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RECOLLECTIONS

How my interest in proteins developed

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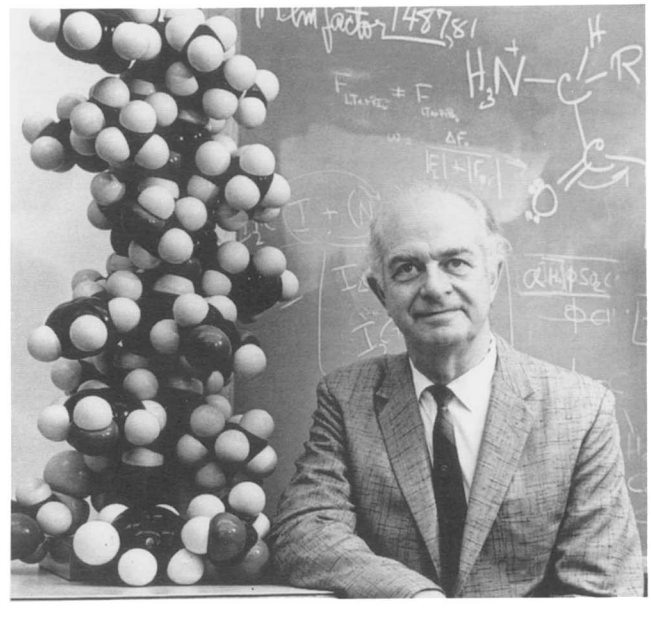
My first memory about proteins goes back to the spring of 1918, during the First World War, when I was a student in a class on camp cookery given by the Home Economics Department of Oregon Agricultural College as a contribution to the war effort. Students in the class were, like me, receiving some military training in the Reserve Officers Training Corps.

I remember making a loaf of bread and also learning something about the macronutrients, proteins, carbohydrates, and fats, but nothing about vitamins—it was too soon after the discovery of vitamins for them to get mentioned in the course. I continued my studies, except for one year, 1919–1920, during which I was a full-time instructor, teaching quantitative analysis. I received my degree of Bachelor of Science in chemical engineering in June 1922, and in September 1922 began my graduate work at the California Institute of Technology. I soon started research on the determination of the structure of crystals by the X-ray diffraction method, supervised by Roscoe Gilkey Dickinson, who was the first recipient of a Ph.D. from the California Institute of Technology (1920). He was then a National Research Council Fellow, but he later became a member of the staff (Professor of physical chemistry). My interest at that time was in minerals, intermetallic compounds, and other inorganic substances—I had not cared much for organic chemistry when I had a one-year course at Oregon Agricultural College. I continued to be interested in the structure of inorganic substances and in applying quantum mechanics to chemical problems, such as the nature of the chemical bond, for about 10 years. Beginning in 1930, I expanded my experimental work to include the determination of the structure of gas molecules by the diffraction of electrons.

Many of the substances that my students and I investigated by electron diffraction were organic compounds. My theoretical work had also extended to encompass compounds of carbon. In the early 1930s, I had formulated a quantum-mechanical theory of the tetrahedral carbon atom, extended, in a simplified form, to inorganic complexes. In the course of our determinations of the structure of carbon compounds, it was possible for my



Professor Linus Pauling. (Courtesy of Linus Pauling Institute of Science and Medicine.)

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Linus Pauling is widely recognized as one of the greatest scientists of the twentieth century. His contributions range from quantum mechanics, nuclear physics, and mineralogy to chemistry, biology, and molecular medicine. His classic monograph on *The Nature of the Chemical Bond* (1939) is generally considered one of the top scientific books of all times. He has authored over 1,000 publications, mostly on scientific subjects.

A native of Oregon (1901), Linus Pauling received his education in Oregon and at the California Institute of Technology (Ph.D. in chemistry and mechanical physics), where he subsequently served for 22 years as head of the Division of Chemistry and Chemical Engineering. Since 1973 he has been Research Professor at the Linus Pauling Institute of Science and Medicine in Palo Alto, California.

