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STANFORD MOORE

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A Biographical Memoir by

EMIL L. SMITH AND C. H. W. HIRS

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STANFORD MOORE, Nobel Laureate in Chemistry in 1972, was born in Chicago, Illinois, when his father, John Howard Moore, was a student at the University of Chicago Law School (J.D., 1917). His father was a graduate of Westminster College in New Wilmington, Pennsylvania, and his mother (née Ruth Fowler) of Stanford University. His parents were married in 1907 and had met at Stanford. It is alleged that this was the origin of the given name of the son.

The education of Stanford Moore began at the age of four at a progressive school in Winnetka, Illinois. When he was six, his father moved to a teaching position in the Law School at the University of Florida; he later accepted a position with Mercer University in Macon, Georgia, where the boy attended the local public schools. In 1924 J. H. Moore became professor of law at Vanderbilt University, where he was to serve on the faculty until his retirement in 1949; he died in 1966 at the age of eighty-five.

In Nashville, Stanford was a student at Peabody Demonstration School, which was operated by the George Peabody College for Teachers. He was an outstanding student for the seven years he attended the school, and he maintained a straight A average. Initially his interests were in English and science, and he was fortunate to encounter a teacher, R. O.

Beauchamp, who aroused his interest in chemistry. In 1931 he entered the College of Letters and Science of Vanderbilt University, considering a career in aeronautical engineering or chemistry. Unable to decide between the two fields, he pursued both the liberal arts and the basic subjects of the engineering curriculum during his first two years. In his third year at Vanderbilt, he came under the influence of Arthur William Ingersoll, who Stan later credited as stimulating his enthusiasm for organic chemistry and molecular structure. As a result, Stan changed his major subject to chemistry and graduated from Vanderbilt in 1935 with the bachelor of arts degree, summa cum laude, and was a recipient of the Founder's Medal as the outstanding student in his class. Surprisingly, the Stanford Moore who tended to lead a rather reclusive life in later years and generally avoided nonscientific social activities was a socialite as an undergraduate. He was active in several clubs, president of his fraternity, and, as president of the student council, the organizer of the senior prom.

In the autumn of 1935, Stan entered the Graduate School of The University of Wisconsin with the support of a fellowship from the Wisconsin Alumni Research Foundation. Although he chose to major in organic chemistry, he elected to do his thesis research with Professor Karl Paul Link, a member of the Biochemistry Department at Madison. It was significant for Stan's later development that Link had spent some time—years earlier—in Graz, Austria, in the laboratory of Fritz Pregl, one of the pioneers in the development of microanalytical methods. Link required all of his students to master these microanalytical techniques. In retrospect it is apparent that Stan's background, first in engineering and then in microanalysis, had an important effect on his later collaborative work with William H. Stein in the development of important new methods of automated analysis.

Moore's thesis research (ultimately summarized in five papers) included a study of the reaction of *o*-phenylenediamine with various monosaccharides. The products of this reaction, a series of benzimidazoles, proved to be readily isolated as stable crystalline solids that lent themselves well to the identification of various monosaccharides. These derivatives continue to be so used.

With the completion of his doctoral dissertation (1939), it was clear that Stan's future was to be in biochemistry. Two attractive options were available: a four-year fellowship at Harvard Medical School and an invitation to become a research assistant in the laboratory of Max Bergmann at the Rockefeller Institute for Medical Research in New York. Bergmann had been one of Emil Fischer's outstanding collaborators, and he had continued after the First World War to make a series of notable contributions in protein and carbohydrate chemistry while at the Kaiser Wilhelm Institut fuer Lederforschung in Dresden. With the rise of the Nazi dictatorship in the early 1930s, Bergmann accepted an offer to join the staff of the Rockefeller Institute, and moved there in 1934. It was through Link's friendship with Bergmann that the invitation for Stanford to go to Rockefeller was presented. As Stan commented later, "The question of whether it would be wiser to go on to medical school or to enter immediately into chemical research was resolved in favor of the latter."

