Spring 1982

Elegant Molecules: [Dr. Stanford Moore]

Fulvio Bardossi

Judith N. Schwartz

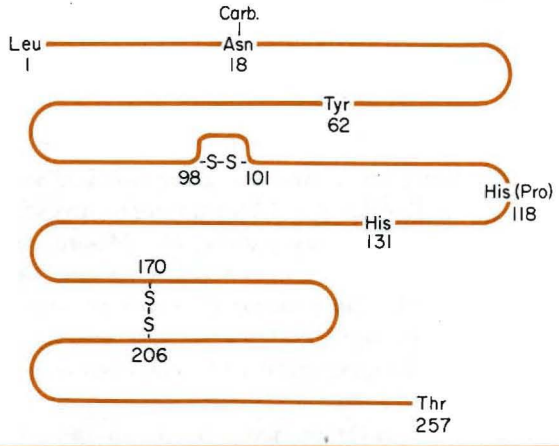


Diagram of special features of bovine pancreatic deoxyribonuclease.

THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

SPRING 1982

Elegant Molecules

Asked how he happened to become a biochemist, Professor Stanford Moore attributes the choice to gifted teachers of science and a vocational counselor who advised him that there was no future in aeronautical engineering. "But," he adds with a smile, "I've never regretted the choice. I can imagine no life more fascinating or more rewarding than one spent exploring the elegant and complicated architecture of organic molecules."

In 1963, Dr. Moore and his Rockefeller University colleague, William H. Stein, reported that their laboratory had determined the chemical structure of the first enzyme and largest protein to be decoded up to that time: pancreatic ribonuclease. In 1972, Dr. Moore and Dr. Stein shared the Nobel Prize in Chemistry with Christian B. Anfinsen of the National Institutes of Health for their contributions to the understanding of the interrelationships of the structure and activity of the ribonuclease molecule.

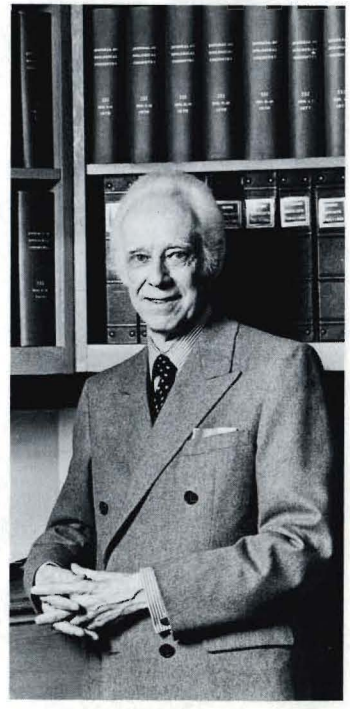
Dr. Stein died in 1980. At his memorial service, the late Philip Handler, then president of the National Academy of Sciences, characterized the 40-year Moore-Stein partnership as "surely among the most productive cooperative endeavors in the history of science."

The research continues today under Dr. Moore's leadership.

FROM THE GREEK *PROTEIOS:* PRIMARY

Proteins comprise the largest part of the solid matter of living cells. Some proteins, such as the hemoglobin that carries oxygen in red blood cells, are involved in transport and storage. Many hormones, like insulin, are proteins; they are chemical messengers that coordinate body activities. Immunoglobulins, the master molecules of immunity, are proteins. (In the same year that Dr. Moore and Dr. Stein received their Nobel Prize, Professor Gerald M. Edelman of The Rockefeller University shared the Nobel Prize in Physiology or Medicine for determining the chemical structure of an immunoglobulin, work facilitated by technology refined in the Moore-Stein laboratory.) All known enzymes are proteins. Enzymes, thousands of them, are the catalysts of innumerable body processes. They can speed the rates of chemical reactions more than a millionfold.

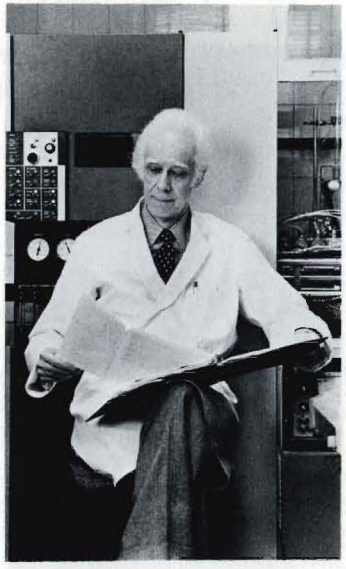
Chemically speaking, proteins are large—they are termed macromolecules. There are proteins with molecular weights that exceed 100,000 (based upon the weight of a hydrogen atom as 1). Proteins are constructed primarily from smaller molecules, about 20 kinds in all, called amino acids. Two or more amino acids linked together form what is called a pep-



Stanford Moore

tide. A protein is a very large, structurally complex polypeptide chain. Some smaller peptides, like their larger relatives, also handle important jobs. One of the most heralded events in the neurosciences in recent years was the discovery of endorphins, natural opiates in the brain. The endorphins are peptides.

