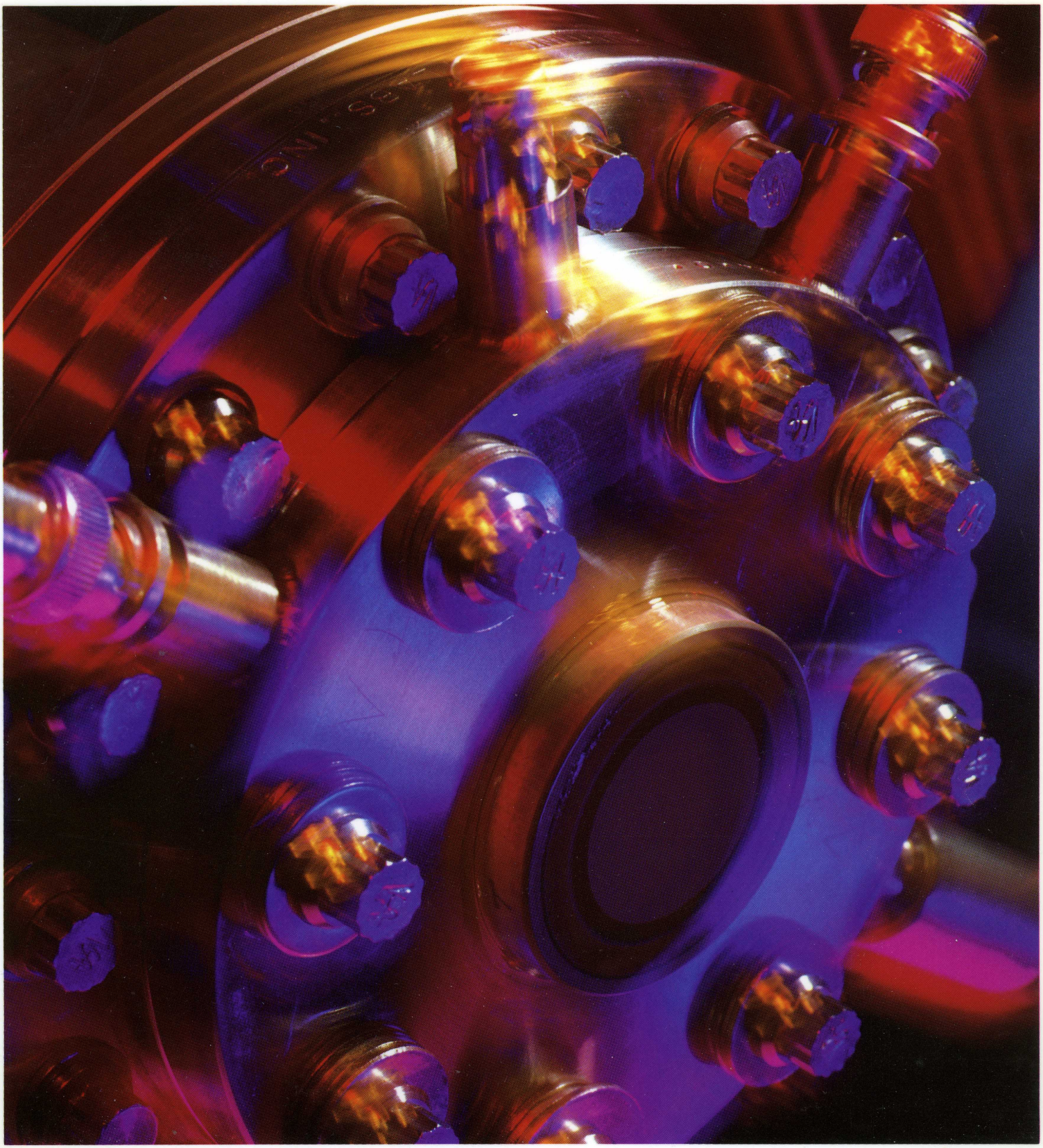
SEARCH

THE ROCKEFELLER UNIVERSITY MAGAZINE



SEARCH

VIEWPOINT

UNIVERSITY AS ORGANISM

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Like many of the living systems scientists study, The Rockefeller University is a complex organism. Just as cells work together to form organs, and organs band together to form systems, the university is composed of people and groups of people perfoming their specialized duties intricately and in concert. If we are to prosper as an organism, we must nourish and nurture our component parts.

Rockefeller has evolved in its nine decades of life from a specific focus on medical research and informal teaching that required narrowly defined support systems to a broad-based research and educational community that seeks to advance knowledge in many aspects of medicine, biology, mathematics, chemistry, and physics. Nearly 700 scientists in scores of disciplines pursue this knowledge. Most of them, including 250 postdoctoral fellows, are involved as well in the teaching process, providing a first-rate graduate education to a rigorously selected student body that numbers 150.

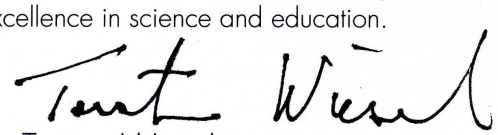
But while the university's job is research and teaching, to accomplish its goals requires much more than scientists and teachers. About 900 members of our community support the research and academics in uncountable ways. And they, too, are constantly training, working daily to find better, more efficient ways to contribute to the organism's success.

Their tasks are multitudinous. There are administrators, grounds keepers, purchasing agents, technicians, a glassblower, security guards, carpenters, painters, plumbers, fund-raisers, computer programmers, and electronic maintenance specialists. There are grant-getters, communications professionals, accountants, writers, engineers, food service workers, nurses and other medical staff, pharmacists, laundry workers, mail clerks, printers, photographers, artists, custodians, editors, and publicists, to name just some. And they all must work together smoothly, interacting in myriad ways with their co-workers to assure that our university reaches its full potential.

In our recent effort to ensure that the organism as a whole recovers from tough times, we have demanded much of this group of individuals, as we have from our researchers and students. Fortunately, the tide has begun to turn. Because of the sacrifices of our staff, and through the generosity of good friends, our resources are increasing. While the university still has a way to go to regain its health, the process of recovery has begun.

Like all forms of life, we at Rockefeller must grow if we intend to survive and flourish, particularly in these difficult days. Building on initiatives begun by David Baltimore, we must expand our financial resources still more so that we can renew our faculty and reinvigorate this institution. And we must also recognize and reward the dedication of our staff—the cells and groups of cells that make the organism work.

With the cooperation of our board of trustees, faculty, and staff, I know we can realize our dreams of excellence in science and education.



Torsten Wiesel
President, The Rockefeller University

MAKING MOLECULES FLY: BRIAN CHAIT AND THE MASS SPECTROMETRY LAB

by Sam Flamsteed

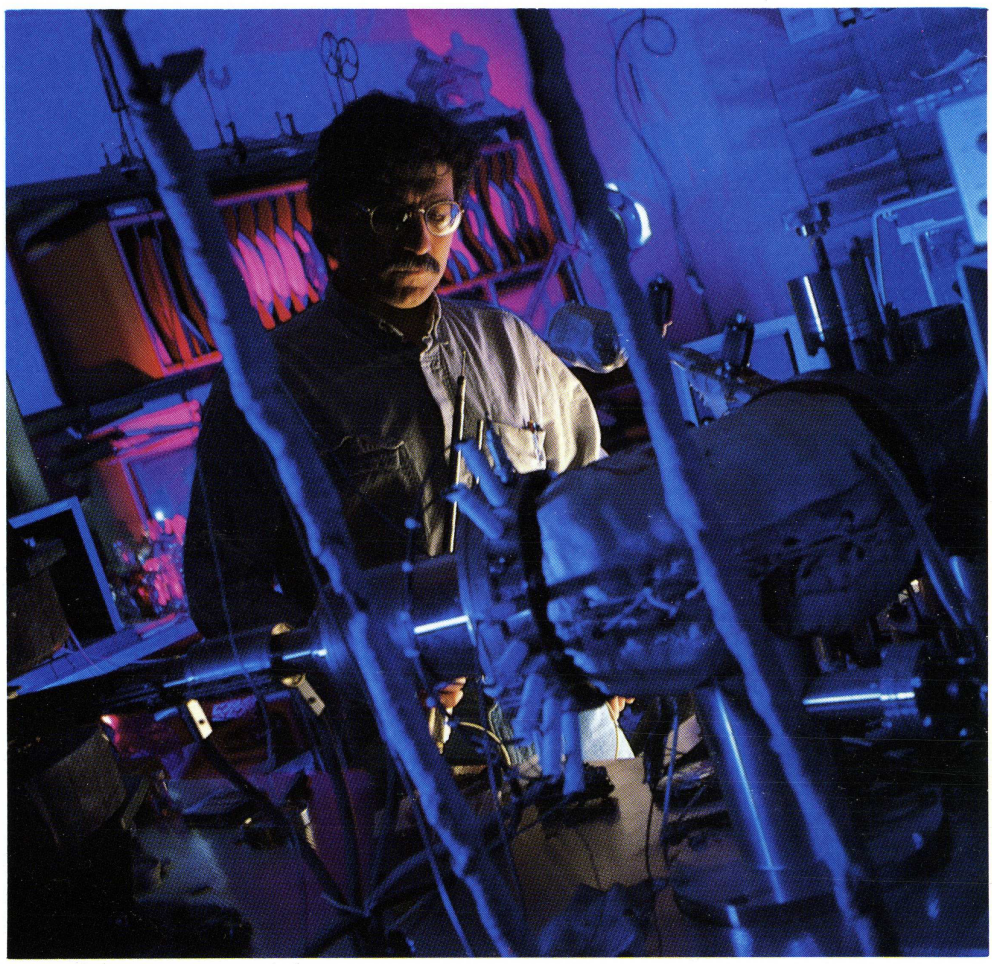
Brian Chait is the head of the mass spectrometry laboratory at Rockefeller, a facility that also serves as the National Institutes of Health's National Resource for the Mass Spectrometric Analysis of Biological Macromolecules. In plain English, what he really does is figure out how to make molecules fly.

"Mass spectrometry," explains Chait, "is the art and science of weighing naked molecules accurately. To do that, you have to do something unnatural: you have to separate them from their environment, separate them from their surroundings. In this lab, we're especially interested in biomolecules—proteins and nucleic acids, for example—and these tend to be bonded very tightly to other molecules. You have to perform a small miracle to get them loose without damaging them."

Who cares how much a molecule weighs? "It's actually an extremely important question," says Chait. "Weighing a molecule can be a real help in understanding how it's built and ultimately how it behaves." For example, he explains, there may be 100,000 different proteins in the human system, each of which has a specific function. "Biologists really need to understand each of these fantastic machines and structural units in all their exquisite detail, but so far they have identified and understand just a small proportion." Mass spectrometry is a way of identifying, classifying, and elucidating the structures of the proteins, important steps toward that understanding.

Another job mass spectrometry can do—and which Chait's lab does regularly—is to weigh molecules artificially created in synthetic chemistry and biotechnology labs, to see whether the finished product matches the specifications set by its designer. "Our activities range from the construction of novel mass spectrometers and fundamental research in mass spectrometry and gaseous ion chemistry to the development of new methodology for measuring molecules and investigating biological systems. We collaborate with biologists at Rockefeller as well as at other institutions—more than seventy research groups every year."

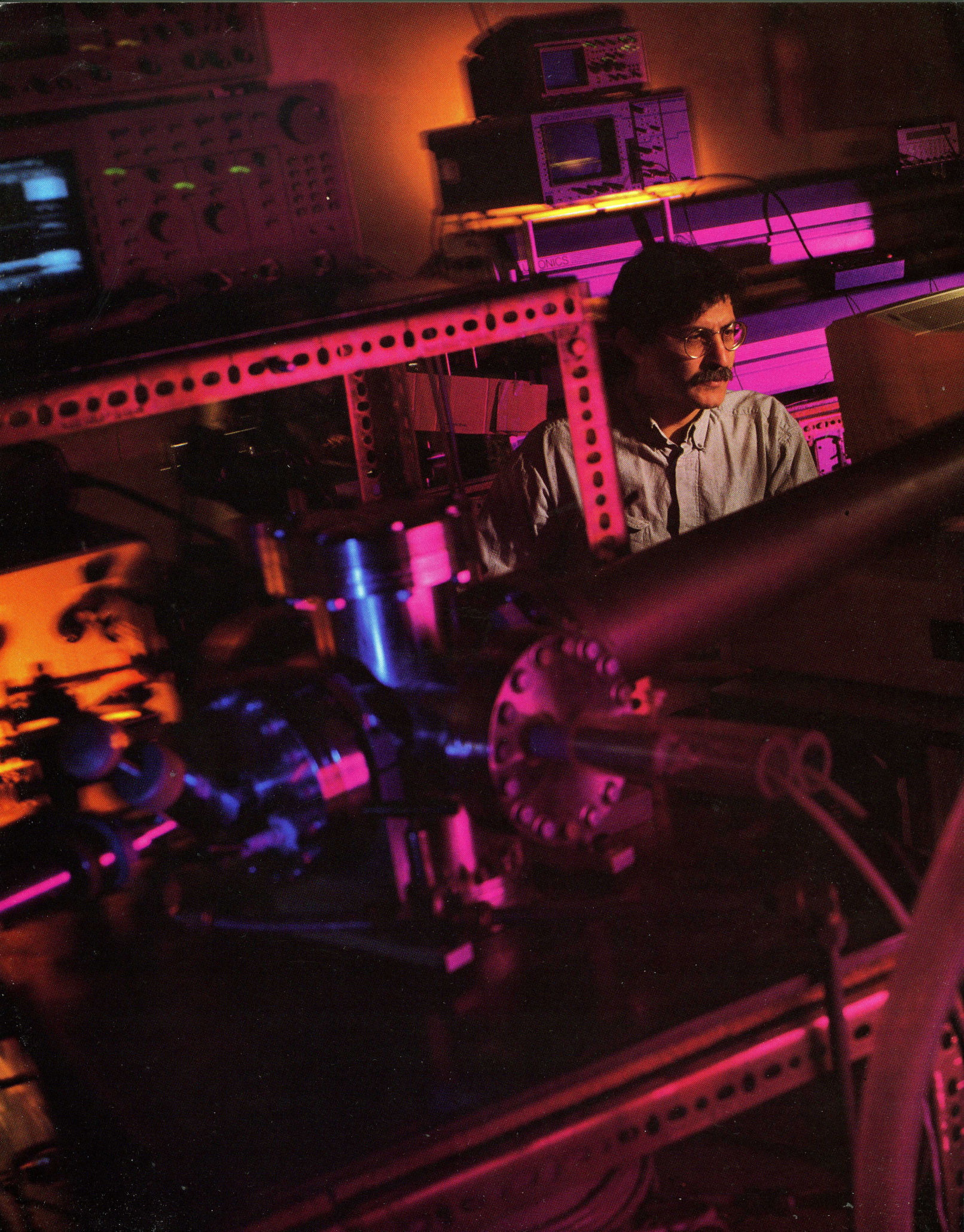
ROBERT REICHERT



Brian Chait prepares a sample for insertion into a mass spectrometer.

