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WILLIAM H. STEIN

1911—1980

A Biographical Memoir by

STANFORD MOORE

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Biographical Memoir

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William H. Stein

WILLIAM H. STEIN

June 25, 1911–February 2, 1980

BY STANFORD MOORE

WILLIAM H. STEIN began his autobiographical sketch for the 1972 volume of Nobel lectures as follows:

> I was born June 25, 1911, in New York City, the second of three children, to Fred M. and Beatrice Borg Stein. My father was a businessman who was greatly interested in communal affairs, particularly those dealing with health, and he retired quite early in life in order to devote his full time to such matters as the New York Tuberculosis and Health Association, Montefiore Hospital, and others. My mother, too, was greatly interested in communal affairs and devoted most of her life to bettering the lot of the children of New York City. During my childhood, I received much encouragement from both of my parents to enter into medicine or a fundamental science.

His early education was at the Lincoln School of the Teachers College of Columbia University. It was a so-called "progressive school" of the time; in addition to fostering interest in the creative arts, music, writing, and sports, the curriculum included well-taught courses in chemistry, physics, and biology, with field trips that he enjoyed. From those years, he used to recall that his first scientific project as a student was as an avid collector of moths and butterflies. At sixteen, he transferred to a preparatory school in New England, Phillips Exeter Academy, which offered a demanding educational experience that he felt strengthened his work habits and his precision of writing.

In 1929 he matriculated at Harvard, as had his father and his older brother before him. He majored in chemistry; the scientific background thus achieved led him to spend one year as a graduate student at Harvard in chemistry. It was suggested to him, however, that he might enjoy the developing subject of biochemistry more than organic chemistry *per se*. As a result, in 1934 he transferred to the Department of Biological Chemistry at the College of Physicians and Surgeons, Columbia University. He found the environment most challenging. Hans Clarke, the chairman, had succeeded in gathering a stimulating faculty and a group of unusually gifted graduate students from around the world. Nearly a dozen of those students became outstandingly productive biochemists.

During his graduate-student days, in 1936, William Stein married Phoebe Hockstader. His wife and their three sons—William H., Jr.; David F.; and Robert J.—were to be invaluable resources for a creative scientist throughout a career in which science and family were intimately interwoven. Stein lived on Manhattan most of his life, with an interlude in Scarsdale, New York, while the children were of school age. He enjoyed summer retreats both at Cos Cob, with opportunities for tennis and swimming, and, in the later years, at Woodbury, in Connecticut.

William Stein completed his Ph.D. thesis in 1937, under the guidance of E. G. Miller, Jr.; the subject was the amino acid composition of elastin. Thus began a lifelong concern with the chemistry of proteins. His first experiment was the preparation of elastin from the ligamentum nuchae of the ox. In the course of applying some of the gravimetric methods of the time, he used two precipitants that had been developed by Max Bergmann—potassium trioxalatochromiate for glycine and ammonium rhodanilate for proline. He was introduced to these methods by Erwin Brand at Columbia, who had worked with Bergmann in Germany.

Bergmann had arrived in the United States from Dresden in 1934 to become a member of The Rockefeller Institute for Medical Research in New York. When Stein completed his studies for the Ph.D. degree at Columbia in 1937, it was a logical progression for him to move southward on Manhattan to join the Bergmann group. Again, Stein found himself in an exceptionally stimulating environment; Bergmann was attracting a talented international group of postdoctoral assistants, many of whom became prominent biochemists. Among the current Academy members from this group are Joseph S. Fruton, Emil L. Smith, Klaus Hofmann, and Paul Zamecnik.

There were two main lines of investigation in the Bergmann laboratory: the specificity of proteolytic enzymes and the structural chemistry of proteins. Stein initially applied his talents to the task of trying to improve gravimetric methods for amino acid determination. His first contribution to methodology was his introduction of the concept of the solubility product method in an attempt to permit quantitative results to be obtained with reagents that gave sparingly soluble salts of amino acids. Stanford Moore joined the Bergmann laboratory in 1939, arriving via Vanderbilt and Wisconsin. Bergmann suggested that Stein and Moore pool their efforts to see whether the solubility product method could be developed into a practical analytical procedure. Through careful attention to the details of gravimetric analysis, using two of the reagents introduced by Bergmann, they were able to determine glycine with 5-nitronaphthalene-1-sulfonic acid as the precipitating agent and leucine with 2-bromotoluene-5-sulfonic acid. The method was applied to hydrolysates of egg albumin and silk fibroin. But the future was to provide methods that were to be less tedious and more micro.

