

Classics

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The Fruits of Collaboration: Chromatography, Amino Acid Analyzers, and the Chemical Structure of Ribonuclease by William H. Stein and Stanford Moore

Photometric Ninhydrin Method for Use in the Chromatography of Amino Acids
(Moore, S., and Stein, W. H. (1948) *J. Biol. Chem.* 176, 367–388)

Chromatography of Amino Acids on Starch Columns. Separation of Phenylalanine, Leucine, Isoleucine, Methionine, Tyrosine, and Valine
(Stein, W. H., and Moore, S. (1948) *J. Biol. Chem.* 176, 337–365)

William H. Stein (1911–1980) graduated from Harvard in 1929 with a major in chemistry. He then spent a year as a graduate student in chemistry at Harvard but transferred to the Department of Biological Chemistry at the College of Physicians and Surgeons, Columbia University, to study biochemistry. He completed his thesis research on the amino acid composition of elastin in 1937 and joined Max Bergmann at The Rockefeller Institute for Medical Research in New York. Stein's initial project with Bergmann was to improve gravimetric methods of amino acid determination. In 1939, Stanford Moore (1913–1982), a graduate of Vanderbilt University who had just earned his Ph.D. from the University of Wisconsin, joined the Bergmann laboratory. In what marked the beginning of one of the longest and most fruitful collaborations in science, the two postdoctoral fellows pooled their efforts to develop the gravimetric methods based on the solubility product of salts of the amino acids into a practical analytical procedure.

Stein and Moore's research was soon interrupted by the war. To help the war effort, Moore enlisted to serve as a technical aid on the National Defense Research Council in Washington to coordinate academic and industrial projects on chemical warfare agents. Stein, on the other hand, remained in Bergmann's laboratory to aid in studies on the physiological actions of vesicant war gases at the molecular level. In 1944, Bergmann died of cancer, but the laboratory work continued until 1945 after which most of the lab members moved on to other positions. Moore and Stein, however, were offered an opportunity by the Director of The Rockefeller Institute, Herbert S. Gasser, to continue, on a trial basis, Bergmann's work on amino acid analysis.

The two immediately started working with the premise, developed in the Bergmann laboratory, that establishing the amino acid compositions of proteins was the first step towards the determination of their chemical structures. Earlier studies on chromatographic fractionation techniques by A. J. P. Martin and R. L. M. Synge and the work by Lyman C. Craig on countercurrent distribution mentioned in a previous *Journal of Biological Chemistry* (JBC) Classic (1) inspired Stein and Moore to explore the use of partition chromatography in determining protein composition. They settled on furthering a method developed by S. R. Elsdon and Synge that used potato starch as a column matrix and eluants of various two-phase mixtures of alcohols and aqueous organic acids to separate amino acids and peptides.

The two JBC Classics reprinted here describe how Stein and Moore used starch columns to separate and quantitate phenylalanine, leucine, isoleucine, methionine, valine, and tyrosine in synthetic mixtures of amino acids and hydrolysates of β -lactoglobulin and bovine serum albumin. The first Classic discusses the starch column procedure, the photoelectric drop-

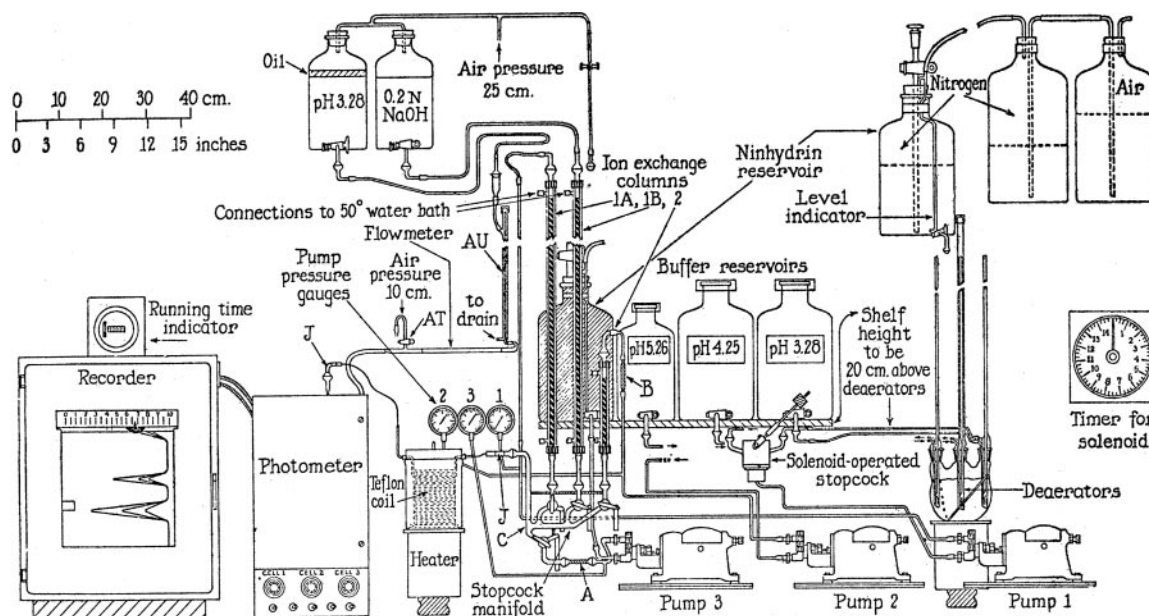


FIG. 1. Schematic of the automatic recording apparatus used in the chromatographic analysis of mixtures of amino acids. (Reprinted with permission from Ref. 6.)

counting fraction collector developed by Stein and Moore (the prototype for the fraction collectors in wide use today), and the quantitation of the amino acids.

