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The Rockefeller University

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SEARCH

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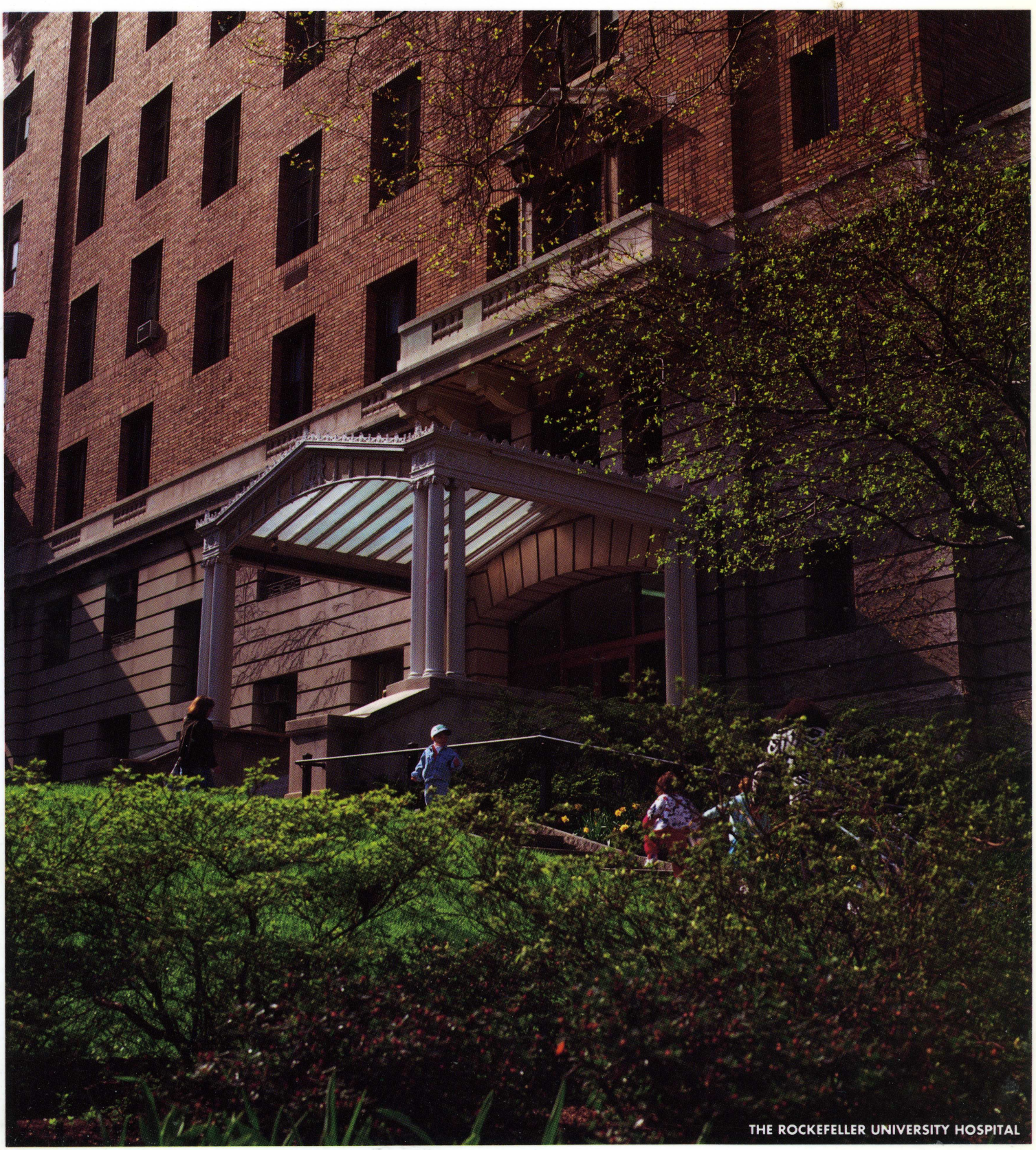

THE ROCKEFELLER UNIVERSITY HOSPITAL

SEARCH

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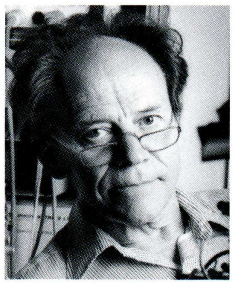
VIEWPOINT

THE ROCKEFELLER HOSPITAL AND THE FUTURE OF CLINICAL RESEARCH

Torsten Wiesel
President, The Rockefeller University



Despite the phenomenal strides in basic biomedical research over the past decade, the emergence of AIDS, antibiotic-resistant tuberculosis, and certain virulent forms of hepatitis has rudely reminded the scientific and health care communities that research at the lab bench is not enough—it also requires observation and attendance at the bedside. Physician/scientists who think about both clinical and basic research often have insights that escape researchers whose attention is confined to one of these domains alone. Combining clinical and basic research can bring about faster application of laboratory ideas and testing of bedside hypotheses, crucial for those who suffer from diseases with near-hopeless prognoses.

Unfortunately, the past several decades have witnessed a decline in medical scientists interested in integrating basic science and medicine. Perhaps because of the enormous power and attraction of molecular biology, few M.D.-Ph.D. students have been able to tear themselves away from their electrophoretic gels and transgenic mice in order to work with human patients. There is, as a consequence, an overwhelming national need for superbly trained physician/scientists as we enter an age of molecular medicine—an era that requires coupling laboratory experimentation with careful bedside attention.

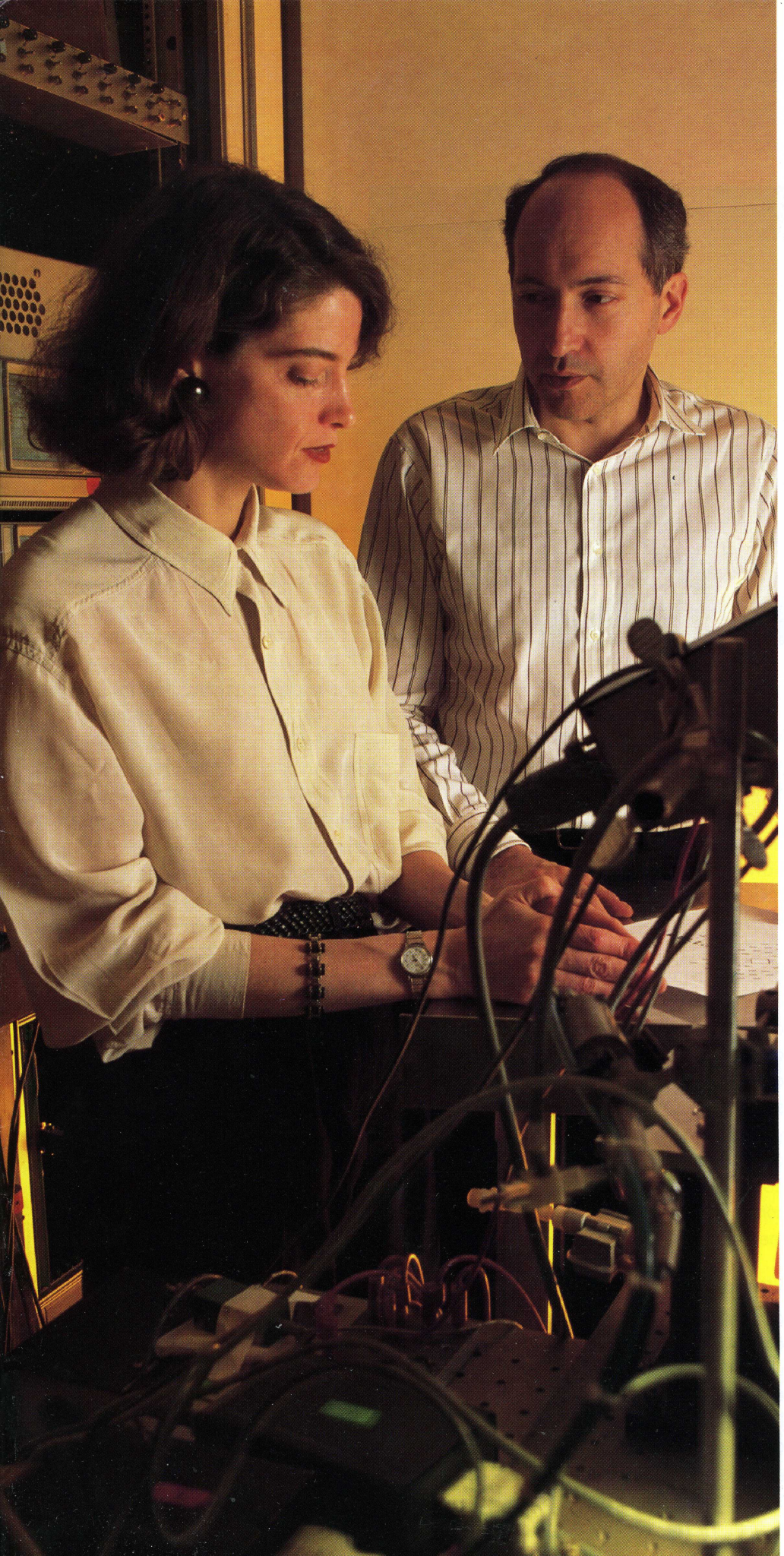
The Rockefeller University, through its hospital, is in a unique position to help fill this need and lead a revitalized approach to biomedical research into the twenty-first century. Since its founding in 1910, the hospital has been a permanent part of the efforts to integrate clinical studies of patients with fundamental biological research. Under the hospital's present leadership of Zanvil A. Cohn, vice president for medical affairs, and Jules Hirsch, physician-in-chief, the university will take full advantage of the opportunities of this new period in medicine.

The founders of Rockefeller wanted a special kind of hospital—a place where new methods would be originated rather than where existing ones would merely be applied. The hospital was the first clinical research center in the United States in which human disease could be studied in a setting of rigorous scientific inquiry. The 30-bed facility served as the model for the 500-bed hospital opened by the National Institutes of Health in Bethesda in the 1950s, as well as for the 74 General Clinical Research Centers that took root across the country. There is no doubt that the Rockefeller hospital has shaped the character of biomedical research in the U.S.

This tradition continues and must be developed further to take full advantage of new and exciting developments in molecular medicine.

- We are establishing new hospital-based labs, headed by junior and senior scientists who will introduce clinical research in fields like the neurosciences and gastrointestinal studies, in addition to existing labs in arteriosclerosis, AIDS, and other areas.
- Our Clinical Scholars Program is being revitalized. We need to train physicians to integrate bedside observations with advanced biomedical research techniques. This program is important because in order to apply scientific discoveries to the patient, we must have skilled doctors familiar with both domains—the laboratory and the clinic.
- Finally, we plan to collaborate in our clinical studies with the hospital of Cornell University Medical College. This is important not only for our own hospital, but also as a way to foster and strengthen our relationship with surrounding institutions.

The future of medicine lies in facilities like our hospital, and the aim of these new programs is to assure that The Rockefeller University will continue to make strong and integrated efforts for the benefit of science, medicine, and patient care.



ROBERT REICHERT

THE DYNAMIC BRAIN

by Geoffrey Montgomery

The six vertical lines in the figure on page five appear tilted. Each of the middle verticals seems to slant away from its left neighbor and toward its right one. Yet, as measurements with a ruler will prove, these six Leaning Towers of Pisa—called the Zollner illusion—are in fact perfectly parallel and straight-standing. That they appear to lean is purely an illusion—but an illusion that, according to Rockefeller neurobiologist Charles Gilbert, may be a manifestation of neural connections in the brain fundamental to perception, memory, and even our ability to recover from nervous system injuries.

ROBERT REICHERT

Charles Gilbert

Since the early 1970s, Gilbert has worked with Rockefeller president Torsten Wiesel, who also heads a laboratory at the university, to define in ever-increasing detail the structure and function of the cells composing primary visual cortex, the credit card–sized region of cells behind the back base of our skulls that is the best understood area of the higher mammalian brain. Primary visual cortex, or V1, serves as the higher brain's "window" for all the information about the visual world before our eyes.

Yet, of course, we have no little green man inside our heads to sit behind the V1 window and analyze the scene before us; the nerve cells that make up the cortical window (or "map") must themselves perform this analysis. Regions of cells within V1 analyze light coming from only a small portion of the visual field, as if the visual scene is tiled into millions of tiny panes, each covering only a small portion of the visual field, called the cell's "receptive field."

Further, cells within visual cortex are activated only by specific kinds of visual stimuli, such as an edge of an object oriented at a vertical angle. All objects are bounded by edges, and the visual cortex first breaks such objects up into a series of short, oriented line segments. Thus, when you gaze at a vertical contour, only a limited population of brain cells whose receptive fields span this area of the visual field fire in a vigorous fashion—specifically, those cells that like vertically oriented lines.

Another important function for the visual system is that all the tiny line segments of which an object is composed must be put together into a unified percept of that object. Visual illusion may reflect the visual system's attempts to perform such perceptual integrations. What the visual illusion of the Zollner figure indicates, however, is that such vertical-sensing cells can be fooled if a vertical bar is surrounded by hatched lines of a slanting orientation. "We know from a long history of perceptual studies," says Gilbert,"that our perception of orientation, as well as color, movement, and other visual attributes, can be influenced by the context in which the attribute is presented." Your teeth appear whiter when you have a suntan, not because of any change in your dentition, but because the context in which your teeth appear—your sunbrowned chin and cheeks—makes the teeth seem whiter by contrast.

