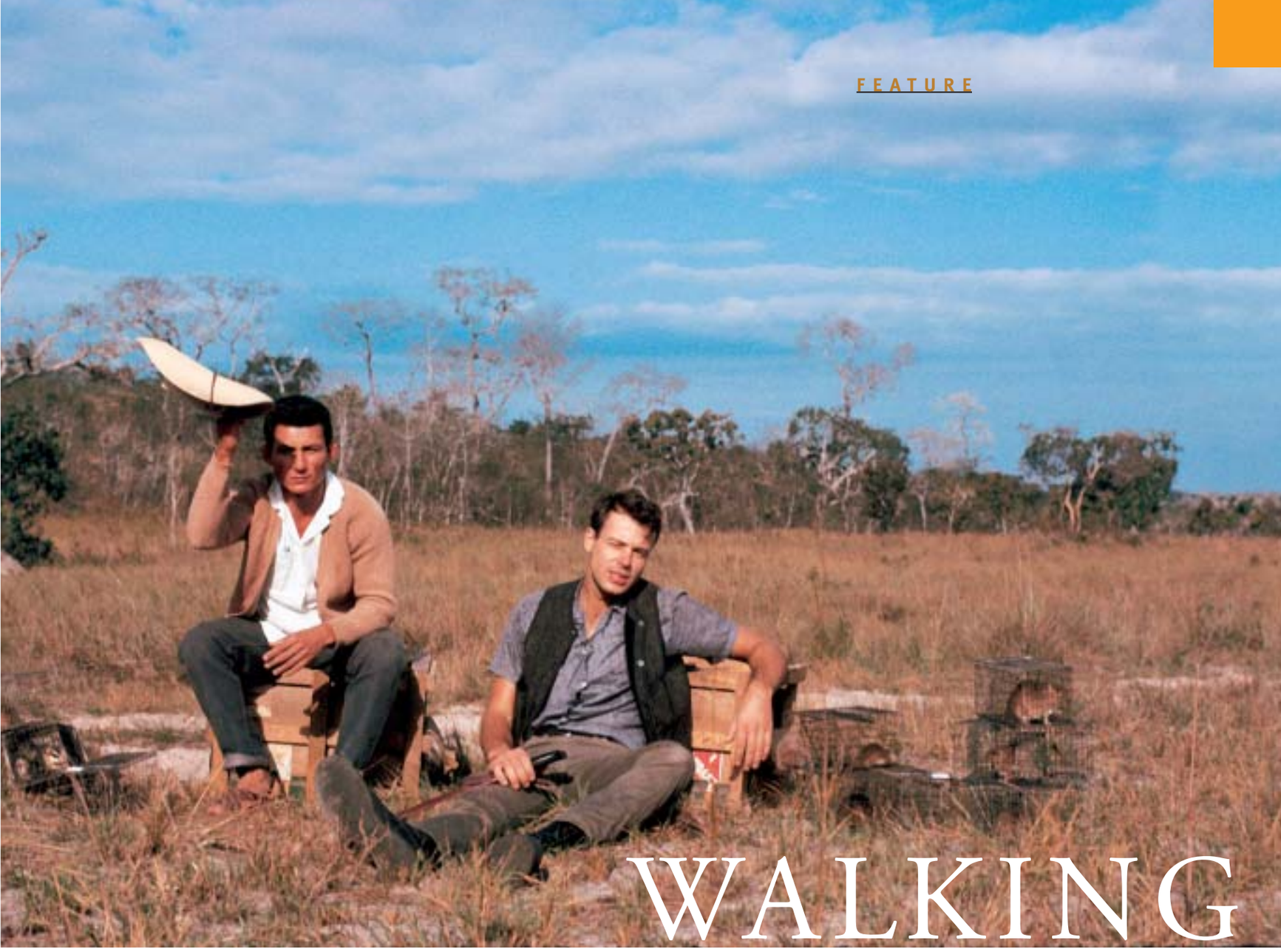


WALKING BEFORE DAWN

HOW A KNOWN POLLUTANT BECAME THE NEXT BIG THING

BY ERICA LLOYD

It's not clear how they got lost, but the air commandos who were transporting the research team had been drinking the night before. They were somewhere over the billion-acre Amazon rain forest, and the C-46 was running out of gas.