Not surprisingly, when something goes wrong with the body's protein chemistry, trouble can follow. There are more than 100 hereditary diseases known to involve specific protein defects. Parkinson's disease and phenylketonuria (PKU), which can lead to mental retardation, result from enzyme deficiencies. The replacement of one specific amino acid by another in one of the polypeptide chains of the hemoglobin molecule results in sickle-cell anemia.



Stanford Moore in the laboratory.

Joshua Lederberg, president of The Rockefeller University, recently wrote: "Almost everything we attempt in rational medicine is connected with the structure and behavior of proteins. Our hopes for radical interventions to prevent or reverse such complex processes as cancer or aging can scarcely outpace that knowledge."

In 1939, when Stanford Moore arrived at what was then The Rockefeller Institute for Medical Research, very little was known of detailed protein structure. Recommended by his professor at the University of Wisconsin, he had been invited to join the laboratory of Max Bergmann, to which another recent Ph.D. in biochemistry, William Stein, had come two years earlier. Bergmann was working on techniques for separating individual amino acids from hydrolysates of proteins. He assigned his two juniors the task of developing ways for determining the precise quantities of each of the amino acids from which a protein is built.

"Dr. Bergmann's goal," says Dr. Moore, "was to find out—because we really didn't know then—whether proteins actually had specific structures. Could we write a formula for them? To do that, we had to find out precisely what they were made of."

Bergmann, who died in 1944, never saw the full realization of his goal. The laboratory's research on protein structure had been suspended with the entry of the United States into World War II, during which time the group worked with the Office of Scientific Research and Development investigating properties of chemical warfare agents. Dr. Moore, sent to Washington to facilitate liaison between science and the military, wound up with the Operational Research Section in the Pacific theater. At the war's end, Dr. Moore and Dr. Stein resumed their collaboration, now with a laboratory of their own, eager to explore some "interesting developments" that had occurred in the interim, particularly in the field of chromatography.

SEPARATING THINGS

Scientists use a variety of techniques to separate, identify, and purify chemical compounds. One of those techniques, chromatography, was introduced early in the century by a Russian botanist, Michael Tswett. In his original procedure, a petroleum ether extract of green leaves was poured into a glass tube packed with calcium carbonate and clear solvent was added. The leaf pigments, adhering differently to the calcium carbonate because of their various molecular characters, separated into bands of different colors as they moved down the column. The bands could then be washed out of the column, color by color. Tswett's column was later adapted for colorless compounds through the use of specific methods of detection and visualization.

Chromatography emerged as a major tool of biochemical science after 1941, when English researchers A.J.P. Martin and R.L.M. Synge introduced what they called partition chromatography, which made it practical to separate water-soluble compounds such as amino acids and peptides. Drs. Stein and Moore, among others, appreciated the potential of partition chromatography for the quantitative separation of amino acids. Following a suggestion by Dr. Synge, they began by using a column of moist potato starch with organic solvents. When a mixture of amino acids was passed through the starch column, and fractions (each composed of a given number of drops) were collected, the amino acids were washed off serially. Thus began a period of innovation and invention—