The basic idea behind mass spectrometry is deceptively simple. First, take a sample of the material you want to weigh and turn it into a gas of free-flying molecules. Ionize the molecules, adding or stripping a few protons from each to give them a positive or negative electric charge. An electric field then sends the molecules flying down a vacuum tube. When they smack into the far end, measure how long it took them to make the trip. And that's it. The time of flight is determined by a molecule's mass (heavier molecules take longer), and, says Chait, "if you do the timing accurately enough, you can measure the mass quite

SAM FLAMSTEED is a free-lance writer whose work appears frequently in *Discover* magazine.



MAKING MOLECULES FLY

Left: *Chait takes results on a mass spectrometer constructed at The Rockefeller University*

accurately as well. We can get the timing down to a fraction of a nanosecond."

"In the early days," explains Chait, "it was used mostly by physicists who were studying the chemical elements. Later on, it was used by chemists, for example petroleum chemists who were studying hydrocarbon molecules for the oil industry." By the late 1960s, though, the technique was being applied to fragile organic molecules, and the standard, brute-force gasification and ionization methods had to be changed. "For years, the way you did it was by heating the sample and then bombarding the vaporized molecules with a beam of energetic electrons," says Chait. "Unfortunately, this treatment tends to destroy the very molecules you want to study."

It is these two problems—gasification and ionization without destroying the molecules—that make mass spectrometry as much an art as it is a science. A major breakthrough on the second part of the problem came from Frank Field, Chait's predecessor at the lab, with the development of chemical ionization. "What you do," says Chait, "is produce another ion—say, protonated methane or isobutane—that reacts with the one you want to study and ionize." Not only does this avoid electron bombardment of the sample, but the chemical reaction can be tailored to generate very little heat, and thus produce a gentle form of ionization.

That still leaves the first problem: how do you evaporate the sample in the first place? "Fortunately," says Chait, "this field is full of marvelous chance discoveries." In the mid-1970s, two nuclear chemists named Ronald MacFarlane and David Torgerson, working in Texas, were weighing disintegration products from highly unstable nuclei and noticed extra bumps in their data: some other species were being inadvertently weighed at the same time. It turned out that some of the energetic nuclear disintegration products were knocking into molecules of vacuum-pump oil and other contaminants inside the spectrometer, and gasifying them. The chemists realized what was happening, and started using fission fragments from the radionuclide californium-252 to evaporate and ionize samples. "The surprise," says Chait, "was that these massive, highly energetic nuclear fragments can sometimes evaporate large, delicate molecules without damaging them."

It was at this point that Chait, himself, entered the field of mass spectrometry, largely by chance. Trained as a nuclear physicist at Oxford, the South African native was working in Manitoba on a project measuring the protein content of wheat samples by irradiation in a cyclotron. The funding ran out, so he and his mentor, Ken Standing, looking for another project, tried building a mass spectrometer like the one the Texans had invented.

The funding soon ran out for that work as well (though not before Chait and Standing constructed a novel instrument that could evaporate biomolecules by bombardment with low-energy ions produced in a hot filament.) When Chait heard that Frank Field was attempting to do similar work, he brought his family to New York. "I expected to be here for a year," he says. That was in 1979; three years ago Field retired, and Chait took over the lab.

Since then, he and his colleagues have continued to improve on mass spectrometry. Building on the pioneering work of others, the Rockefeller lab developed yet another technique for evaporating molecules gently: they dilute the samples into another substance known as a "matrix." Then they zap the mixture with an ultraviolet laser. "This is going to revolutionize the field yet again," says Chait, "because with this method we can probably measure most proteins, we can measure proteins in the face of large amounts of impurities, and we can look at samples with lots of different proteins in them and study them all. We realized the power of the technique the day my colleague Ron Beavis went up to the supermarket and bought some full-fat, homogenized milk, and tried analyzing that. In one shot, we were able to characterize all the major proteins in this very crude biological mixture. We're presently trying to develop a new means to sequence proteins—figure out exactly the order of the constituent amino acids much faster than you can with conventional techniques. We're also just beginning to tackle the question of whether we can sequence DNA by mass spectrometry," he says.

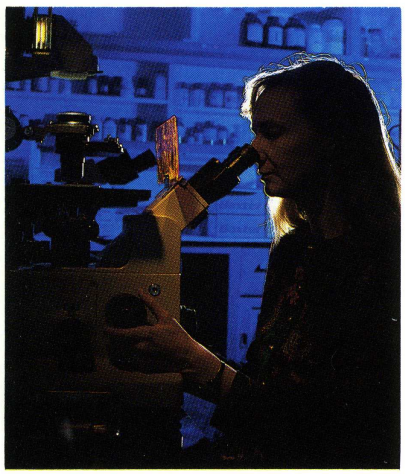
The laser instrument was patented, and a scientific equipment company is now manufacturing it. Meanwhile, scientists in Chait's lab continue to look for innovative ways to do mass spectrometry. One technique now being explored is "electrospray ionization" in which molecular samples are sprayed into the machine in an ultrafine mist, not unlike that produced by a sneeze. There is, evidently, no end to the things Chait will try in the search for ways to make molecules fly.

ROBERT REICHERT

SPY VERSUS SPY:
SCIENTIFIC "COUNTERESPIONAGE" OUTSMARTS BACTERIAL STRATEGIES

by Susan Blum

ROBERT REICHERT



Elaine Tuomanen

All good spies know the rules of espionage: fit in, speak the language, and never arouse suspicion. The precepts are the same for bacteria, nature's consummate foreign agents. Over the eons, the pathogens have learned to penetrate cellular societies more tight-knit than the CIA, and evade defenses far more formidable than the Pentagon's.

Rockefeller scientist Elaine Tuomanen is fas-cinated by the machinations of bacterial operatives. Among the pathogens she studies is *Bordetella pertussis*, the microorganism that causes whooping cough. More children in developing countries die of whooping cough than of any other bacterial disease, and despite the availability of a vaccine, children in developed nations continue to be afflicted, as well.

Tuomanen and her Rockefeller colleagues have found that bordetella acts as a spy by infiltrating the body's cellular address-recognition system. Composed of an interconnecting network of cell surface molecules, this system ensures that cells of the same organ stick together and that mobile cells, such as those of the immune system, reach their appropriate cellular targets.

Molecules known as glycoproteins and glycolipids are an essential part of the cell adhesion and recognition system, for each sports a distinctive sugary lattice that helps give a cell its particular address. In 1985 Tuomanen and her colleagues discovered that two of bordetella's cell surface proteins—pertussis toxin (PT) and filamentous hemagglutinin (FHA)—can decipher lung cells' sugar-coated street signs. This fluency in the dialect of the human body enables bordetella to adhere to lung cells and colonize them.

Recently, Tuomanen and her colleagues discovered that PT is not only a skilled linguist, but a crafty mimic, as well. The scientists found that PT molecules have co-opted the structure and function of human selectins, molecules that link up with cell surface sugars to direct white blood cells around the body. When PT's selectin analogue is active, bordetella moves to macrophages, a type of white blood cell whose usual job is to eat and destroy invading pathogens. Bordetella subtly subverts this function, prompting the macrophage to ingest the bacterium but leave it intact. Once snugly inside the macrophage, bordetella remains hidden from all the body's defenses.

To enter macrophages, bordetella's PT protein teams up with a region of FHA. The stratagems the bacterium uses are a testament to the cleverness of the bug; how those stratagems were uncovered, a testament to the value of scientific collaboration.

Tuomanen knew that FHA contains a region known as the RGD domain. In many mammalian proteins, this domain is a recognition site for integrins—molecules that are crucial players in cell–cell adhesion systems. But what, Tuomanen wondered, was bordetella doing with its RGD domain on the protein FHA?

Scientists don't leave their work when they leave the lab. The question nagged at Tuomanen, even when she took a break to watch her young daughter play outside The Rockefeller University Children's School. There, she would sometimes run into another parent, Rockefeller researcher Samuel Wright. Wright is an expert on how integrins facilitate transendothelial migration, the process by which white blood cells move out of the bloodstream and across blood vessel walls to reach a site of infection. As the researchers learned more about each other's work, intriguing hypotheses began to take shape. "Sam's an integrin person, I'm a bacteria person, and the bacteria forced us together," Tuomanen says.

Pursuing the connections experimentally, the scientists discovered that the complex cooperative interactions between cell surface molecules on bordetella and white blood cells mimic the molecular mechanisms by which white blood cells adhere to, and pass through, blood vessel walls. By exploiting these mechanisms, bordetella can sneak into the macrophage and hide out from immune system surveillance.

But even out in the open, the pathogen has found ways to subvert the body's defenses. Its PT protein serves not only as an adhesion molecule, but also as a toxin that circulates through the body. The selectin analogue region of these circulating particles latches onto white blood cells before the cells can hook up with selectins on blood vessel walls. Derailed, the immune system cells float aimlessly through the bloodstream, rather than rush to the site of infection.

Clearly, bordetella is a multitalented spy. But, says Tuomanen, "It is not all fear and dread. Once you penetrate the spy's

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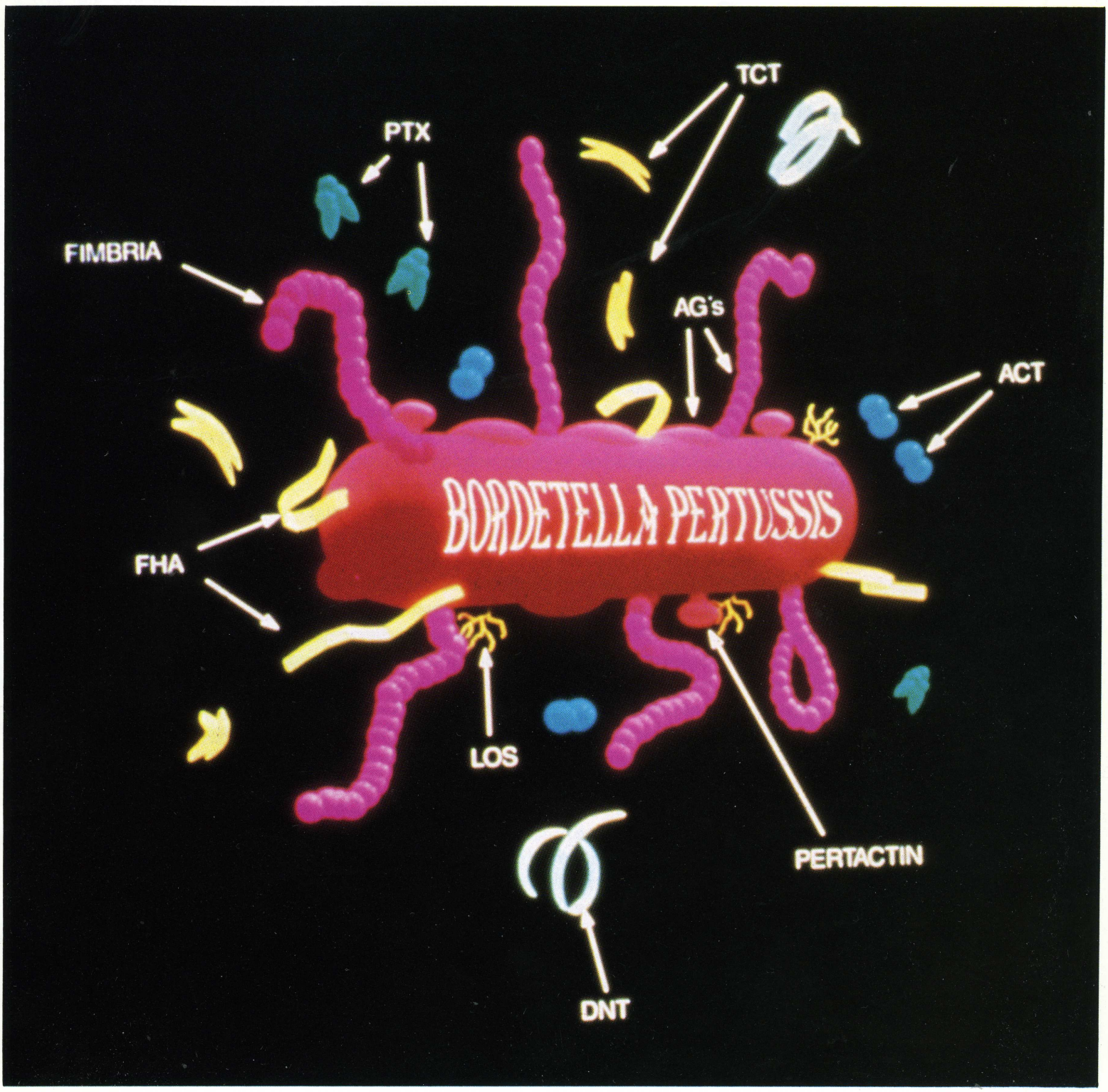
SUSAN BLUM is a science writer in The Rockefeller University Public Affairs Office.

Below: *Many different cell surface components of the bacterium,* Bordetella Pertussis, *contribute to its ability to cause whooping cough*



COMPUTER-GENERATED ILLUSTRATION BY DR. W. NEAL BURNETTE. REPRINTED WITH PERMISSION FROM BIO/TECHNOLOGY.

OUTSMARTING BACTERIA

strategies, you can be a counterspy."

Potential countermoves include constructing better vaccines. Knowing how bordetella adheres, vaccines might be made that keep the bacterium from sticking to lung cells. Properly engineered, such vaccines might be safer than current ones. They might also wipe whooping cough off the face of the earth. "Humans are the only hosts that harbor bordetella. If you can keep the bug from sticking to human cells, you can eradicate the disease," Tuomanen says. In the meantime, she adds, knowledge about how bordetella adheres is also suggesting improved treatment methods. For instance, studies in her lab have shown that bordetella can be washed from the lung when lured by sugars that bind its surface molecules even more strongly than those on lung cells.

As Tuomanen and her colleagues pry open bordetella's cache of secrets, they are learning not only about the bacterium, but also about us. Having spied on humans for millennia, the bugs know us very well—and what they've learned can help us.

For instance, in order to colonize lung cells, bordetella had to learn how to read the lung's address. It did so by evolving a PT protein domain that recognizes the sugary molecule on the lung cell's surface. "With this protein domain in hand, we now have a 'taxi' that can get us to the lung whenever we want," Tuomanen says. Even more exciting, her findings provide a general framework for building cabs that can be used to deliver substances—such as drugs—to specific targets anywhere in the body. In the same way, the mimicry strategies devised by bordetella to waylay white blood cells may someday be harnessed to halt inflammation in diseases such as arthritis.

Unmasking their mimickry is just one way of learning from bacterial spies; cracking their codes is another. The codes are carried in components of the pathogens' cell walls—elaborate external skeletons composed of sugars and amino acids unique to bacteria. Each code elicits a symptom of a bacterial disease.