John Hibbs Jr. (right) in Bolivia in 1964 on an animal trapping quest to find the source of a mysterious and deadly virus. He is sitting with Einar Dorado, a colleague who contracted the virus and died.

COURTESY HIBBS

Hibbs (center) and others from the Middle America Research Unit testing animals to learn how far the virus had spread.

Captain John Hibbs Jr. (MD '62) and the others put on their parachutes. It didn't look good. At least Hibbs had a pistol and a survival kit—a few fishing hooks, matches, a knife, and scarce few other necessities in a waterproof package put together for him by his kid brother. Jimmy had given him the kit as he was preparing for this assignment with the U.S. Special Forces (Airborne). As it turned out, Hibbs didn't have to rely on the foresight of his 14-year-old brother back home in Pennsylvania to get him through. That day in 1964, the crew managed to figure out where they were with the help of the sun and the sextant. More important, they were able to land safely.

Hibbs had finished his internship at the University of Oregon when drafted into the army; while serving with the special forces in Panama, he volunteered to help the Middle America Research Unit investigate an outbreak of hemorrhagic fever centered around San Joaquin, Bolivia. The fever was killing 60 percent of the people who'd contracted it. Hibbs' mobile field research lab was sent into the surreal landscape of the Paraguayan Chaco and into the Brazilian Amazon to trap and tag animals to decipher the source of the infection and how far it had spread. Their charge covered a vast wilderness comparable to the land mass stretching from the Mississippi to the Pacific. There, Hibbs was treated to plenty of made-for-movie characters—from hung-over commandos to Indians running in rivers to catch piranhas for dinner. All part of the job. Hibbs expected the assignment would bring adventure, something that had always called to him. What he hadn't expected was the intellectual ride microbe hunting would prove to be. His best finding was discovering that science was "true high adventure," as he says. Under the direction of Karl Johnson, the team discovered that mice from Bolivia and the chaco carried persistent Machupo virus infection, the cause of the fever. In the middle of the last century, expanding human settlements and climate cycles had brought the mice, notably their feces and urine, into contact with human food.

As an undergraduate at Dartmouth College, Hibbs majored in history. He later enrolled as a medical student at the University of Pittsburgh, where his father, John Hibbs Sr. (MD '34), a scholarly internist in the Uniontown area, had also gone to school. (As a boy in Uniontown, Hibbs Jr. had to walk through his father's waiting room to get to the family living quarters upstairs.) At Pitt, he was bowled over by the caliber of the faculty. Hibbs studied pathology under department chair Frank Dixon and his faculty—"Many of them were founders of modern immunology," he notes. Hibbs soaked up their enthusiasm for understanding the mechanisms of disease. Likewise, Klaus Hofmann and his group made chemistry come alive. Despite his admiration for Dixon and Hofmann, the wings of Scaife Hall where basic science investigation was pursued seemed like foreign territory.

"When you're learning—mastering—knowledge that already exists," notes Hibbs, "it's a very different thing than trying to learn something new about nature."

Since his time in the South American wilds, Hibbs has learned about the joys of, he says with a broad smile, "carrying on a dialogue with nature." When he talks about his work, it's as though this fit 67-year-old is speaking about a secret club, and he can't wait for you to join. Learning details of the world around him is a high. As a child, he loved spending days with his grandfather, who had a farm in the mountains near his family's rural Pennsylvania home. Maybe he would be a farmer, too, the boy thought for a while. His grandfather taught him the name of every plant in the countryside; Hibbs has done the same for his three children. Despite his thrill-seeking inclinations, there's little bravado in Hibbs' gentle demeanor. He lives in Utah now, and his voice echoes the evenness of a western plain, of a boy who once dreamt of plows and sweet-smelling earth. One colleague described him as a meticulous hound dog when it comes to his research, reticent to publish findings until he has thoroughly fleshed out the ambiguities. (The same care and attention is afforded to his patients at Salt Lake City's veterans and university hospitals; the house staff recognized Hibbs with the Outstanding Teacher Award four times.)