While such simple brightness-contrast effects may be mediated by neural connections in the retina, the best candidate for the neural connections underlying the distortions caused by such tilting illusions as the Zollner figure are the long-range horizontal connections that Gilbert and Wiesel discovered in visual cortex in 1977. The axons of the horizontal connections span across wide regions of the cortex, connecting cells with similar orientation preferences, and allowing contextual visual information lying outside a cell's classical receptive field to influence its response to visual stimuli. It was Gilbert and Wiesel's proposal that the horizontal connections may play a modulatory role in visual perception that set the stage for research associate Judith Hirsch's use of a painstaking technique to study these synaptic connections in greater detail.

By surgically removing snippets of mammalian visual cortex, and bathing this cortical slice in a dish containing nerve-supporting solution, Hirsch has been able to place microelectrodes within single cells and selectively activate the horizontal inputs that these impaled neurons receive. "The cortical circuit seen laid out in an anatomical illustration," says Hirsch, "doesn't look like a very flexible structure. But examining the horizontal connections physiologically [by increasing the number of inputs activated or the level of activation of a cell, and electrically recording the consequences] reveals that these connections behave dynamically. Their actions are not static."

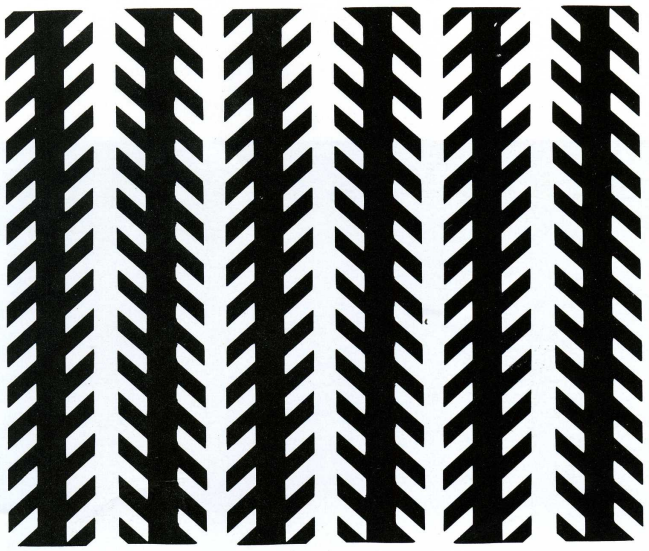
Gilbert and Wiesel's studies have shown that the visual context in which a stimulus appears could exert both immediate and lasting changes on a V1 cell's normal responses. Using the slice preparation, Hirsch has been able to illuminate the events underlying both kinds of changes. She has found that the immediate modulatory effects of the horizontal input is dependent on a cell's state of activation, which is in accordance with earlier findings that horizontal input is recruited by a cell only when the cell is

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

visually stimulated. "It's a tidy way for a nerve cell to decide what to pay attention to," says Hirsch.

Further, within the slice preparation, Hirsch has been able to induce long-term potentiation (LTP) in the horizontal synapse—an increase in synaptic strength that lasts for hours and that, in the hippocampal region of the brain, is believed to underly our ability to store new memories. Both such immediate and lasting changes mediated by horizontal input make sense from a functional standpoint, says Gilbert. "There is a need for plasticity in our processing of information on a moment-to-moment basis, as our glance moves around the room, taking in different visual environments. That's something that has to be updated constantly on a subsecond time scale." The long-term changes induced by Hirsch in the slices may, on the other hand, "be more akin to processes in which information must be acquired and remembered over hours," Gilbert continues.

The most exciting new finding about the horizontal connections, described by Gilbert and Wiesel this past March in *Nature* magazine, concerns far more radical changes in cortical cell function, however. By using a laser to make a small lesion in the retina, Gilbert and Wiesel report they were able to cut off all visual input to a small area of primary visual cortex. "Yet immediately following the lesion," says Gilbert, "the receptive fields of cells at the boundary of this silent area expand tremendously in size"—as if the lesion, by blocking direct visual input from the eyes, has acted to unveil the horizontal inputs from adjoining parts of the cortex, which now play a primary role in constructing the cell's receptive field. Further, Gilbert found that over a period of months, the silent area at the center of the lesion underwent a reawakening—and physiological studies of the reawakened cells' visual sensitivities pointed to the probable role of horizontal connections in this recovery of function. Thus, in addition to the changes mediated by horizontal connections lasting seconds and hours, says Gilbert, "there also seem to be changes which can occur over a much larger time scale, which instead of involving normal perception and memory processes, may be more related to events underlying recovery from a brain injury."



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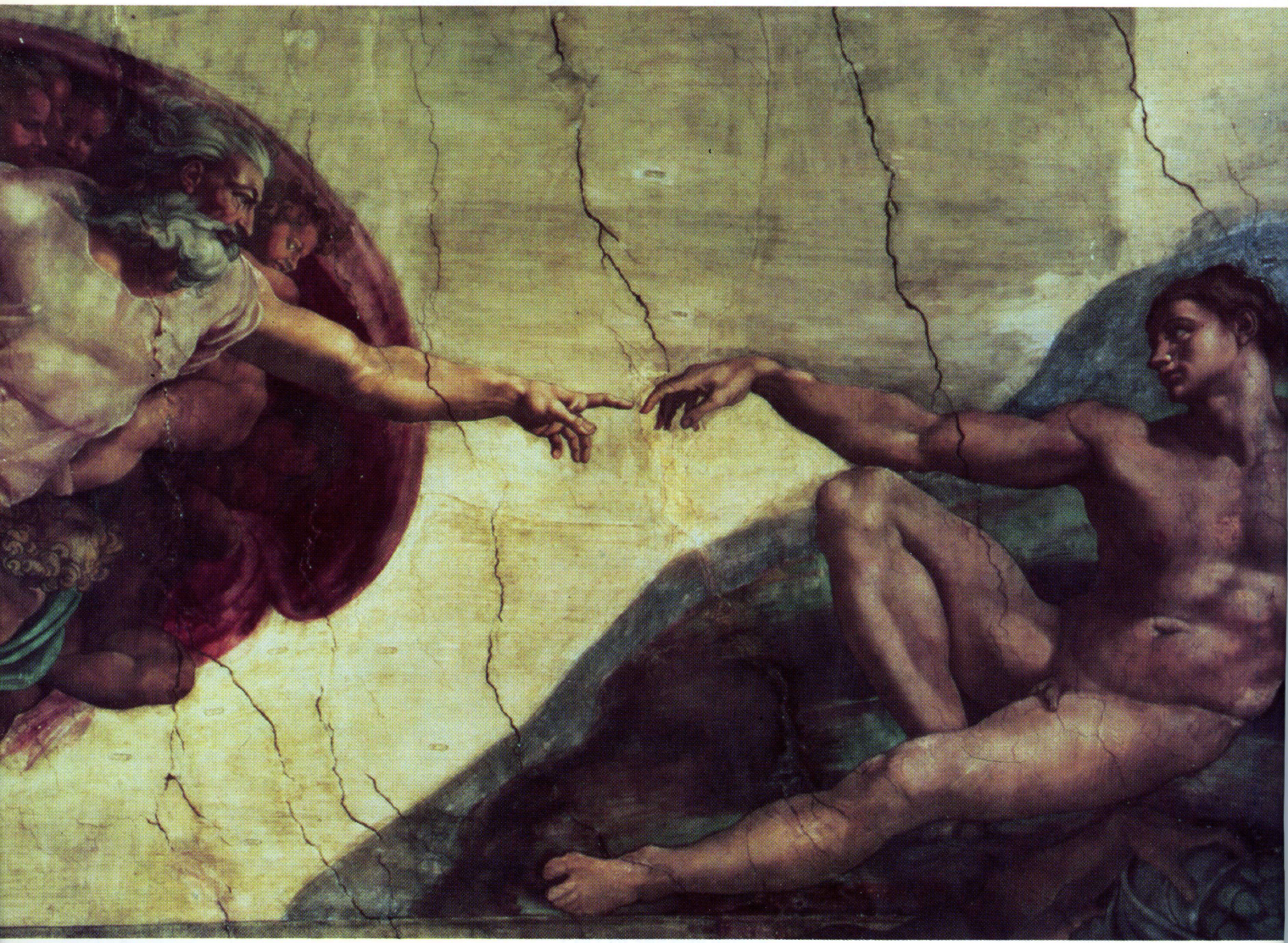
THE SIX VERTICAL LINES IN THE FIGURE ABOVE APPEAR TILTED. EACH OF THE MIDDLE VERTICALS SEEMS TO SLANT AWAY FROM FROM ITS LEFT NEIGHBOR AND TOWARDS ITS RIGHT ONE. YET, AS MEASUREMENTS WITH A RULER WILL PROVE, THESE SIX LEANING TOWERS OF PISA—CALLED THE ZOLLNER ILLUSION—ARE IN FACT PERFECTLY PARALLEL AND STRAIGHT-STANDING. THAT THEY APPEAR TO LEAN IS PURELY AN ILLUSION.

"The question is," says Hirsch, "how do the horizontal connections, which normally serve a predominately modulatory role, now come to act as the principal conveyors of information to these lesioned areas?" One possibility is the synaptic strengthening seen by Hirsch in her tissue slice experiments. A second is the ingrowth of new horizontal nerve fibers to the lesioned area—a possibility now being examined by postdoc Corinna Darian-Smith in the Gilbert lab.

These findings are helping to lead to a sea change in the way scientists think about how the cortex operates. Rather than analyzing the visual scene in an atomistic and fixed fashion, cells bring together information over a much larger area of the visual field, and are capable of dynamic changes in their properties. This capability can serve many functions, says Gilbert, from the integrative needs of object perception, to the learning and recall of visual images, to recovery of function following brain lesions.

HOW DID LIFE BEGIN? THREE VIEWS



Michelangelo's "The Creation of Adam," detail from the Sistine Chapel, the Vatican.

Since the earliest times, humankind has been concerned with our origins—where, when, and how life began. Our own age, like those before us, has spawned an interest in this topic from a range of serious scientists, and, as might be expected, they have come up with a range of (mostly) serious answers. Here are three of the most current views on this weighty topic being discussed in scientific circles, as espoused by Rockefeller scientist Christian de Duve (the "thioester world"), a group of molecular biologists (the "RNA world"), and the biochemist Francis Crick, co-discoverer of the double helix structure of DNA (the "alien" view).

THE SULFUROUS CRADLE OF LIFE

by Matt Clark

To Rockefeller biochemist and cell biologist Christian de Duve, the appearance of life on earth is no accident. It arose through a long series of small steps. And these, given the circumstances that prevailed on our planet four billion years ago, had a fairly high probability of occurring.

As a starting point, de Duve, who won the Nobel Prize in 1974, recalls the widely publicized experiment performed in 1953 by Stanley Miller in the University of Chicago laboratory of another Nobel laureate, Harold C. Urey. Miller put together a gaseous mixture of hydrogen-containing molecules thought to be important for early life; it included ammonia, methane, water, and hydrogen. He then exposed the mixture to a series of electrical discharges, as if to mimic the lightning bolts emerging from the frequent storms thought to occur in the early days of planet Earth.

The result was the formation of a number of organic compounds associated with life, including certain amino acids that are the building blocks of proteins. Some scientists claim that life could not have begun in such a way because most of the hydrogen in the atmosphere at the time would have already boiled away into space as the Earth cooled down.

De Duve calls on experiments of Rockefeller scientists David Mauzerall and Zofia Borowska to explain how an "abiotic world" could have acquired the necessary hydrogen atoms to put together the compounds essential for life. First, there was plenty of water in the primeval seas. Then, de Duve notes, there was a very useful substance, ferrous

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MATT CLARK is a free-lance science writer and former medicine editor of *Newsweek*.

LIFE IN THE RNA WORLD

by Susan Blum

To contemplate life's origin and evolution is to contemplate information transmission from one generation to the next. In cells, information is encoded in genes made up of deoxyribonucleic acid, or DNA. The code is transcribed from DNA into a closely related molecular intermediary known as messenger RNA (mRNA), from which it can then be translated into protein.

Proteins—long chains of amino acids linked together—are the workhorses of the cell. They serve as its architectural elements, as its gatekeepers, and as the enzymes that catalyze its biochemical reactions. And therein lies the crux of the mystery of life's origin. DNA cannot be replicated or translated into protein without enzymes, but those enzymes are themselves proteins, coded for by DNA. Which came first, the molecules that hold the information or the ones that do the work? Even more perplexing, how could one possibly come before the other?

One solution to this conundrum would be a molecule that is both informational and catalytic. Such a molecule is RNA, say proponents of what is known as "the RNA world."

In this world view, RNA preceded both DNA and protein. RNA was able to duplicate itself faithfully enough to survive from one molecular "generation" to the next, but imperfectly enough so that mutations could occur and be selected for when they conferred an advantage. Eventually, RNA also evolved the ability to recombine portions of itself, thus enhancing variation and hastening evolutionary change. The RNA world gave way to the

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

IS THE ORIGIN OF LIFE EXTRATERRESTRIAL?

by John Langone

In the never-ending effort to explain life's mysteries there have been times when reputable scientists seem to have...well...come unglued. Strong in speculation, short on data, they churn out scenarios that, like the classic tabloid and Hollywood approach, go right for the gut instead of the mind.

Consider this. Billions of years ago, an unmanned spaceship loaded with bacteria is launched from a far-distant galaxy by a higher civilization bent on sowing the universe with the seeds of life. Eventually, the sturdy ship finds Earth, and crashes into the warm, primordial ocean. The microorganisms are released, begin to multiply, and, presto, life as we know it on our planet begins.