Part of Tuomanen's research focuses on the codes in the pneumococcus, a bacterium that causes pneumonia and meningitis. Before the advent of penicillin, pneumococcal meningitis killed all of its victims, most of them children. The introduction of antibiotic therapy in the 1940s improved survival rates, but not enough; forty years after penicillin's first use, one of three treated youngsters still died of meningitis.

Tuomanen, herself a pediatrician, was determined to learn why the rate of death and serious complications from meningitis remained so high. She and Rockefeller colleague Alexander Tomasz discovered that antibiotics, which blow the pneumococcus apart like a bomb, create millions of cell wall fragments—a process releasing the code that summons white blood cells to the scene. The result: a sudden, massive, and potentially destructive migration of white blood cells across the blood vessels and into brain tissue.

Tuomanen and her colleagues demonstrated that administering steroids along with antibiotics calms white blood cells' overreaction, while still allowing the pathogens to be destroyed. This demonstration provided the basis for clinical trials worldwide. In 1990, the researchers' strategy was adopted as policy by the American Academy of Pediatrics; since then, the death rate among children treated for meningitis has declined to under ten percent.

But steroids are big therapeutic guns, and Tuomanen wants to find better tactical weapons. A promising approach is to use an antibody, developed by Sam Wright, which masks an integrin on the surface of white blood cells. The masking blocks transendothelial migration, and thus prevents inflammation. Experiments with animals have proved dramatically successful, and Tuomanen and Wright are now working with a pharmaceutical company to bring a drug based on this strategy to market for use in humans. Like the experimental anti-inflammatory techniques emerging from the work with bordetella, the antibody-based strategy may prove useful in a wide range of diseases.

Other useful therapies may emerge by cracking the code pneumococci use to slip through the "blood-brain barrier." The pneumococcus is one of only three known bacteria able to sneak into the brain through this barrier, the natural fortification the body erects to protect the brain from pathogens circulating in the blood. Tuomanen and Tomasz are homing in on the cell wall components that let the pneumococcus pierce this normally impenetrable shield. Once they have found it, they believe, it may provide a new tool to deliver drugs to combat diseases—such as brain cancer, Alzheimer's disease, and AIDS—where the blood-brain barrier has proved a formidable challenge.

Bacterial cell wall components—unique to bacteria—and bacterial cell surface proteins—sometimes near-perfect mimics of their human host—are two very different devices used by bacterial spies to infiltrate and conquer. But scientists are using their own powerful surveillance techniques to thwart the bacteria's stratagems. The battle of wits set in motion by nature's intelligence agents is turning out to be more intriguing than any spy story crafted by human hand.

A SCIENTIFIC PROFILE

PRESIDENT TORSTEN WIESEL— SEVENTH HEAD OF ROCKEFELLER

by Geoffrey Montgomery

[Note: The Board of Trustees has appointed Dr. Wiesel to serve as president of The Rockefeller University for a term of three years beginning January 1, 1992.]

It was late one night in the summer of 1958, and Torsten Wiesel and David Hubel were getting nowhere. In a small, dingy, windowless basement lab at the Wilmer Institute of Johns Hopkins Medical School, the two young scientists had been trying for a month to make sense of the most complex structure in the known universe: the cerebral cortex of the mammalian brain. That day, as on several previous days, they had implanted an electrode into the visual cortex of an anesthetized cat to record the response of a single cortical cell to patterns of light.

But nothing they did could get the cell to fire impulses in a consistent manner. Using a modified ophthalmoscope equipped with a slide projector, they alternately projected a bright spot and a dark spot onto different parts of the cat's retina. To project the bright spot they used a brass rectangle with a hole drilled in it; the dark spot was made by a black dot glued to a glass microscope slide. Wiesel had spent the last three years recording from cells in the cat's retina with these same spot stimuli, and knew first-hand that retinal cells were attracted to them. They fired like mad when they saw spots. But cells in the visual cortex, the area of the brain behind the back base of the skull to which retinal neurons project *via* the optic nerve and thalamus, seemed unmoved.

Then, about four hours into their fruitless recording session, Hubel and Wiesel finally began to get some sputtering responses from their cortical cell. The ophthalmoscope's slide projector was pointed towards the retina's midperiphery. They concentrated their efforts here; perhaps this was the retinal region that fed into the cell in the cortex from which they were trying to record. As they put the black-dotted glass slide into the slot of their projector, it got stuck. They unjammed the slide, pushed it slowly into the slot—and suddenly the cell burst alive. A rapid *pop-pop-pop* sound fired from the audiomonitor like a machine gun.

"The door to all the secrets," as Wiesel would later call it, had been opened.

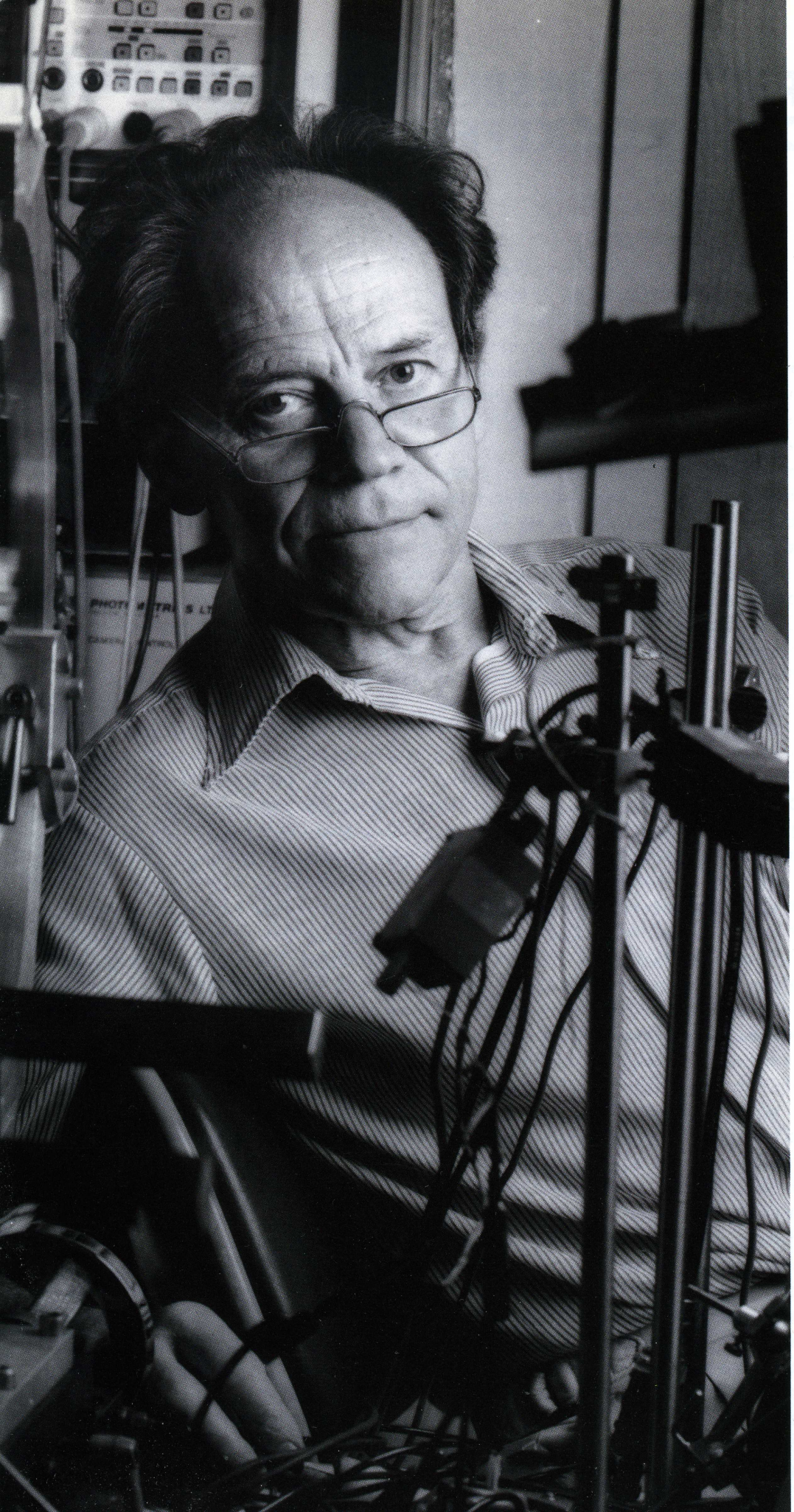
"What had happened was the edge of the glass slide had cast a shadow on the retina," Wiesel remembers. As this shadow-edge moved slowly across a particular portion of the visual field, the cell in the visual cortex had responded by firing a burst of action potentials. By rotating their projector, Hubel and Wiesel soon found that the cell responded only if this shadow-edge was oriented at a particular angle. Such specificity of neuronal response was unheard of. This discovery of what came to be known as "orientation-selectivity" is generally regarded as the most remarkable finding ever made about the cells constituting the cerebral cortex. It led Hubel and Wiesel to an astonishing series of investigations that have revolutionized understanding of the higher brain's structure, function, and development. This work, which garnered Hubel and Wiesel a Nobel Prize in 1981, has provided the basis for new strategies of ophthalmological surgery for infants born with cataracts and other congenital eye disorders. Since winning the Nobel Prize, Wiesel and his colleagues have been involved in a "second wave" of discoveries in visual cortex that may prove as illuminating as the first.

Torsten Wiesel was born in Uppsala, Sweden, in 1924, the youngest of five children. His interest in the brain grew out of his upbringing: his father, Fritz, was chief psychiatrist and head of Beckomberga Hospital, a mental institution located on the outskirts of Stockholm. After earning his medical degree at the Karolinska Institute in 1954, Wiesel entered the neurophysiology lab of one of his professors, Carl Gustaf Bernhard. There he worked for a half year on problems related to epilepsy before going to the Johns Hopkins Medical School lab of Stephen Kuffler in 1955.

Kuffler, building on the work of H. Keffer Hartline, had just published his pioneering studies of the "center-surround" organization of retinal receptive fields—that is, the preference retinal ganglion cells exhibited for light or dark spots of a particular size. For three years Wiesel extended Kuffler's studies of the cat retina. "Steve became a sort of role model for many of us who worked with him," says Wiesel. "He was very understated, with a good sense of humor, and yet he had a very incisive intelligence and a great intuitive sense in his way of doing science." In 1958 Wiesel was joined in his dingy basement lab by David Hubel, a young Canadian neurophysiologist. Thus began one of the longest and most brilliant collaborations in biology. "David and I, in many ways," says Wiesel, "became like two brothers."

By September 1958, soon after the moving shadow of the glass slide had ushered Hubel and Wiesel into the undiscovered country of visual cortex, hints of broader organizing principles began to emerge. A large percentage of cells in primary visual cortex, they found, were tuned to respond to cells of a particular orientation. Moreover, cells in the same cortical location had equivalent orientation preferences. This reminded both researchers of a recent finding by Vernon Mountcastle of Johns Hopkins that somatosensory cortex seemed to be arranged in "columns"; an electrode plunged vertically down into the cortex's six layers encountered cells with similar functional properties. A columnar organization of orientation preference also made sense from the classic

GEOFFREY MONTGOMERY has been a contributing editor of *Discover* magazine, and is currently working on a book about vision and the brain.



ROBERT REICHERT

PROFILE OF TORSTEN WIESEL

anatomical studies of Lorente de Nó, who had found that most connections in cortex are made between local clusters of cells that are vertically aligned. Hubel and Wiesel found that the center-surround properties of retinal ganglion cells discovered by Kuffler were preserved when they were projected to the thalamus; but once this visual input reached the cortex, it was transformed. Inputs from center-surround cells somehow became combined along particular orientation axes within particular cortical columns. Every small segment of visual field represented by primary visual cortex contained multiple orientation columns, each packed with cells attuned to edges of slightly different orientation. In a recording session in 1961, by which time Hubel and Wiesel had moved with Kuffler to Harvard Medical School, the two scientists set out to systematically explore the arrangement of this crystal-like columnar structure in an anesthetized spider monkey they called George.

The recording session began at 8:00 p.m. "I can still picture the experiment vividly," remembers Wiesel. By angling their electrode at about forty-five degrees relative to George's cortical surface, and advancing the electrode a mere fifty micrometers into the cortex per recording, Hubel and Wiesel were able to sample orientation cells from neighboring vertical columns. "What we found," Wiesel says, "was an arrangement of amazing regularity." Hubel manned the slide projector—which by now projected onto a screen facing the animal—while Wiesel mapped the cell's receptive field on a sheet of paper scrolled across the screen. The first cell from which they recorded fired best to lines oriented at precisely twelve o'clock midnight. The second cell, from a neighboring orientation column, fired at 11:40. The third was even closer to eleven o'clock. And the pattern kept continuing: the line of optimal response

shifted backward by inexorable ten-degree steps, like the hour hand of a clock slowly reversing in time—eleven o'clock, ten, nine, eight, seven, six. "It seemed as if it would never end," says Wiesel. The experience was at once tedious and mesmerizing; neither Hubel nor Wiesel moved from his seat. Halfway through the session, on the twenty-fifth cell, the hour hand of orientation preference changed direction and began to shift clockwise. Now, and until the fifty-third and last cell was recorded at 1:00 a.m., time and line of pre-ference ticked forward together. It was perhaps the most spectacular experiment in Hubel and Wiesel's twenty-year collaboration.