Dr. Pauling has received numerous honors, including two Nobel prizes (1954 and 1962), 48 honorary degrees, the Presidential Medal of Merit, and the National Medal of Science.

In this brief Recollection, Dr. Pauling recalls how his interest in proteins developed and how the alpha helix and beta pleated sheet structures of proteins were discovered.

students and me to investigate molecules such as benzene and to obtain much experimental evidence for the theory of resonance in chemistry. For example, the structural information supported the idea that the structure of benzene is a hybrid of the two Kekulé structures (two different ways of introducing three double bonds), with some contribution also from other valence-bond structures.

In the early 1930s, my research on the determination of the structure of the sulfide minerals by X-ray diffraction was supported to some extent by the Rockefeller Foundation. One day, when I was visiting the Rockefeller Foundation headquarters in New York City, Warren Weaver, who was in charge of research grants in chemistry, said to me that the Rockefeller Foundation in fact had no interest whatever in the structure of the sulfide minerals; instead, at that time their interest was largely in biochemistry. I thought about this for some time, and then submitted an application for a larger grant to permit me to investigate hemoglobin by determining the magnetic susceptibility of the substance and its compounds, such as with oxygen. This larger grant was made, and Charles Coryell, my research assistant, and I measured the magnetic susceptibility of hemoglobin and some of its compounds. The results answered a number of questions about how the oxygen molecule is attached to the iron atoms in hemoglobin and about how the electronic structure of the iron atoms changes on combination with oxygen, carbon monoxide, or other small molecules.

We had not had any experience in handling proteins. Although we were able to investigate solutions of hemoglobin and oxyhemoglobin without trouble, I had the feeling that we should have a biochemist with hemoglobin experience associated with us in our work. I had read an interesting paper by Anson and Mirsky in which they reported studies on the denaturation of hemoglobin and other proteins. On another visit to New York City, I went to the Rockefeller Institute for Medical Research and talked with Alfred Mirsky about his possible interest in coming to the California Institute of Technology for a year. He expressed an interest, so I immediately went to Simon Flexner, Director of the Rockefeller Institute, to ask him if he would assign Mirsky to the California Institute of Technology for a year or two, to work on our hemoglobin project.

This was a fortunate arrangement. Not only did Mirsky teach me how to handle proteins in the laboratory—they are far more delicate than inorganic substances—but he also gave me a great amount of information about the properties of proteins and especially about denaturation of proteins. The result of this collaboration was that within a few months we were able to publish our paper on a theory of the structure of native and denatured proteins. We suggested that the polypeptide chains in native proteins had a well-defined configuration and that they were held in place by interatomic forces, van der Waals forces, interaction of electrically charged groups, and especially formation of hydrogen bonds. We said that an increase in temperature or the addition of substances that would break hydrogen bonds would cause the protein molecule to unfold to some extent, loosening some segments of polypeptide chains that were not held in well-defined configurations, and that the product of this process was the denatured protein. In particular, if the pH of the solution was near the isoelectric point of the protein, these unfolded segments of polypeptide chains would get entangled with one another, fastening the molecules together and ultimately leading to the formation of a coagulum. Moreover, loss of the native configuration would destroy the characteristics of the protein, such as the ability to combine reversibly with oxygen and other molecules, the ability to crystallize, and enzymatic activity. I think that this was the first modern theory of native and denatured proteins.

The experimental method of measuring the magnetic susceptibility of hemoglobin compounds permitted the detailed investigation of the chemistry of hemoglobin, such as determination of equilibrium constants and rates of reaction, and led also to the discovery of a number of new compounds of hemoglobin itself and of heme, the iron-porphyrin prosthetic groups in the molecule. For example, one interesting experimental result involved the determination of equilibrium constants of hemoglobin with alkyl isocyanides containing alkyl groups of different sizes and determination of the equilibrium constants for heme with these groups. We found that the equilibrium constants of ethyl, isopropyl, and tertiary butyl isocyanides with heme were essentially the same, but the constants with hemoglobin fell off rapidly as the alkyl side chain became larger. The interpretation of this result, we said, was that the heme groups were buried within the hemoglobin molecule, a conclusion that turned out to be correct when the structures of myoglobin and hemoglobin were determined many years later.

