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A Fine Playground: [Dr. Norton Zinder]

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THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

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"All depends on keeping the eye steadily fixed on the facts of nature, and so receiving their images as they are. For God forbid that we should give out a dream of our own imagination for a pattern of the world."

FRANCIS BACON

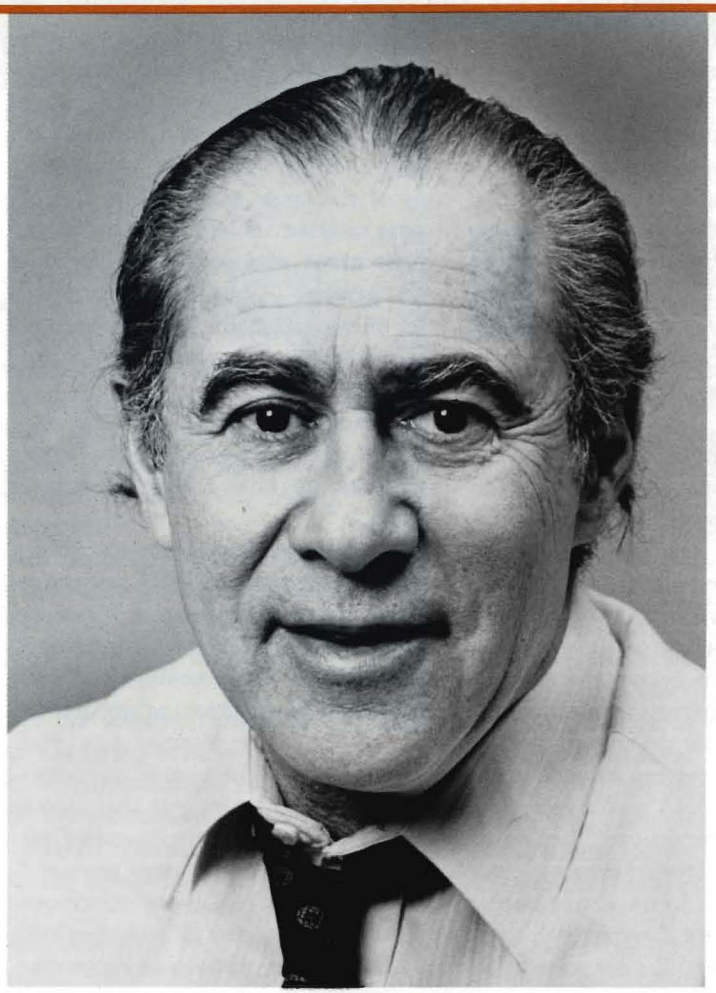
A Fine Playground

Bacteriophages — "bacteria eaters" — are tiny viruses that make their living by infecting bacteria. The late Max Delbrück, one of the pioneers of molecular biology, described bacteriophage research as "a fine playground for serious children who ask ambitious questions."

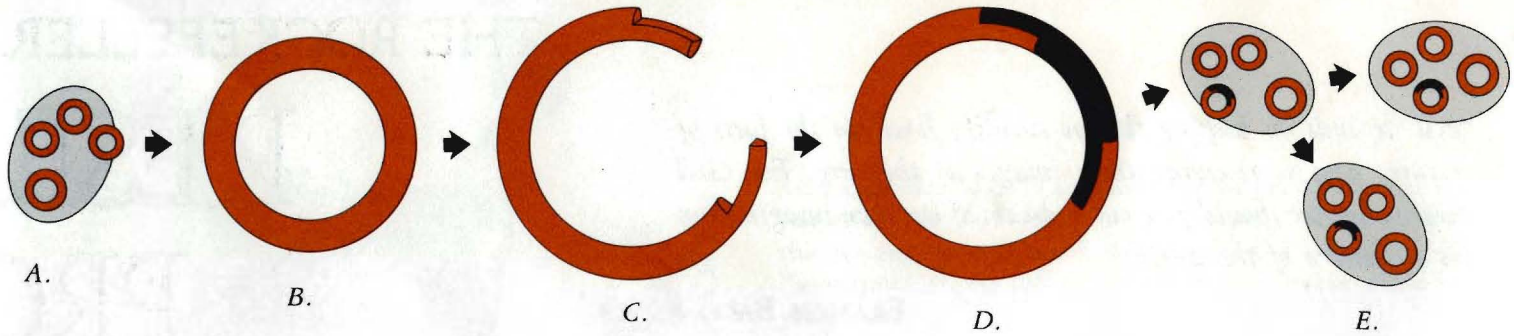
In 1952 Norton Zinder, a graduate student at the University of Wisconsin, reported in his doctoral dissertation that bacteriophages can pick up genes from one bacterium and deposit them in another. Molecular biology was in its infancy. The identification of DNA as the genetic material had been reported only eight years earlier and was still being debated. Watson and Crick had yet to build a model of the double helix structure of DNA. And the universality of the genetic code to all living beings was years from being established. "A lot of people didn't even believe bacteria had genes," Dr. Zinder says.

