In the University of Pittsburgh Department of Surgery, they are tracking a phantom suspect. This ghost has given former presidential candidate Bob Dole a new career in TV commercials. (It is the basis for Viagra, the much-hyped magic potion for erectile dysfunction.) It also appears to play a key role in virtually every aspect of everyday physiology, from control of...
blood pressure to wound healing to microbial infection, even to childbirth.

Therapeutic applications have saved lives in the operating room and given support to those with breathing problems. It also may help dialysis and coronary-bypass patients.

Yet this phantom comes and goes before it can readily be detected.

As molecules go, nitric oxide, or NO, is both elusive and unique, which helped it remain terra incognita until recently. Most substances enter cells via specific receptors on the cell’s surface, as a key fits into a keyhole; nitric oxide needs no receptors. It does its work as a gas, readily passing through the permeable cell barriers. NO also has an extremely brief half-life, a mere six seconds. It materializes in quick puffs and is gone soon after.

The molecule has captured the imagination of thousands of researchers. A torrent of 30,000 (and counting) NO papers has poured out of laboratories, mostly in the last five years; Science magazine crowned it “Molecule of the Year” in 1992 and an Indian journal upped that to “Molecule of the Millennium.”

Much of the basic biology of this ghost substance has been unveiled, surprisingly, at the laboratory benches housed in a surgical department—Pitt’s, to be exact. Former department chair Richard L. Simmons points out that surgeons’ research interests are normally pegged as short-lived. In a marked departure from that stereotype, the Pitt team has zeroed in on pure science as the best route to identifying NO’s benefits to patients.

“It is unusual,” says Simmons, who gave up the department chair in 1998 after 11 years, “for a surgery department to maintain such a devoted and unremitting focus on any problem in basic science.”

That dedication by a small group of surgeons has produced a series of research firsts. To name only a few of the most important, Pitt surgeons were first to show that NO could be made in human liver cells (previously it had been identified only in mouse immune-system cells); first to show that it was made also by the heart; first to describe its role in facilitating organ transplant rejection; first to show that it could be used for therapy in blood-vessel disorders. “And lots more,” Simmons says. Perhaps the two most attention-getting discoveries came in the early ’90s from Timothy Billiar, the tall, reserved Nebraskan who led the research effort at Pitt and last fall succeeded Simmons as department chair, and David Geller, now the Samuel P. Harbison Assistant Professor of Surgery and a faculty member at the Thomas E. Starzl Transplantation Institute. Billiar et al. identified the human gene that turns on inducible nitric oxide synthase (iNOS), which is a key enzyme for triggering production of NO throughout the body. Geller cloned the gene. That success opened the way for expression of a recombinant human iNOS. (Recombinant, meaning Geller applied the same gene-splicing technology—invented, in part, by Pitt grad Herb Boyer—that made possible treatments such as the now widely used form of insulin that has replaced animal-derived insulin. The other often caused allergic reactions in diabetics.) Pitt now holds four patents based on the cloned gene, and pharmaceutical companies worldwide are assiduously using them to pursue a wide range of therapeutic applications.

Oddly, although it combines the two most common elements in the earth’s atmosphere—nitrogen and oxygen—little was understood about nitric oxide as recently as 15 years ago. What was known cast NO as an environmental villain, a particularly noxious product of auto exhaust. In the days before catalytic converters were mandatory, for example, German foresters and Greens vociferously blamed auto-generated NO for killing the spruces and firs of the beloved Black Forest.

And yet physicians had been using nitric oxide therapeutically for a century without completely understanding why. That was in the form of nitroglycerin, a vasodilator often prescribed for angina pectoris (the acute chest pain that comes from heart muscles not receiving enough blood) and other cardiac conditions. Nitroglycerin expands the diameter of blood vessels and increases blood flow to the heart.

In 1977 Ferid Murad, a pharmacologist/physiologist at the University of Texas–Houston, discovered that nitroglycerin opened coronary arteries by releasing NO. He theorized that the body itself could stimulate the release of NO to regulate blood vessels. But he had no experimental evidence to back up his theory. Then in 1980 Robert F. Furchgott of the State University of New York Health Science Center at Brooklyn found that the endothelium, the inner lining of the blood vessels, released a substance that relaxed the vascular muscles and increased blood flow. He called the substance endothelium-derived relaxing factor, or EDRF.