Although the work on polypeptides by Emil Fischer around 1900 had been published, and most chemists considered proteins to consist of polypeptide chains, there were some people who were skeptical. One was the English mathematician Dr. Dorothy Wrinch, who had developed a three-dimensional structure for proteins different from the polypeptide-chain structure. When Dorothy Wrinch came to the United States in the spring of 1938, with support from the Rockefeller Foundation, Warren Weaver wrote to me at Cornell, where I was giving the George Fisher Baker Lectures in Chemistry, asking me to invite her to give a seminar and make a report to the Rockefeller Foundation about my opinion of her work. When she gave her seminar, several pertinent questions were raised by participants in the meeting, and I had a lengthy discussion with her the next day. My report to the Rockefeller Foundation was to the effect that I thought the evidence for her "cyclol" structures was quite weak. Then a paper was published by Dorothy Wrinch and Irving

Langmuir, stating that strong evidence for the cyclol structure had been obtained by analysis of X-ray diffraction photographs of insulin. My associate in Pasadena, Professor Carl Niemann, who had been interested in the structure of proteins for a number of years, suggested that he and I publish a paper summarizing the evidence for the polypeptide-chain structure of proteins. This paper was published later that year.

In the meantime, I had become interested in an effort to analyze the X-ray diffraction photographs of alpha-keratin (hair and related proteins) and beta-keratin (silk and stretched hair) that had been published by Bill Astbury of the Royal Institution in London (later in Leeds University). The photographs of alpha-keratin seemed to show a repeat in the distance 5.1 Å, which would correspond to two residues in a polypeptide chain extended along the length of the hair. I felt that I knew enough about the structure of polypeptide chains to be able to determine the structure of alpha-keratin by analyzing Astbury's diffraction photographs and similar ones made in our own X-ray laboratory, and I spent a good part of the summer of 1937 in this effort. From the theory of resonance I could predict that the carbon–nitrogen bond in the main chain of the proteins would have some double-bond character stolen from the carbonyl groups, and that as a result the polypeptide chain would consist of planar groups joined to one another at the alpha-carbon atoms. Some electron diffraction and X-ray diffraction studies of amides supported this conclusion and provided values of the bond lengths and bond angles. Also, I was sure that the N–H and O=C groups would form hydrogen bonds extending in the direction of the axis of the keratin molecules. Despite my effort to use this information to find a structure that would repeat with two amino acid residues in 5.1 Å, I was unsuccessful. At the end of the summer, I had reached the conclusion that there was some structural principle involved in proteins that had not yet been recognized.

Although some papers had been published describing X-ray diffraction studies of crystals of amino acids and simple peptides, not a single such structure had been determined by 1937. Dr. Robert P. Corey, who had worked with Ralph Wyckoff, one of the early X-ray crystallographers in the United States, at the Rockefeller Institute of Medical Research, came just at that time to spend a year with me in Pasadena. He and Wyckoff had been interested in proteins and had done some X-ray work. He and I agreed that the time had come to make a vigorous effort to determine the structure of crystals of amino acids and simple peptides. Within little more than a year, he had determined the structure of a cyclic bipeptide, piperazine, and he and a graduate student, Gus Albrecht, had determined the structure of the simplest amino acid, glycine. Corey and other postdoctoral people and graduate students in Pasadena continued the work, with much success, and by 1948 had found the correct structures of about a dozen amino acids and simple peptides. No correct structures had been published by any other group of investigators.

This work had been interrupted by the Second World War. For several years Professor Corey, some postdoctoral people and visiting professors, graduate students, and I, myself, had been pretty much tied up by working on war projects. When the war ended, the research on amino acids and simple peptides was resumed, and it soon became clear that all of the structures conformed to the structural principles that I had been making use of in 1937. My conclusion in 1937 that there was some undiscovered structural principle characteristic of proteins had turned out to be incorrect.