His faculty advisor and collaborator in this research was a young assistant professor named Joshua Lederberg (later president of The Rockefeller University). "My thesis examination," Dr. Zinder recalls, "was essentially a conversation between Josh and me. The four other professors in attendance, while expert in the traditional bacteriology of the day, were frankly baffled by what we were saying."

They were talking about a process called transduction, nature's model for what we now know as recombinant DNA technology. The development of modern molecular genetic research and genetic engineering had its origins in observations



Norton Zinder



SCHEMATIC REPRESENTATION OF HOW RECOMBINANT DNA TECHNOLOGY WORKS

A. *Bacterium containing plasmids, circular molecules of DNA found outside the chromosomes.*

B. *Plasmid, now removed from bacterium, into which a segment of foreign DNA will be introduced.*

C. *The plasmid is cut open with specific restriction enzymes.*

D. *Gene (black) spliced into plasmid.*

E. *Recombinant DNA plasmid reinserted in bacterium, which produces daughter cells containing cloned gene.*

of the natural mechanisms of genetic transfer and recombination in the cells of microorganisms.

Genes encode the instructions that control the activities of living beings: the synthesis of proteins, which comprise the major structural and regulatory molecules in cells, and the creation of new beings. Recombinant DNA technology has provided a tool for research on the structure and function of genes more powerful than any that has ever before been available. Not just for the study of the genes of microbes, but of those of every species of living creature including man. Today, biologists are pinpointing the genes responsible for diseases. They are mapping the human genome — our genetic package. A new biotechnology based on recombinant DNA science is beginning to mass-produce critically important biological molecules for medicine, agriculture, and industry.

Dr. Zinder, now John D. Rockefeller Professor of The Rockefeller University and head of the University's genetics laboratory, has an ingenious way of illustrating how DNA technology works. He uses strips of Velcro.

He attaches two strips of equal size down their length to represent the double strands of a segment of DNA. Sticking their ends together to form a ring, he makes a plasmid. Plasmids are small, circular extra molecules of DNA that float around in bacteria outside the chromosomes. In recombinant DNA technology, plasmids are often used as transducing agents. In his demonstration, Dr. Zinder opens the plasmid ring and attaches its ends to the ends of the strands of a particular gene's DNA and closes the enlarged ring. "If I now put this into a bacterium," he explains, "I'll have a little factory churning out identical copies—clones—of the gene and of the gene's product."

In an article entitled "From Genetics to Genetic Engineering," written thirty years after the discovery of transduction, Dr. Zinder stated: "We have had two revolutions in molecular genetics in my lifetime. Both were dependent on the same two components — a knowledge of DNA structure and a sophisticated ability to move genes about. The first depended on natural processes and hence was limited. The second transcends species barriers and is without limit." Both began for Dr. Zinder in unsettling circumstances.

OUT OF THE FRYING PAN

Some years ago, Dr. Zinder was invited to speak at the bicentennial celebration of Columbia University's College of Physicians and Surgeons. He began his lecture by thanking the school for rejecting his application for medical studies in 1947 because otherwise "I might not have had the pleasure of addressing you today." Telling the story he remarks with relish, "you wait a lifetime for an opening line like that."