At this stage, the research on amino acid analysis was interrupted by the war years. The laboratory was engaged

under contract to the Office of Scientific Research and Development to look for possible therapeutic agents for vesicant war gases through study of the physiological mechanisms of action of mustard gas and the nitrogen mustards. Stein was a coauthor of a series of fundamental papers concerned with the chemistry of the reactions of mustard gas and related compounds with the functional groups of amino acids and peptides.

During the war years, in 1944, illness took the life of Max Bergmann at the age of fifty-eight. The members of the laboratory carried the war work to completion in 1945, at which time most of them moved on to other positions.

For three years, Moore had been out of the laboratory serving the Office of Scientific Research and Development in administrative capacities in Washington and on other wartime assignments. Stein and Moore debated whether to accept appointments elsewhere or to ask the Director of The Rockefeller Institute, Herbert S. Gasser, whether he would give them a chance to see what they could accomplish on the Rockefeller scene. Gasser offered the two young investigators an opportunity, on a trial basis, to initiate a research program that might merit continued support.

With that challenge, they started with the premise, born of the Bergmann years, that accurate establishment of the amino acid compositions of proteins is a first step toward progress in determination of their chemical structures. In 1945 it was possible to take a completely new look at the problem of amino acid analysis. The renaissance in chromatography stimulated by A. J. P. Martin and R. L. M. Synge in England, together with Lyman C. Craig's development of liquid-liquid countercurrent distribution in his laboratory just down the corridor from the Bergmann department at Rockefeller, brought to the attention of biochemists the potential resolving power of multi-plate separations. After

weighing the possibilities for speed, resolution, simplicity, and quantitativeness, Stein and Moore decided to try column chromatography. Thus began several busy years of close collaborative effort on the development of methods and equipment.

After initial experiments in which the fractions were collected by hand, a photoelectric drop-counting fraction collector was built to expedite the collection of the effluent in a series of small fractions of precise volume; it was the prototype for the commercially built fraction collectors based upon this principle that became widely used in biochemistry. Then a simple and sensitive quantitative method for measuring the concentration of amino acid in each tube was needed. The ninhydrin reaction had been introduced by Ruhemann in 1911. The blue-colored product is sensitive to oxidation; in initial trials the results did not obey Beer's law. When the reaction was carried out anaerobically, the yield was improved and linearity was approached, but such a procedure was inconvenient. An oxygen-free environment in solution in an open tube was attained by including a dissolved reducing agent, such as stannous chloride or the reduced form of ninhydrin (hydrindantin). A water-miscible organic solvent (first, methyl Cellosolve, and later, dimethyl sulfoxide) was added to keep the blue-colored reaction product (diketohydrindylidene-diketohydrindamine) and hydrindantin in solution. This method of measurement was first used to monitor the peaks eluted from columns of potato starch operated with *n*-butanol-water as the solvent system, a type of chromatogram first tried by Synge. With a neutral solvent, the use of a preliminary wash with 8-hydroxyquinoline was found essential to prevent metals in the starch from distorting the separations. Over a period of several years, quantitative systems with starch columns were developed for determining all of the common amino acids of protein hydrolysates and were

applied to the analysis of β-lactoglobulin and bovine serum albumin in 1949. The results were welcome, but it took two weeks to run the three starch columns required.

Faster analyses became possible when finely powdered ion exchange resins became available for chromatography. With sulfonated polystyrene resins, several years of exploratory chromatograms led to the use of buffers of different pHs at different temperatures for the serial elution of all of the common amino acids of proteins and of physiological fluids. In the early 1950s, the time for each analysis was reduced to about five days.

With an efficient chromatographic method at hand, the next stage was to render the process automatic. This project in instrumentation was undertaken in cooperation with Darrel Spackman and led to an automatic amino acid analyzer in 1958. The eluent was pumped through the resin bed at several atmospheres of pressure. The ninhydrin color was developed in the flowing stream and the optical density was recorded potentiometrically; a hydrolysate was analyzed in an overnight run. Subsequent academically and commercially introduced improvements have utilized finer resins and higher pressures and have attained sixty-minute analyses. The resulting amino acid analyzers found a worldwide market and represented the first widely used form of high-performance liquid chromatography.