When Stan joined Bergmann's group, he became involved in one of the principal concerns of the laboratory, the structural chemistry of proteins. Of particular interest was the development of methods for the gravimetric estimation of the amino acid composition of proteins by utilizing selective precipitants. This approach had been given new impetus two years earlier when William H. Stein joined the laboratory and showed that aromatic sulfonic acids possess desirable prop-

erties in that regard. Whereas earlier workers had concentrated on precipitants that formed highly insoluble salts with amino acids (the prototype was flavianic acid, used by A. Kossel and R. E. Gross in 1924 for the isolation of arginine), the research in Bergmann's group emphasized the fact that salts of extremely low solubility were unnecessary, provided that the precipitant was selective and that the solubility products of the salts were estimated and corrections were applied for the quantity of amino acid remaining in solution.

Bergmann suggested that Stan join forces with Bill Stein to develop the solubility product approach as a routine method for amino acids. There was no way at the time to realize that a scientific collaboration had been initiated that would last for the remainder of the lifetimes of these two young scientists, certainly one of the longest and most fruitful collaborations in the history of all science.

Bill and Stan concentrated their initial efforts on two sulfonic acid reagents—5-nitronaphthalene-2-sulfonic acid for glycine and 2-bromotoluene-5-sulfonic acid for leucine—and showed that good results could be obtained with hydrolysates of egg albumin and silk fibroin. But with the work well under way, the research had to be terminated when the country suddenly found itself at war at the end of 1941.

With the advent of the war, Bergmann's laboratory undertook research for the Office of Scientific Research and Development (OSRD). Their specific mission was to investigate the physiological actions of vesicant war gases (mustard gas, nitrogen mustards) at the molecular level, with the hope of developing therapeutic agents that might be helpful in overcoming the effects of these compounds on the human body. The rationale for the work was that adequate defensive measures for preventing the effects of these toxic compounds, as well as the retaliatory capabilities of the United States and its allies, would inhibit the use of chemical warfare

agents. Fortunately, these agents were not used during the war.

While Bill Stein remained with Bergmann and his colleagues to conduct the research in New York, Stan enlisted in 1942 to serve as a technical aide on the National Defense Research Committee of OSRD to coordinate university and industrial efforts on the biological actions of chemical warfare agents. His base was in Washington, but he made frequent trips to Dumbarton Oaks, where the National Defense Research Committee had its offices. Later (1944), Stan was appointed to the Project Coordination Staff of the Chemical Warfare Service, which was directed by William A. Noyes, Jr. The experiences of the service were summarized in a volume published after the war, to which Stan (with W. R. Kirner) contributed an article on the physiological mechanisms of action of chemical warfare agents. When the war ended, Stan was in Hawaii with the Operational Research Section of the Chemical Warfare Service.

Max Bergmann died of cancer in 1944 at age fifty-eight. The war work of the laboratory, however, was continued by his associates until the end of hostilities in 1945. At that time most of them moved elsewhere, and the department that Bergmann had organized was dissolved. At this juncture Herbert Gasser, then director of the Rockefeller Institute, had the wisdom to offer Bill Stein and Stan Moore space in the former Bergmann department, along with the opportunity—on a trial basis—to continue the work on amino acid analysis that they had initiated before the war.

In the interim the collaborative efforts of A. J. P. Martin and R. L. M. Synge and their associates in England produced novel fractionation techniques, notably partition chromatography. Bill and Stan were aware of this research, although during the war journals arrived from England rather irregularly. When their collaboration was renewed in 1945, they

decided to explore the possibilities afforded by partition chromatography for determining the amino acid compositions of proteins. Their work took place in parallel with that of Lyman C. Craig, whose laboratory was located on the same floor and who had been exploring the potential of countercurrent distribution in the fractionation of peptide antibiotics.