Among other important advances, Hibbs' decades of assiduous give-and-take with the natural world have helped to unravel an entirely new principle for signaling in biological systems and the human body. In 1977, pharmacologist Ferid Murad reported that coronary arteries opened in the presence of nitric oxide; he theorized that the body might actually release the gas. But conventional wisdom at the time said nitric oxide was a byproduct of auto exhaust, a pollutant, not something our bodies would generate. Meanwhile, Hibbs, completely independently and in a totally different field, immunology of infectious diseases, was unknowingly putting puzzle pieces in place that would prove Murad was right.

There's some irony in nitric oxide's intellectual history. Inventor Alfred Nobel suffered from heart disease and then died after a stroke in 1896. Despite his doctor's urging, he'd refused to take nitroglycerin—an explosive he thought he knew well because it was a key component to his invention of dynamite.

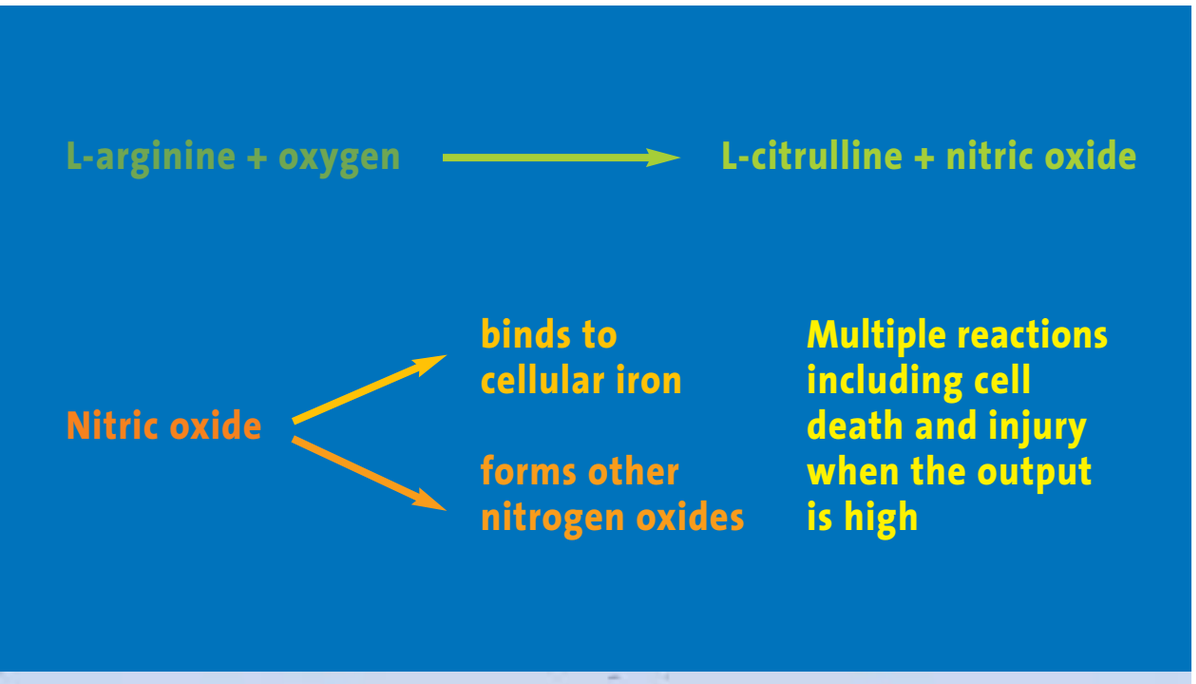
COURTESY HIBBS

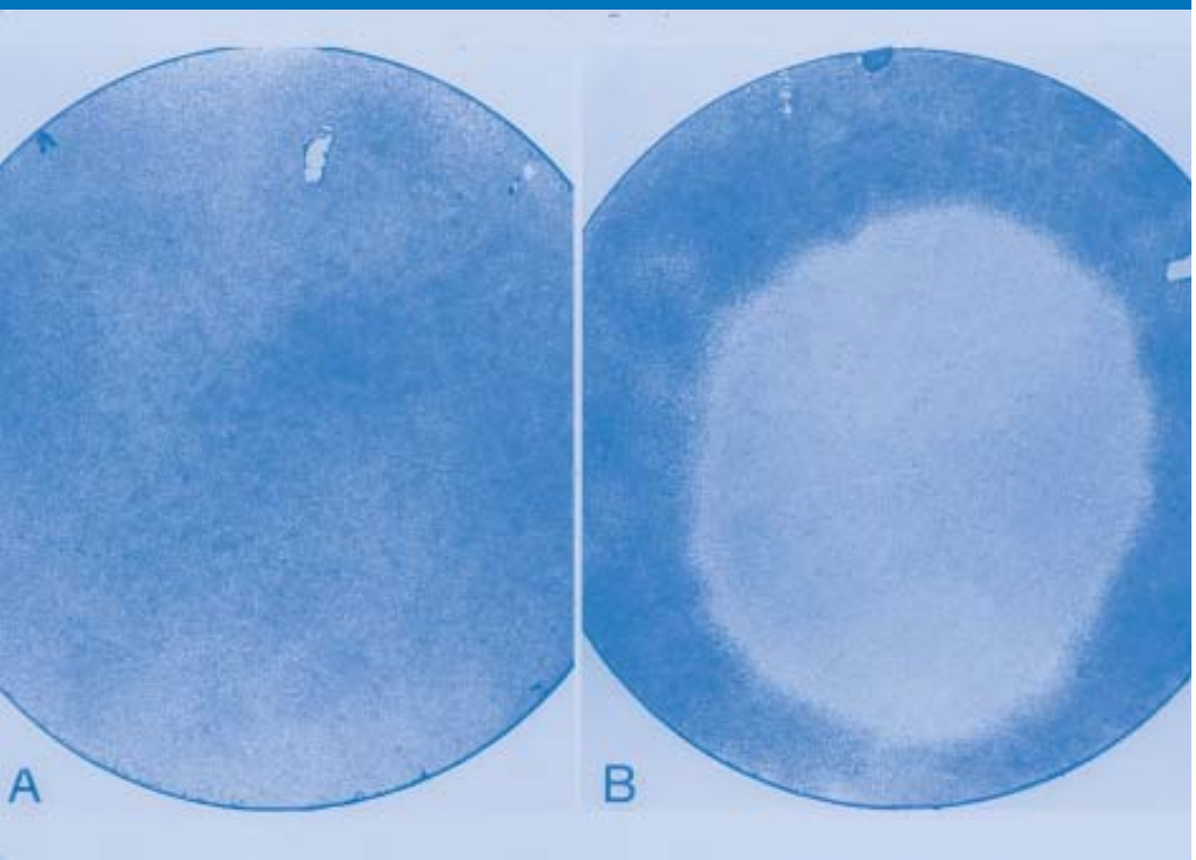
One-hundred-and-two years later, the Nobel Foundation, which Nobel had created from his considerable fortune, recognized Murad, Louis Ignarro, and Robert Furchgott with the Nobel Prize in Physiology or Medicine for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system. Murad had revealed that the century-old treatment for chest pain, nitroglycerin, acts by releasing nitric oxide. From all accounts, that's the tip of the iceberg in terms of nitric oxide's power over biological systems.