To make their procedure quantitative, Stein and Moore needed a method to determine the amino acid composition of their fractions, which is the subject of the second JBC Classic. They used a variation on the ninhydrin reaction, discovered by Siegfried Ruhemann in 1911, in which ninhydrin reacts with NH_2 groups to form a blue product. Because the reaction is sensitive to oxidation, they created an oxygen-free environment in solution by including dissolved reducing agents such as stannous chloride or reduced ninhydrin (hydrindantin). They also added a water-miscible organic solvent, methyl Cellosolve (and later dimethyl sulfoxide) to keep the blue-colored reaction product in solution. The eluate was measured spectrophotometrically, and the concentration of product in each fraction was plotted against fraction number to produce an effluent-concentration curve. The area under each peak gave the amount of amino acid in the sample.

Over the next several years, Stein and Moore developed starch columns for quantifying all of the amino acids in protein hydrolysates and eventually applied their analysis to determine the compositions of β -lactoglobulin and bovine serum albumin (2). Because it took an average of 2 weeks to run the three columns necessary to analyze a single protein, Stein and Moore began to look at ways to decrease analysis time. They employed ion-exchange chromatography with sulfonated polystyrene resins and buffers with different salt concentrations and pH at different temperatures to reduce the time to 5 days by the early 1950s. Next, in collaboration with Daryl Spackman, they rendered the process automatic, creating the first amino acid analyzer in 1958. Fig. 1 shows a diagram of the automated system. Early on, they recognized the impact their discoveries would have in biochemistry and made every effort to provide detailed descriptions of their procedures for use in other laboratories. They facilitated this effort by widely circulating preprints to any biochemists who desired them.

In the early 1950s, Stein and Moore started using ion-exchange chromatography to separate peptides and proteins. Encouraged by their success, they decided to embark on the structural analysis of an entire protein. They chose ribonuclease, an enzyme about twice the size of insulin, which was the first protein to be fully sequenced as a result of the pioneering studies of Fred Sanger in Cambridge, England. They performed their work in parallel with Christian B. Anfinsen. To determine the sequence of ribonuclease, Stein and Moore first hydrolyzed the protein with trypsin and then separated the peptide mixture by ion-exchange chromatography. The peptide sequences were then analyzed by Edman degradation. By repeating this experiment with chymotrypsin and pepsin they were able to deduce the sequence of the entire protein, which they published in 1963 (3). Not content to stop at the sequence of ribonuclease, they also studied the inactivation of the enzyme by iodoacetate and were able to identify

residues in and around its active site. This work on ribonuclease was recognized in 1972 when Stein and Moore were awarded the Nobel Prize in Chemistry, which they shared with Anfinsen.

Stein and Moore, with many students and postdoctoral fellows, studied the structure/function relationships of a number of other proteins including pancreatic deoxyribonuclease, chymotrypsin, pepsin, streptococcal proteinase, ribonuclease T1, carboxypeptidase Y, and pancreatic ribonuclease.

Independently, Stein also applied chromatographic methods to the analysis of physiological fluids including urine and blood plasma, as well as human tissue. In addition to his research, Stein was extensively involved in service to the scientific community including the publisher of JBC, the American Society of Biological Chemists (ASBC, now the American Society for Biochemistry and Molecular Biology). Stein was elected to the ASBC Editorial Committee in 1955 and was chairman of the Committee from 1958 to 1961. He joined the JBC Editorial Board in 1962 and assumed one of the three associate editorships in 1964. Stein eventually became editor of JBC and played an active role in setting up administrative procedures to handle the increasing number of manuscripts the *Journal* was receiving, including organizing a central office at the Society's headquarters in Bethesda. Herbert Tabor, the current editor of JBC, succeeded Stein several years later. Unfortunately, Stein was found to have Guillain-Barré syndrome in 1969 and was a paraplegic for the rest of his life. However, Stein continued to remain actively involved in research at Rockefeller until his death in 1980.¹

Like Stein, Moore was very involved in service to the biochemistry community, including serving as President of the ASBC (1966–67), President of the Federation of American Societies for Experimental Biology (1970–71), and chairman of the Organizing Committee for the International Congress of Biochemistry in 1964. During this Congress he started the custom of inviting eight to ten scientists for breakfast or lunch in his suite so that they could meet their colleagues in intimate surroundings. He continued this practice for another 15 years at both international congresses and ASBC meetings. Moore's loyalty to the Rockefeller University and devotion to biochemistry were ultimately reflected in his will, which bequeathed his estate to the university to be used as an endowment to support an investigator in the field of biochemistry.²

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¹ All biographical information on William H. Stein was taken from Ref. 4.

² All biographical information on Stanford Moore was taken from Ref. 5.