The conjurer of that vision, as outlandish as it is simple in explaining the origin of life, was no sci-fi writer, but Francis Crick, the British biochemist who, with James Watson, discovered the double helix structure of DNA, for which they won the Nobel Prize. Moreover, the suggestion—dubbed "directed panspermia"—appeared, in 1973, in a space journal, *Icarus*, edited by the astronomer Carl Sagan. (It was later expanded in a book, *Life Itself*.)

How could such a man, who had delved so deeply into the complexities of intertwining chains of genes and helped unravel the code within them, come up with a cockamamy explanation like that? If someone had deliberately scattered the seeds of life throughout the universe, why had not these beings, given their superior intelligence, ever contacted us? More

(CONTINUED ON PAGE 12)

JOHN LANGONE, a science journalist and author, was a staff writer and editor at *Discover* and *Time*.

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THE SULFUROUS CRADLE OF LIFE

iron. With the help of ultraviolet light from the sun, the iron would have oxidized in the water—the process in which rust forms when an iron nail is left out in the rain. This process would have released enough hydrogen atoms into the environment to make the amino acids and other building blocks essential for life as we know it today. Layers of "banded iron" in Pre-Cambrian strata laid down between 1.5 and 3.8 billion years ago in many parts of the world support the notion that iron played this critical role.

The next question is how did the constituents of primitive proteins in Miller's lab make the biochemical trek to something as complicated as RNA, the genetic material essential for the assembly of the proteins that comprise our living world?

De Duve rejects the notion put forward by some scientists that RNA arose spontaneously. Attempts to synthesize RNA in the laboratory under "primitive Earth" conditions have largely failed.

Instead, de Duve proposes a transitional step between the abiotic world of Miller-like chemical reactions and the "RNA world," an expression coined by Harvard scientist and Nobel Prize winner Walter Gilbert. De Duve calls this stage the "thioester world." To a biochemist, "thio" means sulfur, an essential ingredient of all living organisms, where it plays many important roles. "The people who have considered the origins of life tend to neglect the role of sulfur. But in early Earth the waters were full of sulfides—the scenery was like Yellowstone Park and the stench of sulfides was everywhere."

De Duve believes that the abundant early sulfur compounds, thiols, joined with the amino acids and other acids to make thioesters. These thioesters, he says, could have done two things:

First, the linkage of amino acids activated as thioesters led to the creation of "multimers," molecules that acted as primitive catalysts for early metabolism, what de Duve calls "protometabolism."

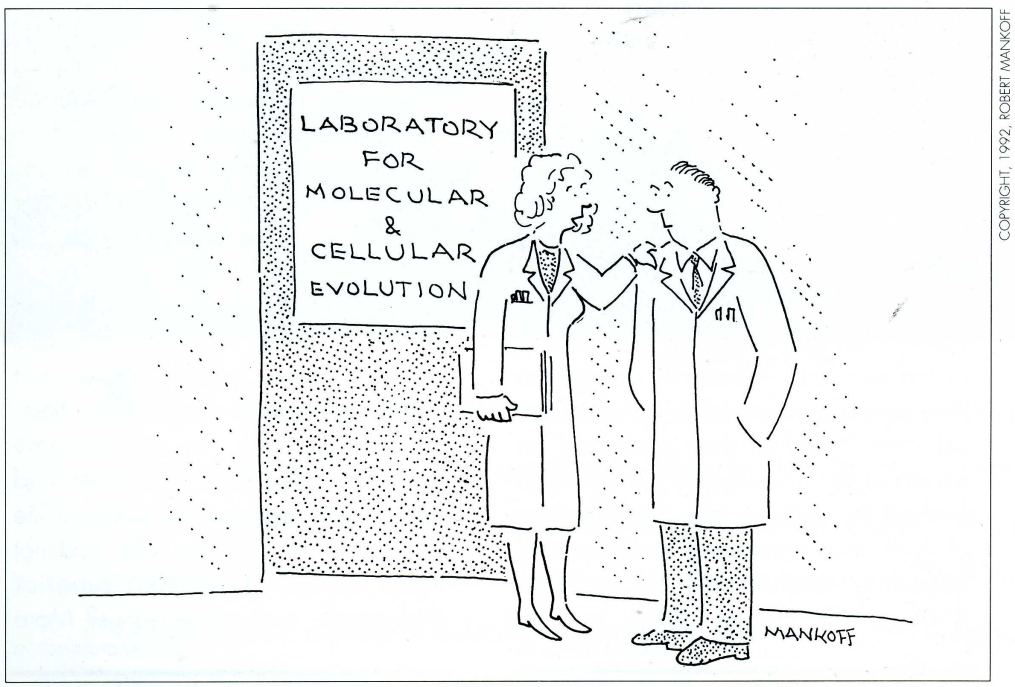
Second, the breaking of chemical bonds in thioesters could have provided the primitive source of energy for this protometabolism. (Today, the phosphorus-containing molecule known as adenosine triphosphate—ATP—is the major source of power for cellular metabolism.)

"With thioesters you can provide, on the one hand, the needed catalysts, and on the other, the needed energy to support a primitive metabolism, which I believe was not very different from what it is today," says de Duve.

According to de Duve, the interactions between the multimer "protoenzymes" and the primitive amino acids and other molecules available in the abiotic world eventually led to the early RNA. "In order to move from Miller's primeval soup to Wally Gilbert's RNA world we need a complex network of reactions catalyzed by multimers," says de Duve. The next question, as he sees it, is how life managed to take over the primitive metabolism first catalyzed by multimers.

"We have the early metabolism catalyzed by multimers that serves to bring about the RNA world," says de Duve. "Then we have the metabolism as it occurs in your cells and in mine today that is catalyzed by new enzymes, more complex proteins made by this new RNA machinery." Did metabolism two grow out of metabolism one, in de Duve's words, "or did life have to reinvent metabolism from scratch"? De Duve rejects the latter hypothesis.

One way in which protometabolism could have generated life as we know it would have involved a controversial



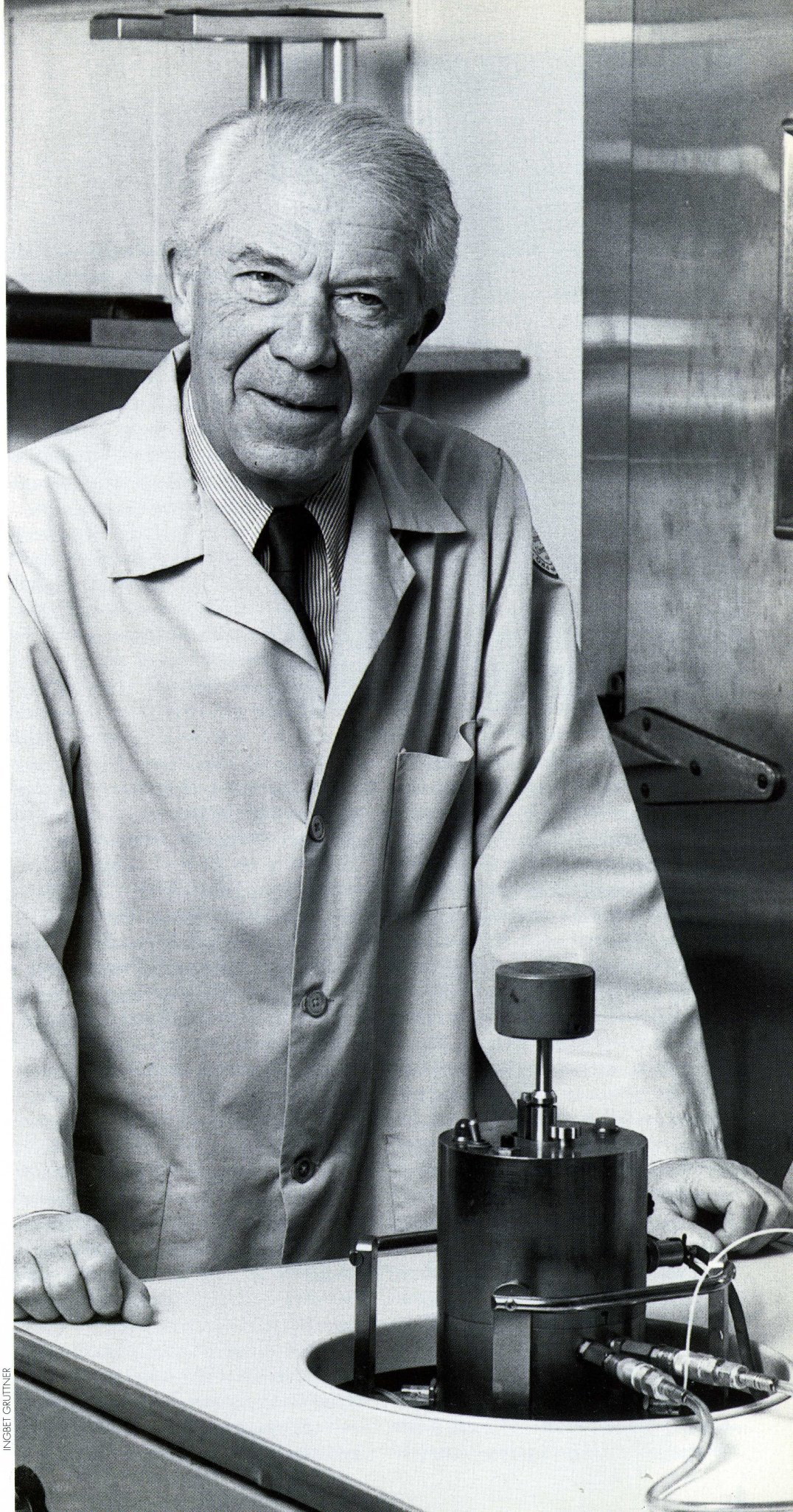
"Look, I'm very fond of you, Edward, but you say 'DNA' and I say 'RNA' so lets call the whole thing off."

process known as "reverse translation." In one scheme, multimers would have produced a primitive RNA; then the RNA would have coded for new proteins. This concept, however, goes against the so-called Central Dogma of Francis Crick (co-discoverer, with James Watson, of the double helix, for which they were awarded the Nobel Prize): that information can pass from DNA to RNA to protein, but once inside a protein, the information "cannot get out again."

Although some critics have suggested as much, de Duve says he doesn't believe in reverse translation (nor does he believe in Central Dogmas).

His model for the further development of life is a system in which the multimers go on doing their job while the new RNA machinery starts making proteins. "This factory is making catalysts—all sorts of peptides, all sorts of proteins," says de Duve. What happened next was a process of Darwinian selection, by which those proteins that turned out to serve a useful function won out over those proteins that had no useful purpose—or, indeed, may actually have been harmful to the system—in the evolution toward new life. "Any protein that happened to do what a multimer used to do would automatically fit into place," he says.

Darwinian selection helped make "protocells," primitive structures that contained tiny RNA genes, and porous membranes with no transport mechanisms, according to de Duve. In time, these evolved into bacterial cells that contained an array of enzymes and coenzymes similar to those of present-day organisms. These early cells would also carry a set of genes made from DNA containing in stable form genetic blueprints of life as we know it.



INGBET GRUTTNER

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LIFE IN THE RNA WORLD

"ribonucleoprotein world" when RNA came to embody the code for proteins and a molecular mechanism evolved for translating that code. Later still, the information encoded in RNA was transcribed "backwards" into the more chemically stable molecule, DNA. The rules of this "DNA world"—in which DNA makes RNA makes protein—have ordered the lives of all cells, from the time of the first putative ancestral cell dubbed the "progenote."

The decade of research between the mid-1950s and the mid-1960s elucidated the details of how DNA's coded information is made manifest in protein. Among the discoveries was RNA's central role in the process of translation. As messenger RNA, the molecule carries encoded information. As transfer RNA, it links that message with its amino acid correlates. And as ribosomal RNA, it serves as a crucial component of the cellular workbenches on which proteins are forged. These discoveries led Francis Crick, Leslie Orgel, and Carl Woese to propose in the late 1960s that RNA preceded protein in evolution; to have done so, they said, RNA must have played a catalytic role. Though intriguing, their speculations languished for lack of information and methods that might put them to the test.

A second wave of thinking about RNA as the first genetic material started to crest in the late 1970s. At that time, studies conducted by James E. Darnell, Jr., now a professor at Rockefeller, and other researchers elsewhere disclosed surprising facts about RNA processing. In eucaryotic cells—cells in which the DNA is sequestered in a nucleus—it turns out that not all the codes transcribed from DNA into RNA wind up being "expressed" as protein. Rather, mRNA is an edited transcript of DNA, from which meaningless intervening sequences, or introns, have been removed. The meaningful mRNA regions—many of which code individually for protein regions, or domains—are then spliced together to embody the message for a final, functional protein. "The discovery that many exons equal protein domains was a revelation about how evolution could have happened," says Darnell, for it showed that RNA chains could have evolved randomly, gradually developing both regions of meaning and a way to excise meaningless messages.

Still, one crucial piece of the puzzle was missing: evidence that RNA could act catalytically. If it could, a scenario could be envisioned in which RNA could self-replicate and self-splice without the help of protein enzymes.

The first such evidence came in 1981. Studying splicing events in a single-celled eucaryote called *Tetrahymena thermophila*, Thomas Cech and his colleagues at the University of Colorado showed that an intron in preribosomal RNA can catalyze the cutting and splicing necessary for its own removal. They named this catalytic RNA a "ribozyme." Two years later, Sidney Altman and his colleagues at Yale showed that RNA could act as a true catalyst—operating on an RNA molecule other than itself, and remaining unchanged in the process. Their studies focused on an unusual enzyme called ribonuclease P. Found in bacteria and eucaryotes, ribonuclease P is composed both of RNA and protein; the scientists found that its catalytic powers reside in its RNA component. In 1986 Cech and his colleagues discovered that a shortened form of the tetrahymena ribozyme also performs as a true catalyst. In 1989, Cech and Altman were awarded a Nobel Prize for their groundbreaking work.