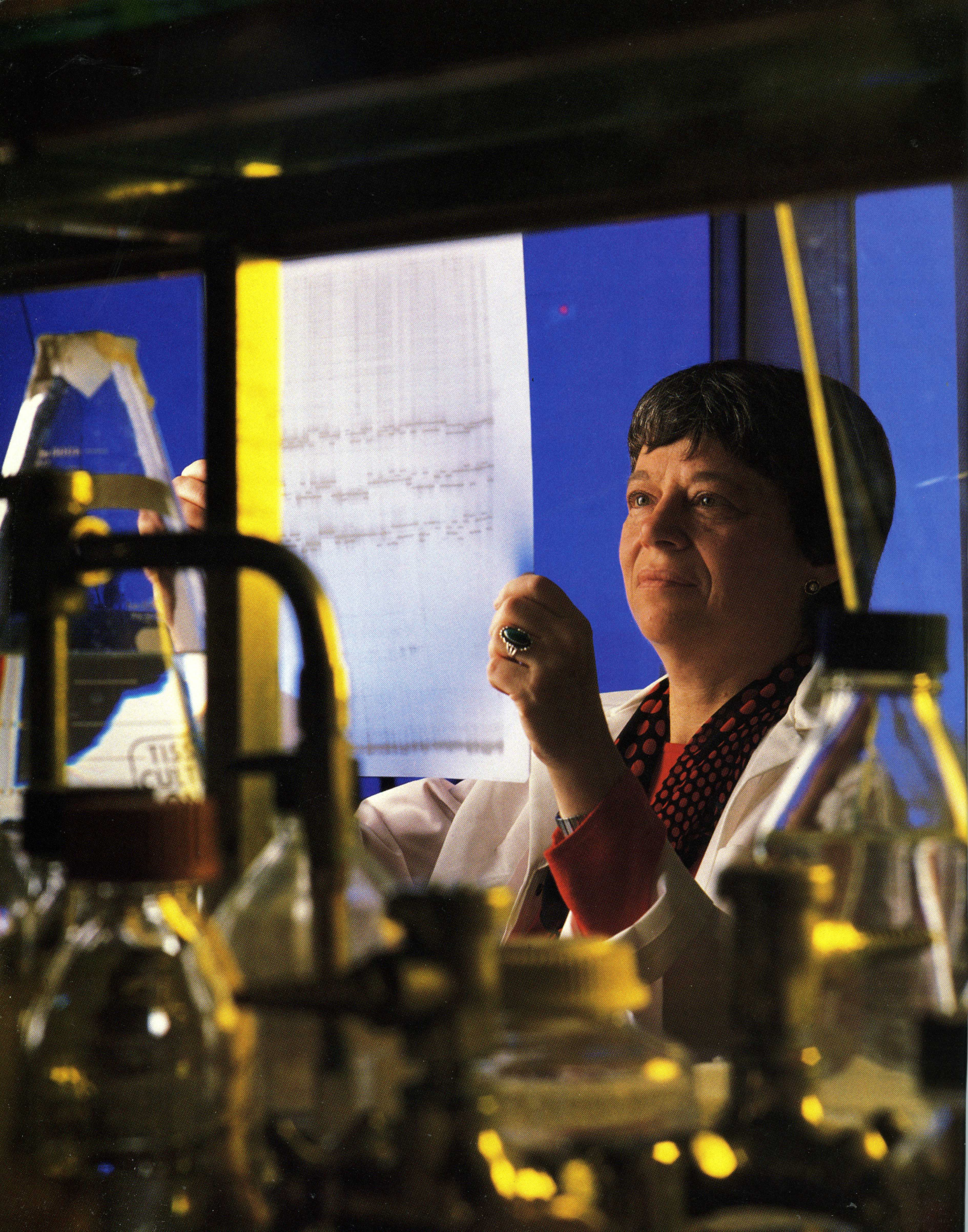
Arising out of George and related studies came one of the most important concepts of Hubel and Wiesel's work: the idea of functional architecture. "You know a functional property like orientation-selectivity is a salient one for an area, not just because you think you observe that property in the response of an individual cell," explains Charles Gilbert, a Rockefeller collaborator of Wiesel's since 1976, "but because it's mapped out in this orderly fashion, in terms of functional architecture."

In the early 1960s, Hubel and Wiesel began their path-breaking studies of the effects of early visual deprivation on the development of primary visual cortex. They found that a second piece of functional architecture—the "ocular dominance columns," a series of alternating stripes in which inputs from the two eyes are segregated—become grossly distorted when infant cats and monkeys are raised with one eye closed. During a "critical period" shortly after birth, the ocular dominance columns stemming from the open eye expanded, crowding out neurons from the closed eye. Since the days of Descartes and Locke, philosophers have debated whether experience is necessary for the development of vision and other mental functions. Hubel and Wiesel provided the first insights into how the brain requires contact with the multiform features of the outside world to become properly wired; and their hypothesis for how such wiring becomes established early in life has provided a paradigm for modern research into the mechanisms underlying both brain development and memory. In the early 1980s, ophthalmological surgeons in San Francisco began to apply Hubel and Wiesel's findings to the clinic. Because we see as much with our visual cortex as with our eyes, infants born with congenital cataracts must have them removed during a "critical period" very early in life, before the ocular dominance columns from their good eye permanently displace columns from the clouded eye. The concept of functional architecture is now helping to save the sight of many children.

In 1983 Wiesel established a lab at Rockefeller in the midst of a second wave of discoveries about the functional architecture of vision. Hubel and Margaret Livingstone found that small, oblong-shaped structures—the "blobs"—stained by the enzyme cytochrome oxidase contain machinery specialized for color processing. In the Wiesel lab, Daniel T'so and Charles Gilbert showed that residing in each blob are color cells of a particular type, and that where a blob borders orientation columns, there are cells selective for both an object's color and form. Moreover, since the late 1970s, Gilbert and Wiesel have studied the properties of neuronal connections that, in contradistinction to the vertical, columnar connections unveiled by classical studies, span horizontally across the cortex, linking columns with similar functional properties. They believe that these long-range horizontal connections are central to several still-mysterious perceptual tasks, such as figure-ground segregation and color perception of natural scenes, that require integration of information across the visual field.

The Wiesel lab, in collaboration with Rockefeller visiting scientist Amiram Grinvald, has also pioneered the application of "optical imaging" to visual cortex—a technique by which the activity of large regions of cortex can be pictured directly on a video screen. Optical imaging has enabled T'so, Gilbert, and Wiesel to discover a fourth kind of functional architecture, one specialized for detecting depth cues, within a second visual area to which primary visual cortex projects. "It is through this unveiling of functional architecture," Wiesel believes, "that you really under-stand the relationship between structure and function in the brain. You start to get that sense now for the second visual area. Things are falling into place."

Yet the relationship between structure and function—the deepest and most fundamental principle of biology—has yet to fall into place in other cortical regions. "We still don't know the functional architecture of auditory cortex and other sensory and motor areas," says Wiesel. Wiesel, who avidly collects paintings and other art objects on his lectures and travels around the world, sometimes wonders whether the picture of functional architecture that has emerged from the visual cortex has fully penetrated the eyes of researchers studying other sensory modalities. "Then again," he says, "they just haven't been able to find the right stimulus, the one that would reveal the organizing principle of these areas." Smiling in his sunny president's office as he recalls that evening in the windowless basement lab thirty-four years ago, when the edge of the glass ophthalmoscope slide cast its angled shadow over the retina and into history, Wiesel adds: "They haven't been lucky enough to find the secret door that leads to all the treasure."



ON THE TRACK OF A RARE DISEASE: STUDYING FANCONI ANEMIA

by Susan Blum

Left: *Arleen Auerbach at work in the laboratory.*

Scampering up a tree, the nine-year-old slipped, fell, and broke his arm. When Rockefeller scientist Arleen Auerbach heard the news, she was, of course, concerned—but she couldn't help being a little bit pleased as well. "It's great that he's now so healthy he's out climbing trees!" she says.

The boy has Fanconi anemia (FA), a rare genetic disease that Auerbach, a member of the laboratory of D. Martin Carter, has devoted her career to studying. FA manifests itself in a baffling array of symptoms. Some of them—such as birth defects and mental retardation—vary from person to person. But one set of problems is tragically common: blood cell abnormalities that lead to aplastic anemia and acute myelogenous leukemia. These fatal consequences of the disease usually develop in childhood or adolescence.

The youngster now climbing trees with abandon is a case in point. By the age of five, he had developed severe aplastic anemia and would probably have died within a year. But thanks to discoveries made by Auerbach and her colleagues at Rockefeller and elsewhere, he is still going strong.

"Until recently, bone marrow transplantation was often the best way to treat the anemia and leukemia resulting from FA," Auerbach reports. The bone marrow—a rich source of blood cell precursors known as stem cells—is collected from a healthy, compatible donor and transfused into the patient, whose own bone marrow has first been destroyed by drugs and irradiation. The donor's stem cells then multiply and develop into all the blood cells the recipient needs.

A healthy sibling with a compatible tissue type is the best potential donor for people with FA. In 1981, Auerbach and her colleagues developed a blood test to determine whether asymptomatic individuals have FA—a crucial first step in identifying healthy siblings as potential donors. The assay can also be used to test a sibling prenatally to determine if the baby will be able to serve as a donor.

Even under the best of conditions, though, bone marrow transplantation for FA still carries risks. For the donor—especially a baby—the risks include general anesthesia and the removal of a great deal of bone marrow. For the recipient, there is the risk of waiting until the donor is old enough to undergo the procedure, and the possibility of graft-versus-host disease, a reaction in which immune cells in the donor's tissue attack the recipient's organs.

Fortunately, there is now a way to bypass the need for bone marrow transplantation. Auerbach and her colleagues have shown that the blood from a newborn's umbilical cord is at least as good a source of stem cells as bone marrow. "You can collect the cord blood at birth, freeze it, and use it when needed at a later time," Auerbach explains. Easier on the baby, the procedure is better for the recipient, too, as it lets the transplant take place sooner and may elicit less graft-versus-host response.

The technique was first attempted in 1988, when cord blood from a newborn baby girl was transfused into her brother. Four years later, the results are clear and gratifying: a desperately ill little boy has been transformed into an energetic grade schooler.

Since that first procedure, additional cord blood transplants have been performed in the United States and abroad, with exciting preliminary results. In addition to its uses in FA, the procedure also holds promise for many other diseases that destroy bone marrow function, including anemias, leukemias, and certain immune deficiencies and genetic disorders.

As compelling as the work on cord blood transplantation is, it is just a part of a multifaceted research program on Fanconi anemia at Rockefeller. For example, FA patients are evaluated at The Rockefeller University Hospital in order to allow researchers to study the wide range of clinical manifestations of the disease.

Another facet of the work involves identifying the genes that cause FA. "Fanconi anemia has so many different manifestations that one gene is probably not responsible for all cases," Auerbach explains, adding that researchers worldwide are cooperating in an effort to locate the constellation of genes that may prove responsible. It is unlikely that all the genes will be found on just one chromosome. Auerbach and her team have tracked one of them to a region on chromosome 20, and are starting to home in on its exact location. "There's a good chance the gene will be found soon," she reports.

The hunt for the FA genes is facilitated by the fact that Rockefeller is the site of the International Fanconi Anemia Registry, a source of referral for molecular and clinical studies. Patients in the Registry provide the blood samples needed for the painstaking work involved in identifying the FA genes.

Finding the genes—and then pinpointing critical mutations—will be a crucial first step in determining the mechanisms underlying the disease. Researchers believe the genes help control essential functions, probably related to DNA processing or repair. But though the hints are tantalizing, "at the moment, we don't have a clue about the basic defects," Auerbach says.

Finding the genes will also make it possible to develop tests to identify disease "carriers"—people who have one faulty and one normal copy of a gene. Carriers cannot now be identified.

It would also be a boon to scientists. Auerbach believes that FA carriers may hold some clues to a wide range of disorders. If two faulty copies of an FA gene clearly lead to cancer, birth defects, and blood disorders, perhaps one faulty copy leads to an increased susceptibility to these conditions. "Once we can identify FA carriers, we can follow them epidemiologically," says Auerbach. "As a result, we may be able to increase our knowledge about diseases that are much more common than Fanconi anemia."

ROBERT REICHERT

TREASURES FROM THE PAST:

THE CASPARY GALLERY SCIENTIFIC INSTRUMENT COLLECTION

Text by Dan Cooper, with Merrill W. Chase
Photographs by Robert Reichert

Carrel-Lindbergh Perfusion Apparatus
In 1938 The Rockefeller Institute was featured in the cover story of *Time* magazine, an article describing the research of Alexis Carrel and his special assistant, the aviator Charles Lindbergh. Their perfusion apparatus was *not* an "artificial heart," for it supplied only soluble nutrients under aseptic conditions, but it kept organs alive in vitro for perhaps two weeks.

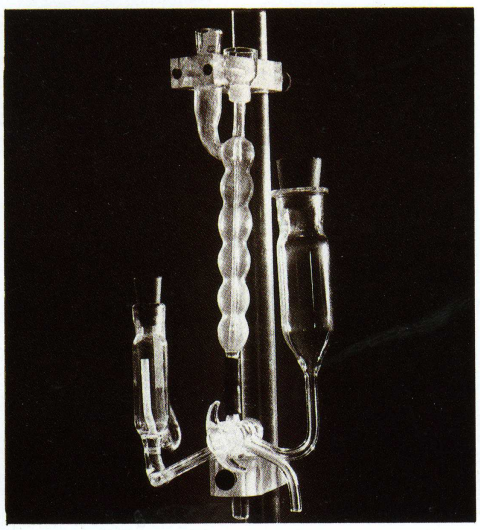
A collection of antique instruments is on display in Rockefeller University's Caspary Gallery. One can find apparatus and instruments that Rockefeller scientists used to make some of their most important discoveries. Many are firsts—the first portable pacemaker and the first practical glass electrode are examples. Some, like the perfusion apparatus of Alexis Carrel and Charles Lindbergh, reflect special moments in the history of this institution. Most are quaint—the kymograph, for example. They're museum pieces, literally, superseded by inevitable improvements in research technique.

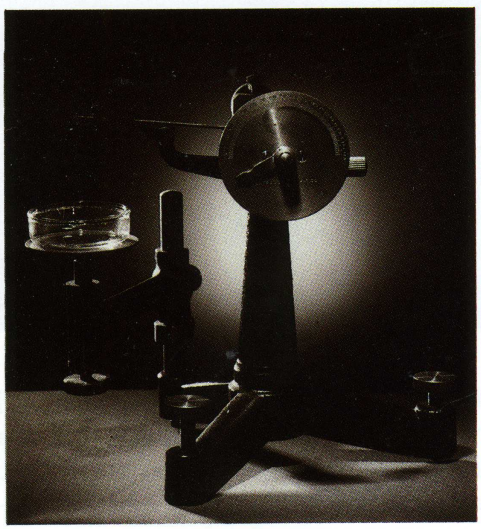
Today's laboratory is often automated; measurements are converted into bits and bytes for the computers that reduce and analyze the data. More scientific instrumentation is purchased; less is built in the university's instrument shop, which can't match commercial economies of scale.

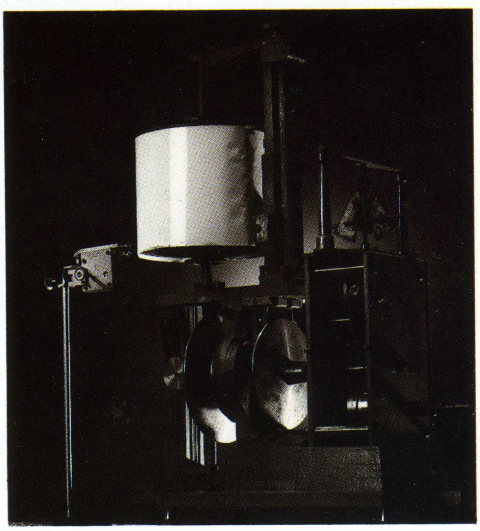
The collection in Caspary carries viewers back to a simpler time. They can imagine the instruments once again in use and evoke the scientists on whose insights and discoveries current researchers build.

Only a few examples are presented here. Visitors will find an excellent catalog, prepared by Rockefeller Professor Emeritus Merrill W. Chase, former Director of Public Information Fulvio Bardossi, and former Manager of Furnishings and Interiors Patricia Berlin, who organized the exhibit in 1976. Visitors can recapture some of the university's great past, and perhaps contemplate a future in which today's apparatus will seem quaint.

ROBERT REICHERT, whose work has appeared in *Time, Life, Forbes, Discover,* and other magazines, is the photographer for the Public Affairs Department at the university. DAN COOPER is an MIT physicist turned science writer who has preserved his doctoral-research apparatus at his home in South Orange, New Jersey. MERRILL W. CHASE, Rockefeller professor emeritus, provided valuable insights based on his work as an organizer of the collection.