"Not that I ever planned to practice medicine," he adds. "I always wanted to do research, but I had this idea that to be able to do it I had to have a medical degree." Bright and energetic, a voracious reader, and a fierce stickball competitor, he breezed through the Bronx High School of Science and raced through pre-med studies at Columbia College, cheerleading at football games and taking twenty credits a semester. He was graduated, at the age of eighteen, with a "gentleman's B" that failed to compete with the flood of medical school applications from returning World War II veterans.

During the following year, he worked as a psychiatric aide at a hospital, and on the advice of his college genetics professor, Francis Ryan, he applied to the University of Wisconsin for graduate studies with Joshua Lederberg, who had preceded him at Columbia but whom he had never met. Months passed. "I was on a fishing trip when I got a telegram from my folks saying I was expected in Madison in two days," Dr. Zinder says. He arrived in the middle of a midwestern summer to

encounter a broiling basement laboratory and "a twenty-three-year-old *wunderkind* who knew everything and assumed I did, too."

The first discovery of a natural mechanism of genetic recombination, called transformation, also provided the first evidence that genes were made of DNA. It was reported in 1944 by Oswald Avery, Colin MacLeod, and Maclyn McCarty, bacteriologists at the hospital of what was then The Rockefeller Institute for Medical Research. They were trying to understand how heat-killed pneumonia bacteria could affect living ones, a phenomenon that had been observed some years before by the British pathologist Fred Griffith. What they found was that the live bacteria possessed the ability to pick up free DNA released from the dead ones.

When Joshua Lederberg read Avery's paper he postponed (and never resumed) his medical studies to pursue its implications. Two years later, working with geneticist Edward Tatum, he discovered a second type of DNA recombination in bacteria — conjugation, a kind of primitive sexual mating. Normally, bacteria reproduce simply by dividing in half. In rare instances, as Lederberg and Tatum found, two bacteria will attach and form a connecting bridge through which one of the bacteria will pass a chromosomal strand to the other. This discovery provided a whole new technique for genetic study.

When organisms mate, their offspring inherit different combinations and distributions of genes. One of the classic ways geneticists exploit this is by mating a normal organism with a mutant, an organism with an alteration in the normal gene arrangement. By comparing offspring it becomes possible to get a handle on what genes are responsible for the synthesis of what proteins by seeing what proteins are lacking or altered when genes are lacking or altered.

At Wisconsin, Dr. Lederberg was trying to expand his conjugation findings to other species of bacteria. Dr. Zinder's job was to make the mutants. Biologists make mutants by exposing organisms to environmental or chemical insults. "Today," Dr. Zinder says, "making mutants is a pleasure. In those days it was an ordeal. Not to speak of the fact that I had barely ever handled a pipette and had never had a microbiology course in my life."

A month later there were no mutants. "Josh was upset. I was furious. In any event, maybe it was the stimulus of the tension, but Josh got this brilliant idea — to use penicillin. Penicillin kills only growing cells. Mutants don't grow unless you give them certain kinds of supplements in the medium. I designed some experiments and they worked. Zappo! We had mutants falling out of the sky."

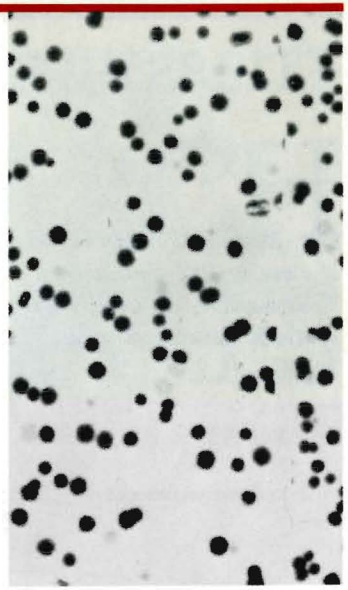
Mating could now be sought in other species. To be sure that the new cells were the product of mating and not just cell division they mixed strains that differed from one another in more than one trait. "We expected to find offspring with a mixture of traits from both parents," Dr. Zinder says. "Instead, they all resembled one parent. At first we thought we had stumbled on an instance of transformation. We tested it with an enzyme that normally kills naked DNA. Nothing changed. Knowing that, bacteriophages became suspect."