Hibbs had never met anyone with so much passion. Anything Jack Remington focused his attention on—windsurfing, rock climbing, skiing, or immunology—he pursued with extraordinary gusto. Remington, an infectious diseases specialist at Stanford University and Hibbs' new adviser, seemed to have more energy than a Pacific gale; and that's a topic Hibbs would have known something about. After his service in the army, Hibbs had returned to Oregon for his residency, then set sail for 10 months in the Pacific as part of a crew of five on a 39-foot sloop on a 24,000-mile journey. On his return, the life of a scientist awaited him, and it proved just as invigorating. Hibbs thrived as a fellow under Remington.

Remington was exploring how our bodies fight intracellular microbes, pathogens that antibodies can't reach because they become protected by the cell membranes they invade. He had shown that mice infected by intracellular microbes experienced an immunity that was not specific to any one disease. In other words, if animals were infected with, say, the bacteria that causes tuberculosis, they developed a resistance not only to the very pathogens they'd been exposed to—*Mycobacterium tuberculosis*—but also to other intracellular pathogens, be they bacteria, viruses, or protozoa. The key to this resistance seemed to be the macrophage, the cellular scavenger in the linings of blood vessels and organs. Hibbs wondered if activated macrophages would work the same way with cancer—that is, if their ability to ward off a gamut of uninvited guests would extend to tumors. It did. When he placed macrophages from mice infected by intracellular microbes with tumor cells, the tumor cells either died or stopped growing. (The macrophages hardly affected normal cells.) And it didn't matter if the tumor cells were from mice, rats, or humans—the activated macrophages from mice still offered resistance.

(This is beginning to sound like a cure for cancer. In fact,





ABOVE LEFT: Who would have thought that nitric oxide, a known pollutant, played a key role in vascular health, immune response, behavior, inflammation, impotence, shock, cancer, memory, and then some? Hibbs delineated the biochemical pathway our bodies use to synthesize nitric oxide. BELOW LEFT: (A) No activated macrophages equals no cytoxicity. The dark area is covered with tumor cells growing normally. (B) Tumor cells don't grow in the white area, where they meet up with activated macrophages. Hibbs discovered this as a fellow in 1970. Determining how the macrophage managed this trick would keep him occupied for a good 16 years.

similar experimental techniques with intracellular microbes have been used effectively in certain melanomas, leukemias, and in a bladder cancer. Yet the approach has limited utility.)

But how did the macrophage provide resistance? What was the mechanism? Hibbs wouldn't be satisfied until he found out.

For the past 33 years, John Hibbs Jr. has maintained a research laboratory supported by the Department of Veterans Affairs and the National Institutes of Health; in addition, he is the chief of infectious diseases for the University of Utah. In 1999, that university recognized him with the title Distinguished Professor of Medicine. The machete he used in the Amazon is now a poker for the fireplace in his home set in wooded hills not far from canyoned wilderness; it's the same home in which he and his wife, Francoise, have resided since they moved to the Salt Lake City area in 1971 (they added on as their family grew). He and Francoise start their days at 5 a.m., hiking in the mountains near the university.

Hibbs expected the assignment would bring adventure, something that had always called to him. What he hadn't expected was the intellectual ride microbe hunting would prove to be.

There is an epitaph devoted to founders of Pitt's Allegheny Observatory that could be a theme for Hibbs' approach to life. It reads, "We have loved the stars too fondly to be fearful of the night." On Hibbs' epic 24,000-mile sail, he learned celestial navigation. The big test came when they were looking for Easter Island, which is only 50 square miles in size. Hibbs became sold on the ancient method when one morning he looked off the bow and—wow!—there was the island, on schedule and exactly where they'd predicted.

Hibbs loved the night shift at the helm. The sun's glare could be disorienting. But how could he not welcome nightfall when, as he puts it, "all of a sudden, the world is old friends and landmarks"?

He is quite comfortable making his way in the dark. His work for more than 30 years has been a gradual and steady progression of seminal observations that began at beaconing sites he first came across under Remington's tutelage. When he set up his own lab at Salt Lake, Hibbs was determined to probe this nonspecific resistance phenomenon more mechanistically. What was it that created the toxic response in tumor cells and microbes?