In the writing of the papers on methodology every effort was made to include all of the details needed for effective use of the methods. This enterprise was facilitated by circulating preprints to biochemists who expressed an immediate interest in using the procedures and who could check the completeness of the experimental directions in advance of publication.

During the early years of our cooperation, Stein and I worked out a system of collaboration that 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 the writing of papers. I never drafted a text that Stein could not improve.

The methodology was developed with the primary aim in mind of opening new approaches to the study of the chemical structures of proteins. After the first four years of the above studies, Gasser decided that the two young investigators were making enough progress to merit being hosts to a postdoctoral fellow. In 1949, they attracted Werner Hirs from Columbia University. A key decision at that time was the choice of the protein to study. In England, Frederick Sanger had his classic studies on insulin well under way, for which he was able to use qualitative methods in large part. The study of longer polypeptide chains would gain from quantitative analyses at each step. And an enzyme would be an appealing subject for study because the structural knowledge could provide a baseline for determination of specific residues involved in the enzyme-substrate interactions. Bovine pancreatic ribonuclease, a protein about twice the size of insulin, was readily available and had been partially purified at Rockefeller by Dubos and Thompson and by Kunitz. Hirs extended the technique of ion exchange chromatography to ribonuclease on a polymethacrylic acid resin.

In 1952 Gasser decided that Stein and Moore qualified as members of The Rockefeller Institute for Medical Research; the title became professor when the institution assumed its role in graduate education as The Rockefeller University under the administration of Detlev Bronk.

The research on chromatographically purified RNase A

was then extended, with Hirs, to the development of methods for the ion exchange chromatography of peptides obtained by tryptic and chymotryptic hydrolysis of the chain in which the four disulfide bonds had been split by oxidation. J. Leggett Bailey joined the project to study the peptides liberated by pepsin. Thus began the collection of data from which a sequence for the enzyme could be deduced, with the invaluable additional aid of the sequential degradation reaction newly introduced by Pehr Edman in Sweden in 1950.

At the time that these studies on ribonuclease were begun, Christian B. Anfinsen and his associates at NIH also turned to ribonuclease as an appropriate molecule for structural study; the combined results from the two laboratories (experiments in progress were freely discussed) expedited the solution of the problem. The determination of the final sequence, to which Darrel Spackman and Derek Smyth were contributors at Rockefeller, also depended upon a key observation at NIH by Erhard Gross and Bernhard Witkop, obtained through the application of their ingenious method of cleavage at methionine residues by cyanogen bromide. Thus, for the first time, the chemical formula of an enzyme could be written.

Derivatization experiments were then undertaken at Rockefeller in order to identify residues at or near the active site. Iodoacetate was the first reagent studied. The enzyme was known to be inactivated by the reagent; at that time rapid reaction with iodoacetate was thought of primarily as an indication of –SH groups. When it was established that ribonuclease did not have any –SH groups, an evident task was to ascertain what was happening. Through experiments begun by Gerd Gundlach, amino acid analysis was used to show that, depending upon pH, the reagent could alkylate primarily methionine, histidine, or lysine residues in the enzyme. Arthur Crestfield showed that in thirty minutes at pH 5.5, the principal reaction was with the imidazole group of

one of two specific histidine residues, yielding a carboxymethyl group either on the 1-nitrogen of histidine-119 or on the 3-nitrogen of histidine-12. Through Robert Heinrikson's data on the effect of carboxymethylation of the ε-NH_2 group of lysine-41 on these reactions, it was possible to conclude that in the three-dimensional structure of the enzyme the reactive nitrogens of histidine-12 and histidine-119 were about 5 Ångstroms apart at the active site of the catalyst and that the ε-NH_2 group of lysine-41 was 7-10 Ångstroms from nitrogen-3 of histidine-12. These three-dimensional predictions, made on chemical grounds, were borne out by the subsequent x-ray crystallographic analyses of Frederic Richards and Harold Wyckoff at Yale.

One of the questions posed to George Stark was whether these two uniquely reactive histidine residues would still be especially reactive toward iodoacetate if the molecule were unfolded in 8 M urea. As expected, they are not. But in one of these experiments he detected, by amino-acid analysis, a side reaction that turned out to be carbamylation of lysine residues by traces of cyanate in the urea to give homocitrulline. This observation served as one of several reminders that, as demonstrated in 1828 by Wöhler, ammonium cyanate and urea are in equilibrium. One of those thus reminded was Anthony Cerami, then a student in another laboratory at Rockefeller. Some years later, when he heard that urea was being administered to patients with sickle cell anemia, he wondered whether cyanate merited consideration as the possible active agent in such an experiment. He elicited the cooperation of James Manning, who had recently joined the Stein and Moore laboratory. From the investigations of the two young men, there grew a decade of research on the effectiveness of the carbamylation of hemoglobin S in converting the molecule to one of almost normal physiological function.