It took six years more for Louis J. Ignarro of the University of California, Los Angeles, another pharmacologist, to capture the ghost. Ignarro, using spectroscopy, identified the relaxing factor as nitric oxide. Furchgott, Murad, and Ignarro received the 1998 Nobel Prize in Physiology or Medicine for discovering “an entirely new principle for signaling in the human body.”

In 1987, as Furchgott and Ignarro caught the attention of the scientific world, Richard Simmons came to Pitt from the University of
Minnesota. Billiar, four years out of medical school, came with him as a research fellow. Simmons, “a transplanter by trade,” quickly saw that nitric oxide, with its ability to dilate blood vessels, might offer clues to one of the most dismaying and unfortunately all too familiar problems of the operating room—the postsurgical shock and loss of blood pressure that can lead to massive organ failure and death. Geller, who joined the effort a few years later, had more than once seen these devastating effects: “Patients get sick and then very sick and end up in surgical IC units; they develop sepsis or disseminated infection throughout the entire body, and go into multisystem organ failure, which eventually does them in; they succumb and die.”

Simmons explains how the research offensive came about. “I am a catalyst by nature—and curious.” He began to recruit a team to carry out the work.

“The power structure in the field of surgery is such that a chairman has tremendous power,” he says. “Therefore, people in the field with ambition tend to be drawn to the chair’s interests. A smart chair takes advantage of that opportunity. You get to choose the smart ones. You support the careers of the smartest and most productive and are rewarded for that in turn.”

By 1988, Simmons was starting to see the returns on his recruiting efforts in the form of research results; by 1993 he was flooded with NO breakthroughs. “We didn’t at first focus on NO,” Simmons says. “It just happened to be the answer to questions we were asking about organ failure in sepsis. That caused us to ask more questions. If you keep asking why, you sometimes are lucky.”

Nitric oxide actually plays a couple of basic roles in human biology, each of which is controlled by the expression of different genes. When it’s released by an enzyme known as constitutive nitric oxide synthase (cNOS), it often becomes a neurotransmitter, one of the chemical messengers that carries impulses from nerve cell to nerve cell. That enzyme is present in minute quantities in the body at all times to carry out this role. Other times, it’s turned on by inducible nitric oxide synthase (iNOS)—the enzyme Billiar and Geller have come to know about as well as anyone—and it is produced when the body is stressed by inflammation, say, or widespread infection. Then it is turned out in great quantity—up to 1,000 times more than when it’s produced by cNOS. This latter, often crisis, mode of nitric oxide is what has captivated Billiar and Geller.

Billiar led the way in unraveling the complexities of the molecule. As early as 1989, he was explaining that NO was derived from L-arginine, one of the amino acids that are the building blocks of life. He also identified the role of growth-regulating cytokines in the generation of NO. His curriculum vitae lists 24 pages of publications, almost all on NO, and nine pages of invited lectures.
After Timothy Billiar found the gene, David Geller (above) cloned it.

around the world. Within seven years he rose from assistant professor of surgery to associate professor to Watson Professor of Surgery to George Vance Foster Professor and chair of the department.

“Tim is recognized as one of the world’s leaders in this area,” Simmons says, praising his protégé. “He is always invited to meetings, national and international, on anything to do with NO—a testimony to the importance of his work.”

In contrast to Billiar, who is often so soft-spoken that you must lean forward to catch what he is saying, the ebullient Geller comes bounding from his office, hand outstretched. “I’m Dave Geller!” he says, without waiting for you to be ushered in by an assistant. He sits at a cluttered desk and scoops up a pencil-sized apparatus, clicks it, and hands it to his visitor. Tiny wire hooks pop out at the base.

“For radiofrequency ablation of liver tumors,” he explains, inviting the visitor to click it again and then pointing to the slender wires. “I’m a liver surgeon. I spend half my time doing liver transplants, half removing liver cancers. With this, we click it into a liver cancer and these 10 little tines, or hooks, come out and burn out the cancer.