As a starting point, Bill and Stan decided to develop a column chromatographic method based on work by S. R. Elsden and Synge (1941), who had demonstrated that useful separations of amino acids and peptides could be obtained with potato starch as the matrix and various two-phase mixtures of the lower alcohols, such as *n*-butanol, with aqueous organic acids as the eluant. To render the procedure quantitative, a suitable micro method for the determination of amino acids in the column effluent was required. To this end, Bill and Stan studied the ninhydrin reaction, known since its discovery in 1911 to result in the formation of colored products from all amino acids. They discovered that reproducible yields of the product could be obtained when the reaction was conducted in the presence of a reducing agent, initially stannous chloride.

To monitor the progress of the separations effected on the starch columns, the eluate was collected in small fractions of equal volume; these were treated with ninhydrin under reducing conditions, and the colored products were measured spectrophotometrically. The concentrations of colored product in each fraction were plotted against fraction number to obtain a so-called effluent-concentration curve. The area under each peak on such curves gave the amount of amino acid in the sample.

Initially the fractions were collected manually, but the labor involved quickly led to the design and construction of an instrument in which each drop of effluent from a column was

made to interrupt a light beam incident on a photocell, thereby incrementing a counter. Drops were collected into spectrophotometer tubes. When a predetermined number of drops had been collected, a turntable advanced to bring a new tube into line. Although this instrument was not the first fraction collector to be described, it became the prototype for the commercial instruments that soon appeared thereafter in laboratories around the world.

With these developments it became possible to refine the chromatographic procedures themselves. In the methods ultimately described in 1949, three runs were required to determine all the amino acids in a protein hydrolysate. Bill and Stan described the application of the method to the determination of the compositions of beta-lactoglobulin and serum albumin. The three runs required a total of less than 5 milligrams of protein, with a standard error of less than 5 percent, a remarkable achievement at the time. Recognizing the impact this methodology would have in biochemistry, Bill and Stan went to considerable lengths to provide detailed descriptions of all the necessary steps for the successful application of their procedures in other laboratories. Most of this information was circulated in the form of preprints, well in advance of publication, to anyone desiring access to it. They were to repeat this service to the biochemical community many times in subsequent years as improvements in methodology were made.

In 1949 Herbert Gasser decided that Moore and Stein had demonstrated the competence as independent investigators that he had hoped they would develop. As a result the research budget for their laboratory was increased substantially. This permitted the recruitment of postdoctoral associates and additional technical assistants over the next several years, increasing the scope of the research. Space limitations preclude mention of the numerous students and postdoc-

toral associates who from this time onward were affiliated with the Moore and Stein laboratory. The interested reader is referred to the bibliography of this memoir for further information.

Although the starch column procedures represented a breakthrough of the utmost significance in protein chemistry, there were some limitations. First, there was the slow flow-rate of the columns (one complete analysis of a protein hydrolysate required two weeks). Moreover, a fresh column had to be prepared for each run, and the separations were sensitive to the presence of salts in the sample. Bill and Stan therefore decided to investigate ion-exchange chromatography on sulfonated polystyrene resins as an alternative. They were encouraged by the success attained by S. Miles Partridge in England in the preparative-scale fractionation of amino acids by displacement chromatography on such resins.

Effective separations of all the amino acids in a protein hydrolysate in a single run were quickly obtained by elution with sodium citrate and acetate buffers of increasing pH and concentration at various temperatures, but a great deal of painstaking effort was required to standardize the performance of the columns. The problems were finally overcome when more reproducible resins became available. The successful development of the ion-exchange methodology not only allowed a considerable reduction in the time required for analysis of a protein hydrolysate, but for the first time it permitted reliable analyses of the amino acid content of various physiological fluids: urine, plasma, and protein-free extracts of tissues. These methods also resulted in the discovery and estimation of new components of these fluids.