MacInnes's "Glass Electrode"
Measuring pH (acidity or alkalinity) became a lot easier in the late 1920s when Rockefeller scientists Duncan A. MacInnes and Malcolm Dole developed the first "glass electrode" from Corning 0-15 glass, through which hydrogen ions can pass. The parts were held by lucite rods, the whole shielded in a grounded metal cabinet. MacInnes devised a special stopcock for readings in succession of buffer, unknown fluid, and a following wash. The current was amplified in two stages and pH read from a dial for the very first time. Nonetheless, it gave Rockefeller researchers new ease and accuracy in measuring pH in biomedical studies. Today,

LeComte de Nouy's Tensiometer
The Rockefeller Institute was a pioneer in recruiting physical scientists who could apply their insights and apparatus to the quest for biomedical knowledge. The device in the photo, developed by physicist Pierre LeComte de Nouy, measures surface tension. The dial registers the angle of twist in the wire (and hence the force) that's required to separate the small ring from a liquid surface. de Nouy used it in investigations of the properties of blood serum. The tensiometer shown here is a commercial version; instrument manufacturers have adapted many Rockefeller innovations.

The Kymograph—Catching the Wave
Today most laboratories have a computer and video display to record and instantly analyze the flood of data that research produces. It was not always so. In the past, recording required the use of the kymograph (from the Greek *kyma*, wave). First, researchers had to wrap glazed paper around a drum, then blacken it with soot from a benzene flame. Only then could they capture the motion of a stylus that scratched away some of the soot as the spring-driven drum turned, thus recording an electrical signal or the twitching of a muscle. Later, the trace could be fixed in dilute shellac and photographed.

OFF CAMPUS

ED ZIFF'S LOVE AFFAIR WITH DNA

by Philip DiMauro

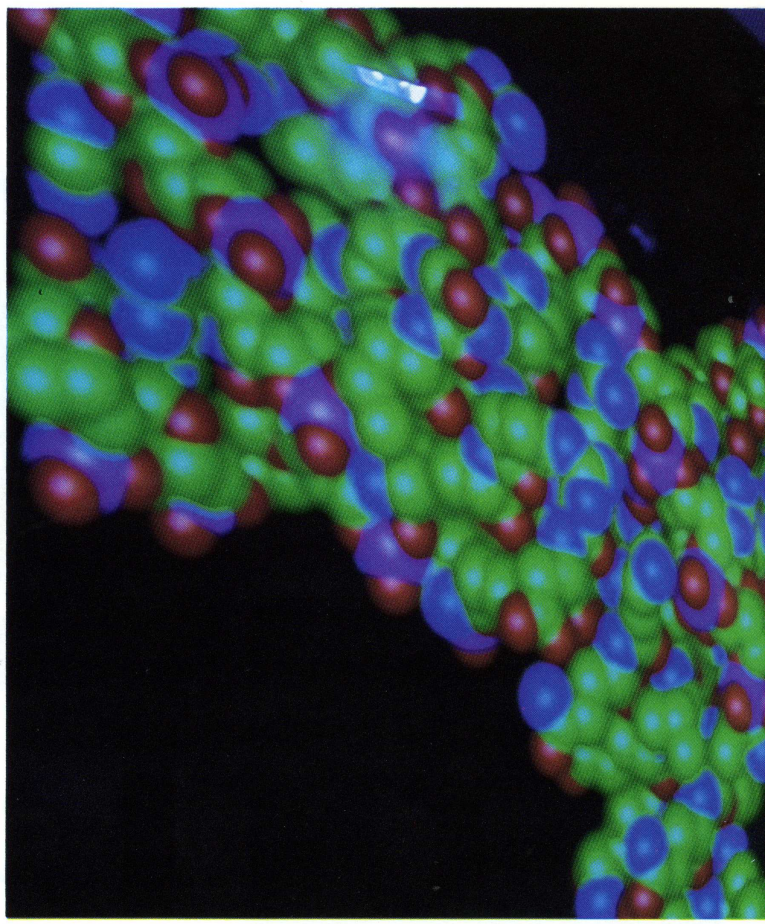
As a faculty member at Rockefeller in the late 1970s, Ed Ziff enjoyed team-teaching the advanced biochemistry course because it helped him to get acquainted with junior faculty members and Ph.D students from different laboratories. Then, as now, the course included a program of guest lecturers and weekly discussion of relevant scientific papers. "It was a tradition to ask the students any question at all, to really put them on the spot," Ziff recalls with a grin. "It was a very effective teaching method."

One victim of this well-intentioned grilling, Michael Greenberg, was nonetheless eager to rejoin his former teacher at the New York University Medical Center in 1983. That was two years after Ziff left Rockefeller to set up the NYU biochemistry laboratory that he leads today as an investigator of the Howard Hughes Medical Institute. Ziff had recognized Greenberg's talent as a scientist, but even he couldn't have predicted that their collaboration would change the course of research in Ziff's laboratory and, in the opinion of some scientists, create a new field of DNA research.

A chemist by training, Ziff became fascinated with the remarkable information-storing capabilities of the nucleic acids, DNA and RNA, during his final undergraduate year at Columbia. He wrote his Ph.D. thesis on nucleic acid chemistry at Princeton, and then journeyed to England in 1970 for post-doctoral work at the Medical Research Council Laboratory of Molecular Biology in Cambridge with Fred Sanger, a founding father of molecular biology and winner of two Nobel Prizes. In Cambridge, Ziff worked out the sequence of the first small fragment of DNA isolated from a phage, a virus that infects bacteria. It was painstaking work. "You have to appreciate that at the time, DNA was a bit like a rat's nest, just horribly complicated," Ziff says.

The phage was an ideal research model because of its relative simplicity. A virus consists of a few genes made from DNA or RNA cloaked in a protein capsule. It infects a cell by invading it and reprogramming the cell's genetic machinery to make more virus particles. In the late 1960s, most of the breakthroughs in genetics were coming from research with bacteria and viruses, but Ziff had already begun to consider the possibility of deciphering the long chains of DNA in the complex genomes of animal cells. The genes in our cells, bound in tight coils of DNA, contain the structural blueprints for the proteins we need to live. When a gene releases the instructions a cell requires to make a specific protein, biologists say the gene is expressed in that cell.

Ziff gained more experience in later work with with a tumor-

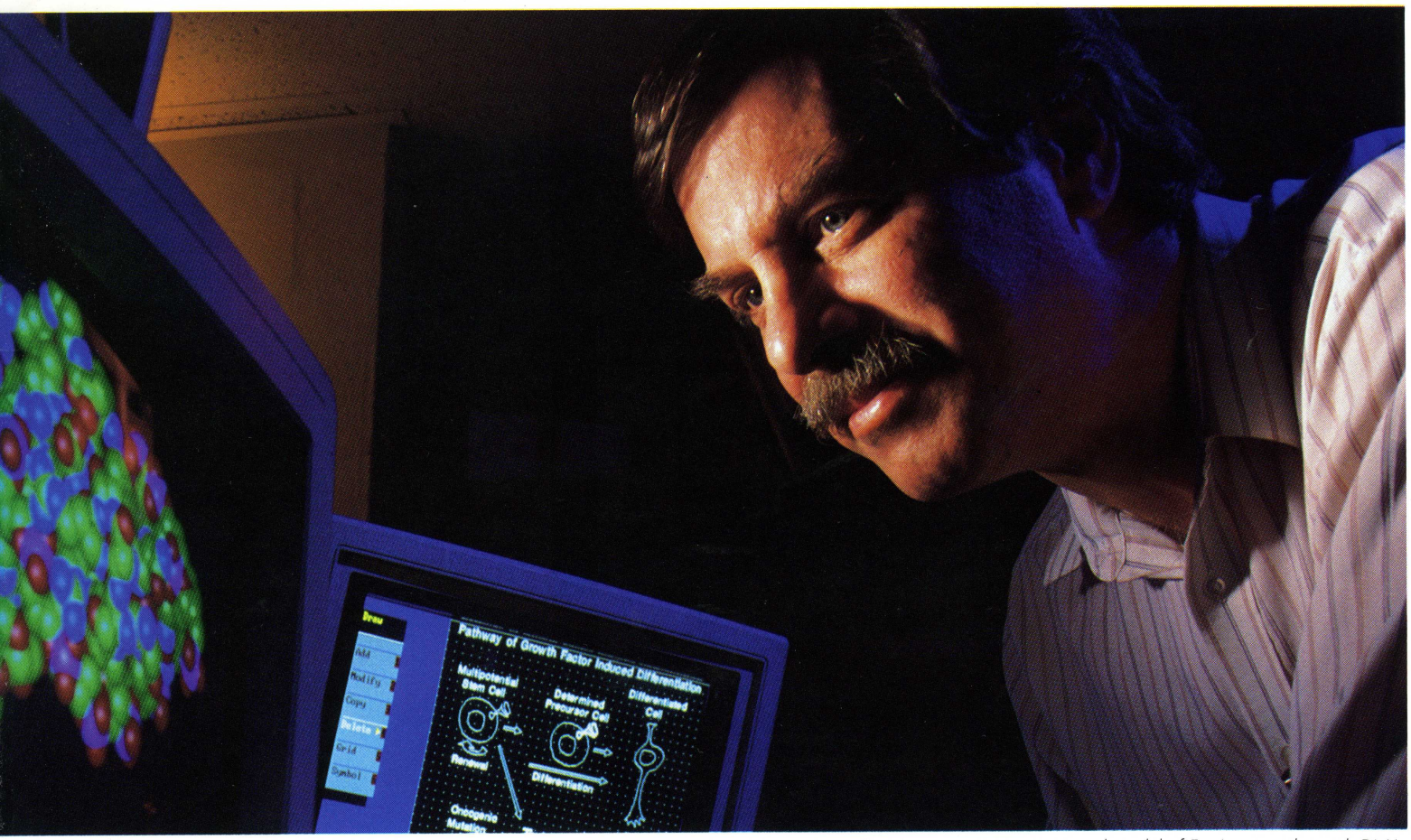


ROBERT REICHERT

inducing virus at London's Imperial Cancer Research Fund (ICRF) laboratories, where he became increasingly curious about the derangement of cellular growth programs that results in cancer. In 1975 Ziff joined the faculty of James Darnell's laboratory—then called the Laboratory of Molecular Cell Biology—where he focused on transcription, a primary step in gene expression during which DNA instructions are copied onto a messenger RNA molecule for delivery to cellular protein factories. With colleague Ronald Evans, he located the precise points in the adenovirus genome where transcription begins. When he moved to NYU, Ziff commenced a study of a protein called E1A that the virus uses to modify its host to its own advantage. E1A works as a communicator between the virus and its host. It was clear that a virus could infect a cell, and even cause a cell to become cancerous, by sabotaging the normal flow of information from the cell's DNA. But how did the normal regulators of cell growth work?

Greenberg and Ziff decided to tackle the problem of growth regulation in animal cells. They designed an experiment to test the theory that growth factors—molecules that cause cells to

PHILIP DiMAURO, a member of The Rockefeller University Development Office, has also worked as a business and entertainment journalist.



Ed Ziff studies a computer-generated model of Fos-Jun complex with DNA.

grow or divide—do their work by triggering gene expression. The two scientists cultured mouse cells in a medium containing growth factors and waited until the cells completely filled the culture dishes and ceased dividing. This "quiescent" state indicated that the growth factor supply was exhausted. Then they added more growth factors to the cells and analyzed the response. One result practically leaped out at them: the level of expression of a gene called *c-fos* multiplied 100-fold in a few minutes.

They had discovered a link between growth factors and gene expression, but the significance of their finding, published in 1984, was much greater. "The experiment pointed to the existence of a pathway that could conduct signals from outside the surface of the cell to a specific gene within moments," Ziff explains, "and the nature of that pathway has been been the subject of a lot of research ever since." c-fos was already known as a protooncogene—a gene which could, if defective, contribute to the onset of cancer. The experiment therefore suggested that certain cancer-causing genes could be regulated by environmental signals. Subsequent work in other labs showed that the Fos protein, which binds to DNA in combination with another protein called Jun, is a transcription factor, a major regulator of transcription of other genes. "What began to emerge from all this work," says Ziff, "was a complicated and sophisticated set of mechanisms that allows cells to respond to their environment."

Later experiments conducted by Greenberg and Debra Leonard in Ziff's laboratory showed that nerve growth factor, which is vital to the development of the nervous system, induces Fos expression in neurons. And some researchers now believe that Fos plays a part in modifying mature nerve cells in response to their environment, which means that this versatile gene may be critical to learning and memory. "To go from studying basic biochemistry to probing the nature of memory is quite a long transit, "says Ziff, "but that's one of the privileges of being a scientist. You can follow your curiosity and explore the greater implications of your own work."

Michael Greenberg is now an associate professor at Harvard Medical School, and Ed Ziff recently began a collaborative study of transcription factor structure with Rockefeller scientist Stephen Burley.

A MOLECULAR BASIS FOR SYNAPSE FORMATION

by Geoffrey Montgomery

Right: *Hui-Quan Han prepares a tissue culture.*

In February 1990, in the small tissue culture room of Paul Greengard's neurobiology laboratory, graduate student Hui-Quan Han looked under the microscope and found that the dishes of nerve cells he had been tending for over a year had become transformed. It was a transformation Han neither expected nor welcomed. In fact, the strange new appearance of his cultured neurons, the bulbous protruberances that had materialized all along their slender fibers, worried him. He was afraid he could no longer proceed with his experiment as planned. It was only after his initial surprise at the new shape of his cells had faded, says Han, "that I began to think that this phenomenon might be significant."

Significant, indeed. For later experiments confirmed Han's second impression that the unexpected bulbous structures represented newly formed synapses—the specialized junctions between nerve cells through which flow all perceptions, movements, memories, and thoughts. By inserting the gene for synapsin IIb, one of a family of four proteins which the Greengard lab has been studying for over two decades, into his cultured neurons, Han had induced his cells to form synapses where none had existed before. Han's finding about synapsin IIb, published in *Nature* in February 1991, has caused something of a sensation among neuroscientists: he has received a vast number of requests from all over the world for reprints of his synapsin article, and more requests are still arriving.