Like all viruses, bacteriophages are simply little packages of genes wrapped up in a protein coat. They survive and reproduce by intruding their genetic instructions into the DNA of the cells they invade, often subverting the instructions the cells need for their own survival. As with other viruses, some bacteriophages are extremely lethal. "Infected cells can disappear in front of your eyes," Dr. Zinder says. Others will lie low for a while, only occasionally bursting out and moving on to other cells.

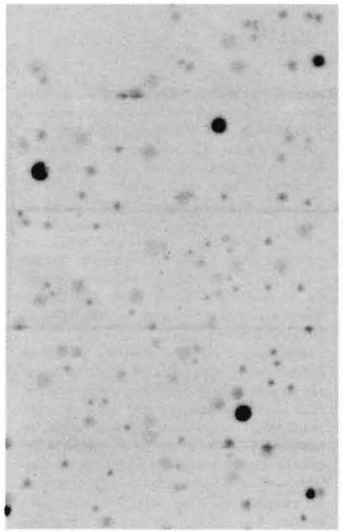
As the two young researchers determined, bacteriophages, protected by their protein coats from the destructive enzyme in the medium mix, were indeed infecting the bacteria. And when they left a cell they were taking with them some of the cell's DNA, which then recombined with the DNA in the cells that they next infected: transduction.

Despite the perplexity of his thesis examiners, Dr. Zinder got his Ph.D. Looking back, he reflects: "It's hard now to remember an era when people didn't comprehend what we now so easily understand as genetics — the genetics of everything. My examiners were good biochemists and microbiologists but they didn't think genetically. To them genetics had to do only with fruitflies and corn. It was a stroke of incredible insight on the part of the department chairman, R. A. Brink, himself a corn geneticist, to have hired Josh."

Dr. Zinder's degree was not, however, in genetics but in



From Dr. Zinder's original 1948 experiments to isolate mutant bacteria using penicillin. Top, the ratio of normal bacteria (black dots) to mutants (white dots) is one hundred to one. After the addition of penicillin, the ratio is reversed, below.



medical microbiology. "Josh and I had decided that that way at the very worst I might wind up doing stool cultures in a hospital, but I'd get a job. At that time, a job in genetics usually had to await the demise of a university's sole genetics professor."

As it turned out, he wound up at Rockefeller, eventually associated with Rollin Hotchkiss (now professor emeritus), who was expanding Avery's findings on transformation. Dr. Zinder continued to refine transduction and to begin intensive studies of the genetic structure of bacteriophages, work that continues in his laboratory to this day.

Dr. Zinder offers a wry footnote to the Wisconsin era of his career. He remembers that in 1951 the physicist Leo Szilard, with "characteristic brilliance and prescience," told him to patent transduction.