Concurrently, the potential of ion-exchange chromatography for the separation of peptides and proteins was developed. It was soon found that certain stable, basic proteins—notably bovine pancreatic ribonuclease and chymotrypsino-

gen, and egg-white lysozyme—could be chromatographed effectively on IRC-50, a polymethacrylic acid resin. The elution of these proteins from the exchanger occurred in a predictable way in response to changes in pH and ionic strength. Somewhat later the successful fractionation of histones from calf thymus was achieved.

Encouraged by these successes, in 1953 Bill and Stan felt the time had arrived to embark on the structural analysis of a protein. It should be recognized that when the events recorded here were taking place, little more was known about the fundamental chemical structure of proteins than in Emil Fischer's time. It was only in 1948, when Frederick Sanger and his students began elucidating the primary structures of the polypeptide chains in insulin, that convincing evidence was at hand to demonstrate that proteins have unique amino acid sequences. Sanger's success with the insulin chains (21 and 30 residues) showed that the structure of a polypeptide could be deduced from the sequences of smaller peptides derived from it by selective, partial hydrolysis with weak acid or enzymes. Sanger's work also emphasized the problems of separating the complex mixtures of peptides that resulted from such hydrolysis. It was clear, however, that the determination of the primary structure of longer polypeptide chains would be difficult, if not impossible, by the methods used with the relatively small polypeptide chains of insulin.

With this background the choice of a protein became a critical decision for Moore and Stein. They elected to investigate the small enzyme ribonuclease, which they had already studied, arguing that knowledge of its structure would almost certainly aid in understanding its enzymic activity. Their work was performed in parallel with that of Christian B. Anfinsen and his colleagues in Bethesda, but the approaches of the two laboratories were different and they functioned on a collaborative rather than competitive basis. Indeed, neither

Bill nor Stan relished competition in science, although they recognized its value in expediting progress.

The investigation of the structure of ribonuclease started with a sample of the oxidized protein that was hydrolyzed selectively with the proteolytic enzyme trypsin. The resulting mixture of peptides was separated by ion-exchange chromatography on a column of sulfonated polystyrene resin by procedures similar to those used earlier for the separation of amino acids. The compositions of these peptides showed that the entire sequence (124 residues) of ribonuclease was represented. To establish how these peptides originated, the oxidized enzyme was next hydrolyzed with chymotrypsin, a protease with a selectivity different from that of trypsin, to produce a second set of peptides that were likewise separated on sulfonated polystyrene. From the known selectivities of trypsin and chymotrypsin, largely elucidated years earlier by Bergmann and his colleagues, the order of the tryptic peptides in the polypeptide chain was deduced. Confirmation was obtained from another set of peptides isolated from a peptic hydrolysate.

As this work proceeded, it was evident that progress was limited by the rate at which amino acid analyses could be performed. With the manual methods then in use, a single run required almost three days and several hundred spectrophotometer readings. Thus, work was initiated in 1956 to develop automated amino acid analysis. It was only after extensive refinement of the instrumentation that the method was published in 1958. With the resins then available, the analysis time was shortened to twenty-four hours and the sensitivity permitted runs on as little as 0.5 micromole. Subsequent developments have resulted in a reduction for the time of analysis to an average of about an hour and increased sensitivity by two orders of magnitude. The benefits to our

knowledge of protein chemistry that became possible with the use of the Moore and Stein analyzer have been incalculable.

It is a testimonial to the standards of excellence of Moore and Stein that both the original fraction collector they devised and the automated amino acid analyzer described in 1958 remain in perfect working order, the former now in the museum at Caspary Hall, the latter still in the laboratory in which it was assembled at the Rockefeller University.

