

NO News Is Good News

A startlingly simple molecule unites neuroscience, physiology, and immunology and revises scientists' understanding of how cells communicate and defend themselves



A decade ago, nitric oxide (NO) was just another toxic molecule, one of a lengthy list of environmental pollutants found in unsavory haunts such as cigarette smoke and smog. Destroyer of ozone, suspected carcinogen, and precursor of acid rain, this gas had a bad reputation.

But over the past 5 years, diverse lines of evidence have converged to show that this sometime poison is a fundamental player in the everyday business of the human body. In 1992, NO was ushered into the pantheon of messenger molecules with a fanfare of hundreds of research papers. This year scientists probed NO's activities in the brain, arteries, immune system, liver, pancreas, uterus, peripheral nerves, and lungs. They found that the molecule is essential to activities that range from digestion and blood pressure regulation to antimicrobial defense.

Nitric oxide also carries important information in the nervous system: In males, it is the messenger that translates sexual excitement into an erect penis. In the brain, nitric oxide may be a long-sought mystery molecule that aids in learning and remembering.

Yet early wariness concerning the power of NO was not ill-founded: This year the molecule's derivatives were found to damage DNA in human cells, and it is implicated in nerve damage left by strokes.

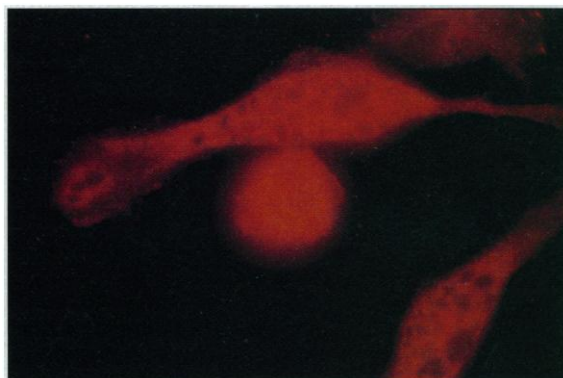
In 1992, the nitric oxide breeze wafting through the scientific world strengthened into gale force winds, stirring up potential drug development and propelling hundreds of heretofore-unrelated scientists together into a new research field.

NO wonder. Not to be confused with its cousin, laughing gas or nitrous oxide (N_2O), nitric oxide is the smallest, lightest molecule—and the first gas—known to act as a biological messenger in mammals. The molecule has one unpaired electron, making it a free radical that avidly reacts with other molecules. In the presence of oxygen, NO may vanish a few seconds after it forms, although its lifespan in the human body is unknown.

No one expected such a bizarre molecule to be important in physiology, and the earliest research reports were greeted with disbelief. But in the late 1980s, scientists in distinct disciplines—immunology, cardiovascu-

lar physiology, and carcinogenesis—suddenly realized they were studying the same molecule. Like a squirt of some powerful perfume, a puff of nitric oxide spurs different cells into an array of different activities, from communication to defense to regulation.

A thousand times NO. In 1992, scientists probed the reasons behind these multiple personalities. One significant clue: the biochemistry of nitric oxide manufacture. Cells rely on various forms of an unusual enzyme called NO synthase (NOS) to do the job, and a



NO trace. Immunohistochemical staining tracks the presence of NOS and lights up NO-making macrophages.

single cell may have two kinds of enzyme—constitutive and inducible—that produce NO for different roles. Constitutive enzymes are ever-present citizens of the cell, always available to make brief puffs of NO for delicate tasks like neurotransmission. In contrast, inducible enzymes are goaded into action more slowly by other cellular messengers. But over a period of days they can produce at least 1000 times more NO for cellular defense. In 1992, scientists cloned the genes for a string of these enzymatic forms, including constitutive NOS from human and bovine endothelial cells, and inducible NOS from mouse macrophages and human liver cells.

Understanding this unusual enzyme is crucial to designing drugs to turn NO on and off. For example, a handful of molecular cofactors are tightly bound to NOS and help the enzyme accomplish its task of stripping five electrons from one amino acid (L-arginine) to produce NO and another amino acid. This year biochemists discovered that one such molecular helper is heme, the iron-containing compound that carries oxygen and makes blood red. Since heme is a known electron acceptor, the new knowledge gives biochemists a start on figuring

out how the enzyme works.

NO cure for heartache. This year, clinical applications of NO knowledge bloomed in several directions at once, but much effort focused on nitric oxide's role as the body's own blood pressure police. In blood vessels, NO is released by endothelial cells on the inside of the vessel wall, migrates to nearby muscle cells, and relaxes them. This dilates the vessel and lowers blood pressure.