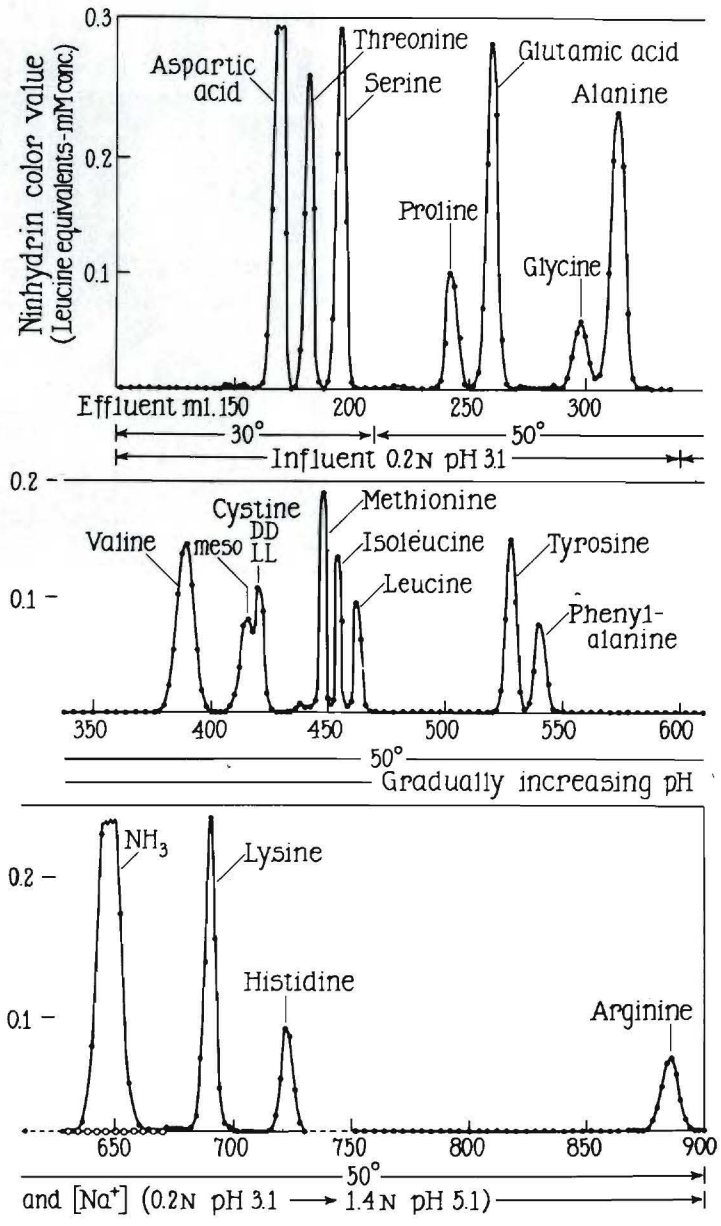
the "tooling up," as Dr. Moore has called it—that has had far-reaching effects on laboratory technology.

The starch chromatogram was, indeed, a great improvement over earlier methods of separation. The drop collecting, however, as Dr. Moore recalls, was "a tedious and time-consuming process, and I, as the bachelor, always got the night shift. Bill and I decided that some simple automation would be desirable." Drawing upon the skills of The Rockefeller Institute's instrument makers, they fashioned an automatic fraction collector, which, unattended, could collect and count fractions, moving from tube to tube. (The photoelectric eye that they used was adapted from one originally designed to center the transparent wrappers on packages of chewing gum.) About this time the laboratory also made quantitative the use of ninhydrin, a reagent that yields a blue color, for estimating the extremely small amounts of amino acid in each of the fractions. The intensity of the color given to a fraction by the reagent is proportional to the amount of amino acid that it contains.

For a number of years, Dr. Moore and Dr. Stein and their group searched for a better adsorbent than starch. They found it in one of the newly developed synthetic ion-exchange resins. (Ions are electrically charged atoms or groups of atoms, a category into which amino acids fall. Ion exchange has been used extensively in industry, as in the process of water softening.) With ion-exchange chromatography, refined through manipulations of aqueous solutions, acidity, and temperature, the laboratory was able to obtain separations of high resolution.

The crowning achievement of their instrument-making days was the amino acid analyzer, invented in collaboration with Darrel Spackman, a young member of the laboratory. With it, the procedure became completely automatic. The analyzer is now manufactured and, with commercially introduced improvements, has become standard equipment for analyzing purified proteins, physiological fluids, and foods in laboratories all over the world.

"Before the war," says Dr. Moore, "I spent two years determining two amino acids in two proteins by manual methods.



The amino acids in an acid hydrolysate of ribonuclease A, determined manually by ion-exchange chromatography (1954).