The complete covalent structure of ribonuclease was published in 1963, the first such structure for an enzyme. It was then decided to investigate the inactivation of ribonuclease by iodoacetate. In a series of investigations in which the progress of the reaction at different pH values was followed by amino acid analysis, they showed that inactivation at pH 5 was the result of carboxymethylation on nitrogen-1 of histidine-119 or nitrogen-3 of histidine-12, but not at both sites of the same ribonuclease molecule. Inactivation at lower pH values was found to be caused by reactions with methionine residues; at higher pH by reaction with lysine-41. In this way they were able to propose that histidines-12 and -119 are close to one another in the active site of ribonuclease. This proposal, which proved to be central in further investigations of ribonuclease, was subsequently confirmed in other laboratories by x-ray analysis, and it permitted interpretation of kinetic studies and nuclear magnetic resonance work elsewhere that led ultimately to a detailed explanation of the mechanism of action for the enzyme.

The work on ribonuclease was recognized by the awarding of the Nobel Prize in Chemistry for 1972 to Moore and Stein, jointly with Anfinsen. Other honors are listed elsewhere.

As the number of students and postdoctoral associates in

the Moore-Stein laboratory expanded, the scope of the work enlarged correspondingly. Here we can only mention some of these investigations. These included the determination of the amino acid sequence of pancreatic deoxyribonuclease; investigation of the reaction of cyanate ion with proteins; structural studies with pepsin; the mechanism of action and structure of streptococcal proteinase; studies of the sequence and the active site of ribonuclease T1; the isolation of 2′,3′-cyclic nucleotide 3′-phosphohydrolase and its inhibitor from brain; studies on ribonuclease inhibitors; and many studies on modifications of pancreatic ribonuclease. In addition there were many investigations by younger associates and colleagues in their laboratory at the Rockefeller University on which the names of Moore or Stein do not appear.

From the foregoing and the appended bibliography, it is evident that the collaboration of Moore and Stein continued at the Rockefeller even after Bill Stein suffered a crippling paralysis in 1969. The biographical memoir on William Stein was the last publication submitted by Stanford Moore, just a month before his own death. In this memoir Moore comments, "During the early years of our cooperation, Stein and I worked out a system of collaboration which lasted for a lifetime. Stein combined an inventive mind and a deep dedication to science with great generosity. Over a period of forty years, we approached problems with somewhat different perspectives and then focused our thoughts on the common aim. If I did not think of something he was likely to, and vice versa, and this process of frequent interchange of ideas accelerated progress in research. It also helped in writing papers; I never drafted a text that Stein could not improve." We include this quote to indicate that we cannot attempt to judge which of the two was responsible for specific contributions to their joint accomplishments and those with their collaborators.

With the exception of the war years (1942–45), Moore was away from the Rockefeller for only a year, beginning in 1950. He spent half the time in Brussels, Belgium, setting up a laboratory devoted to amino acid analysis, and half the year in Cambridge, England, sharing a laboratory with Frederick Sanger when the work on the amino acid sequence of insulin was proceeding. Stan felt that this year in Europe was important both in his development as a scientist and in furthering his interest and collaboration in international scientific activities.

Stan served the community of biochemists well as an editor, as an officer of the American Society of Biological Chemists, and as chairman of the Organizing Committee for the International Congress of Biochemistry held in New York in 1964. The Congress was a memorable event in its organization of scientific presentations and its gracious and efficient hospitality. During this Congress Stan began the custom of inviting eight to ten guests for breakfast and lunch in his suite each day, so that fellow scientists could meet in intimate surroundings with their colleagues. He continued this practice for another fifteen years at international congresses and at the annual meetings of the American Society of Biological Chemists. Only his declining health forced a termination of this custom.

Stanford Moore was intensively and single-mindedly devoted to science. He consciously avoided activities that did not involve science and scientists. This lifelong bachelor was an early riser, and he was at his desk or in the laboratory throughout the day and on the weekends. Yet he was a gracious and hospitable host to his scientific friends and associates. Nevertheless, he was a conscientious citizen, and for about a year in the 1960s he served on a grand jury in New York hearing testimony on the activities of organized crime (the famous case of the Cosa Nostra). Typical of Stan, after a

day of hearings, he would spend the evenings and the weekends in the laboratory to keep up with his scientific work.