Understanding this process opens the door to a host of new drugs. Indeed, faults in the NO system may be the guilty parties in some familiar cardiovascular diseases, possibly even essential hypertension and atherosclerosis. In 1992, scientists raced to develop a new class of chemicals that can release NO more controllably and successfully tested them in cell cultures and in mammals.

In a handful of extreme cases in 1992, physicians have used NO inhibitors to save lives. Septic shock, a leading cause of death in U.S. intensive-care wards, is somehow related to too much NO: Life-threatening low blood pressure occurs when the body pours out nitric oxide in response to a bacterial infection. This year, nitric oxide inhibitors successfully brought several patients' blood pressure out of the danger zone in a few minutes. Also this year, Phase I clinical trials were begun to test NO inhibitors as an adjunct to interleukin-2 (IL-2) therapy for stubborn cancers of the skin and kidney. IL-2 rouses the immune system but also triggers a dangerous flood of nitric oxide; physician-scientists hope NO inhibitors will keep blood pressure up while IL-2 helps kill the cancers.

The power of NO. As a defensive weapon, NO appears to work in at least two ways: by inhibiting key metabolic pathways to block growth, and by killing cells outright. In the first mode, NO attacks susceptible iron groups in certain enzymes, including those that synthesize DNA and help cells respire. When those enzymes are crippled, cells cannot grow and divide; this may be an important part of NO's antitumor function.

This year it was shown that NO can also combine with oxygen to eventually produce potent cellular assassins such as the hydroxyl radical, $\cdot\text{OH}$, and nitrogen dioxide. Such pathways may be behind NO's antibacterial properties, which are the basis of the centuries-old practice of curing meat with nitrite-

rich salts. In 1992, scientists investigated the possibility that NO is a primitive defense against a whole range of microbes and worked to understand how host cells defend themselves against this internal killer.

In the brain, NO's split personality emerges at full strength, as the molecule acts as either messenger or killer depending on conditions. For example, evidence gathered this year and last suggests that neurons containing NO synthase may actually kill their neighbors during a stroke. These NOS neurons—themselves eerily resistant to stroke damage—apparently may become overstimulated by the excess neurotransmitter released during a stroke and flood nearby cells with toxic amounts of NO. In mice and cultured nerve cells, NO inhibitors can reduce damage significantly; scientists are avidly pursuing the therapeutic implications.

NO neurotransmitter. In neuroscience, startling discoveries of NO's myriad roles are emerging at a rapid pace. This year nitric oxide has proven itself privy to a host of delicate body functions—yet it continues to defy the normal rules of neurotransmission.

This evanescent molecule sometimes does the work of a neurotransmitter but doesn't look or act like any other such messenger. When a neuron fires, it releases neurotransmitter from special storage vesicles into a gap called the synapse; the receiving cell picks up the neurotransmitter and is activated. But NO has no special storage facilities, and no special release mechanisms. Made when and where it's needed, it seems simply to diffuse out of the producing cell.

Most neurotransmitters are composed of amino acids or a string of peptides, which couple with precisely configured receptors on the surface of cells. Compared to this model, NO is positively promiscuous. It needs no receptor gates, passing through membranes like a ghost. Its known targets are enzymes deep within cells, and it carries its message to any and every cell within reach.

NO sex. So what makes this odd molecule so similar to a neurotransmitter? One of the best examples comes from sex. This year, scientists proved definitively that in males, NO translates sexual excitement into potency by causing erections. Key pelvic nerves get a message from the brain and make nitric oxide in response. NO dilates blood vessels throughout the crucial areas of the penis, blood rushes in, and the penis rises to the occasion. If NO synthesis is blocked—as was done in experiments in live rats this year—those all-important blood vessels never dilate, and the penis stays limp. With 10% of the male population suffering from impotence, researchers are hotly pursuing this line

of research in hopes of clinical applications.

In addition to being found in the genitalia, nitric oxide also carries out its duties as neurotransmitter throughout the lungs and gut. For example, during digestion, the gut begins a set of systematic wavelike contractions—peristalsis—that move food through the stomach and intestines; NO triggers the relaxation component. This process is blocked in some infants with a potentially lethal condition called infantile hypertrophic pyloric stenosis.

This year, scientists found evidence that lack of NO is the culprit in that disorder.

Learning to say NO. Back in the brain, a series of experiments done in late 1991 and throughout 1992 suggests that nitric oxide may help cells to store and retrieve information—the keys to learning and memory.