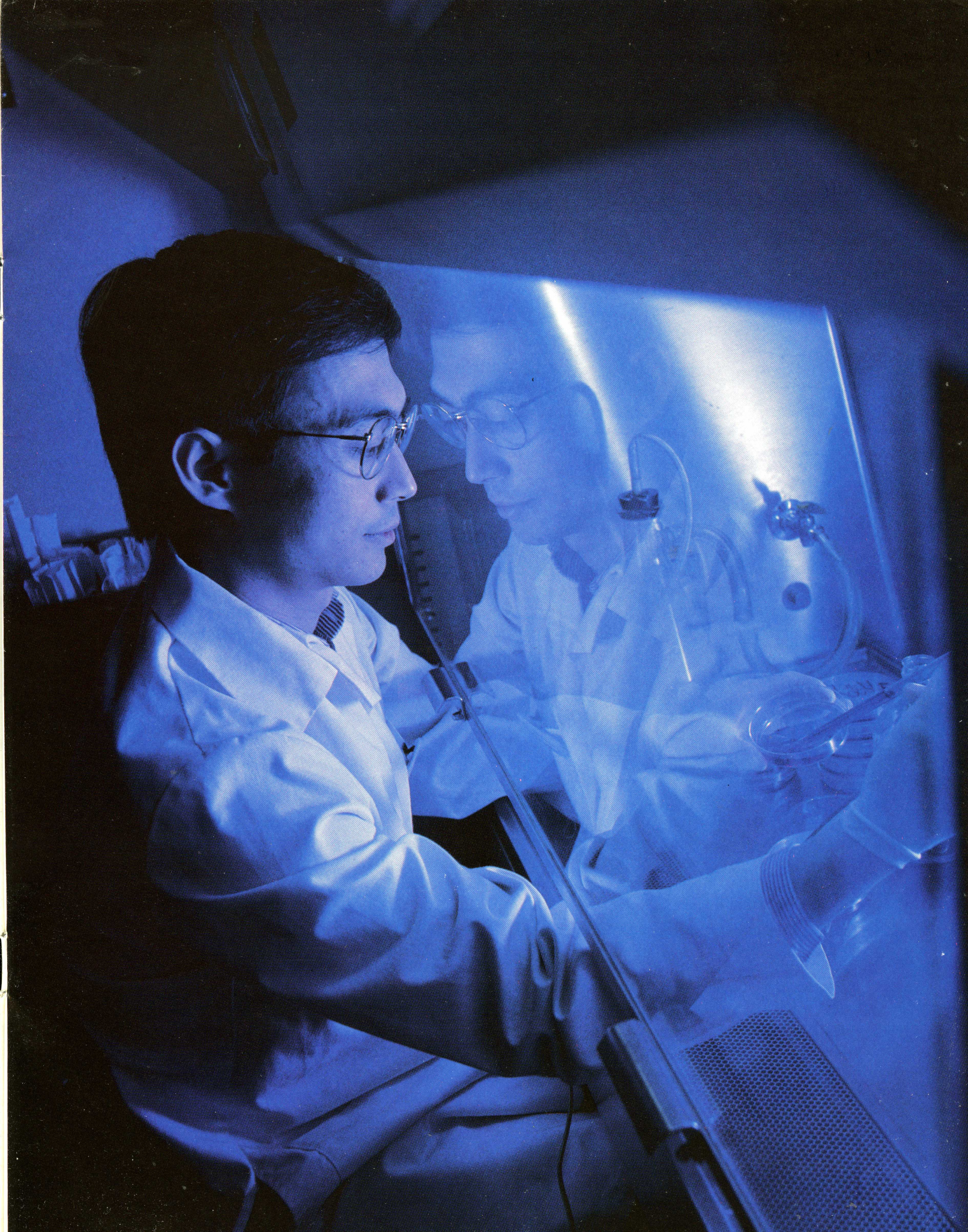
The origin of Han's fascination with synapsin is rooted in his native China, where, after graduating from medical school, he entered the Chinese Academy of Sciences as a Ph.D. student to pursue his interest in neuroscience. His former mentor in China, T.P. Feng, achieved an international reputation nearly a half-century ago for his discovery of post-tetanic potentiation, in which a neuron that has been stimulated at high frequency retains a short-term "memory" of this activity. The neuron reacts to later low frequency stimulation with enhanced neurotransmitter release; its signaling power has been temporarily strengthened. Feng continued to study the phenomenon, but "he described it as a black box," Han remembers. "He said there are some molecular mechanisms that may be important, mechanisms that someone needs to find. And so I have always kept that in mind."

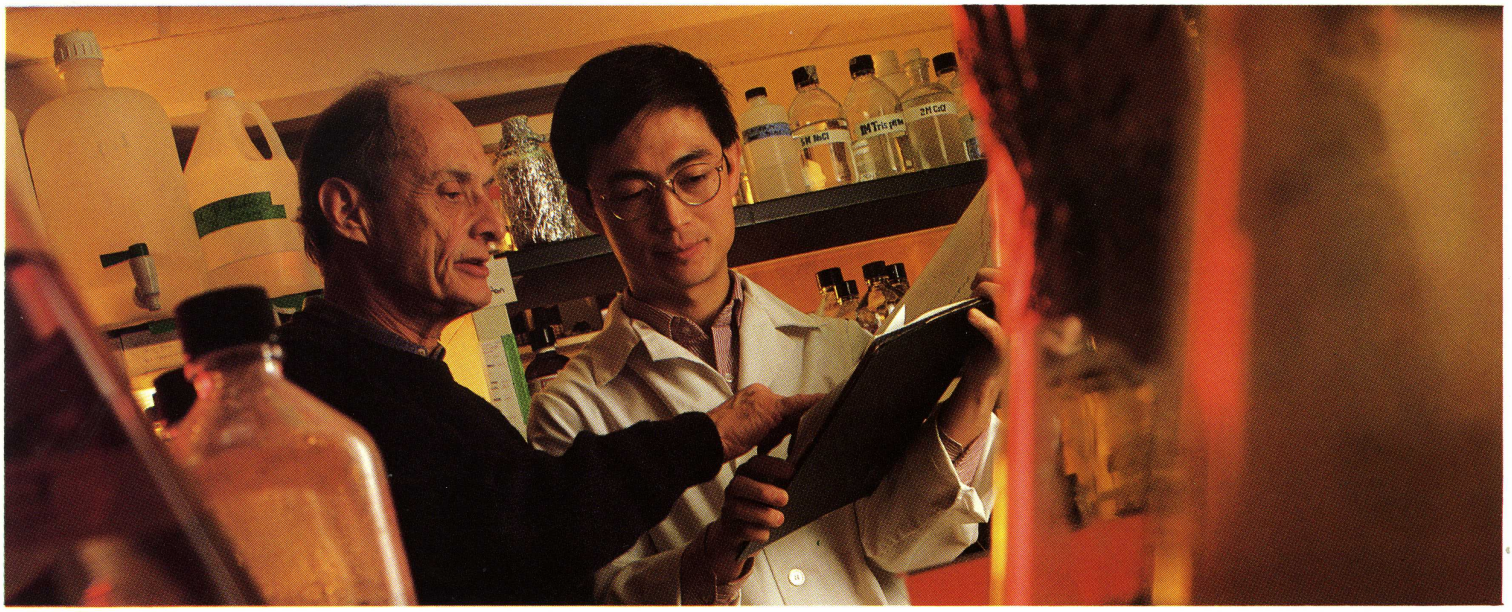
In 1986, shortly before he was to have completed his doctorate with Feng, Han received a fellowship offer from Harvard Medical School. It was an offer Han felt he could not refuse. His mentor, Feng, had trained at England's top physiology lab. "And I thought, to be realistic, to be able to do cutting-edge science, I had to go to a top lab, too." Once at Harvard, Han began to study the Greengard lab's pioneering work on phosphorylation, synapsin, and the regulation of neurotransmitter release. "The work of that lab had made it clear that the synapsins might play a key role in the phenomenon of post-tetanic potentiation." In 1987 Han entered Rockefeller as a doctoral candidate. "I immediately went to Paul and told him that I came here to try to learn a little bit about synapsin and try to become his student. And Paul said," Han remembers with a laugh, "'You're welcome.'"

Greengard, in collaboration with Rodolfo Llinas, had recently made a crucial discovery regarding synapsin's function. They found that if they injected dephosphorylated synapsin I into a squid giant axon, neurotransmitter release was inhibited. But if into the axon they instead injected a kinase—an enzyme that adds a phosphate group to dephosphorylated synapsins and other dephosphorylated molecules—neurotransmitter release was enhanced. Synapsin I was apparently acting as a molecular switch controlling neurotransmission. In its dephosphorylated state the synapsin acted as a kind of glue to hold back packets of neurotransmitter from release sites at the synapse. In its phosphorylated state the synapsin glue lost much of its binding power, freeing the transmitter packets for release in the event of a nerve impulse. These data were compatible with the idea that the reversible regulation of transmitter release could explain post-tetanic potentiation.

Because of his vast admiration for the experiment underlying this model, Han decided to do a modified version of the squid axon injection experiment with a purely mammalian system. Instead of injecting synapsin I protein into a squid axon, he would use recombinant DNA technology to insert the gene for synapsin into a line of cultured mammalian nerve cells. The gene would then allow the neurons to manufacture large amounts of synapsin protein.

But what line of neurons to use? After a year of intensive literature and laboratory research, Han began to focus his attention on a little-used mammalian neural cell line called NG-108. As Han intensified his study of NG-108 cells, he found they were the most suitable ones for his work. Unlike most other nerve cell lines, NG-108 could differentiate into





Han and mentor Paul Greengard study data from an experiment.

cells with many mature neural characteristics. In addition, if they were co-cultured with muscle cells, NG-108 cells formed functional synapses—a feature that would allow Han to repeat the squid axon injection study of how synapsin managed to alter the efficacy of synaptic transmission.

Yet once the long, laborious process of transfecting his NG-108 cells with the synapsin IIb gene was completed, Han was confronted with the unexpected transformation of his cells. His control cells looked the same; but the transfected cells had sprouted small bulbous masses along their fibers. Han had prepared himself for the worst: that his procedure might give the cells "some weird new morphology." But upon reflection, he recalled how the scientific literature referred to such bulbs by a variety of terms: varicosities, synaptic boutons, nerve terminals. What all those varied names meant was that this presynaptic structure was proving to be of central importance to neural development and function.

Finally, after Han had seen these bulbous structures repeatedly and convinced himself that they could be real and meaningful, he went to Greengard and described what he had found. Greengard, greatly intrigued, said immediately: "Let's go take a look." Master and apprentice ambled down the hall and crowded together into the cramped tissue culture room. Han watched as Greengard looked at one set of cells after another under the microscope. The minutes ticked by, one hour, two hours: and Greengard was still looking. Greengard remembers the experience of scrutinizing the cells vividly. "This particular cell line normally never makes synapses onto each other. And yet to someone who's seen nerve terminals, that's what these bulbous structures looked like." Based on the correlation between levels of synapsin protein and neural development, Greengard had predicted in print that the synapsins might be involved in synapse formation; yet before Han's experiment, no evidence of a causal relationship existed. Watching Greengard peering with great concentration at his NG-108 cells, says Han, "I knew Paul was excited deep inside; but outside he stayed calm."

Indeed, Greengard and Han agreed that they should keep calm and quiet about the phenomenon until Han could confirm that what looked under the optical microscope like a synapse was a synapse. "I had to do electron microscopy, quantitative analysis of these small structures, biochemistry," says Han, "and use all the information to draw the conclusion that they really were nerve terminals." Han's follow-up studies, as well as a related series of experiments on Xenopus toads, all confirmed that synapsin can, indeed, induce the formation of new synapses. This remarkable finding has opened a new window on the fundamental mechanisms underlying synaptogenesis. As the noted cell biologist Regis Kelly of the University of California, San Francisco, wrote in his commentary on Han and Greengard's *Nature* paper reporting the discovery: "The beauty of the work described by Han et al. is that it encourages conjectures about synapse formation, while at the same time offering an experimental system in which to test them."

"I consider myself very lucky," says Han, who will be continuing his synapsin research in the Greengard lab at Rockefeller as a postdocoral fellow. "I felt strongly about coming to this laboratory in the hope of contributing to the understanding of the molecular basis by which nerve cells communicate with each other. I feel that I made the right choice." In acknowledgment of Han's scientific skills, Greengard wrote a letter to Han's first great teacher, T.P. Feng, praising the superb training Han had received in China. And in the acknowledgment to his doctoral thesis, Han wrote that he will never forget the many intense hours that he and Greengard spent looking at the NG-108 cells that day in the cramped tissue culture room—mentor and student learning something new about synapsin, together.

FROM ROCKEFELLER'S PAST

H. KEFFER HARTLINE AND THE DISCOVERY OF LATERAL INHIBITION

by Geoffrey Montgomery

H. Keffer Hartline

One day in the late 1930s someone flicked on the light switch in H. Keffer Hartline's lab. Hartline generally worked in the dark, measuring the response of single optic nerve fibers to small spots of light in the giant compound eye of the horseshoe crab, Limulus. What happened when the overhead lights came on had happened many times before: the optic nerve fiber's response to the small spot of light, as measured by an electrode in contact with the nerve fiber, drastically diminished. But why should flooding the room with light decrease instead of increase the fiber's firing pattern? Hartline had never paused to ponder this question. "I have no idea how often I had noticed this [phenomenon] unthinkingly," he later recalled, "without grasping its perversity." But once his mind became fixed on the optic fiber's unusual response to light, Hartline was launched on perhaps the most extraordinary line of inquiry in his illustrious career.

From the very beginning of his career Hartline's research centered around light. Instilled with a love of natural history by his father, a biology teacher, Hartline in 1923, at the age of twenty, published his first paper: a report on the light-avoiding behavior of pill bugs, or wood lice. In the decades that followed—at the University of Pennsylvania, at Johns Hopkins, and from 1953 to 1983 at Rockefeller—Hartline inaugurated a new way of studying how nervous systems represent the visual world. Using a new technique he developed—microdissection by hand—to record from the eyes of a remarkable range of species, including the 350-million-year-old "living fossil," the horseshoe crab Limulus, Hartline laid the foundation for modern single-cell visual physiology, an achievement for which he received the Nobel Prize in 1967. Hartline's earliest studies of Limulus convinced him that the retina, the sheet of nerves lining the back of the eye, is hardly a passive projector of pictures to the brain. The retina of even as archaic a creature as Limulus is instead an astonishingly sophisticated image-processor; it is where the brain's interpretation of the images begins. And nowhere was this lesson more beautifully demonstrated than when Hartline began his investigation of why of a single Limulus optic nerve fiber drastically reduced its response when the overhead lights went on.

The Limulus eye is composed of a thousand individual photoreceptors, called ommatidia. And until the day the lights went on Hartline had assumed that the thousand individual ommatidia functioned independently; if a single receptor "saw" light, it sent an impulse to the brain, no matter what its neighbors saw. But Hartline soon found this was not the case at all. The ommatidia talked to each other over a network of connecting nerve cables. Specifically, if many neighboring photoreceptors saw bright illumination—as when the overhead lights went on—their optic fibers barraged each other with inhibitory signals. As Hartline and Rockefeller colleague Floyd Ratliff (now a professor emeritus at the university) discovered over two decades of investigation, the result of this lateral inhibition is manifold: the retina is wired to respond not to regions of uniform darkness or light, but instead to areas of contrast, to the edges and contours of shadows and objects.

It is clear that lateral inhibition is central to human vision as well. When walking over desert floor, for instance, we pay little attention to the endless uniform expanse of sand and sky; but we will immediately notice the leaves of a palm tree piercing the uniform horizon or the shadow of a circling vulture crossing our path. Our survival hinges on places where light patterns change. And it is these changes in the visual world that lateral inhibitory mechanisms are ideally suited to detect.