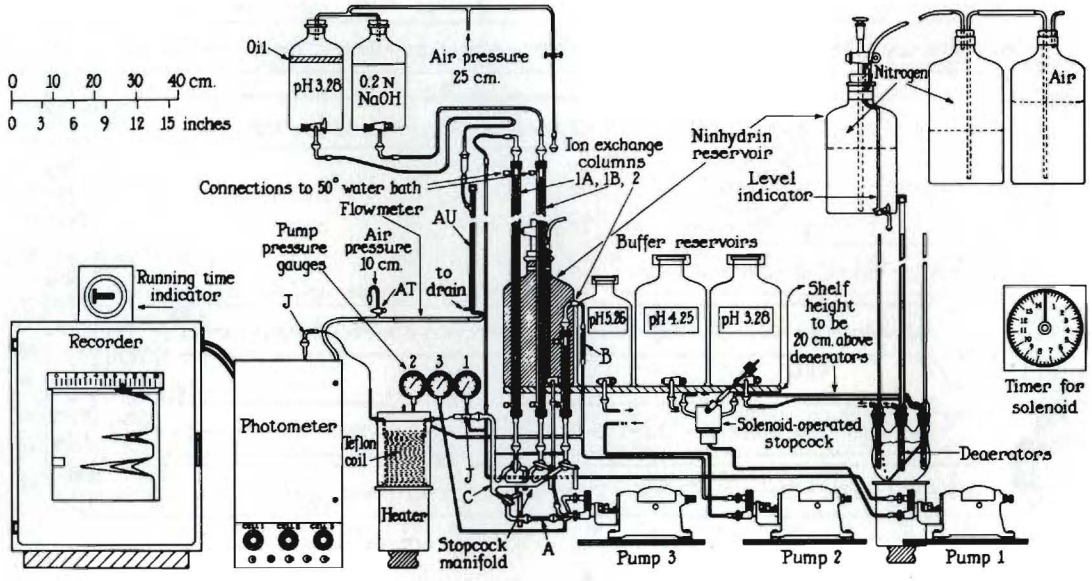
With the analyzer, eighteen amino acids can be determined in an hour. Among our early applications, conducted with Harris Tallan, another of the many talented young collaborators we've had the good fortune to have with us over the years, was the identification of two new amino acids in urine, the measure of one of which is useful in disease diagnosis, and of two previously unidentified amino acids in the brain." Earlier, while still using the starch column, the laboratory had determined the quantity of each of the free amino acids of blood plasma. James Manning, a current member of the laboratory, began his research at Rockefeller by developing a method for separating optical isomers of amino acids (molecules that are mirror images of one another) on the analyzer.

RIBONUCLEASE

In the postwar years, new tools and new information spurred a renaissance in biochemical research. Lyman Craig at Rockefeller introduced counter-current distribution, another separation method, which helped Vincent du Vigneaud at the Cornell University Medical College to achieve the first structural formula for a peptide hormone, oxytocin, which has a molecular weight of 1,007. In England, Frederick Sanger, drawing in considerable part upon the paper chromatography methods introduced by Martin and Synge, made the breakthrough to the determination of the sequence of the hormone insulin, which has a molecular weight of 5,733. Their pioneering efforts earned Nobel prizes for Sanger and du Vigneaud. Their techniques, however, were not fully suitable for the study of longer polypeptide chains. The quantitative technology then being developed in the Moore-Stein laboratory made it possible to go up the molecular scale.

"By 1950," says Dr. Moore, "we started looking at the literature for a suitable protein. We wanted one that would be roughly twice the size of insulin and that could be obtained in reasonable quantity and homogeneous form."

Their choice turned out to be bovine pancreatic ribonuclease. This protein from the pancreas of cattle was the appropriate size; it had been made plentifully available from commercial sources as a by-product of meat processing; and it had been partially purified some years before by René Dubos and R.H.S. Thompson at Rockefeller and isolated in crystalline form by Moses Kunitz, also at Rockefeller. And the protein was an enzyme.