Since those original discoveries, numerous other examples have been found of ribozymes' abilities to self-cut, self-splice, and self-elongate, and evidence is accumulating that they allow RNA to self-replicate, as well. These are capacities that would have been necessary for life to evolve in a proteinless, RNA world. But are ribozymes really up to the job?

Judged on a number of quantitative standards—for instance, the rate of acceleration of chemical reactions, and a feature scientists call "catalytic perfection"—many ribozymes "compete well with your better protein enzymes," Cech says. Qualitatively, he adds "the extant proven examples of ribozymes show a much more limited versatility than one sees in protein enzymes, but the study of ribozymes is a much newer field, and we don't yet know the full extent of their capabilities." Indeed, he reports, current research in his lab and the lab of Harry Noller at the University of California at Santa Cruz indicates that ribozymes are "much more versatile than people might have thought just a few years ago."

Not only can some RNA catalysts match protein's powers, but in at least one respect ancient ribozymes may have even surpassed them. "Proteins aren't as clever as they're cracked up to be. They make and break bonds and transfer chemical groups, but they don't move electrons around," says Yale University's Alan Weiner, a molecular biologist who has written extensively about the RNA world. To accomplish the electron shuttling necessary for biochemical reactions, current-day proteins rely on molecules called cofactors. Weiner is impressed with the observation, first made by the biochemist H. B. White, that "cofactors have an RNA handle on one end, and the other end looks a lot like a nucleotide" —a component of nucleic acids. Thus, cofactors may be what Weiner and his colleague, Nancy Maizels, term a "molecular fossil"—a remnant of a world in which RNA catalysts controlled primitive metabolic events.


ROBERT REICHERT

James E. Darnell, Jr.

Those who entertain the notion of an RNA world do not claim it solves all the mysteries of life's origin. For one thing, there is the question of how RNA came into being: the synthesis of RNA out of the primitive "organic soup" strikes some scientists (RNA-world proponents and opponents alike) as unlikely or even impossible. One alternative scenario proposes that a simpler RNA-like molecule preceded RNA on life's stage. To Darnell, Noller, and Cech, this idea has merit; Weiner is less convinced. "The proposed molecules are so close to RNA that it is hard to tell, given our current understanding of prebiotic chemistry, whether they would be any more likely to arise spontaneously," he says, proclaiming himself "an agnostic" on the question of RNA precursors.

But whatever their notions on what preceded RNA, those who posit an RNA world agree on one thing: there were no reproducible, information-laden proteins. They say that while short strings of amino acids (called peptides) may have arisen randomly, and even been potentially useful, there was simply no way to perpetuate them, and thus no way to perfect them through evolution. "Peptides cannot make directed peptides. Protein as template to produce more protein has never been demonstrated," says Darnell, contrasting this with nucleic acids, which are replicated by means of a complementary DNA or RNA template.

Rockefeller professor emeritus Christian de Duve, who contests the notion of an RNA world, proposes that when small, useful catalytic peptides emerged randomly, their information was stored and perpetuated through the interactions of these protoenzymes with the substrates upon which they acted.

This scenario is impossible, Weiner counters, because it proposes that meaningful peptides were stabilized by what they did. "It's like saying that your hands will be stabilized by hard work," he says. "But in fact, what happens to all tools that are used is that they get worn down.

"If a peptide is doing work—say, cleaving a bond—it's going to be at 'ground zero' for a chemical reaction, right where protons or electrons are being added or taken away," Weiner continues. "So rather than being stabilized by the hard work they're doing, these catalysts would be destabilized. In fact, it would be the unemployed and useless peptides that would be the most stable." In short, he says, de Duve's theory "attempts to make substrate-induced stability of the enzyme replace the ability of genetic information to be stored."

There is another objection to the "protein-first" view of the world. "If you say there were nontemplated primitive proteins that started everything going, then once you have RNA, you have to do it all over again and evolve sequences that code for functional protein," Noller says. "I don't know any way of telling the RNA what sequences are going to give it a terrific catalyst; there's no way, starting with a protein, to go backwards into nucleic acid. It seems to me," he concludes, "that rather than having two implausible scenarios, one is enough."

Healthy skepticism about any origin-of-life theory aside, Noller is encouraged by recent findings emerging from his lab and Cech's. These results may help illuminate one of evolution's deepest mysteries: the development of the system that translates information from the language of nucleic acids into the language of proteins.

During translation, the incipient protein is built up one amino acid after the other on the ribosome, a complex cytoplasmic machine composed of both RNA and protein. The process culminates in the formation of peptide bonds that link each amino acid to its neighbors on the growing protein chain. Despite decades of research, the enzyme that catalyzes peptide bond formation has remained elusive. Now work in the labs of Noller and Cech is closing in on the target.

Researchers in Cech's lab have found that a ribozyme can catalyze reactions at the carbon center of amino acids, and Noller and his colleagues have shown that the equivalent of peptide bond synthesis continues on ribosomes after virtually all their protein components are stripped away. These results indicate that the enzyme that catalyzes peptide bond formation may well be a ribozyme. Should this prove true, says Darnell, it will be the "Holy Grail" for those who believe in the RNA world. For if ribozymes can catalyze reactions not only on RNA but on amino acids as well, then RNA could have done everything necessary to pave the way for life as we know it today.

(CONTINUED FROM PAGE 7)

IS THE ORIGIN OF LIFE EXTRATERRESTRIAL?

important, even if there were such a genesis, how could it shed light on the origin of life? How did the higher civilization, which fired off the encapsulated bacteria, itself evolve? "Crick hypothesized life's origins on some unnamed planet," huffed Sidney Fox, director of the Institute for Molecular and Cellular Evolution at the University of Miami, "partly because he could not show how DNA could have come into being without other DNA."

Still, Crick's notion bears listening to because no one can confidently dismiss it out of hand, given the lack of any firm scientific explanation of how life emerged anywhere. Furthermore, speculation about life's extraterrestrial origins has a long history, one not always driven by kooks and quacks, but by prominent scientists, including one other Nobel laureate. And there is also Crick's own rationale for formulating his theory of directed panspermia, along with his honest admission about it. "Every time I write a paper on the origin of life," he has said, "I swear I will never write another one because there is too much speculation running after too few facts; although I must confess that in spite of this the subject is so fascinating that I never seem to stick to my resolve. The kindest thing to say about directed panspermia is to concede that it is indeed a valid scientific theory, but that as a theory it is premature."

The idea that life on Earth grew out of life somewhere else in the cosmos may have surfaced first with the brilliant British physicist, William Thomson, Lord Kelvin. When he wasn't working out the absolute scale of temperatures that bears his name, or investigating the oscillatory nature of electrical discharges, he was thinking in more popular terms. "The hypothesis that life originated on Earth through moss-grown fragments from the ruins of another world may seem wild and visionary," he told the British Association for the Advancement of Science in 1871. "All I maintain is that it is not unscientific."

Some years later, in 1908, August Svante Arrhenius, a Swedish chemist who won the Nobel Prize for his studies of chemical reactions, tried to give substance to Lord Kelvin's suggestion. Arrhenius proposed that life-bearing planets ejected microorganisms that drifted through space as spores; driven along by radiation from the stars, the spores touched one world after another, including Earth, where they took root to jump-start life. The theory was called "panspermia," for "seeds everywhere."

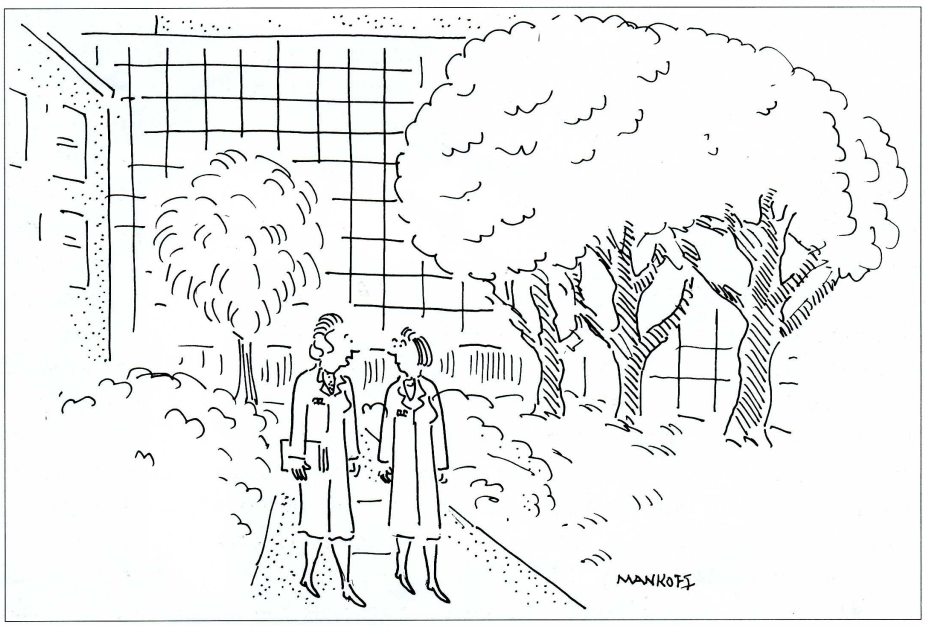
Over the years, several variations on the seed theme emerged, most of them hinging not on full-blown microorganisms wafting their way from outer space, but on chemicals deposited here when all the elements—including some of the key ingredients of life—were created in the cataclysmic Big Bang fifteen to twenty billion years ago. In 1936 the Russian biochemist A. I. Oparin, one of the first to postulate that life had a cosmic origin, theorized that organic compounds, the forerunners of more complex life forms, could have formed easily from primeval raw materials. The turbulent environment of the young Earth was a perfect laboratory, a seething chemical soup, stabbed by lightning, bathed in radiation from the sun, and blasted by volcanic activity. Indeed, an impressive experiment conducted in 1953 by Stanley Miller, then a chemist at the University of Chicago, demonstrated how it might have happened. Miller circulated a mixture of ammonia, methane, hydrogen, and water—components of the Earth's primitive environment—past an electrical discharge; in a week he had produced amino acids and other molecules that are found in living systems today.

But while the elements found in the early atmosphere may have been the precursors of life, they were not life itself, and the idea persisted that perhaps life arrived a little farther along than in a raw chemical state. A few scientists speculated that meteorites carrying organic material—including amino acids and several key ingredients of DNA—had fallen on the primitive Earth, the larger ones exploding on impact. This could have changed the organic matter to carbon monoxide and hydrogen, and later, when the fireball cooled, the gas could have recombined and produced a fresh supply of organic material, the forerunner of life as well as our oil deposits.

But for some scientists this process was still a bit too slow. British astronomer Fred Hoyle was one who would throw his weight behind the old theory of panspermia. Hoyle had coined the term "Big Bang" to differentiate it from his own theory of a "steady-state" universe, the proposition that the universe had no beginning, but has been in its present state indefinitely. Hoyle was also a science fiction writer, and several of his notions about biochemistry smacked of it: interstellar dust that was made up of life-bearing spores and prebiotic molecules, freeze-dried bacteria and algae in stardust, and comets that absorbed biological material and ferried it to earth. Most biologists ridiculed these ideas, arguing that any seed organisms would

be quickly killed off by lethal doses of radiation in space. Critics also maintained that if panspermia were a fact of life, then the moon and planets in our solar system—strongly presumed to be without life—would also have been landing sites for spores. Biologist Lynn Margulis, commenting on one of Hoyle's popular books, *Life Cloud*, said, "The book is flamboyantly irresponsible. Its theme, moreover, is entirely contrary to the considerable opinion of most workers in the field, if origins of life can be considered a field. The book is wanton, amusing, promiscuous fiction."

Crick's theory of directed panspermia—encapsulated spores deliberately sent to Earth by someone—got around the radiation obstacles that the random panspermia of Arrhenius and Hoyle would face. And although the higher civilization notion required, in Crick's own estimation, that one "leave behind quantitative considerations and allow our imagination a somewhat freer hand," the way the microorganisms could grow on Earth was logical. "A fairly typical bacterium, such as *E. coli*, is about one micron wide and two microns long," he wrote. "Thus, a billion of them could be packed into a volume of a few centimeters. They can be frozen alive and most of them will survive when they are eventually unfrozen. In this frozen state they can persist almost indefinitely without any serious loss. At a very low temperature, such as that of space, many of them might survive for well over 10,000 years. They would be almost immune to impact shock and other similar hazards. Best of all, if they fell into a prebiotic ocean they would thrive, especially since many can survive with little or no oxygen. In fact, some bacteria can grow on such a simple medium that almost any prebiotic soup would allow them to survive and multiply rather effectively, provided it is not too cool. Moreover, they do not need to be huddled together. A single bacterium, under favorable circumstances, could infect a whole ocean."