The cellular basis for learning and memory is thought to rely on strengthening the connection between sending and receiving neurons, the presynaptic and postsynaptic cells. One way to do this is a process called long-term potentiation (LTP), in which repeated firing causes postsynaptic cells to respond more strongly the next time they receive a signal. One theory for how it works invokes the “retrograde messenger,” a mysterious molecule that would travel backward across the synapse and enhance the release of neurotransmitter in the presynaptic cell. Such a messenger would have to pass lightly from cell to cell without benefit of either release machinery or receptors—just like nitric oxide.

So in the past 2 years, neuroscientists have rushed to test nitric oxide. Several labs have now performed the crucial experiment and gotten positive results: In rat hippocampal neurons, LTP can be prevented by inhibiting NO synthesis. Also this year, scientists injected NO inhibitors into the brains of live rats and found that the rats could no longer learn a water maze.

But not everyone is ready to crown nitric oxide as retrograde messenger. One missing piece in the puzzle: Thus far, no one has been able to detect NOS reliably in the hippocampal neurons that are supposed to be releasing NO. A few more experimental replications will increase everyone's confidence that the effect is real. Expect intense, focused effort—and a torrent of papers—to resolve this debate next year.

NO class? In the brain, nitric oxide science is in the discovery phase, as neuroscientists test NO in a variety of systems. It's far too soon to put all the pieces together, but NO is clearly a crucial part of our neurochemical system.

Some scientists speculate that nitric oxide is merely the first of a soon-to-be-discovered

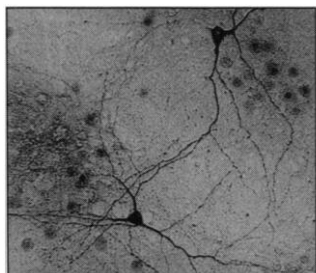
new class of signaling molecules—gases that swiftly pass through a cluster of cells and then vanish. Preliminary evidence hints that one candidate for such an airy messenger may be carbon monoxide, another molecule that today has a shady reputation. Stay tuned for new developments next year.

Despite this year's burst of research, scientists have a long way to go before they understand nitric oxide's paradoxical nature. On page 1898 of this issue, Stamler *et al.* argue that the uncharged, free radical form, NO[•], may not be responsible for all of the myriad activities attributed to “nitric oxide.” Other, intermediary compounds containing the NO group may also play a role. That view is by no means a consensus opinion, but the debate itself bespeaks a new attention to basic chemistry, especially oxidation and reduction reactions. As Stamler *et al.*'s paper illustrates, NO's conceptual contribution has made itself keenly felt this year. This molecule's Cinderella story—from vaguely noxious gas to powerful queen of communication and defense—offers a classic example of nature's continuing power to surprise.

And the runners-up are...

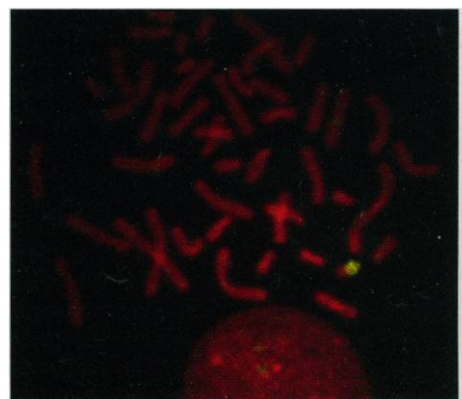
In 1992, science surged forward, often in unexpected directions. Below are nine advances that deserve to share the spotlight—and remember, they may be composed of billions of molecules. Any elegant technique or unusual corner of the universe may qualify, if it has earned a place in scientific history this year.

Chromosome countdown. In a landmark year for the Human Genome Project, scientists in 1992 completed high-resolution physical maps for two of the 24 human chromosomes, Y and 21. These maps demonstrate that the genome project—which celebrated its second birthday this year—is living up to



NO resistance. NOS neurons (gray) live as others (black) die.

TED DAWSON



New maps. Chromosomes Y (yellow) and 21 have been completed.

WEN-LIN KUO

its promise. The goal of mapping the entire human genome by 1995, once considered overly ambitious, is now clearly within reach.

Each map is itself a scientific coup. The Y chromosome is the essence of maleness, responsible for dividing our species into two

sexes. Chromosome 21 has been linked to genetic disorders such as Down's syndrome and familial Alzheimer's.