Another lab had repeated Hibbs' activated macrophage experiments but shown that macrophages from normal mice could kill tumor cells when infused with type 2 interferon. Hibbs saw for himself how well this worked with his bench experiments. Then, working with Brice Weinberg and Harold Chapman, fellows in his lab, he saw that almost every lab chemical they added to the serum with type 2 interferon ended up inciting the process and killing more tumor cells. Something was off. *Everything* couldn't be an immune activator. The investigators became suspicious that the commercially acquired supplies they'd been using were contaminated and took on the unpleasant task of visiting a slaughterhouse to collect blood themselves to use as serum. At a local slaughterhouse, they saw workers putting blood into stainless steel buckets, amid the bile and guts around them, wearing the same clothes they had worn as they performed all the other tasks required of them that day. Hibbs asked them what they were doing. Well, they were collecting blood for a commercial serum supplier. It struck Hibbs that these guys knew as much about sterile techniques as he knew about flying a 747 (nil). The scientists became convinced they were likely to find bacteria in the serum they'd been buying. They were right. It was full of endotoxin, a byproduct of bacteria. Every batch was loaded with the stuff. Then they tested other chemicals they'd been using; those contained endotoxin as well. When they removed the microbial products and tried the interferon experiments again, the macrophage wasn't nearly as potent. From there, Hibbs' lab demonstrated that type 2 interferon primed the macrophage, but the macrophage wasn't activated until it came into contact with microbial products. (The mice Hibbs had infected back in Remington's lab produced interferon as part of their immune response.)

This told the scientific world a couple of other important things as well: The results that labs everywhere had been reporting—across disciplines—could have been affected by the very serums and chemicals they'd used. Also, the macrophage deserved recognition as an extraordinarily sensitive immune sentinel. Macrophages were positioned throughout the body, ready to detect and act on the presence of microbes. And their response could be subtle or quite violent (as in the case of the contaminated serum and also septic shock, the biomedical community would learn later).

Hibbs poked and prodded further. He discovered that an analogue of the amino acid L-arginine inhibited the macrophage's ability to kill tumor cells. Further, L-arginine itself was absolutely essential for macrophages to be toxic to pathogens and tumor cells. Eventually, he pieced together the biochemical pathway that created the macrophage's toxic effects. He demonstrated that in an enzymatic reaction, L-arginine was somehow converted to citrulline (another amino acid). But at the time, an L-arginine-to-citrulline reaction was not understood to take place in the human body outside the urea cycle. And the reaction Hibbs saw was different from what happened with urea. There was another oddity: When Hibbs tracked the pathway, he couldn't account for where a nitrogen atom had gone. It seemed to have vanished from the equation.

In the meantime, biochemists at the Massachusetts Institute of Technology were interested in how nitrite-enriched foods affected the body. They were concerned that nitrite (the salt of nitric acid) was carcinogenic. MIT's Steven Tannenbaum had discovered that our bodies somehow synthesize nitrates (a similar salt). This seemed to happen only in the presence of microbes. When checking one study subject's urine, Tannenbaum noticed his nitrate levels had spiked. The subject happened to have a fever. This prompted Mike Marletta and Dennis Stuehr, astute young scientists in the same lab, to treat mouse macrophages in tissue culture with endotoxin, resulting in the production of nitrite and nitrate. They thought maybe the salts were a byproduct of inflammation. Hibbs read about these findings in January 1986.

That's when it all came together for him. Hibbs realized where the wayward nitrogen atom had gone, nearly ending a 15-year-long chase for the elusive mechanism that rendered the activated macrophage so potent. It made sense: a nitrogen oxide must be the missing biochemical link—responsible for

killing the tumor cells and Remington's intracellular pathogens. This conclusion was fortified by another finding. With the help of Donald Granger and Jean-Claude Drapier, inspired fellows in his lab, he'd learned that the activated macrophage had damaged tumor cell mitochondria, causing iron to leak out of the cells. Nitric oxide was known to perturb iron metabolism.