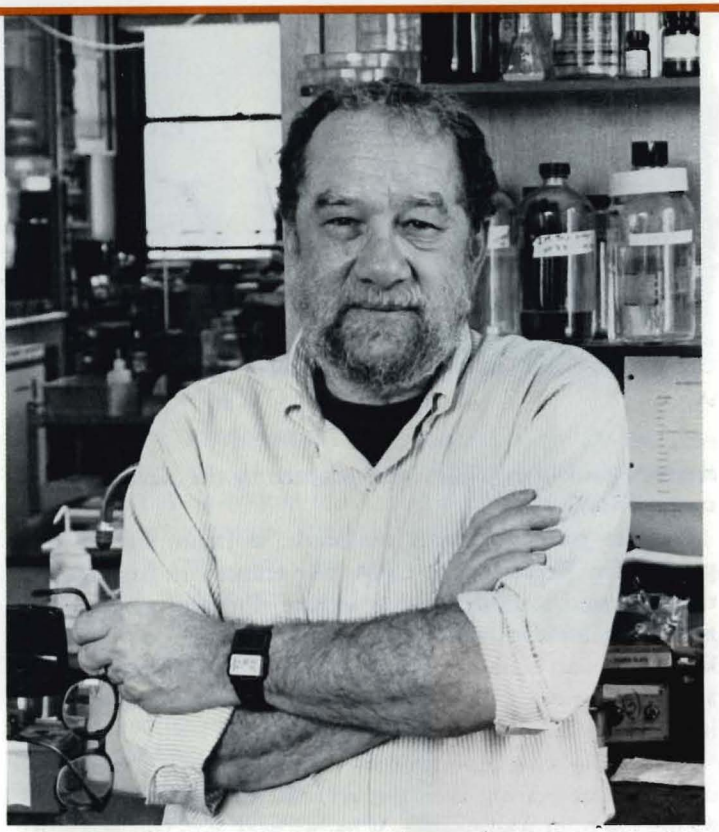
INTO THE FIRE

Recombinant DNA technology across species came to fruition when it became possible to cut DNA at specific sites using chemical scissors called restrictions enzymes. "We knew what we were looking for," Dr. Zinder says. "We had the theory. All we needed to do was find the enzymes."

They were found, in 1970, by Johns Hopkins biologist Daniel Nathans. "Dan's lab was working on one enzyme," says Dr. Zinder. "My lab was working on another. His enzyme recognized a specific site and cut it. Mine recognized a specific site and cut somewhere else. Why? I don't know why. Because that's how God made it."

A decade earlier, when Dr. Nathans was at Rockefeller, he and Dr. Zinder collaborated on experiments that helped to explain the mechanisms of the genetic code, the system whereby the instructions encoded in DNA are translated into the synthesis of proteins. The message, written in a four-letter chemical alphabet, is copied from DNA to RNA, another nucleic acid. Messenger RNA relays it to the cell's protein-making plants, where each "word" of the message instructs the cell to make an amino acid, the small molecules that, strung together, build proteins.

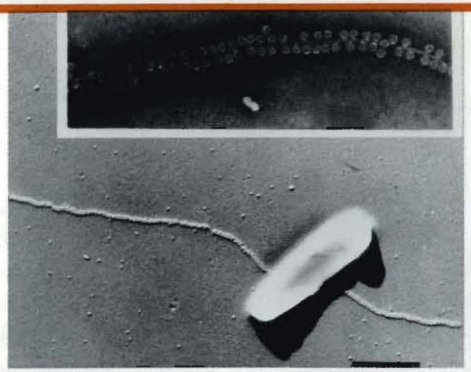
The first protein synthesized in a test tube was made using a bacteriophage as the messenger. Phage f2, as it is called, is one of a very few viruses, and the only such creatures in nature, whose genes are made of RNA rather than DNA. Discovered in Dr. Zinder's laboratory, phage f2 provided, he says, "a powerful check" on the fidelity of the in vitro system being used to develop the genetic code.



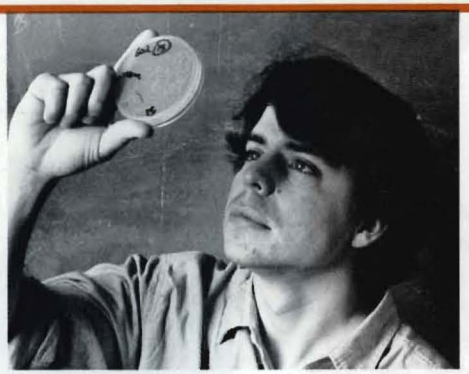
Professor Peter Model, co-leader of the genetics laboratory, whose bacteriophage research is helping to explain how genes express themselves, control one another, and cooperate to create new organisms. Currently, Dr. Model is concentrating on questions of how virus proteins are inserted into the membranes of host cells, what makes them stay there, and how new viruses are assembled at the host cell's surface.