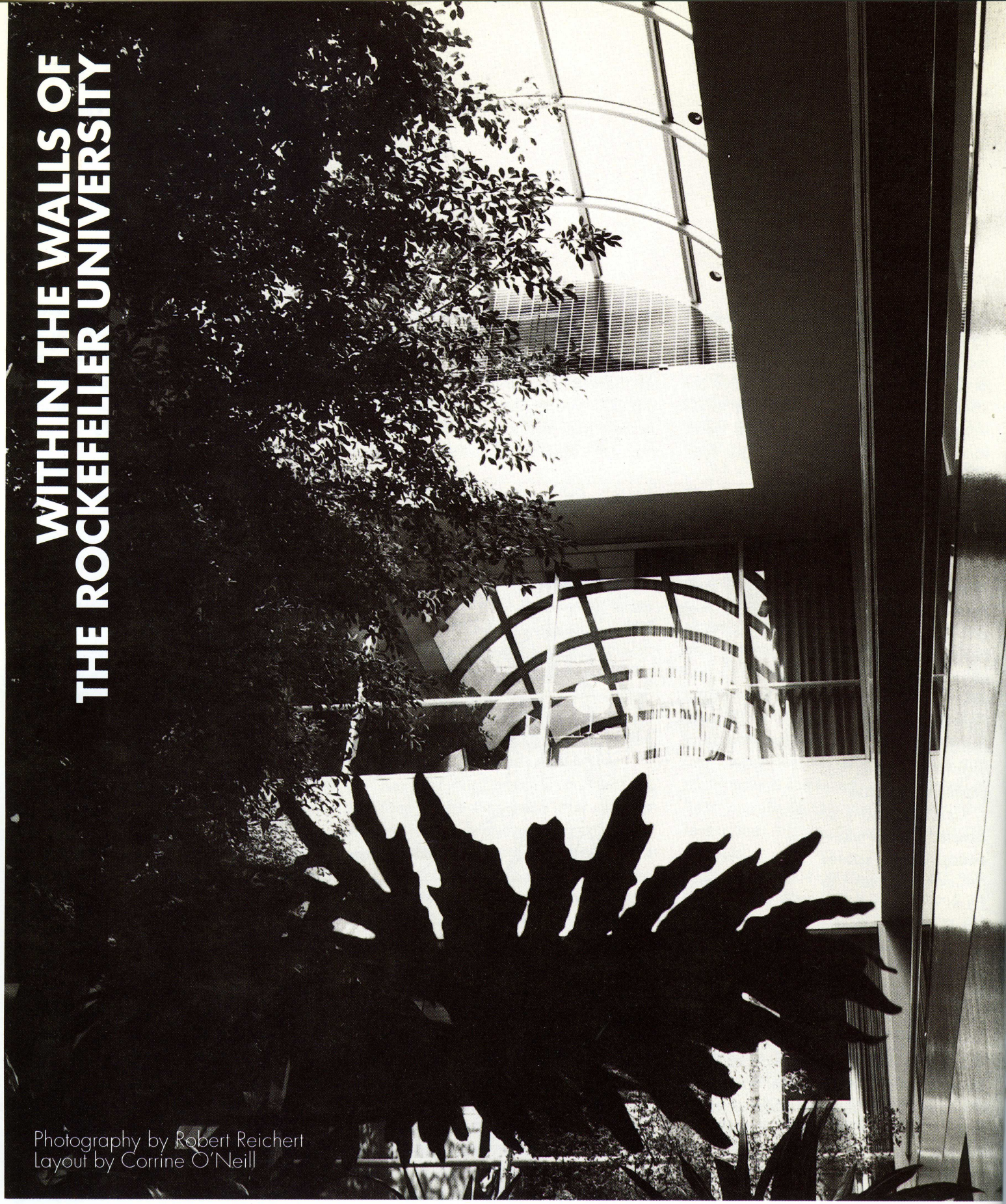
"I don't place much stock in the 'panspermia' explanation of the origin of life. 'Panovia' makes more sense."

Crick maintains that two facts led him (and his partner, California chemist Leslie Orgel), to directed panspermia. One was the uniformity of the genetic code, which suggested that at some stage life had evolved through a "small population bottleneck." The other was that the age of the universe appears to be more than twice the age of Earth, thus allowing time for life to have evolved twice over from simple beginnings to highly complex intelligence.

Still, one gets the impression from Crick's relaxed observations on the subject that he is somewhat skeptical about it all. "The chief difficulty in writing a popular book about the origin of life is that it is mainly a problem in chemistry, mostly organic chemistry," he concluded years after he first proposed the theory. "And almost all laymen dislike chemistry. The object of my book was not to solve the problem of life's origins, but to convey some idea of the many kinds of science involved in the problem, ranging from cosmology and astronomy to biology and chemistry. I myself had a rather detached view of directed panspermia—I still have."

Or, as Crick once told chemist Robert Shapiro of New York University: "We thought of this theory but were not all that sold on it. The object is to give the intelligent person an idea of what the problem is, and this is just a tag to sing it on. Everybody, as they say in California, can relate to certain ideas and things like coming on an unmanned rocket...."

WITHIN THE WALLS OF THE ROCKEFELLER UNIVERSITY



Photography by Robert Reichert
Layout by Corrine O'Neill

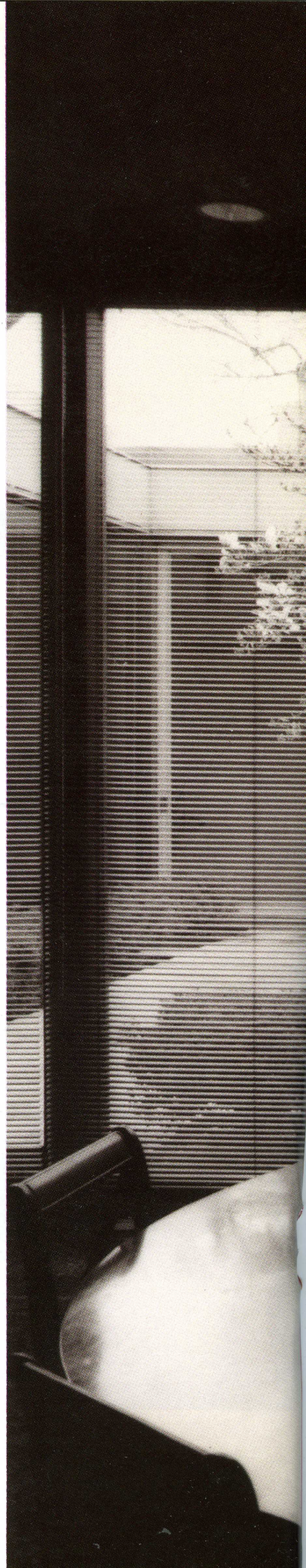
SMITH HALL ANNEX ATRIUM

The vaulted skylight atrium in the Smith Hall Annex features a garden filled with ficus, ferns, and stone outcroppings which can be seen from within the building on three different levels. Originally designed by Coolidge, Shepley, Bulfinch and Abbott architects in 1931 as a facility for laboratory animals, the Annex was restructured into office space in 1984.

Scientific discovery flourishes when researchers can work in surroundings that reflect an institution's professionalism, purpose, and history of achievement. This photofeature, focusing on a few of the interior details of the buildings on campus, provides a glimpse of some of the special features that contribute to the unique character of The Rockefeller University.

DOORFRAME IN WELCH HALL
This hand-carved wooden doorframe adorns the entrance to the Welch Hall Library Periodicals Room, which served as a dining and assembly hall until the library was renovated in 1973. Designed by the architects Coolidge, Shepley, Bulfinch and Abbott, the building, which is attached to the east side of Founder's Hall, was completed in 1929.

SIMON FLEXNER'S DESK
This desk belonged to Simon Flexner, The Rockefeller Institute's first director (1902–1935). It is still situated in the office in Founder's Hall once occupied by Flexner. Today, both the office and the desk are used by David J. Lyons, the university's vice president for business and finance and treasurer. In 1958, when Caspary Hall was built, Rockefeller's third head, Detlev W. Bronk (1953–1968), made Caspary the official site of the president's office, which it remains today.



INNER COURTYARD OF PRESIDENT'S HOUSE
Open to the sky is the glass-enclosed interior courtyard of The Rockefeller University President's House, designed by Harrison and Abramovitz and completed in 1957. The magnolia tree in the center is visible from all sides of the main floor of the house.

ROBERT REICHERT is the photographer for the university's publications. He is an editorial photographer whose work has appeared in *Time, Life, Forbes, Discover,* and other magazines. CORRINE O'NEILL is head of graphic design at the university.

"SO MANY THINGS REMAIN TO BE DONE"

BECOMING PROFESSOR EMERITUS DOESN'T MEAN RETIREMENT FOR BRUCE MERRIFIELD

Text by Bonnie Kaiser
Illustrations by Irving Geis

Editor's note: Bruce Merrifield, the John D. Rockefeller, Jr. Professor at the university, becomes professor emeritus this month, but the energetic scientist has no plans to retire from the laboratory and intends to embark on new paths to discovery as long as the challenges remain. Writer Bonnie Kaiser reflects on the man and his science.

My first impression of Bruce Merrifield dates from a ceremony honoring him in 1970. Presenting the award was Tom Bruice, a chemist at the University of California, Santa Barbara. Tom recalled first meeting Merrifield in the late fifties and being deeply impressed with the scientist, yet highly skeptical that a novel idea of Merrifield's, growing out of his research, would work. The gathering of scientists laughed knowingly in appreciation of Merrifield's triumph. These scientists, who picked future Nobel laureates the way others picked thoroughbred horses, predicted that although Merrifield himself never would have thought it, someday the world would also recognize his triumph.

Recognition did come to Merrifield in the form of the Nobel Prize in Chemistry in 1984 for his novel idea, the development of a simple and ingenious method for synthesizing peptides and proteins, a technology that helps scientists precisely penetrate and manipulate biological molecules. The Nobel-granting body, the Royal Swedish Academy of Sciences, said of his work that it created "completely new possibilities in the field of peptide and protein chemistry, as well as in the field of nucleic acid chemistry where other researchers have applied Merrifield's ideas." It is interesting to note that one continuing grant, Public Health Service Grant DKO1260, "Solid Phase Synthesis of Peptides and Proteins," which was first awarded July 1, 1956, has supported this work.

Merrifield said, however, with characteristic modesty, "Some are dubious when I say I did not ever expect such a thing—but it is surely true. And I still do not know how it happened, but I am grateful. I am also grateful that my wife, Libby, was with me when the news came and could share the whole thing, including the trip to Sweden and the award ceremony." Libby and Bruce have been married forty-three years. She was a fourth-year graduate student in zoology at UCLA when they wed. She spent twenty-five years raising six children and doing some teaching before Bruce drafted her back into research. "It was my wife, Libby, of course, who held everything in the family together," says Merrifield. "It was a sacrifice of certain of her ambitions, but I think she feels amply rewarded—at least I hope so."

INGBET GRUTTNER

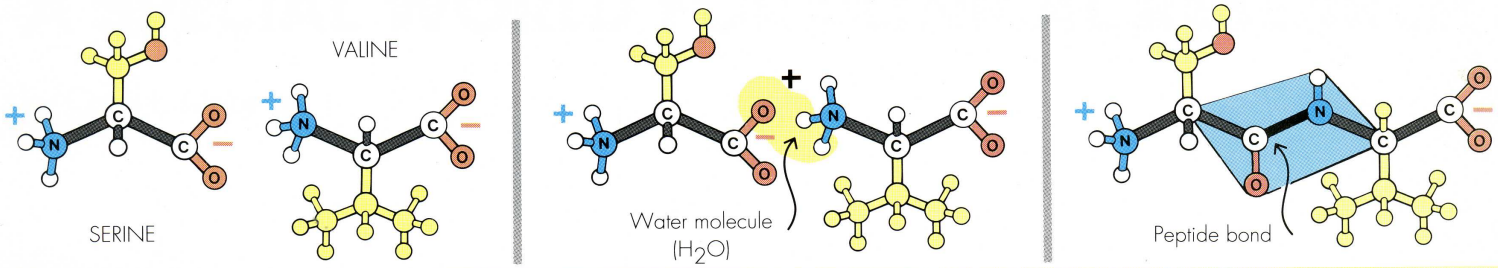
Bruce Merrifield

I have more recent impressions of Merrifield, too. Earlier this year we were in his office. Libby had just finished discussing her latest results, and we were chatting about children and career and choices. Bruce had kindly lent me a draft of his autobiography. Having read that along with recent reprints helped me realize for the first time that he invented solid phase synthesis in order to do what he really wanted to do: study the chemistry and biochemistry of peptides and proteins. For that he needed a reliable method for synthesizing peptides quickly, in high yield, and of high optical and chemical purity. He looked at me as though it were the most obvious thing in the world. But then, anyone who picks up his doctorate one day, as he did, marries the next, and embarks on a romantic cross-country honeymoon drive on the third day in order to accept an invitation to work at The Rockefeller Institute has a very special approach to life.

To help understand the chemical as well as the biochemical synthesis of peptides and proteins, it is instructive to back up to 1943 and Bruce Merrifield's first job. Fresh out of college, he worked as a technician, cleaning cages and feeding rats and chickens. Not much for a UCLA grad with a bachelor's degree in chemistry. Serendipitously, this led to his participation in a significant experiment designed to show the importance of essential amino acids in protein biosynthesis. Proteins, and their shorter counterparts, peptides, are molecules made up of individual units called amino acids. These amino acids are not stored in the body; they are synthesized as needed. However, the "essential" amino acids cannot be synthesized and must

BONNIE KAISER, who holds a doctorate in biochemistry from The University of Chicago, is the science outreach coordinator at The Rockefeller University. She is the widow of Emil Thomas Kaiser, a former professor of bioorganic chemistry and biochemistry at The Rockefeller University, and the daughter-in-law of Emil Kaiser, a scientist in the laboratory of Bruce Merrifield. IRVING GEIS is a molecular artist who has been an illustrator for *Scientific American* for thirty-five years. He is also the coauthor of three textbooks on chemistry and biochemistry.