Yet the same contrast-detecting machinery endowed by evolution to horseshoe crabs and humans can create regions of contrast where none exists. Lateral inhibition, Ratliff and Hartline showed, can lead to visual illusions. In the 1860s Ernst Mach, an Austrian physicist and philosopher, noticed that the edges of half-shadows appear to be bordered by light and dark bands that have no physical reality. Mach deduced that these illusory "Mach Bands" were the result of inhibitory interactions among retinal nerves—a prediction confirmed a century later in the eye of Limulus. Mach Bands were also noted by another group of nineteenth-century observers: the Neo-Impressionist painters, in whom Ratliff became intensely interested (see "Endnotes," page 27). In his seminal 1965 study, *Mach Bands: Quantitative Studies on Neural Networks in the Retina*, Ratliff analyzed the interrelationship between visual perception as studied by neurophysiologists and that studied by sensory psychologists, and by philosophers and painters.

Whoever accidentally flicked on the lights in Keffer Hartline's darkened lab not only illuminated the path to the discovery of lateral inhibition, but to one of the few genuine marriages between the visions of scientists and artists in our time.

SEX AND THE SINGLE BRAIN

AN INTERVIEW WITH BRUCE McEWEN ON DIFFERENCES IN OUR MOST COMPLICATED ORGAN

By Susan Blum

Is anatomy destiny?

Freud thought so. Determined by the most basic physical differences between men and women, the destiny posited by the Viennese psychiatrist reflected (and helped reinforce) repressive societal notions of "proper" roles for the two different sexes.

After holding sway for almost a century, Freud's theories gradually fell into disrepute, replaced by the idea that destiny is determined by social rather than biological factors. Now the theoretical pendulum is swinging again—though this time not so widely.

Recent studies have reopened the question of biology's influence on behavior. But rather than emphasizing differences in the sex organs per se, as did Freud, these modern-day studies focus on the brain, an organ that is turning out to differ according to sex—and, perhaps, even to sexual preference.

Still controversial, and ringed with caveats, the current studies exploring possible links between brain and behavior are conducted with a much more sophisticated understanding of the molecular underpinnings of anatomy, and a much greater awareness of the social, environmental, and idiosyncratic factors that also affect behavior.

Within this subtler context, the new studies hold the potential to liberate rather than repress. A case in point is the research of Simon LeVay, a neurobiologist at the Salk Insitute who found that an area of the brain is different in homosexual and heterosexual men.

LeVay's report appeared in the journal *Science* last fall. According to Bruce McEwen, a Rockefeller scientist who studies the effect of hormones on the brain, "the immediate significance of the report was more political than anything else. It let people in the gay community say, 'look, this is a biological feature of who we are.' When there is a possible biological substrate, it makes a difference in people's attitudes."

Though the political implications of the study may be clear, the scientific ramifications are not. As the author of the report himself points out, the findings raise many more questions than they answer. For instance, are the differences in the brain region a cause or a consequence of homosexuality? What might account for the anatomical differences? And how might these differences actually be affecting behavior?

There are many intriguing hints, but no solid answers. The area under consideration is a region known as the interstitial nucleus of the anterior hypothalamus-3, or INAH-3. In LeVay's studies, autopsies showed that the INAH-3 was more than twice as large in heterosexual as in homosexual men. Previous studies had already shown this area to be more than twice as large in heterosexual men as in women.

What might be influencing the size of the INAH-3? McEwen reports that a similar area, or nucleus, in the rat hypothalamus is affected by sex hormones. "By analogy to the rat, we can say it is possible that hormones are at work in this part of the human brain, but we just don't know yet," he says. Should hormones be shown to be involved in the human brain, he adds, there will still be many more questions than answers. "Numerous studies have shown that the blood levels of hormones are the same in heterosexuals and homosexuals. So what would account for the different hormonal effects in the brain? They would have to be very localized, and, perhaps, limited to a very short time period during prenatal development or in childhood."

Questions of fundamental causation aside, how might the INAH-3 be affecting behavior? McEwen explains that in animals such as monkeys, levels of sex hormones are important in sexual motivation—that is, in deciding and then demonstrating who is an appealing mate. "We call this aspect of behavior 'proceptivity,'" McEwen explains. "In animals, it is related to behaviors that lead members of the opposite sex to become interested in one another."

In rats, the region similar to the INAH-3 is normally larger in males than in females and is one of the nuclei in the hypothalamus known to regulate typically "male" and "female" sexual behaviors. McEwen speculates that the INAH-3 may play a similar role in humans. He cautions, however, that the region—no bigger than a grain of salt—is probably not solely responsible for sexual motivation. "If you make a lesion in the equivalent area of a rat's brain, there is no change in the rat's sexual behavior. Only when the lesion is much larger do you start to see disruptions. This has been a problem in rat studies, and it's a problem when you start to think about humans, too. Of course, no one makes experimental lesions in human brains, so we may never know exactly what the INAH-3 does."

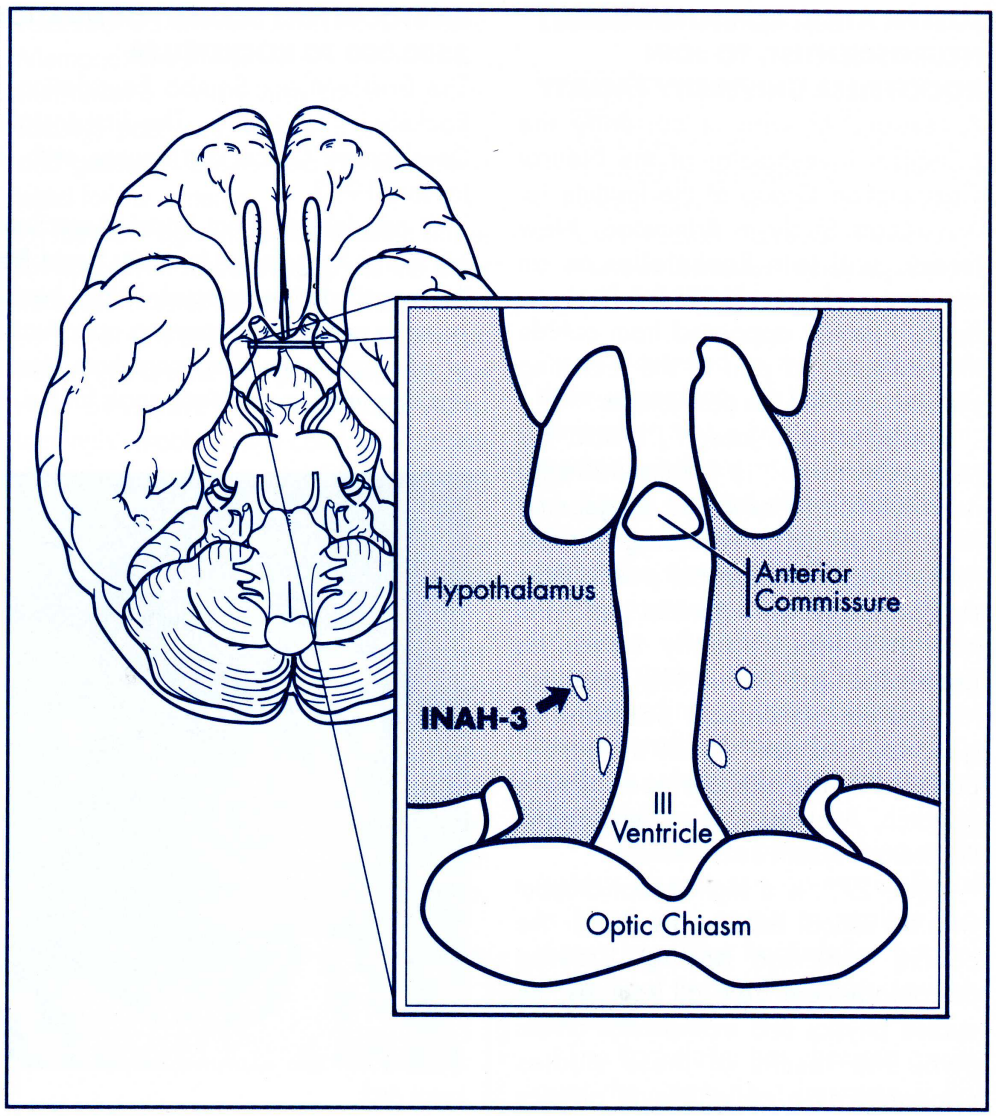
Sexual motivation is just one of the complex behaviors that might be mediated by sexually determined differences in brain structure, known as sexual dimorphisms. Learning abilities, verbal and spatial skills, and propensities for particular mental and neurological disorders may all reflect hormonally influenced anatomical differences that develop prenatally or early in life.

In humans, the most commonly studied dimorphic structure is the corpus

callosum, a bundle of nerve fibers connecting the right and left cerebral hemispheres. Studies have shown that regions of this structure are different sizes in men and women. Scientists speculate that these differences may relate to differences within the cerebral cortex, the brain region responsible for integrating information, and may help account for the fact that, on average, women are more skilled at verbal tasks, while men do better at spatial ones.

Other studies have pointed to hormonal fluctuations that can influence adult brains. For instance, research in McEwen's lab has shown that during the rat's estrous cycle there are changes in nerve cell interconnections in the hypothalamus and in the hippocampus, a brain region involved in learning and memory. And researchers elsewhere have found that performance on tests of spatial ability varies with hormonal fluctuations. Men tend to do better on the tests in the spring, when their testosterone levels are lower, than they do in the fall when their testosterone levels peak. Women's test performance fluctuates more frequently, peaking each month during the part of the menstrual cycle when estrogen levels are lowest. Women also produce testosterone—though not as much as men—and those with the highest testosterone levels do best on the tests.

MARGARET RYON



The hypothalamus, located deep within the brain, is a region involved in controlling sexual behavior. A recent study reported that a nucleus in the hypothalamus, the INAH-3, is larger in hetrosexual men than in homosexuals.

McEwen points out that knowledge about sexual differences can be important in the diagnosis and treatment of disease. Males, for example, have a fourfold higher incidence of dyslexia and learning disorders, and recover more slowly from some kinds of strokes than do women. Understanding how their brains differ from those of females may improve treatment prospects for these conditions. On the other hand, estrogen makes women more susceptible to the effects of certain neuroleptic drugs, such as those used to treat schizophrenia. A greater focus on this difference might lead to better therapeutic regimens for women, who suffer more severe side effects from the drugs than do men.

Though discoveries about sexual dimorphism in the brain must not be dismissed, they must never be used to legislate, McEwen asserts. "It would be absurd to say, 'Let's only let men be map readers for the military because they're good at spatial relationships, and let's only let women give speeches because they're better verbally. You have to look at individual capabilities, which vary over a wide range," he insists. Moreover, he adds, inherent differences may well be overcome by training, encouragement, and experience.

"Anatomy is not destiny," McEwen sums up. "It may sometimes bias, but it never determines."

CAMPUS NEWS

JOSEPH ATICK, COMPUTATIONAL NEUROSCIENTIST, TO JOIN ROCKEFELLER UNIVERSITY FACULTY

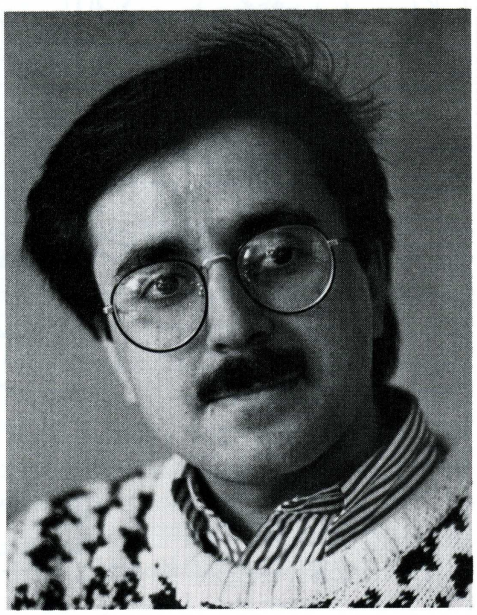
A researcher who is currently the principal investigator of the Neural Computation Group at the Institute for Advanced Study in Princeton, New Jersey, will join Rockefeller as an assistant professor. He is the first new faculty member appointed from outside the university as part of the recruiting program initiated this past summer.

The scientist is Joseph J. Atick. His research area is computational neuroscience, a new and burgeoning field concerned with finding testable mathematical theories that predict how the nervous system organizes itself, and how it computationally solves its perceptual problems. His research focuses on the sensory pathways in general and the visual system in particular. The ultimate goal of such research, Atick says, is to understand the nervous system as a whole.

Atick, 27, is a high school dropout who left school because he found the course work too boring. Braving discouragement from all sources, he studied physics and mathematics on his own. The results of these studies appeared as a college-level physics textbook Atick completed at the age of sixteen. The book attracted the attention of Stanford University physicist Stanley Hanna, who invited Atick to explore the possibilities of studying there. After a round of tests, Atick was admitted to Stanford as a graduate student in physics. He received his Master's degree in 1985 and his Ph.D. two years later. He joined the Institute for Advanced Study as a postdoctoral fellow in 1987 and was made a long-term member in 1988.

Atick trained as a high-energy physicist, which gave him a "frame of mind," and then moved into the neurosciences, inspired by his enduring fascination with the nervous system. "My work has always been driven both by experimentation and by first principles," he says, adding that Rockefeller, where exploration is shaped by the same two considerations, will provide a congenial atmosphere for his research.

"Rockefeller is a unique place, which has always had researchers at the frontiers of experimentation," he says. "To be close to where the action is in the neurosciences will stimulate my work, and drive me toward my goals. I'm very enthusiastic about all the possibilities for collaboration."



RANDALL HAGADORN

Joseph Atick

BRISTOL-MYERS SQUIBB FOUNDATION PLEDGES $500,000 TO ROCKEFELLER

The Bristol-Myers Squibb Foundation, Inc. recently pledged $500,000 to The Rockefeller University. The first installment of $250,000 was received by the Development Office last January, with the second and final payment scheduled for January 1993.

"I am delighted that Bristol-Myers Squibb Foundation chose to make this generous gift to The Rockefeller University," said President Torsten Wiesel. "An unrestricted gift of this magnitude is enormously helpful because it can be flexibly applied to meet our most pressing needs and promising opportunities. We are delighted to have the partnership of such an outstanding corporate leader. Gifts such as this one are important for ensuring that our ambitious goals for the university are achieved."

THE SEARCH IS ON

Five Rockefeller search committees, each under the leadership of a senior university scientist, are seeking candidates for appointment to new positions as heads of laboratories at the assistant, associate, and full professor levels. The committees placed ads in a variety of scientific publications and have received more than 200 responses to date.