To map the chromosomes, scientists cloned overlapping pieces of DNA, each tagged with one or more known sequences, and then put the tags in order. Thus the vast stretches of unknown DNA that make up the chromosomes have been divided into manageable chunks, bounded on each side by known sequences.

Also this year, independent groups of scientists completed two separate genetic linkage maps of the whole genome, linking molecular markers with inheritance of specific traits.

All this information will speed the work of understanding known genetic diseases, as well as the genetic components of common afflictions such as cancer and heart disease.

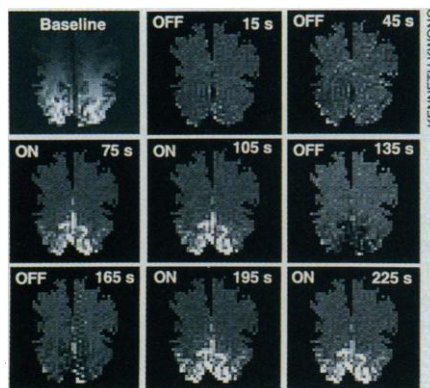
There's still much to be done, of course—these chromosomes are charted by maps, not by complete sequences. Those going gene hunting must still do some searching—but now they know where to look.

Resourceful ribozymes. Ribozymes—RNA molecules that can serve as catalytic enzymes—greatly expanded their known repertoire of activities this year, showing promise as therapeutic agents and spurring a flurry of research in both companies and universities.

When first discovered, ribozymes' tasks were restricted to processing RNA molecules. But in 1992, nearly conclusive evidence suggests ribozymes can also build bridges between amino acids to form proteins, and can make and break the bonds that link amino acids to RNA. This offers a new view of how proteins are made in the cellular structure called a ribosome. Biologists once thought proteins themselves performed the catalytic labor, while RNA merely supplied a supportive scaffold. This year's results suggest the reverse—the proteins are the scaffolding, and the ribozymes do the work.

Also this year, scientists co-opted the power of natural selection to coax ribozymes to perform new chemical tricks in the test tube (see article on page 1910). Ribozymes' ability to "evolve" new functions—as well as their newly expanded catalytic talents—are consistent with the "RNA world," the theory that early life relied on RNA to perform many roles in the biological stage.

The more practical implications of all this enzymatic activity have not been lost on the biotechnology industry. Companies old and new have pounced on the opportunity to develop specific ribozymes that could slice the RNA of pathogens such as HIV while



Making movies. MRI tracks changes in the human visual cortex as lights flicker.

leaving host cells intact.

MRI makes its move. Magnetic resonance imaging (MRI) has long offered multiple benefits to medicine and science, because it offers a non-invasive way to peer inside the body. But in 1992, the technology leaped forward with the development of functional MRI, which allows scientists to track blood flow in the brain in real time. This year

subjects stepped into a large magnet and stared at lights or squeezed objects, while scientists made real-time movies of the changes in their brains. Scientists even told subjects to imagine a bright light—and watched the visual cortex light up.

For years this kind of functional imaging—where scientists match thought, deed, or disease to corresponding brain activity—has been the domain of PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computer Tomography). Now MRI, which does not require injections of tracer elements as these techniques do, offers a third alternative.

Functional MRI uses magnetic fields and radio waves to track changes in blood flow and blood oxygenation. And it's becoming quicker and easier all the time. Fans insist functional imaging doesn't even require a state-of-the-art MRI machine but could be done on the 2000 conventional machines in use in hospitals worldwide. Though Hollywood needn't worry about competition just yet, the age of MRI movie-making has begun.

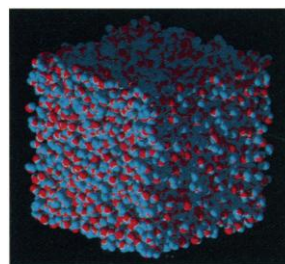
X marks the spot. For more than a decade, circumstantial evidence has been piling up for an extra-terrestrial villain in one of geology's great whodunits: the Cretaceous-Tertiary extinction, when the dinosaurs and

many plants and sea-dwelling invertebrates vanished. In 1992, new evidence has brought one chapter of the story—the search for a likely impact crater—to a close.

Two years ago, geologists honed in on a giant subterranean crater, 180 km in diameter, near the town of Chicxulub in Mexico's Yucatan peninsula. This year, using lasers and high-resolution mass spectrometers to measure isotopes of argon, two groups of researchers independently dated molten rock from the crater at 65 million years ago—right at the Cretaceous-Tertiary boundary. Samples from Haiti and northeastern Mexico showed that tektites—beads of glass forged in the fiery heat of an impact—were exactly the same age as the crater and similar in chemistry.