A LOOK AT THE WORK THAT EARNED BRUCE MERRIFIELD THE NOBEL PRIZE— THE MERRIFIELD METHOD OF SOLID PHASE SYNTHESIS



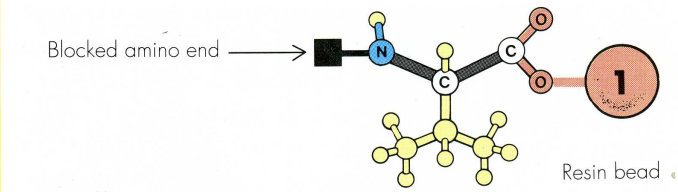
LINKED AMINO ACIDS FORM A PROTEIN (POLYPEPTIDE) CHAIN

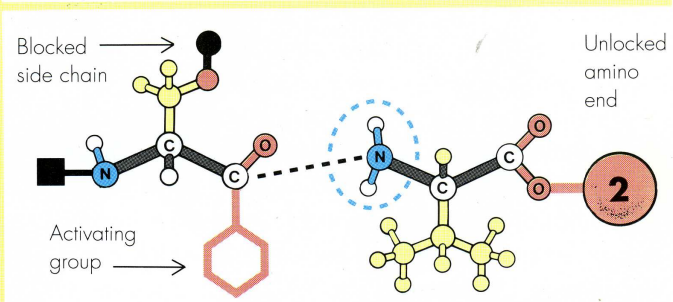
Proteins (also called polypeptides) and peptides (their shorter versions) are built up by linking amino acids together through peptide bond formation.

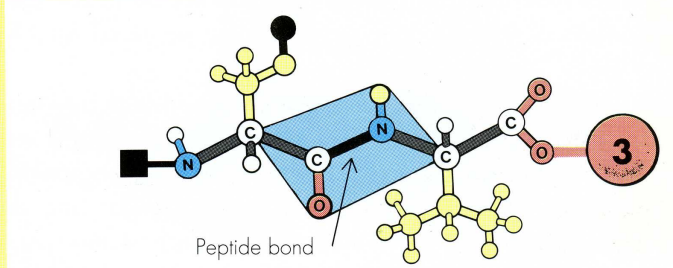
STEPS IN SOLID PHASE PEPTIDE SYNTHESIS

In general, solid phase synthesis begins with insoluble resin beads (the solid phase) which have been prepared to permit chemical bond formation with the carboxyl group of the first amino acid.

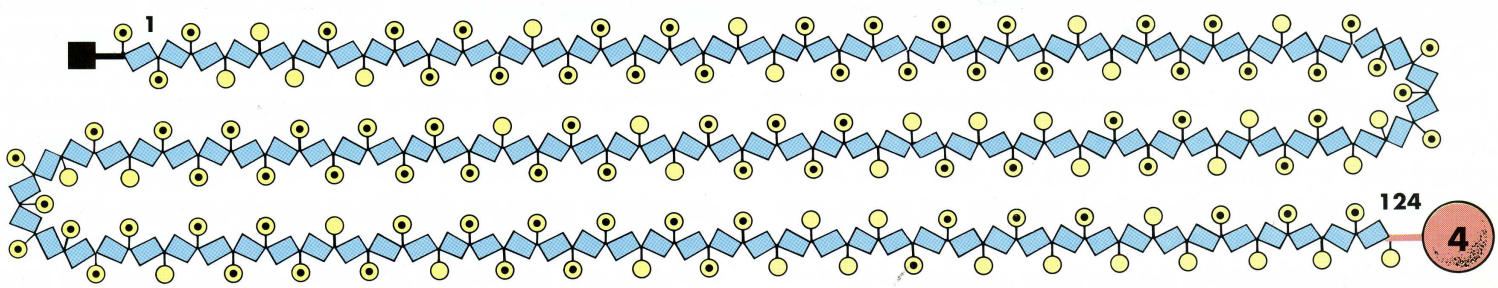
1. In this way, the first amino acid, with its amino acid end blocked, is anchored to the resin bead. Blocking groups on the alpha-amino end, and reactive side chains, prevent unwanted reactions.
2. The alpha-amino group is then unblocked. The product is neutralized and is ready to couple with a second blocked amino acid. The carboxyl group of the second amino acid is activated such that coupling to form a peptide bond will occur.
3. The peptide bond is formed.
4. Steps two and three are repeated until the peptide of desired length is synthesized.





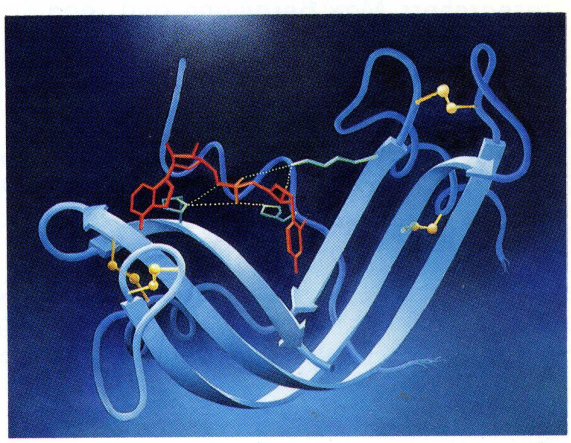


LEGEND
Steps 1-3: Side chain–blocking group (usually a benzyl group)—black circle Amino acid–blocking group (tert-butyloxycarbonyl)—black square Carboxyl-activating group (derived from the dicyclohexyl-carbodiimide coupling agent)— open red hexagon
Step 4: Blocked side chain—open circle with black dot inside
Regular side chain—open circle Amide plane (blue) contains the peptide bond



The protein is cleaved from the resin bead and all blocking side chain groups are removed. Next, the protein is purified and tested for activity.

In 1969, using the automated method he had developed, Bruce Merrifield and his colleague Bernd Gutte made synthetic ribonuclease A, an enzyme composed of 124 amino acid units. This protein was chosen because it was a small and stable enzyme of known amino acid sequence and three-dimensional structure. With the demonstration that the synthetic ribonuclease showed a close chemical and physical resemblance to the native protein, and that it was a true enzyme, the power and vitality of the Merrifield method of solid phase synthesis was established.



RIGHT: *The sequence of amino acids in a polypeptide chain is sufficient to determine the three-dimensional structure of the protein, in this case ribonuclease S, shown in this painting.*

BRUCE MERRIFIELD BECOMES PROFESSOR EMERITUS

come from the diet. The mechanism of protein biosynthesis was not known at that time. Proteins had been thought to arise not from the joining together of individual amino acids, but possibly from reassembly of peptides resulting from partial digestion of dietary proteins. This experiment lent some support to the idea that cells might make proteins using amino acids as the starting material, and, most importantly, showed for the first time that all essential amino acids must be present at the same time for protein synthesis and growth to occur.

Thus, by preparing diets for laboratory animals, he helped show something about processes happening at the molecular level, a level that was still hidden from scientific view. Appreciation for the knowledge one must have in order to design simple yet elegant experiments which could yield rich information led him back to UCLA for doctoral studies with Professor M. S. Dunn, where he made important discoveries in the field of nucleic acid research. He received his Ph.D. in 1949.

After graduate school, Merrifield was invited by Rockefeller scientist D. W. Woolley to join his laboratory and work on finding and characterizing peptide growth factors for bacteria. There was a need to synthesize many peptides and develop variations on them. The institute provided all the inspiration Merrifield required. The halls of Rockefeller imbued him with reminders of great scientific discoveries. The elegant dining room in Welch Hall encouraged fertile lunchtime conversation with his colleagues. And, Merrifield gratefully acknowledges, he was inspired by the proximity of two eminent Rockefeller researchers, future Nobelists William Stein and Stanford Moore, who were using a resin for chromatography along with quantitative assays to quickly and accurately analyze proteins for amino acid content. He could meander into their lab and watch Moore tinker with his analyzer like a kid with his car. Early Rockefeller researchers Max Bergmann and Leonidas Zervas had discovered the first readily removable alpha-amino blocking group (the carbobenzoxy group) which was used as standard practice in peptide synthesis. And then there was the brilliant Wayne Woolley, himself. Merrifield's earliest recollection of him is of a thirty-five-year-old researcher, blind as a result of juvenile diabetes, carefully and expertly preparing diets for his laboratory animals. The discovery and commercialization of the peptide hormone insulin had come in time to save Woolley's life, yet it could not prevent his eventual blindness. Having Woolley as a mentor to provide intellectual guidance and encouragement was very important to Merrifield. "It would be very difficult for most young investigators nowadays to work at that level of involvement without a mentor," he says.

The work Merrifield was doing on peptide growth factors relied on an effective yet time-consuming and difficult chemical synthesis. Merrifield realized that synthesizing the many peptides required for his study would be easier and faster if there were a new method of synthesis, so he developed one—chemical synthesis on a solid matrix, a totally novel technology. Many labs raced to use the Merrifield method, though not all were successful. It appeared so deceptively simple: put the amino acids, reagents, and solvents into their containers; toss some resin beads into a little glass reaction vessel with a glass filter at the bottom; turn on the automatic synthesizer; go out for the day; cleave the finished peptide off the beads; and submit results for publication by nightfall. It is not quite that simple. Consider the problem of overall yield. Each step in a synthesis must be checked for completion using very sensitive reagents and most steps can be controlled to give 99.9 percent yield. Taking care to control and check each step can give a product in very high yield and of high purity. The remainder is a mixture of peptides of various shorter lengths which have to be separated out. Much of the work that continues in methodology is to increase overall yield and purity.

As Merrifield becomes professor emeritus this month, culminating a career at Rockefeller that spans forty-three years, he is looking ahead, not back. He continues the wonderful tradition of lunching with his group. Spontaneous discussions begin over the latest news—whether it is destined to appear in *The Journal of Biological Chemistry* or *The Wall Street Journal*. The scientific talk is just as fertile as ever; for example, the group is expecting a visiting graduate student to arrive from abroad and they are considering a project for him. Merrifield asks Senior Research Associate Cecille Unson-O'Brien whether the visitor can work with her on the amino acids on either side of the nine-position of the glucagon antagonist—studies that may hold promise for diabetes sufferers. It is agreed, and what began nearly half a century ago when two young researchers coaxed their jalopy from California to New York comes full circle. Even at the beginning of his sixth decade of scientific work, Merrifield is seeking new challenges. Retirement, as most people think of it, is not in the picture. As he states in his autobiography, "...now it is time to return to reality and resume my research because so many things remain to be done."

THE ROCKEFELLER UNIVERSITY HOSPITAL: A SPECIAL WORLD FOR CLINICAL RESEARCH

by Carol L. Moberg

Charles Dickens evoked the clinical and social complexities of disease when he called tuberculosis "a disease which medicine never cured, wealth never warded off." Over a century later, tuberculosis, which was thought to have been brought under control, threatens to become epidemic. Today, in The Rockefeller University Hospital, tuberculosis is just one of many diseases under fresh and intense scrutiny. In fact, the hospital may well be unique in the United States for studying tuberculosis in both a laboratory and clinical setting.

These tuberculosis studies are part of an invigorated clinical research program at the hospital. Recently, Rockefeller moved beyond the debate over the cost and complexity of maintaining this difficult type of biomedical research. Instead of debate, several efforts are in place to mobilize the next era of scientific medicine and to mentor the next generation of clinical researchers.

ROBERT REICHERT

A young patient with epidermolysis bullosa (EB) receives daily care from D. Martin Carter and Dorothea Caldwell-Brown. This lifelong, inherited blistering disorder of the skin provides substantial opportunities for clinical studies. One result has been to harvest in the laboratory skin cells that successfully cover disfiguring wounds.

In February, the university's board of trustees elected two senior physician-scientists to head Rockefeller's thirty-bed hospital and its scientific programs. Zanvil A. Cohn was appointed vice president for medical affairs, and Jules Hirsch was named physician-in-chief. In reaffirming the university's commitment to the hospital, President Torsten Wiesel said, "These distinguished professors will head a major effort to rebuild and strengthen the hospital, from the standpoint of both patient care and scientific discovery."

A MISSION IN CLINICAL RESEARCH

Eighty-two years ago, the Rockefeller hospital led a revolution in medicine with its novel mission to study disease as it actually appears in human beings under conditions favorable to treatment and to scientific investigation. There was no precedent in the United States for a research hospital staffed by full-time physician-scientists to study disease at the bedside and in their own laboratories. Even today, the only patients admitted are those selected for specific diseases under study, and there is no charge for their care.

Rufus Cole, Rockefeller's first physician-in-chief (1909–1937), fostered this mission. "It is through the observation of patients," he believed, "that clues are obtained as to the proper direction" of laboratory experiments. At the hospital's seventy-fifth anniversary, in 1985, Rockefeller's president at the time, Joshua Lederberg, observed that disease presents bolder experiments on humans than scientists would ever dare perform. Clinical investigations, he said, "are an absolutely indispensable part of

CAROL L. MOBERG, research associate at Rockefeller, is co editor with Zanvil A Cohn of *Launching the Antibiotic Era* (The Rockefeller University Press, 1990). Her current projects include writing an annotated bibliography of the works of René Dubos.

GIRAUDON/ART RESOURCE, NY

Treatment of tuberculosis at the Hôpital Saint-Louis in Paris, by E. Loëvy, 1890. Most of what physicians learned a century ago about diagnosing and treating tuberculosis is no longer applicable.

the way we develop our fundamental knowledge about the biology of the human organism." When a hypothesis is checked against the entire fabric of disease, Lederberg added, "it is nature that sets the stage and not the confines of preconceived theoretical notions."