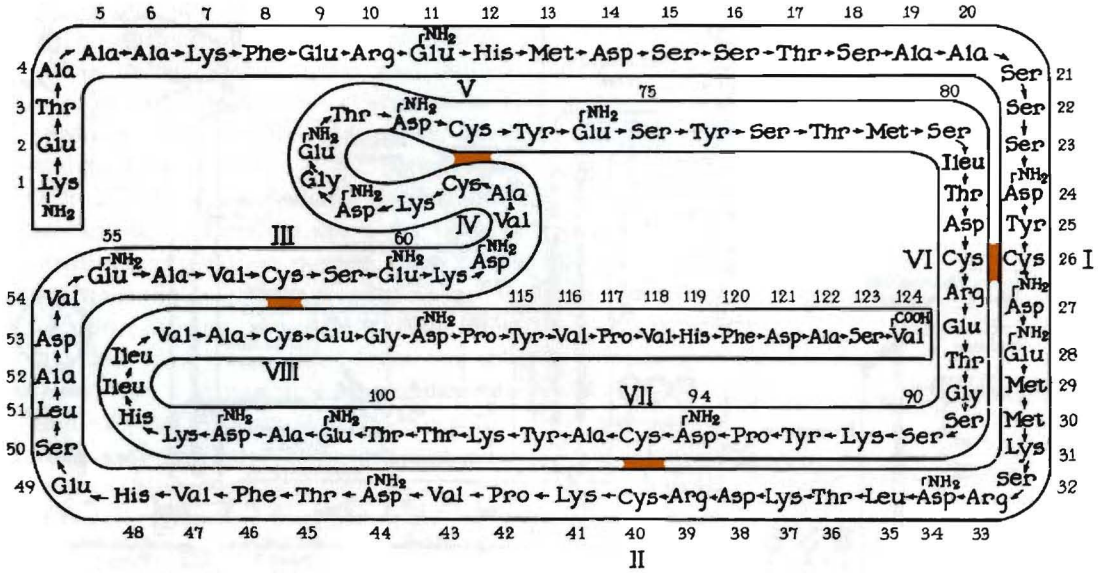


Schematic diagram of the initial design of an automatic amino acid analyzer (1958).

"We wanted to take on an enzyme," Dr. Moore explains, "so that when we had the structure worked out we could relate it to the catalytic mechanism—in the case of ribonuclease, its action in speeding the process of digesting RNA."

Using an ion-exchange column, Werner Hirs, who was the first postdoctoral fellow to join the laboratory, accomplished further purification, achieving an extremely homogeneous product, which they labeled ribonuclease A.

The next problem was to split the protein into peptide segments, separate them chromatographically by ion exchange, and quantify each fragment, work facilitated by knowledge of the mode of operation of protein-splitting enzymes elucidated earlier by Max Bergmann and his colleague Joseph Fru-



The sequence of amino acid residues in bovine pancreatic ribonuclease; the disulfide cross-links are color-coded (1963).

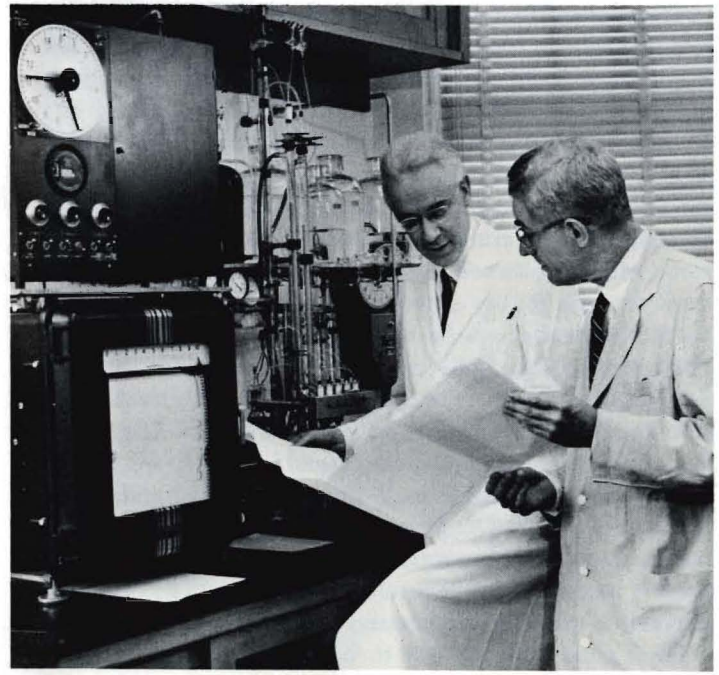
Ala	*Alanine*
Arg	*Arginine*
Asp	*Aspartic Acid*
Asp-NH_2	*Asparagine*
Cys	*Cystine*
Glu	*Glutamic Acid*
Glu-NH_2	*Glutamine*
Gly	*Glycine*
His	*Histidine*
Ileu	*Isoleucine*
Leu	*Leucine*
Lys	*Lysine*
Met	*Methionine*
Phe	*Phenylalanine*
Pro	*Proline*
Ser	*Serine*
Thr	*Threonine*
Tyr	*Tyrosine*
Val	*Valine*

ton. The determination of the actual sequence of amino acids within the peptide fragments was aided invaluably by a chemical reaction that can clip off one amino acid unit at a time, a procedure developed by a Swedish scientist, Pehr Edman.