“If we can’t cut ‘em out, we burn ‘em out.”

Geller is certainly a preeminent researcher as well as surgeon. He has 60 NO publications on his own CV, holds a career development award from the American College of Surgeons and a five-year $500,000 research grant from the National Institutes of Health (NIH). After conducting liver research at NIH, he came to Pitt as a 25-year-old intern, quickly immersing himself in the nitric oxide work. His iNOS cloning triumph came before he was 30. Billiar calls him “the world’s leading authority on the iNOS gene.”

Geller continually emphasizes the department’s view that, despite its commitment to basic science, Pitt surgeons remain surgeons first and foremost.

“My research interests stem from what I do clinically as a surgeon,” he says.

“Many problems we see in the operating room, if we could understand the pathophysiology or molecular biology and the basic mechanisms responsible, maybe ultimately we could find a way to prevent or find novel strategies to treat patients.”

Thus, the vexing problem of postsurgical shock remains at the top of the agenda for the dozen or so department “worker bees,” as Simmons calls the NO researchers. Billiar is the primary investigator (working with Bruce Pitt of environmental and occupational health, Tony Bauer of medicine, Brian Harbecbt of surgery, Mitch Fink of critical care medicine, and others) on a major, nationally funded effort that’s looking into this complex problem. Department researchers are also investigating NO’s apparent role in apoptosis, or programmed cell death. “We are one of many labs all over the world looking into this question,” Billiar says, noting that understanding NO’s link is hugely important. “It’s a big field of research,” he notes. For instance, researchers think that understanding NO’s role in cell-death programming might lead to new cancer
Nitric oxide has an extremely brief half-life, a mere six seconds. It materializes in quick puffs and is gone soon after.

therapies down the road—however, no one knows how NO might halt cancer growth, it may actually stimulate it.

NO zaps infections as well; elsewhere at Pitt, researchers are studying how the immune system employs it in diseases such as tuberculosis (no one understands exactly how the TB process works). Also under investigation is NO’s possible role in neurodegenerative diseases such as Parkinson’s and Alzheimer’s as well as rheumatoid arthritis and osteoarthritis. Too much NO production triggered by inflammation may have a role in tissue destruction in arthritic joints.

On a more basic level, Geller is investigating, through an NIH grant, the on/off signals of the iNOS gene. “We have a fairly good idea of what turns on the gene. Almost nothing is known about the molecular mechanisms that turn off the gene. In certain settings you want to overexpress the gene because it would be beneficial; in other settings it would be clearly harmful, and you want to turn it off or prevent its being activated.” To give an example of an NO too-much/too-little tightrope walk that occurs naturally in our bodies: The cardiovascular system relies on a little NO to keep blood pressure in check, yet an excess can bring about catastrophic collapse.

A clinical trial of NO in kidney dialysis patients is in the startup mode, directed by vascular surgeon Edith Tzeng, assistant professor of surgery (who, Billiar says, in his low-key way, “happens to be my wife”). In dialysis, a U-shaped arteriovenous shunt linking arteries and veins is permanently implanted, usually in the arm, and blood is withdrawn via the vein, cleansed of water and impurities and then returned via the artery. The shunts open narrow and allow clots to develop, or they close up altogether and collapse within a year or so after implantation. In earlier research, Tzeng, with Pitt’s Larry Shears, had shown that NO inhibited the growth of smooth muscle in the vascular walls; it is this growth that reduces the diameter of the blood vessel and hampers blood exchange. In the trials, supported as part of a $14 million cardiac gene therapy grant, she will infuse the cloned iNOS gene when the shunt is implanted to determine if the vessels will remain open. Later, the investigation may be extended to coronary-bypass and angioplasty patients, whose vessels also may close some years after the procedure.

Reflecting on the NO furor of the past decade, Simmons notes that worldwide research interest in the subject has started to plateau. There is no sign of a slowdown at Pitt, however, where surgeons continue to chase the shadow.

As part of a huge gene therapy grant, Edith Tzeng is infusing the iNOS gene to see if it will stop blood vessel walls from narrowing. By the way, she determined that nitric oxide can hamper growth of smooth muscle on vessel walls.

William Kiester contributed to this article.