Historically, observations by Rockefeller scientists about hospital patients have led to a clearer understanding of life processes. For example, Oswald Avery's search for bacterial virulence in pneumonia patients led to the demonstration in 1944 that DNA is the genetic material and resulted in a revolution in biology. Similarly, the work of Maclyn McCarty (the hospital's physician-in-chief from 1960 to 1974) and Rebecca Lancefield on rheumatic fever, and Henry G. Kunkel on chronic liver disease, led to new developments in clinical immunology. In still another area, Donald Van Slyke's studies of pulmonary and kidney failure resulted in the invention of many of today's most important diagnostic tools of biochemistry.

CURRENT HOSPITAL-BASED RESEARCH

Rockefeller's role in clinical investigation links what many believe is an expanding gap between basic science and applied medicine. A recent case study demonstrates how an unprecedented degree of thoroughness in observing a few patients leads to help for many.

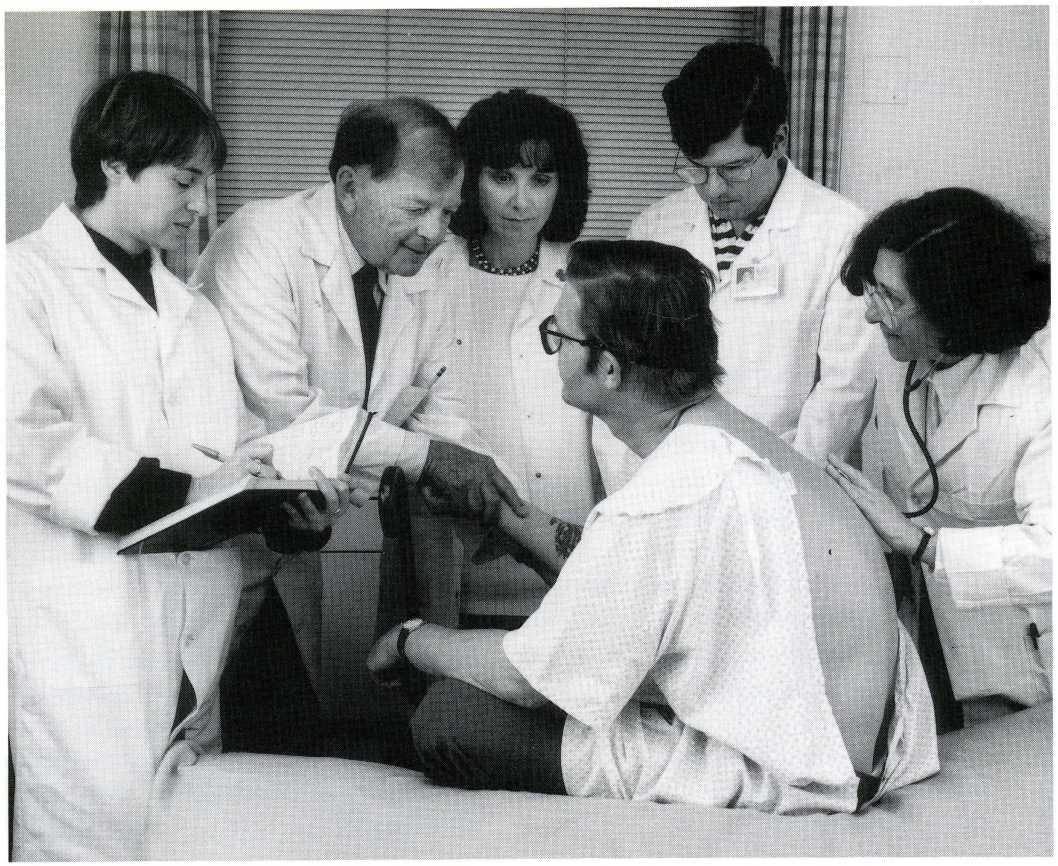
A Rockefeller physician observed that an ordinary skin test for tuberculosis in leprosy patients caused the surface layer of skin to thicken. In the laboratory, tests determined which immune cells and lymphokines (products secreted by these cells) were stimulating skin growth. After leprosy patients were given local inoculations of natural, growth-promoting factors that had been identified, there was a rapid body-wide reduction of leprosy bacilli and noticeable physical improvement. As a result, other experimental therapies to bolster the body's own defenses are currently being expanded to patients with tuberculosis, AIDS, psoriasis, and chronic wounds. These studies by young faculty members Alice Gottlieb, James Krueger, and Gilla Kaplan, who work with senior physician-scientists Zanvil Cohn, Ralph Steinman, and D. Martin Carter, carry on Rockefeller's tradition of patient-oriented research.

Just as research interests of the university have broadened over the years, so, too, have clinical investigations in the hospital acquired new dimensions. Disorders under study today are approached using modern sciences of immunology, metabolism, genetics, nutritional pharmacology, and behavior. Some current works in progress fall into various stages in the research process.

Long-term comprehensive studies continue at the hospital on obesity and atherosclerosis, as researchers examine multiple links in the disorders. The laboratory of Jules Hirsch looks at how obesity develops and why it leads to diabetes, hypertension, and heart disease. The patient-oriented studies on eating behavior and weight control, composition of fat tissue, and the control of appetite by chemicals in the brain are now leading to a search for obesity genes in animal models.

The laboratory of Jan Breslow (the hospital's physician-in-chief from 1991 to 1992) looks at both genetic and environmental influences of atherosclerosis, in which fat-rich cells form plaques that eventually clog arteries and lead to heart and cerebrovascular disease. The group has examined some seventeen genes responsible for this plaque and also conducts dietary studies to determine the interaction of fats, exercise, and drugs to prevent and control the disease.

Other studies characterize mechanisms of disease. Problems of addiction are of long-standing interest at Rockefeller where Vincent Dole conceived the methadone maintenance program. Now, Mary Jeanne Kreek's lab is finding biochemical bases of behavior in opiate and cocaine addicts by studying receptors



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Today, another plague of tuberculosis threatens. Clinical research in the Cohn-Steinman laboratory focuses on the basic interactions of the tubercle bacillus with the body's immune cells.

and neuropeptide gene expression in areas of the brain involved in perpetuating addiction and in hormone release in reproduction and stress response.

Still other studies focus on the pathogenesis of a disease. The Cohn-Steinman laboratory recently identified an early step of the immune system response to the HIV virus that causes T cell lymphocyte depletion in patients with AIDS. Now the group is monitoring patients who demonstrate the flu-like symptoms that accompany the conversion from HIV negative to HIV positive.

Another type of research is producing new therapies to prevent or treat disease. The Carter laboratory has refined a technique for growing in culture skin cells that, when returned to patients, heal mutilating wounds. Attallah Kappas (physician-in-chief of the hospital from 1974 to 1991) and his laboratory have designed a safe, inexpensive drug that prevents severe jaundice in premature babies and treats newborns in developing countries who are at risk for brain damage.

Rockefeller scientist Elaine Tuomanen has developed a vaccine against whooping cough that prevents bacteria from adhering to walls lining the respiratory tract, and an antibody therapy against meningitis (developed with Rockefeller researcher Samuel Wright) that eliminates the destructive effects of the inflammation in the brain.

As early Rockefeller pathologist Theobald Smith has written, clinical research works at "the undefined boundaries and frayed edges of scientific medicine."

NEW INITIATIVES

In planning for its future, the leaders of the university's hospital have set ambitious goals. According to Hirsch, one goal is to study "the many new diseases brought on by the plague of disorders related to behavior, life-styles, and environmental pollutants—addiction, nutritional disorders, violence, depression (apathy), and the inability to work and live creatively." In addition, new heads of laboratory and new independent researchers are being sought to introduce clinical studies in neuroimmunology, autoimmune disorders, diabetes, and Alzheimer's disease. Another goal is translating molecular biology from the laboratory bench back to the patient.

An immediate task, according to Cohn, is the revitalization of the Clinical Scholars Program. "Diseases are multifactorial, complex situations, from which we get only hints," he says. "We need to prepare minds to recognize which clues to follow in order to reach the larger principles of human biology and disease mechanisms."

The Clinical Scholars Program is now recruiting a young generation of physician-scientists for a three-to-five-year apprenticeship in the hospital. Full financial support, including salary and laboratory facilities, will come from new endowments. Active searches are in progress for physicians completing their residency and for new recipients of M.D.-Ph.D. degrees who want to consider careers in clinical investigation. Clinical Scholars will be faculty members and full colleagues in the Rockefeller community of scientists. In the fall, Cohn intends to launch several informal groups of mentors and Clinical Scholars in which to share ideas and experiences.

With major new commitments to preserve the hospital's unique tradition of clinical research, its leaders are preparing to continue addressing human disease in the coming era of molecular medicine. According to Hirsch, "The special nature of human disease calls out for intensive clinical investigation to bring the latest tools of biomedicine to bear on illness. We also want to demonstrate again how curiosity, questioning, and careful investigation at the bedside can generate a new set of basic sciences for the complex illnesses that will be of central concern to medicine in the next century."

FROM ROCKEFELLER'S PAST

ENDURING IDEALS

SIMON FLEXNER AND THE EARLY YEARS OF THE ROCKEFELLER INSTITUTE

by Robert Applebaum

ROCKEFELLER UNIVERSITY ARCHIVES

Simon Flexner

In 1901 The Rockefeller Institute for Medical Research was founded by John D. Rockefeller (shortly after his first grandson died of scarlet fever) as a place where scientific investigation into infectious diseases could be combined with clinical care and study of patients with these ailments. A year later, Simon Flexner became its first director.

The new institute soon opened its first laboratories under the stewardship of Flexner, who plotted its course for three formative decades as it matured into a major presence on the frontiers of medical inquiry, combining patient-oriented care and research with investigations at the laboratory bench. Not only did The Rockefeller Institute rapidly attain a stature on par with the Pasteur Institute in Paris and Berlin's Koch Institute for Infectious Diseases—Europe's premier research facilities whose traditions Flexner modified to a particularly Ameri-can form—but it also inspired the creation of other investigative institutions through-out the United States and became a training ground for a corps of physicians and scientists staffing those fledgling research departments.

Indeed, many of the philosophic tenets that Flexner brought to bear at The Rockefeller Institute—above all, those that gave free reign to scientists and fostered individual creativity—still define the nature of the modern-day Rockefeller University, which assumed its present name in 1965 after expanding several years earlier in 1954 to include graduate education.

"Flexner's legacy continues to be important," says Maclyn McCarty, a professor emeritus at the university. "Flexner had a seminal role from the outset and set a successful pattern here. He was farseeing. He had vision."

Peyton Rous, a scientific colleague of Flexner's at the institute, was also struck by his ability to sense the shape of things yet to unfold. Simon Flexner "had vision of the sharpest, seeing the future implicit in the present; and he acted upon what he saw," wrote Rous in a 1949 profile of Flexner's life for the Royal Society of London. "From youth, as if with foreknowledge, he made himself ready for the needs of a coming time, moulding himself for its purposes, almost in detail."

The son of an itinerant peddler, Simon Flexner received a rudimentary education at the University of Louisville Medical School before moving on to the Johns Hopkins University in 1890 to study under William Henry Welch, whom Rous credits with having brought "modern scientific medicine" to America. Within two years, Flexner was named associate professor and became Welch's first assistant, spending the remainder of the decade immersed in the study of pathology, bacteriology, immunology, and related fields both in Baltimore and in laboratories throughout Europe. He also headed a Hopkins commission to study diseases in the Philippines, afterwards accepting an appointment as professor of pathology at the University of Pennsylvania and subsequently becoming the director of The Rockefeller Institute.

Trained during an era in which the relationship between infectious diseases and illness was just becoming apparent, Flexner was a member of an elite group of American pathologists, headed by William Welch, whose agenda called for proving that "pathology was no mere addendum to the clinic but a dynamic science in its own right," according to Rous.

In the view of Daniel Fox, a historian and president of the Milbank Memorial Fund, a New York–based philanthropic organization, Welch was the "American guru" for a new system of medical inquiry and belief that espoused an educational model designed to disseminate knowledge from the laboratory to medical practitioners in the field. A willing convert, young Simon Flexner gained quick entrance to Welch's inner circle, Fox says, and became a "true believer" well before the founding of The Rockefeller Institute.

"For four thousand years there was fundamentally no method in medicine that was guaranteed to lead to better health," Fox explains. "In the last four decades of the nineteenth century the method is there: Knowledge comes from the principles of physiology and cellular pathology and the study of bacteria as a causal factor in disease. Knowledge comes from the lab bench [as well as] from looking at patients. And once that knowledge comes from the lab, it must be tested in a

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teaching hospital. Finally, the diffusion of knowledge comes through a modern medical school. It is a pyramid determining the image of health care in the twentieth century."

As The Rockefeller Institute took shape under Flexner's guidance and with Welch heading its scientific advisory board, it became the ideal vehicle for spreading the newfound gospel, Fox says, embodying the doctrine of the medical vanguard in its "pure, distilled form." With its labs and research hospital, and its role in preparing scientists to fill posts throughout the nation, the institute was "headquarters for the theory and distribution" of a modern scientific system of medicine in the United States.

Though Flexner modeled The Rockefeller Institute after the great European laboratories, American notions of pluralism and egalitarianism were woven into the underlying fabric of the research facility. In place of rigidly defined departments that could stagnate over time, Flexner built the institute around relatively easy-to-set-up, and equally easy-to-disband lab groups, each headed by a senior scientist. Inherent in this structure was a conscious desire to depart from the European custom of organizing research institutes around one remarkable man—such as the Pasteur, Koch, and Ehrlich Institutes. In Flexner's words, stated in a biography of William Welch coauthored with his son: "Thus [The Rockefeller Institute] was not confined in its growth by the interests, however great, of a commanding [scientific] personality; it could look forward to a broader foundation of science. Furthermore, its usefulness could not be so seriously impaired by the death or retirement of one man."