In 1973 the first genetic chimera was made by means of recombinant DNA science. But the thrill of achievement was soon tempered by concern. An experiment had been proposed in which a tumor-causing virus was to be cloned in a human intestinal bacterium. Some scientists began to ask whether such experiments posed a danger. Was it possible that harmful and uncontrollable recombinants might inadvertently get loose from the laboratory? In 1974 a committee of the National Academy of Sciences called for a temporary moratorium on certain kinds of experiments. Dr. Zinder was among the committee's eleven members. In March of the following year, he joined an international group of one hundred and forty molecular biologists at a meeting in Asilomar, California. Working around the clock for four days, they hammered out a set of

Postdoctoral Fellow Bénédicte Michel from the Institut Jacques Monod, in France, whose research focuses on the regulation of DNA synthesis in bacteriophage.



A.



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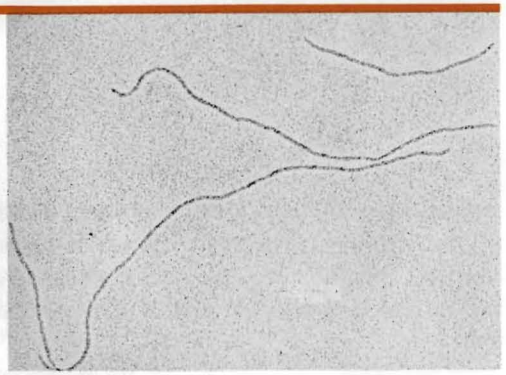

C.

A. *The human intestinal bacteria* E. coli. *The hairlike projection, or pilus (magnified in the inset), is covered with RNA bacteriophage in which the genetic material is made of RNA instead of DNA. The first RNA phage was discovered in Dr. Zinder's laboratory and was an important tool in research on the genetic code.*

B. *David Russell, who presented his Ph.D. dissertation this winter on a biochemical mechanism that acts to prevent DNA replication in microorganisms.*

C. *Filamentous phages currently under study in Dr. Zinder's laboratory. Because this kind of bacteriophage is a filament it can come in many sizes and thus can carry foreign DNA.*

research guidelines, which were adopted by the National Institutes of Health.

But the genie was out of the bottle, as James Watson later put it. The Recombinant DNA War erupted in headlines in the press and in heated public debate. Pressure mounted for restricting legislation. Dr. Zinder found himself on the front lines of the battle, an angry David armed with a Velcro slingshot and a temperament inclined to what a student of his diplomatically terms "animated discussion."

"The thing that mobilized us," says Dr. Zinder, "was local communities getting into the act. We would have accepted regulation from Washington. What we were afraid of, and what we came very close to having imposed on us, were laws in New York saying one thing and laws in California saying another. You can't do science that way. You can't compete. You can't collaborate. What was most discouraging was that the people writing the local legislation didn't know the first thing about the science they were proposing to regulate."

The discussion continued — with senators, with congressmen, with governors, with legislative aides, with reporters, with environmentalists, with public action groups, with other scientists — rising to a crescendo in 1976 and 1977. When the dust settled there was no legislation.

"There are still skirmishes," says Dr. Zinder, such as the recent disputes about modified bacteria being used to prevent plants from freezing. But the concerns that scientists had first expressed were eventually resolved through research that provided detailed understanding and showed recombinant organisms to be no more dangerous than the components of which they were made.

In 1982 Dr. Zinder, along with biologists Paul Berg of Stanford University and Maxine Singer, then with the National Institutes of Health, received the first award for Scientific Freedom and Responsibility given by the American Association for the Advancement of Science. It was presented to them for their leadership during the DNA debate.

The recombinant DNA controversy was not Dr. Zinder's first experience with science and public policy. In 1973, during the Nixon administration's "War on Cancer," he was asked by the National Cancer Board to be chairman of a committee to review the Special Virus Cancer Program of the National Cancer Institute. The report the committee submitted early the following year was highly critical. Not of the need for viral cancer research, but of the manner in which the program was being administered largely through contracts for specifically targeted goals.