The structure of ribonuclease, as worked out in the Moore-Stein laboratory, is shown in the schematic illustration. When amino acids link together, each component loses a molecule of water. Hence, amino acids in polypeptide chains are called "residues." The enzyme consists of 124 residues of 17 different kinds of amino acids—1,876 atoms. Its molecular weight is 13,683. From Christian Anfinsen, working concurrently with ribonuclease, it had been learned that the molecule is one long chain, unlike insulin, which has two chains. The chain is joined at four places—the disulfide bridges—as determined by Darrel Spackman.

The illustration is two-dimensional, but nature is three-dimensional. While the sequencing work was going on, the Moore-Stein group and other laboratories were conducting chemical experiments on ribonuclease activity, which in turn shed some light on the molecule's shape. The substrate, the substance on which an enzyme acts, normally fits neatly into a trough on the enzyme surface. Through chemical manipulations of the various amino acids in the molecule, it was found that ribonuclease activity stopped when either of two histidine residues (at positions 12 and 119) was modified. This implied that those residues must be near one another when the enzyme is in its natural conformation. That conclusion was later confirmed by scientists who determined the full three-dimensional architecture by the physical technique of X-ray crystallography.

After completing the determination of the chemical structure of ribonuclease, the laboratory went on to decipher pancreatic deoxyribonuclease, which is about twice as large. This protein is an enzyme that catalyzes the breakdown of DNA.



Stanford Moore and William H. Stein at the amino acid analyzer in 1965.

THE NEXT TWENTY YEARS

Protein chemists, at Rockefeller and elsewhere, have continued to delve deeper and deeper into the mysteries of the "elegant and complicated" molecules. One of the areas in which such progress is yielding exciting new insights is neurochemistry: investigations now underway in Dr. Moore's laboratory encompass basic studies on enzymes that act on nucleotides in the brain and in the eye. James Manning and his associates have developed an independent program exploring many facets of the biochemistry of sickle-cell hemoglobin, of fetal hemoglobin, and of inhibitors of bacterial enzymes. These projects have therapeutic objectives.

In work that he refers to as "ribonuclease revisited," Dr. Moore explains that the enzyme that was the subject of the laboratory's first inquiry continues to be of prime interest. "When we began working with it," he says, "ribonuclease was recognized only as one of the catalysts in the digestive tract. We and others later found that ribonucleases of similar molecular design are present in almost every mammalian cell, in combination with inhibitory proteins that serve to regulate the catalytic activity." Peter Blackburn has had a key role in studies of a ribonuclease inhibitor first isolated in pure form from the human placenta. The research bears upon the possible function of ribonuclease activity in normal protein biosyntheis and in malignant disease. "In that connection," Dr. Moore says, "it seemed to us that it would be informative to see whether we could make the enzyme *more* active than the native molecule. We found that we could speed activity toward some substrates by cross-linking one ribonuclease molecule to another or to other organic compounds known to have special affinity for RNA. Also, by coupling the protein to carbohydrates, it has been possible to direct the enzyme toward tissues whose cells have receptors for sugars, particularly the liver cell." Such experiments lead to ways through which basic information can grow into potentially practical means of targeting enzymes, or proteins with drugs attached to them, to the liver. Current experiments concern cancer and malaria. Cooperation is being rendered to several laboratories at Rockefeller in the application of the principles of amino acid and protein chemistry to other problems of clinical concern, such as cystinuria and cystic fibrosis.

In his fifth decade of research, Stanford Moore reflects: "When I was a graduate student I never dreamed I would live to see the chemical structure of a protein. When I was a 'post-doc' I never expected to see the three-dimensional architecture of an enzyme. I think that the next 20 years are going to be even more exciting in biochemistry, as it becomes possible to learn more about the intricate synergisms of living systems." □

Above, Peter Blackburn at an instrument for high-speed liquid chromatography.

Left, Wanda Jones, assistant for research, and James Manning with a model of the alpha-chains of human hemoglobin.

RESEARCH PROFILES is published four times a year by The Rockefeller University. It is written and edited by Fulvio Bardossi and Judith N. Schwartz. This is issue Number 8, Spring 1982. Inquiries should be addressed to the University's Public Information Office, 1230 York Avenue, New York 10021 or phone (212) 570-8967. Photographs, pages 1, 2, and 6, Ingbert Grüttner; page 5, Sam Vandivert. Printed in the United States of America.