Innovative for the early 1900s, this framework has endured the century, as has Flexner's custom of encouraging lab heads to pursue their own lines of research rather than imposing an agenda from the top. Flexner "believed that scientists who had a record of discovery might be argued with, but would have to be backed, if they insisted, in projects of which he disapproved," writes his son, James Thomas Flexner, in *An American Saga*.

This Flexner-inspired practice of "looking for the best people, generously funding their work, and leaving them alone" while "trusting all will come to good," says David Lyons, Rockefeller University treasurer and vice president for business and finance, "has been part of our culture from the beginning and hasn't changed."

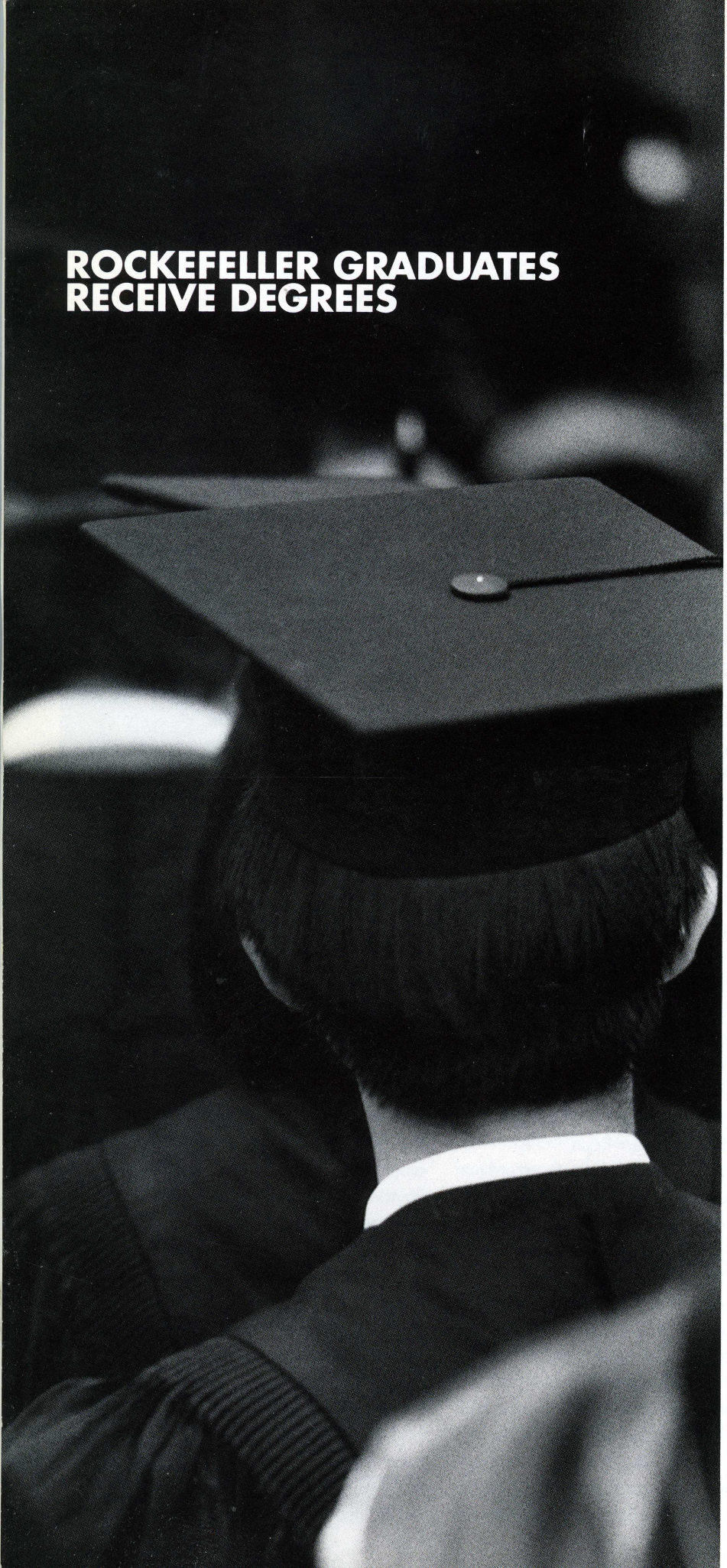
Since the institute's inception, creating a nurturing environment for research and discovery has also meant little pressure to watch the clock or concern with the oft-invoked maxim of "publish or perish." The effect of this philosophy, Peyton Rous used to point out, is that "a person at [Rockefeller] has no reason to do anything trivial," recalls former university president Frederick Seitz. Concerning Flexner's libertarianism Rous writes: "He looked upon individuality as the mainspring of enterprise in thought as in all else. Individuality throve on freedom; he would give it to all who could stand it."

For university scientist Bruce Merrifield this freedom has proven invaluable. "Three years without publication would be deadly somewhere else, but it can be done under our system," notes the Nobel laureate, referring to a lengthy period during which he struggled to test and develop his innovative ideas for the chemical synthesis of peptides and proteins. "The lab arrangement here functions as a protective shield, and the leeway it provides made all the difference in my work. At Rockefeller the labs are organized and headed by individuals whose only duty is to do their research. Within reason, there is no interference."

At the time of Simon Flexner's retirement in 1935, more than 150 Rockefeller-trained veterans had gone on to become professors in scores of American universities. "It was crucial for a young research-oriented doctor or Ph.D. to spend two or three years at The Rockefeller Institute as part of his or her basic training," explains Darwin Stapleton, director of The Rockefeller Archive Center, about an era that lasted from the teens through the 1930s. "Flexner was one of the great talent scouts. He learned who was up and coming, and invited promising people to join the institute. It was understood they would come for a few years and move on. There were only a limited number of permanent positions."

Much of the early success of The Rockefeller Institute is due to the leadership of Simon Flexner. "During the fifty years of his personal effort medicine emerged into the sharp light of science," Peyton Rous comments. Flexner "helped this happen, and he did vastly more. He revealed the existence in the unconsidered human commonality of latent abilities to discover, and he showed that these could be called forth by fostering individual initiative and giving it scope. The planners of The Rockefeller Institute had thought of it as a purposeful utilization of human strength; but they had not known how to come at the strength, much less how to bring it to bear. Flexner did both."

ROCKEFELLER GRADUATES RECEIVE DEGREES



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Fourteen graduate fellows and seven biomedical fellows are being awarded Ph.D. degrees in The Rockefeller University's thiry-fourth graduation ceremony, Thursday, June 18, 1992. The graduates completed their doctoral research in many different laboratories on campus.

The graduation ceremony itself takes place in Caspary Auditorium. The degrees are conferred by university president Torsten Wiesel, after each student's adviser speaks about the laboratory research that student has completed. Preceding the ceremony is a luncheon for students, their advisers, family, and guests, hosted by President Wiesel and attended also by the university's deans and trustees. A reception follows the ceremony.

This year's degree recipients include graduate fellows Nathan Bahary (advisers: Jeffrey Friedman and Rudolph L. Leibel), Mary Baylies (Michael Young), Robert Camp (Ellen Puré), Lisa Croner (Ehud Kaplan), Elizabeth de Beus (Günter Blobel), Ioannis Giannakis (Mark Evans), Ran Jia (Hidesaburo Hanafusa), Robert Kovelman (Robert G. Roeder), Kent Nastiuk (David Clayton), Marina Picciotto (Paul Greengard), Anne Louise Prieto Cruse (Kathryn L. Crossin and Gerald M. Edelman), Rafael Yuste Rojas (Torsten Wiesel and Lawrence Katz, Duke University), and Yuhang Zhao (Hidesaburo Hanafusa).

Biomedical fellows receiving degrees are Mary Burrous (Peter MacLeish), David Edwards (Ehud Kaplan), Anne Hermanowski-Vosatka (Samuel Wright), Jeffrey Krane (D. Martin Carter and James Krueger), Ruta Nonacs (Ralph Steinman), John Seykora (Alan Aderem and Jeffrey Ravetch), and David Sternberg (Hidesaburo Hanafusa).

Rockefeller was founded as an institute for medical research in 1901, and became a university in 1954 under the leadership of Detlev W. Bronk. The first Ph.D. degrees were awarded to graduate fellows in 1959, and in 1965 the institute's name was changed to The Rockefeller University. Since the first graduate degrees were awarded, the university has conferred Ph.D. degrees on more than 550 students.

In 1972, The Rockefeller University joined with Cornell University Medical College to offer a joint M.D.-Ph.D. degree to a select group of biomedical fellows. Today, the program is Tri-Institutional, including The Rockefeller University, Cornell University Medical College, and the Cornell University Graduate School of Medical Sciences, which in turn includes the Sloan-Kettering Institute.

ENDNOTES

AN AGE-OLD MYSTERY: HOW DID LIFE BEGIN?

by Christian de Duve

The origin of life has always been an object of wonder and fascination, though not of rational inquiry. There were two ready answers to the mystery: creation, as exemplified by the Book of Genesis; and spontaneous generation, essentially an accepted fact until the end of the seventeenth century. Then, starting with the experiments of the Italian biologist Francesco Redi, the debate about spontaneous generation was launched. It raged for two centuries, until Louis Pasteur finally settled the matter. After that, until the early 1950s, the problem of the origin of life was relegated to the realm of the unknowable and viewed by most scientists as unworthy of serious pursuit.

There were some notable exceptions: the Soviet biochemist Alexander Oparin, the acknowledged father of the field, who first published a booklet on the origin of life in 1924 and later expanded it to a full-size book that went through numerous editions; the British geneticist J.B.S. Haldane, who addressed the topic in a brief, but influential, essay published in 1929 by *The Rationalist Annual*; and the British physical chemist and crystallographer John D. Bernal, one of the founders of biophysics. It is of possible historical relevance that all three were confirmed Marxists, militant defenders of dialectic materialism. One may wonder to what extent ideology had something to do with their desire to explain life as a naturally emerging phenomenon in the evolution of the Earth. It certainly had with Oparin, whose book is peppered with references to the philosopher Friedrich Engels. Rumor even has it that he was set on the problem by the Party.

Attitudes began to change by the end of the Second World War. Biochemistry had made important advances and opened the possibility of formulating the problem of the origin of life in concrete terms. But hardly any biochemist saw the subject as appropriate for experimental investigation. Then the bombshell fell. In 1953, Stanley Miller, a young chemist working in the laboratory of physicist-turned-cosmologist Harold Urey, attempted to reproduce conditions that Urey, an authority on the origin of planets, believed might have prevailed on the surface of the primitive Earth. Miller took a simple reducing gas mixture (CH_4, H_2, H_2O, and NH_3), assumed to simulate the prebiotic atmosphere, and exposed it to repeated electric discharges, taken to mimic prebiotic lightning flashes. After a few days, several amino acids and other typically biological organic compounds had accumulated in the water phase. Not since

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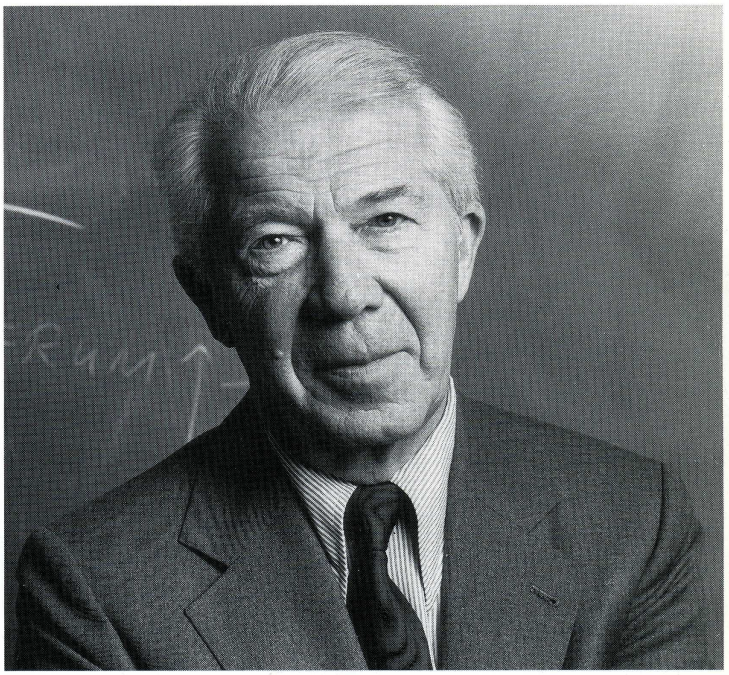
INGBET GRUTTNER



Friedrich Wöhler synthesized urea in 1828 had a chemical experiment been hailed as a comparable milestone. After the organic world, the prebiotic world had been freed from the vital spirit and had entered the laboratory.

Since this historical experiment, the field has veritably exploded. In the last three decades, the origin of life has been the subject of dozens of books, scores of essays, thousands of articles, relating an enormous amount of experimental and theoretical work. Periodicals devoted exclusively to the subject have been founded. Textbooks dedicate whole chapters to it. The reason for this upsurge of interest is simple. We have come to know enough about life to draw the basic blueprint according to which all extant living organisms are constructed. Scientists faced with the blueprint (or, rather, with their own version of the blueprint, because they tend to see life through different glasses, depending on their fields of specialization) find the problem of how the plan materialized almost inescapable. This turned out to be my case as well.

But I must add a warning. If not considered totally outlandish any more, the field still remains largely confined to speculation. When it comes to events that happened several billion years ago, hard data are scarce and, perforce, are supplemented by reasoning and imagination, if not blind faith. Yet, life did start somewhere, sometime, somehow. Trying to reconstruct the events that led to its birth holds almost irresistible fascination, especially now that we have available so much new knowledge on the nature of life and so many new tools for digging into the past and approaching the problem.

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