With data linking impact debris to the Chicxulub crater, and both to the pivotal \$65 million date, many former skeptics now admit that something big crashed to Earth at the end of the Cretaceous era in Mexico. And so the next phase of research has begun: finding out what happened after the crash, and whether more mundane processes such as climate change served as accomplices in the death of the dinosaurs.

Parallel power. Supercomputer users are accustomed to rapidly changing technology. But 1992 was something special: the dawn of the era of a new style of supercomputer, the massively parallel machine. This year nearly a dozen supercomputer companies introduced or announced plans for massively parallel computers. Scientists used these machines to tackle an array



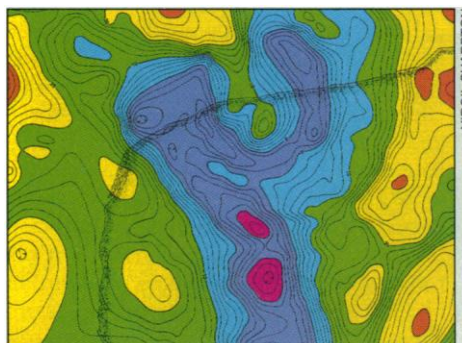
Seeing parallel. A parallel machine models water.

of complex problems, from imaging the surface of a silicon chip to deciphering the structure of an ion channel protein.

Most of today's supercomputers use more than one processor—the Cray Y-MP has eight, for example—but massively parallel machines represent a new type of computer architecture, in which a small army of processors can dissect and work huge problems at awesome speeds. Devotees foretell a thousand-fold increase in peak computer speed in the next few years.

What kinds of science will these new machines solve? Any problem rich in data and complexity—the "grand challenges" of science, including the mysteries of molecular interactions, protein folding and structure, properties of materials, climate change, and the evolution of galaxies.

Bench scientists can take heart in another landmark this year: jumps in the speed of computer communications. Today scientists can communicate on Internet, with each other and with supercomputers, at speeds up to 45 million bits per second, a goal achieved 1 year ahead of schedule. Next year, expect speeds of 155 million bits per second.



Heavy hitter. Gravity data outlines the Yucatan's 65-million-year-old crater.

Nitrogen news. One of the triumphs of the Industrial Revolution was the Haber process, which allows chemists to create ammonia, NH_3 , from nitrogen gas, N_2 , by using strong-arm tactics of high temperature and pressure. But scientists have long known that nature does it better. Certain bacteria in the roots of plants perform the same reaction at atmospheric pressure and temperature, in the process making nitrogen available to other life forms.

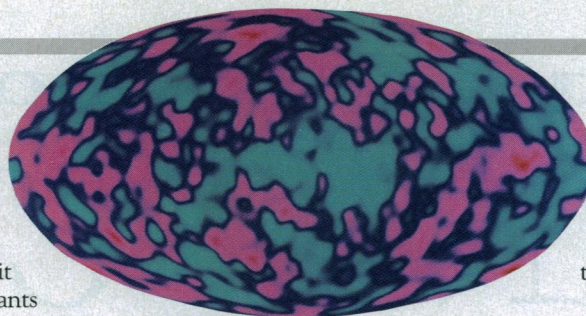
How do these bacteria do it? Nineteen-ninety-two saw the start of the era in which chemists and biologists can begin to answer that question. This year, scientists revealed the structure of nitrogenase, the enzyme responsible for the enviable abilities of nitrogen-fixing bacteria.

During the past decade, two independent groups of scientists have carried out the painstaking work needed to deduce the enzyme's complex structure. Their results show that nitrogenase is essentially two complicated proteins, an iron protein and an iron-molybdenum protein, which work together to catalyze the reaction. One surprise was the molybdenum: chemists had long thought this transition metal crucial to the catalytic process. But the molybdenum atoms turn out to be so thoroughly tied up with other bonds that they seem unlikely candidates for the actual site of nitrogen reduction.

Finding the structure set off a flurry of theorizing—and experimentation—by chemists who think they can already see how electrons might be persuaded to take the crucial steps: from the iron protein, to the molybdenum-iron protein, and finally to N_2 . However the electrons make their journey, the structure of nitrogenase has begun a new age of exploration for a new generation of chemists.

Space tracks. Cosmology, a field rich in theory but poor on experiment, this year received a rich inoculation of data from a satellite seeking the seeds of galactic structure. Called the Cosmic Background Explorer (COBE), the National Aeronautics and Space Administration's probe was sent out to take the temperature of the early universe by measuring the faint afterglow left by the Big Bang.