"Contract research can do some things very well," Dr. Zinder says. "It's fine for developing applications of research. What it's not good for is basic research, when the nature of the problem hasn't even begun to be fathomed. How can you contract someone to come up with an idea, a discovery, in X number of months or years?" The Zinder Report, as it was called, resulted in a reapportionment of research funds with a greatly lessened emphasis on contracts.

Dr. Zinder is again spending time in Washington. A few years ago, he was asked to join a National Research Council committee to advise on the disposal of the country's obsolete chemical warfare weapons. "We have a huge stockpile of these weapons," he says, "all made before 1969 and in various stages of disintegration. How do you get rid of them? Do you dump them in the ocean? Do you burn them up? Do you blow them up? Do you neutralize them chemically? Do you send them to the moon?"

Over a two-year period, members of the committee inspected all the American stockpiles in the United States and abroad

Drs. Model and Zinder in front of a nucleic acid synthesizer, or "gene machine," in which pieces of genetic material are made.

and put together their report. Their suggestion is combustion, which they have deemed to be far less hazardous and cumbersome than any other method. "The main problem," Dr. Zinder explains, "is that a lot of these weapons are configured in bombs and rockets. You have to crack them open or disassemble them by remote control in such a way that they don't explode. It's a very slow, tedious process."

Now chairman of the National Academy of Science's oversight committee for the project, Dr. Zinder will continue to shuttle to Washington over the next several years. "It takes me away from the lab, which I don't like, but I feel an obligation to see it through." Beginning this summer, he will be serving the National Academy of Sciences in another role. An academy member since 1969, he has been elected to its ruling council.

BACK TO BASICS

Starting with Joshua Lederberg, Dr. Zinder has been, he says, "most fortunate" in his associates. At Rockefeller, he "grew up" in Rollin Hotchkiss's laboratory, much as Peter Model, a colleague of more than twenty years, grew up in his, and whose work has provided significant insights into the structure and assembly of the lab's current favorite phage.

Most of the other members of the laboratory are students. "Over the years, my lab has been largely run with students," Dr. Zinder says, "They're smart, they're fun, and they say what they think." He smiles. "They remind me of me. I don't give them orders. We talk about science. I tell them what interests me. They tell me what interests them. Then I let them do what they want to do. They have to be taught a lot technically, but that's the 'how' part, not the 'what.'" "Norton works through suggestion," says Joseph Heitman, a current student. "Sometimes it's very subtle. Sometimes it shifts the whole way you're thinking."

Sometimes it's not so subtle. David Russell, another student, wanted to pursue an idea about a mechanism he thought affected DNA replication. Dr. Zinder didn't think it did. Russell took his chances and went ahead with the research. "Norton went over the work with a fine-tooth comb," he says. The paper was published last year in the prestigious journal *Cell,* with Dr. Zinder's name appended after Russell's in the traditional senior scientist approval slot.

Throughout his career, Dr. Zinder's interest has remained steadfastly focused on basic inquiries into the structure and function of genes. He concentrates on bacteriophages because they are easier to understand and to handle than higher forms. The phages currently under study in his laboratory have, at most, ten genes. The human genome contains a hundred thousand or more. Many of the fundamental ideas about how genes operate, which have made it possible for scientists to dare to approach higher forms, were achieved through such research on microorganisms.

"What basic scientists do," says Dr. Zinder, "is try to understand how living things work. I think it's an acceptable argument that knowledge of itself has intrinsic value. If you want to push me as to what 'good' it does, is it going to cure cancer or something, I can only answer that I don't know. I don't know if it will help us cure cancer, but I can absolutely guarantee you that without it we never will."

What Dr. Zinder's research has provided for science to date is three bacteriophages, each a tool that has contributed to important findings. First there was P22, the phage that uncovered transduction of bacterial genes. The second was RNA phage f2, which helped unravel the details of protein biosynthesis and the genetic code. The current favorite alluded to earlier is the filamentous phage f1, which can provide single strands of any piece of DNA for use in probing and sequencing genes. In the company of a new generation of "serious children" he continues to ask ambitious questions. □

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