The five search committees are:

- Neurosciences, headed by Charles Gilbert
- Chemistry, Biochemistry, and Structural Biology, headed by Robert G. Roeder
- The Cell and Developmental Biology Committee, headed by David J.L. Luck
- The Immunology and Microbiology Committee, headed by Emil C. Gotschlich
- The Medical Sciences Committee, headed by Jan L. Breslow

ROCKEFELLER, NEIGHBORS TO SHARE LIBRARY FACILITIES

A new agreement among the Tri-Institutional libraries enables members of the Rockefeller community to borrow books from Cornell University Medical College and Memorial Sloan-Kettering Cancer Center during a six-month trial period. Similarly, members of the other institutions will be able to borrow books from Rockefeller during that time.

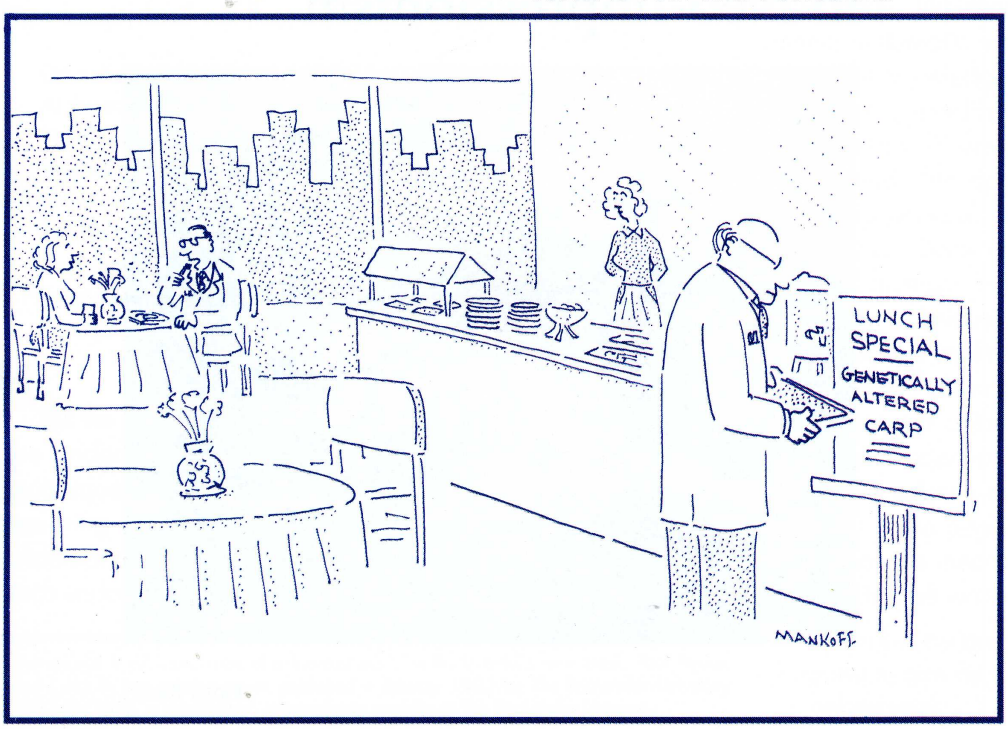
"The librarians—Memorial Sloan-Kettering's Jeanne Becker, Cornell's Robert Braude, and I—decided that the time had come to work more closely together," said Patricia Mackey, Rockefeller librarian. "Pooling our resources will enable each of us to keep costs down and to provide better service to our users. I think we all hope that reciprocal borrowing can become a permanent arrangement."

Cornell's collection, which comprises 96,000 books and 463,000 journals, will be open to anyone from Rockefeller or Memorial Sloan-Kettering who registers with Cornell's book circulation desk. Because of their smaller staffs and more focused collections, Rockefeller and Memorial Sloan-Kettering will limit inter-institutional borrowing to permanent faculty, postdocs, research associates, and graduate fellows who demonstrate a need for the material.

The new agreement will make it easier for Rockefeller researchers to use the other libraries' facilities as well. For example,

- Cornell's and Memorial Sloan-Kettering's on-line computer catalogs are now available on some Rockefeller terminals. (Rockefeller's catalog is on-line at Cornell and Memorial Sloan-Kettering as well.)
- Rockefeller researchers now have the option of opening an account at Cornell for photocopying.
- Photocopy cards for use in the Memorial Sloan-Kettering library can be purchased there at the library desk.

The new agreement builds on a tradition of cooperation among the libraries which dates back to the 1930s. In the 1970s the relationship was formalized by a pact that allowed members of the institutions on-site access to each other's collections.



LETTER TO THE EDITOR

SEARCH welcomes letters from readers and will publish them selectively. Here are some excerpts from a letter we received in response to an article entitled "Heir to a Scientific Heritage: Louis Kunkel Tackles Duchenne Muscular Dystrophy," which appeared in the Fall 1991 issue.

I was interested in the article on the three generations of Kunkels. I clearly remember that when I was a young medical virologist at Rockefeller, Louis O. Kunkel told me about resistance to plant viruses across the lunch table—a wonderful place to widen your mind. Henry was an example to me of someone who used and developed clinical studies to arrive at a deeper understanding of basic biology, and he personally helped me to use new methods while I was at the Institute (1951-54).

I have recently been looking at the history of my own corner of British science and came to realise that there, too, The Rockefeller had an important influence over several generations....

The Rockefeller has changed greatly over the years, but because it has repeatedly opened its doors and its community to foreigners, like myself, it has enriched the development and education of generations of British scientists and helped us to contribute to medical science with (we hope) something of the quality and style we saw during our days in New York City.

Sincerely,
David Tyrrell
MRC AIDS Directed Programme
PHLS Centre for Applied Microbiology and Research
Porton Down, Salisbury

THREE NEW BOOKS PUBLISHED BY ROCKEFELLER UNIVERSITY FACULTY

One of the grandest of Rockefeller traditions is the array of books—ranging from science to philosophy—written by its faculty. In keeping with this tradition, three emeritus professors, Floyd Ratliff, Abraham Pais, and Christian de Duve, have recently published, respectively, books on Neo-Impressionism, the eminent physicist Niels Bohr, and the origin of life.

Floyd Ratliff became interested more than twenty years ago in the technique of divisionism (a variation of pointillism) used by the Neo-Impressionist Paul Signac. The result of this interest is a book, *Paul Signac and Color in Neo-Impressionism*, a ground-breaking examination of the artistic technique in terms of modern scientific theory of color, published by The Rockefeller University Press in January 1992.

Ratliff, whose academic work includes biophysics, physiological psychology, and the history and philosophy of science, has been a major contributor to the scientific understanding of vision—that view of the world that comes to us by way of changing patterns of light and shade imaged on the retina of the eye. Throughout his career, he has thought and written about the relationship between visual science and the visual arts.

Ratliff's book also includes the first English translation of a work by Signac: *From Eugène Delacroix to Neo-Impressionism.*

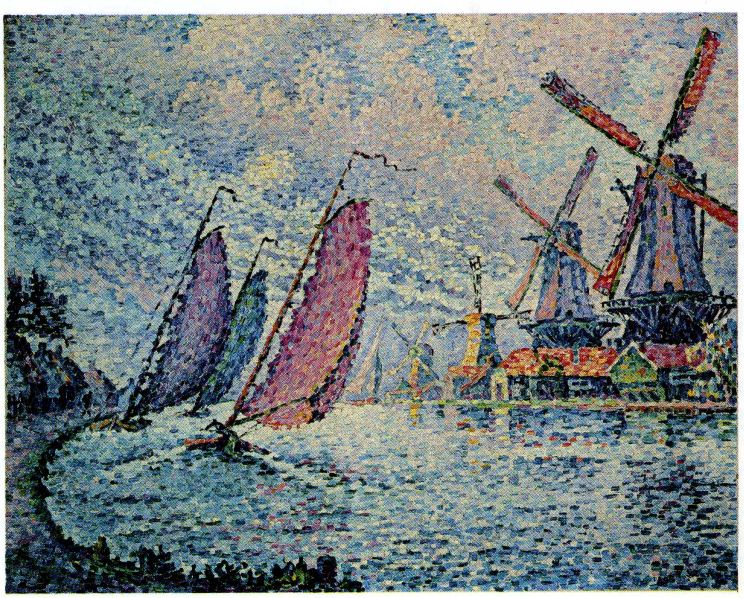
Abraham Pais, a theoretical physicist, published *Niels Bohr's Times: In Physics, Philosophy, and Polity* (New York: Oxford University Press) in October 1991. Bohr played a major role in shaping the theory of the atomic nucleus, and he decoded the atomic spectrum of hydrogen—an achievement that marks him as the founder of the quantum dynamics of atoms. His concept of complementarity, which provides the philosophical underpinning for quantum theory, qualifies him as one of the twentieth century's greatest philosophers, as well as one of its most eminent physicists. Pais's book illuminates Bohr's life and thought.

Pais is also the author of *Subtle Is the Lord...*, a biography of Albert Einstein. Published in 1983, this book was a national bestseller and was awarded *The New York Times* Best Book of the Year award, and was the winner of the American Book Award for Science that year. Pais's own work in physics is also remarkable. He is one of the founders of the scientific discipline devoted to the exploration of the invisible subatomic world populated by a host of infinitesimal particles called "hadrons" and "quarks," as well as to the search for a theory that identifies the ultimate building blocks of matter and provides a unified explanation of the forces governing their behavior.

Christian de Duve's latest book, *Blueprint for a Cell: The Nature and Origin of Life* (Burlington, NC: Neil Patterson Publishers, 1991) concerns a topic no less weighty than the origin of life itself. Drawing on his own scientific research, which has centered on the separation and characterization of the different parts, or organelles, of living cells, de Duve provides a critical summary of current theories and speculations on questions such as "How did life begin?" "How did the first cell form and how did it evolve to cover the earth with teeming life?" and "Was life an accident?"

de Duve, a 1974 joint winner with Albert Claude and George E. Palade of the Nobel Prize, shares his time between New York, where he is Andrew W. Mellon Professor Emeritus at Rockefeller, and Brussels, where he is professor emeritus at the Catholic University of Louvain and President of the International Institute of Cellular and Molecular Pathology. He is known for the discovery of two cell organelles, the lysosomes, in which digestive processes take place, and the peroxisomes, which are sites of oxidative metabolism. His studies have yielded new insights into the cellular basis of many diseases.

Paul Signac and Color in Neo-Impressionism



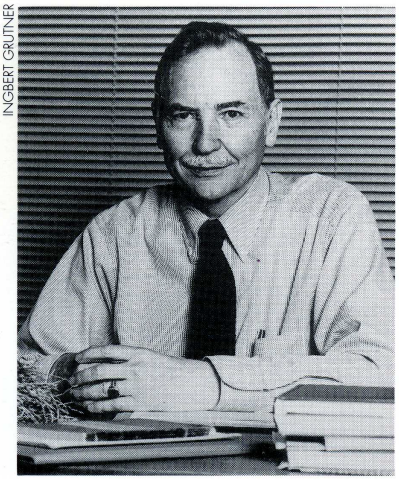
Floyd Ratliff

MUSEUM OF FINE ARTS, SPRINGFIELD, MASS; ROBERT J. FREEDMAN COLLECTION

ENDNOTES

THE SCIENTIFIC THEORY OF COLOR IN NEO-IMPRESSIONISM

by Floyd Ratliff



INGBERT GRUTNER

In Neo-Impressionism the scientific theory of color had an influence on the accepted technique, and the rules of that technique had an influence on the practice of individual artists within the movement. But those influences were not always controlling. The use of small touches of pure color by the Neo-Impressionists may have been inspired by the theory of the optical mixture of color, but in the actual application of those touches on the canvas the theory of the interaction of color turned out to be more relevant, and a strict pointillism almost immediately gave way to a more lenient divisionism. Similarly, the movement with its fairly well defined rules of technique attracted many adherents. But in actual practice, each and every painter put his or her own stamp on the technique and in the end many scattered far and wide.

Every artist is subject to many and diverse influences but, in the final analysis, art is individual. And that is as it should be.

The triumph of color. There are those who fear understanding, fear that knowledge destroys, and fear that when the web of mystery around a thing of beauty is disentangled and removed, then beauty itself will flee.

> Do not all charms fly
> At the mere touch of cold philosophy?
> There was an awful rainbow once in heaven:
> We know her woof, her texture: she is given
> In the dull catalogue of common things.
> Philosophy will clip an Angel's wings,
> Conquer all mysteries by rule and line,
> Empty the haunted air, and gnomèd mine—
> Unweave a rainbow.
>
> Keats, *Lamia*, II, 229

But the Neo-Impressionist painter's "cold philosophy" is merely the understanding of the historical and lawful basis of the technique and cannot be seen directly in the painting. All painting rests on the laws of nature whether the painter understands those laws or not. Both Seurat and Signac sought such understanding, unlike the rebellious Matisse, who said:

> My choice of colours does not rest on any scientific theory; it is based on observation, on feeling, on the very nature of each experience. Inspired by certain pages of Delacroix, Signac is preoccupied by complementary colours, and the theoretical knowledge of them will lead him to use a certain tone in a certain place. I, on the other hand, merely try to find a colour that will fit my sensations.

There is no doubt that there are wide differences among indiyiduals in their color preferences, their particular likes and dislikes. As the Russian proverb explains: In taste and in color, comrades pull asunder. But sensations are another matter; the basic psychophysiology of color is more or less the same in everyone, everywhere. And when Matisse finds a color that will fit his sensations, that fit expresses some fundamental truth about color, and the perception of color, which transcends Matisse's own particular idiosyncracies and his own personal experiences. As Alberti (1404-1472) wrote:

> Among colours there are certain friendships, for some joined to others impart handsomeness and grace to them. When red is next to green or blue, they render each other more handsome and vivid. White not only next to grey or yellow, but next to almost any colour, will add cheerfulness. Dark colours among light ones look handsome, and so light ones look pretty among dark ones.

The perceived friendships (and antagonisms) among colors are universal and enduring truths. What was true about color in the 1400s was still true 400 years later in the 1800s, and is still true today. Whether the artist chooses to exploit those truths in some systematic way based on the accumulated artistic and scientific knowledge of centuries past or simply on the basis of his own immediate personal intuition and direct personal experience is irrelevant, as far as color appearances are concerned. The appearance of color is determined by certain enduring physical, physiological, and psychological events and processes. Color is as color does. And the truth about color always triumphs over our necessarily incomplete theories and imperfect techniques.

Color in Neo-Impressionism had broad theoretical bases in both art and science....But, in the end, any artist who wishes to succeed as a colorist must go beyond painters' theories of the brush and scientists' theories of color. In art, practice always takes precedence over theory. For it is the ultimate appearance of color in the resulting work of art itself, and not the underlying theory or the means of its application which is paramount. As Signac himself wrote in 1884:

> I paint like this because it is the technique that seems to me best suited to give the most harmonious, the most luminous, the most *colorful result.*

This excerpt is the conclusion of a five-part essay in Floyd Ratliff's new book, *Paul Signac and Color in Neo-Impressionism*, published in January 1992 by The Rockefeller University Press. Dr. Ratliff, a biophysicist, is a professor emeritus at The Rockefeller University.

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