The occurrence of that primordial explosion is backed up by pervasive microwave radiation, which bathes the universe at exactly the temperature predicted by Big Bang theorists, 3 degrees above absolute zero.



Cosmic kernels. COBE found traces of the structure of the early universe.

But Big Bang theory also suggests that 15 billion years ago the newborn universe was a featureless structure, stretching out evenly in all directions. Today matter is clumped tightly into galaxies like our own Milky Way, with vast reaches of empty space in between. How

did galaxies and star clusters emerge? Cosmologists had predicted slight bumps in the texture of the early universe, which over the eons were strengthened by the force of gravity into major cosmic structures. Traces of these early irregularities should be preserved today as slight variations in the background radiation.

In 1992, after many months of orbit, COBE got the goods: It measured slightly cooler and warmer spots, differing by only 30 millionths of

a degree, in the cosmic microwave radiation. Thus cosmologists' notions of the early universe have been vindicated. More good news arrived this month, when data from a balloon-borne experiment, which scanned a smaller area of sky at a different wavelength, partially confirmed COBE's results. For cosmological explorers, looking up has paid off.

Common antisense.

Scientists have long recognized the potential power of knocking out specific genes with antisense molecules. This year, this benchside technique blossomed into drug development, and antisense compounds were used in humans for the first time.

Antisense molecules prevent cells from translating genetic information into proteins. Nucleic acids have two complementary strands, only one of which—the “sense” strand—codes for protein. The other strand is “antisense,” which can bind to sense molecules and prevent them from being expressed.

Antisense weapons have been crafted to fight leukemia, AIDS, hepatitis, and other diseases. This year scientists used a virus to slip antisense RNA molecules into cells already infected with HIV. The results: HIV infectivity decreased by more than 99% in vitro.

Another antisense technique relies on relatively short synthetic DNA molecules (called oligonucleotides) that are apparently spontaneously taken up by cells, and so need no special means of delivery. This year, the first human trials of these compounds began, in a patient with leukemia and in patients with human papilloma virus, which causes genital warts. And in September, the National Institutes of Health approved the first antisense gene therapy trials, scheduled to begin next year in patients with lung cancer.

Embryonic efforts. In 1992, medicine marched backward to the earliest stages of life, breaking new ground in fetal diagnosis and treatment and gaining greater understanding of the biology of life in utero.

This year, the healing power of fetal tissue was used to treat Parkinson's disease in a handful of studies. The results were encouraging—several patients regained their ability to walk and talk. But transplanting fetal tissue to adults is only the beginning. Because the fetal immune response is not yet fully developed, undeveloped fetuses themselves may be ideal recipients for transplants. In 1992, scientists continued to explore the potential for fetal medicine in animal models, and in some cases performed surgery to correct anatomical defects in utero.

In fetal diagnosis, testing was pushed back to the first few days of life in a procedure appropriately nicknamed BABI (Blastomere Analysis Before Implantation). Coupled with in vitro fertilization and performed at the eight-cell stage, the technique allows physicians to screen embryos for specific genetic

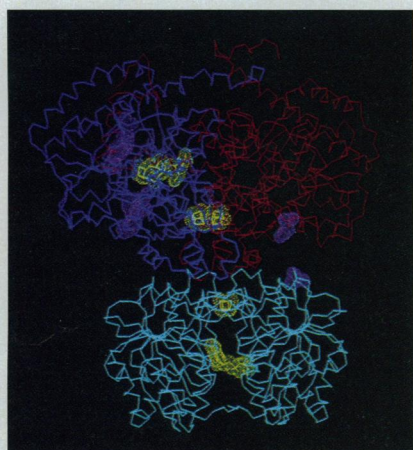
diseases before implanting them into the mother's uterus. In 1992, BABI produced a healthy baby girl who does not carry the gene for cystic fibrosis—even though both her parents do.

Scientists also opened a new diagnostic frontier that may eventually allow fetal testing simply by screening the mother's blood. New techniques can isolate fetal cells from maternal cells in

the bloodstream, and in two cases have identified fetuses with major genetic defects.

Taken together, these developments pave the way for a new kind of medicine, in which patients are diagnosed—and treated—months before they are born.

—Elizabeth Culotta and Daniel E. Koshland Jr.



Nitrogenase unveiled. Two proteins (Fe, bottom, and Mo-Fe, top) work together.



The youngest patients. Fetal medicine forges ahead.