In July 1986, he submitted his findings to *Science*. They were published in January.

Scientific universes were colliding right about then. Independent of Hibbs and the MIT scientists, Louis Ignarro and Robert Furchgott had just concluded that nitric oxide was released in the cardiovascular system. Back in 1980, Furchgott had observed that smooth muscle cells in the cardiovascular system relaxed and dilated in the presence of an agent he called EDRF (endothelial derived relaxing factor); Ignarro and Furchgott independently reported their conclusions that EDRF was nitric oxide at a conference in July 1986, when Hibbs submitted his paper. Ignarro then collected more data supporting his hypothesis, which *Circulation Research* delayed publishing until December 1987, because the data seemed so unlikely. Hibbs showed soon after, in 1988, that nitric oxide, a highly unstable molecule that exists for less than 10 seconds before it's converted to the end products nitrite and nitrate, was indeed the molecule that macrophages used to kill tumors and microbial cells. Marletta reported at the same time his own unequivocal biochemical evidence that nitric oxide was produced biologically.

By then, interest in nitric oxide had exploded in the biomedical community. NO, the shorthand name for nitric oxide, was an overnight sensation. Tens of thousands of papers on the molecule have been written since Ignarro's and Furchgott's presentations. Who would have imagined that a gas—of all things, a known pollutant—passed through cell membranes to play the role of a vital metabolic regulator?

As it turned out, nitric oxide is produced by many cells in the body besides macrophages. We now understand, thanks first to Hibbs, that it's a weapon against infection. It's also a signaling molecule in the nervous system, a regulator of blood pressure, and a gatekeeper of blood flow. Nitric oxide helps us recognize different scents and appears to play a role in memory and behavior. It's considered key to developing better treatments for atherosclerosis, certain intensive care situations, cancer, and impotence (the gas is the magic behind Viagra). It plays a harmful role in sepsis and circulatory shock, so the development of NO-inhibiting treatments is regarded with great optimism. In fact, Pitt's Department of Surgery, a hotbed of NO investigation under chair Timothy Billiar, was the first to show that NO production increased in humans experiencing sepsis. (The department lays claim to many NO "firsts," including identifying and cloning the gene for iNOS, an enzyme that triggers production of nitric oxide in the body. Pitt's nitric oxide beginnings came in the late '80s when Billiar and then-chair of the surgery department, Dick Simmons, collaborated with one of Hibbs' inspirations, Klaus Hofmann.) Hibbs' own work as a NO pathfinder continues to this day.

Murad, in his Nobel lecture, noted Hibbs' contributions and that Hibbs had never been sufficiently recognized for his important observations.

"Many in the field, including myself," confides Billiar, "feel that John Hibbs' contributions to the discovery of the nitric oxide pathway were significant enough to warrant sharing the Nobel." He was nominated for the prize.

Each day, Hibbs walks before dawn, before the aspens play their games with light and shadows. At his office in the Veterans Affairs building, he works quietly, out of the spotlight. He hasn't published much in the last few years, notes Granger. But, "the best is yet to come," Granger is convinced. His old mentor is piecing together how our bodies adapt to stress metabolically—more on nitric oxide. "I think it's extraordinarily important work," says Granger. "The government should give him a *carte blanche* research grant. I can't think of anybody I'd put above him for scientific and humanistic honors."

Hibbs' current puzzle involves some of the most difficult biochemistry he has ever encountered, "sort of a Lewis Carroll–*Through the Looking Glass* chemistry," Hibbs says. A few years ago, he thought he would retire about now, but the biology he's trying to figure out has just begun to crystallize for him, a culmination of 15 years of effort. He'd like to see it through. "There's really something important there," he says. His grant money runs out this summer, however. And he's not sure that he'll get funding to continue. ■

COURTESY HIBBS

Hibbs on one of many family hikes. This was taken in Idaho; the Teton Range is in the background.