From RNase A, with 124 amino acid residues, attention

was turned to bovine pancreatic deoxyribonuclease; chromatography on phosphocellulose yielded a homogeneous preparation of DNase A, which proved to be a glycoprotein with a single peptide chain of 257 residues. The sequence of DNase A was established in 1973 as a result of several years of researches by Paul Price (as a graduate student), Teh-yung Liu, Brian Catley, Johann Salnikow, and Ta-hsiu Liao. Additional experiments were conducted by Tony Hugli, Bryce Plapp, and Dalton Wang. The result was a thorough knowledge of the chemistry of the enzyme, its existence in four chromatographically distinct forms (A, B, C, and D), and identification of special features of each isozyme.

Stein, throughout his life, in his generous manner, took a special interest in facilitating the careers of scholars whose sojourns in the laboratory made possible the exploration of many facets of the researches. A number of enzymes were the subjects of studies of specific aspects of protein structure and function. For seventeen years, Stein was the principal investigator on a grant from the National Institute of General Medical Sciences, NIH, to study that subject. Some of the enzymes, in addition to RNase and DNase, that the laboratory studied with partial support from that grant were: bromelains (with Shoshi Ota), chymotrypsin (with Denis C. Shaw), pepsin (with T. G. Rajagopalan, T. A. A. Dopheide, and Roger Lundblad), streptococcal proteinase (with Teh-yung Liu, William Ferdinand, Brenda Gerwin, Norbert Neumann, Michael C. Lin, and Michael Bustin, in cooperation with the enzyme's discoverer, Stuart D. Elliott), ribonuclease T_1 (with Kenji Takahashi), 2′,3′-cyclic nucleotide 3′-phosphohydrolase from brain (with Arabinda Guha, David C. Sogin, and Robert J. Drummond), and carboxypeptidase Y (with Rikimaru Hayashi).

Stein took a particular interest in the application of the chromatographic methods to the analysis of physiological

fluids for amino acids. One of his earliest uses of the procedure was in a quantitative study of the major ninhydrin-positive constituents of human urine. He extended his studies to cystinuria, and the laboratory, in collaboration with Alexander Bearn of The Rockefeller Hospital, investigated the aminoaciduria of Wilson's disease. Part of that study required adaptation of the method to the measurement of blood plasma amino acids as well. In cooperation with Harris Tallan, the free amino acids of mammalian tissues were also surveyed in detail. Out of these several studies grew the identification of 3-methylhistidine and tyrosine-O-sulfate as normal constituents of human urine, and the observations that acetylaspartic acid is a major metabolite in brain and that cystathionine is a principal amino acid in human brain. In a study with Alejandro C. Paladini, phenylacetylglutamine was found to be a normal major metabolic product in human urine.

One of the first applications of the amino acid analysis procedure was to human hemoglobin A prepared electrophoretically by Henry Kunkel of The Rockefeller Hospital; the absence of isoleucine was demonstrated to be one criterion for the purity of this protein. That study led to a reexamination (with R. David Cole) of the cysteine content of human hemoglobin.

Stein combined his research efforts at Rockefeller with service in a number of capacities on the national and international scenes. He was a visiting professor at the University of Chicago in 1961, and at Harvard University in 1964. His lectures there, throughout his travels, and to graduate students at Rockefeller conveyed an exciting picture of the horizons that new methods were opening in the study of the chemical structure of proteins. He was a member of the Medical Advisory Board of Hebrew University-Hadassah Medical School, Israel, 1957–70; trustee of Montefiore Hospital in

New York, 1947–74; and member of the Council of the Institute of Neurological Diseases and Blindness of NIH, 1961–66. He was vice-chairman of the U.S. National Committee for the International Union of Biochemistry, 1962–63, chairman, 1965–68, and chairman of the Publications Committee for the Sixth International Congress of Biochemistry held in New York in 1964. He and his wife enjoyed the worldwide travel that was an integral part of his career and the opportunity to host the scholars from many countries who came through New York City in the course of their journeys. His major pastimes were directly related to the life of a scientist with international interests.