It should be noted that none of the methods and instruments designed by Moore and Stein was patented. Personal profit was far from their minds. Indeed, Stan Moore had little interest in personal possessions; his small office and bachelor apartment had the minimally effective furnishings. Stan's obsessive neatness both of person and surroundings was legendary.

In the last two years of life, as his health deteriorated, Stan lived with the awareness of progressive nerve and muscle degeneration from amyotrophic lateral sclerosis. He kept this knowledge to himself as long as possible, but characteristically finished his writing obligations, disposed of many of his personal possessions, and left his papers and files in a meticulously organized condition. He died in his apartment, a short distance from his beloved laboratory at the Rockefeller University where he had spent so many fruitful and satisfying years.

Stan's loyalty to the Rockefeller University and his devotion to biochemistry were reflected in his will, in which he bequeathed his estate "to be used as endowment toward the salary or research expenses or both of an investigator in the field of biochemistry." As Stan stated in a letter to President Joshua Lederberg of the University, which was delivered after his death, "I would like (to the best of my modest ability) to help a young scholar have the same opportunity that I had."

Although Stan had requested that no memorial service be held, his friends and former associates felt that he should be honored. This was done at "A Symposium on Protein Chemistry in Tribute to Stanford Moore" at the Rockefeller University on November 4, 1983, at which former members of the Moore-Stein laboratory presented their latest studies and

others spoke on the contributions of these two memorable individuals.

IN PREPARING THIS MEMOIR we have used the brief autobiographical notes deposited in the Academy files by Stanford Moore. We are indebted to Joshua Lederberg, James M. Manning, and C. R. Park for their helpful suggestions and for supplying some information.

AWARDS, HONORS, AND DISTINCTIONS

CHRONOLOGY

1913	Born, September 4, Chicago, Illinois
1935	B.A., Summa cum laude, Vanderbilt University
1938	Ph.D., University of Wisconsin
1939–42	Rockefeller Institute for Medical Research (later the Rockefeller University): Assistant, 1939–42; Associate, 1942, 1945–49
1942–45	National Defense Research Committee, Office of Scientific Research and Development (later the Chemical Warfare Service)
1947–49	Chairman, Panel on Proteins, Committee on Growth, National Research Council
1949–52	Associate Member, The Rockefeller Institute
1952–82	Professor and Member, The Rockefeller University
1950	Francqui Chair, University of Brussels
1950–60	Editorial Board, *Journal of Biological Chemistry*
1953–57	Secretary, Commission on Proteins, International Union of Pure and Applied Chemistry
1961–64	Chairman, Organizing Committee, Sixth International Congress of Biochemistry, New York (1964)
1968	Visiting Professor, Health Sciences, Vanderbilt University School of Medicine
1970–71	President, Federation of the American Societies for Experimental Biology
1974–82	Trustee, Vanderbilt University
1982	Died, August 23, New York City

SELECTED MEMBERSHIPS

American Society of Biological Chemists (Treasurer, 1956–59; President, 1966–67)
American Chemical Society
Belgian Biochemical Society (Honorary Member)
Belgian Royal Academy of Medicine (Foreign Correspondent)
Biochemical Society (Great Britain)
National Academy of Sciences (Elected, 1960; Chairman, Section of Biochemistry, 1969–72)

American Academy of Arts and Sciences (Elected, 1960)
Harvey Society

HONORARY DEGREES

Docteur *honoris causa*, Faculty of Medicine, University of Brussels, 1954
Docteur *honoris causa*, University of Paris, 1964
Dr.Sc., University of Wisconsin, 1974

AWARDS SHARED WITH WILLIAM H. STEIN

American Chemical Society Award in Chromatography and Electrophoresis, 1964
Richards Medal, American Chemical Society, 1972
Linderstrøm-Lang Medal, Copenhagen, 1972
Nobel Prize in Chemistry, 1972

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