It was not until March of 1948 that I again attacked the problem of determining the structure of alpha-keratin—I might well have attacked this problem a couple of years earlier. In March 1948, I was serving as Eastman Professor in Oxford University. One day I decided to think about the alpha-keratin structure. I did not have X-ray photographs of alpha-keratin with me, and I decided to ignore the X-ray data and depend solely on my knowledge of structural chemistry. I decided to make the assumption that all of the amino acid residues in the polypeptide chain of alpha-keratin are structurally equivalent, with the different side chains not exerting a significant perturbation. I remembered that as a graduate student I had heard Professor Harry Bateman state that the operation that converts an object into an equivalent object anywhere in space is a translation along an axis coupled with a rotation around the axis. I knew that to repeat this operation would give a helix. By making a drawing of a polypeptide chain on a sheet of paper and folding the paper on parallel lines passing through the alpha-carbon atoms, I tried to bring the N–H group and the O=C group into the proper orientation and distance from one another to correspond to the formation of an acceptable hydrogen bond, with the N–H···O distance about 2.8 Å. It took me a couple of hours to find this structure and to make calculations about the repeat distance. In fact, it turned out that there were not two residues, but 3.6 residues in the repeat distance of the helix (the pitch of the helix), and that the pitch could be predicted to have a value close to 5.4 Å.

This was quite satisfying to me, because this structure, which I called the alpha helix, provided a beautifully simple explanation of the properties of keratin. There was a serious difficulty, however: the X-ray photographs seemed to give the repeat distance as 5.1 Å. The discrepancy troubled me to such an extent that I did not publish anything about the alpha helix for about a year and a half, because I did not want to publish an incorrect structure and I could not explain the diffraction photographs.

On my return to Pasadena, I talked with Professor Corey and then asked a visiting professor, Dr. Herman Branson, to check my calculations and to look for other

helical structures. He found only one other structure, the gamma helix, which in fact I had found in Oxford before finding the alpha helix. Then a paper was published by Bragg, Kendrew, and Perutz, in Cambridge, describing a number of helical structures of polypeptide chains obtained in their search for an alpha-keratin structure. From my point of view, all of these structures were wrong, because they did not involve planarity around the nitrogen atom. I thought that it was likely, however, that in the course of time they would learn enough chemistry to see what peptide group had a planar structure, and would discover the alpha helix, so Professor Corey and I decided to publish a short description of the alpha helix and the gamma helix in the *Journal of the American Chemical Society*. We followed this publication by a more detailed description of the alpha helix, with Herman Branson as a coauthor, and by some other papers, including description of the beta sheets, pleated sheets involving parallel extending chains of polypeptides.[1]

When I went to the California Institute of Technology in 1922 as a graduate student, I was fortunate to become a member of a chemistry department in which the determination of the structure of crystals by the X-ray diffraction method was being carried out. This may have been the only place in the world in which X-ray crystallography was being practiced in a chemistry department rather than in a physics department. Bragg, Kendrew, and Perutz were physicists working in a physics department, the Cavendish Laboratory. None of them, I judge, knew very much about structural chemistry. Bragg had talked with Lord Todd, the head of the chemistry department in Cambridge, about their work. Todd says that he told Bragg that the amine group was planar, but apparently Bragg did not understand what he said. I was fortunate in having a good understanding of the two fields, structural chemistry and X-ray diffraction. My recommendation to young scientists is that they get a thorough knowledge of one field and also some knowledge of other fields of science.

[1] *Editor's note:* See *J. Am. Chem. Soc. 72* (1950), 5349, and *Proc. Natl. Acad. Sci. USA 37* (1951), 231, 235, 251, 256, 261, 272, and 282. This series of papers includes a brief discussion of how the 5.1-Å reflection of keratins might arise because of nonuniform structure in the fibers.