The American Society of Biological Chemists drew upon his editorial skill, beginning in 1955, when he was elected to the Editorial Committee. He was chairman of the Committee from 1958 to 1961. He was an active participant in the search that led to the appointment of John Edsall to the editorship of *The Journal of Biological Chemistry* in 1958, upon the retirement of Rudolph J. Anderson. Stein joined the Editorial Board of the *Journal* in 1962. In his drafts of editorial letters he was quick to praise a fine manuscript, to decline an inadequate one, and careful to explain in gracious detail the options for revision when that seemed necessary. Two years later he was asked by Edsall to assume one of the three associate editorships.

As Edsall's ten-year term as editor drew toward a close, Stein was asked by the Council of the Society to consider the position. He accepted and took a leading part in setting up the administrative procedures that would facilitate the handling of the increasing numbers of manuscripts that were being received as the *Journal* grew in size. The changes included the organization of a permanent central office at the Society's headquarters in Bethesda to which all manuscripts would go initially. Stein had earlier had a key role in the

recruitment of Robert A. Harte as full-time executive secretary of the Society; Harte was made business manager of the *Journal* and Edith Wolff was the first executive assistant. A staff was thus established to handle the innumerable business details associated with receiving more than 3,000 manuscripts annually and managing the publication of editorially acceptable texts.

Stein's foresight in centralizing the first step in the editorial process, with the view of facilitating the transfer of the editorship to the next recipient, was tested—tragically—much too soon. He was stricken by major illness in 1969, after a year and a half in the position.

In 1969, while in attendance at an international symposium on proteolytic enzymes being held in Copenhagen, Stein developed a high fever that prevented him from giving the paper he was scheduled to present. A few days later, en route home by air, paralysis began. He barely survived the acute phase of the disease, which was diagnosed as a severe case of Guillain-Barré syndrome. After a year of hospitalization, he remained a quadriplegic. He met this tragedy with great courage and with preservation of his sense of humor. His wife and their three sons helped him immeasurably to meet the almost unbelievable frustrations of a disabled person possessing the intellectual drive of a creative brain that was fully functional to the last day. The Rockefeller University, under the administrations of Frederick Seitz and Joshua Lederberg, was host to Stein's determined endeavors during eleven years of this difficult existence. He, of course, had to relinquish the editorship of the *Journal* in 1969, but he continued to provide welcome advice, both on specific manuscripts and on matters of policy, to Herbert Tabor, his successor.

Stein had the pleasure of seeing the subject of RNase grow in interest rather than taper off. When the laboratory's work

on the enzyme was begun, the protein was viewed as a catalyst of rather limited physiological interest; it was recognized as one of the enzymes of the digestive tract. Work from several laboratories on the presence of RNases of the pancreatic type in most mammalian cells and of a widely distributed specific inhibitor of the enzyme broadened the scope of the subject. Stein followed with enthusiasm the researches of the young associates in the laboratory on the isolation of the inhibitor in pure form from human placenta and the establishment of its molecular properties. He made the uncomfortable journey by wheelchair to his office at Rockefeller whenever he felt able, and he was a valuable consultant to all of the members of the laboratory. On occasion, they would meet at his home for informal seminars on current research. He took an active interest in reading manuscripts and his editorial skill was always helpful. With the stimulating cooperation of Phoebe Stein, their home continued to be the scene of visits by scientists from around the world who enjoyed the opportunity to discuss both biochemistry and the issues of the times, which he continued to analyze with keen perception.

A life of sixty-eight years filled with unusual measures of accomplishment, acclaim, and suffering came to a close on February 2, 1980, when William Stein died suddenly from heart failure at his home in New York.

Among the many honors that Stein received were election to the National Academy of Sciences in 1960 and to the American Academy of Arts and Sciences in the same year. Awards that he shared with Moore were the American Chemical Society Award in Chromatography and Electrophoresis (1964), the Richards Medal of the American Chemical Society (1972), the Kaj Linderstrøm-Lang Award, Copenhagen (1972), and the Nobel Prize in Chemistry (1972), shared also

with Christian B. Anfinsen. Academic honors included D.Sc. *honoris causa* from Columbia University (1973), D.Sc. *honoris causa* from the Albert Einstein College of Medicine of Yeshiva University (1973), and the Award of Excellence Medal from the Columbia University Graduate Faculty and Alumni Association (1973).

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