RECOLLECTIONS

Reminiscences from a life in protein physical chemistry

WALTER KAUZMANN

Frick Chemical Laboratory, Princeton University, Princeton, New Jersey 08544

(RECEIVED September 23, 1992; REVISED MANUSCRIPT RECEIVED October 30, 1992)

For reasons that will become apparent in due course, my story begins with George Bancroft (1800–1891), eminent historian, diplomat, politician and educator, and grandfather of Wilder Bancroft (1867–1953), who in the 1890s brought the infant science of physical chemistry to Cornell from Ostwald's laboratory in Leipzig and van't Hoff's laboratory in Amsterdam [1]. George Bancroft came from a distinguished old New England family, noted for its obstinate rebelliousness—a tradition which, as we shall see, Wilder Bancroft strongly maintained.

After receiving his undergraduate degree from Harvard at the age of 17, George Bancroft went to Germany to study for several years—one of the first Americans to earn a German Ph.D. He worked under one of the foremost German historical scholars, and absorbed the beginnings of the techniques of modern historical research. During his studies in Germany and subsequent travels on the continent, Bancroft met many of the eminent intellectuals of the day—Goethe, Humboldt, Hegel, Byron, Schleiermacher, and Bunsen, among many others.

After returning to America, he began an outstanding

career. He taught for a few years at Harvard, then left Harvard to found the influential Round Hill School in Northampton, Massachusetts. Continuing the rebellious traditions of his family, he became a Democrat (all of the important New England intellectuals before the Civil War had been Whigs). He served as Secretary of the Navy under President Polk during the Mexican War, founded the Naval Academy at Annapolis, and then became American Minister to London from 1846 to 1849.

Later (1867–1873) he served in Berlin as American Minister to Prussia under Presidents Johnson and Grant. These were the fateful years of the consolidation of Prussian power in Germany, including the Franco-Prussian War and the declaration of the German Empire. Bancroft came to know and admire—and misjudge—Bismarck.

George Bancroft, like his grandson, Wilder, had a tendency to form judgments based on what he *wanted* to believe, rather than on the actual situation that existed. He was sure in his own mind that Bismarck's political agenda marked the progress of democracy in Germany. In Bancroft's view, Bismarck and Prussia were to be regarded as the liberators of Europe! Bismarck was "a lover of liberty," a great republican, and champion of humanity. Although an aristocrat, he regarded it as his duty to lead the world movement toward the freedom and unity of peoples. Canny Bismarck, who seemed to enjoy talking with Americans, may have deliberately fostered this view. Partly because of Bancroft's influence, official American policy before and during the Franco-Prussian War was strongly pro-German, though there were other reasons for this as well.

But George Bancroft's principal claim to fame today is his multiple volume *History of the United States*, which enjoyed a large sale and was translated into all of the major European languages. Although these volumes are still recognized for their scholarship, readability, and content of useful information, even contemporary reviewers warned that Bancroft often colored his opinions with what

Reprint requests to: Walter Kauzmann, Frick Chemical Laboratory, Princeton University, Princeton, New Jersey 08544.

Walter Kauzmann is Professor of Chemistry Emeritus at Princeton University, where he received his Ph.D. in 1940. He rose through the ranks to Professor (1960), but in the intervening period he was Westinghouse Research Fellow, and served on the Manhattan Project. At Princeton University, he was Chairman of the Department of Chemistry (1964–1968) and Chairman of the Department of Biochemical Sciences (1980–1982). Dr. Kauzmann is a member of the National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences, and recently was awarded an Honorary Ph.D. degree by the University of Stockholm. He is recipient of the Linderström-Lang Medal (1966) and will receive the Stein and Moore Award of The Protein Society in 1993. His research interests range from quantum chemistry, optical rotatory power, high-pressure physical chemistry, and mechanism of conversion of chemical to mechanical energy, to the physical chemistry of proteins and the history and philosophy of science. Among protein chemists, he is best known for his pioneering studies of the contributions of hydrophobic interactions to the structure of proteins.

he wanted to believe rather than with the objective view that one has the right to expect of a professional historian.

These writings, along with two fortunate marriages and skill in investing, were the source of a considerable fortune that eventually came to Wilder Bancroft. This inheritance had a large impact on American physical chemistry in the early 20th century, as we shall describe below.

George's son, John, was a disappointment, but John's son, Wilder, caught the flame from his grandfather. After graduating from Harvard in chemistry, Wilder followed in his grandfather's footsteps by studying for a Ph.D. in Germany. He spent two years in Ostwald's laboratory in Leipzig, where he absorbed some of the spirit of J.W. Gibbs (whose papers were at that time being translated by Ostwald), and became intensely interested in the phase rule. Unfortunately, Bancroft also absorbed Ostwald's distrust of the atomic theory, and for the rest of his life he resisted the inroads of physics into the science of chemistry—with unfortunate consequences whose effects have been with us until fairly recently. After spending some time with van't Hoff in Amsterdam, Wilder taught for two years at Harvard. He then joined the faculty at Cornell, where he became a center of controversy and eventually the dominant force, pursuing his version of the then youthful science of physical chemistry.

Because he was independently wealthy, Wilder Bancroft was able to found and provide support for the influential *Journal of Physical Chemistry*. Founded in 1896 out of his own pocket, he served as editor until 1933, when financial reverses made it impossible for him to continue its support. He used his position as editor to publish lengthy articles containing his ideas, many of which were out of the mainstream that was being taken by physical chemistry. Indeed, some of his ideas, especially as they represent his thinking about proteins, were simply outrageous, as we shall describe below.

As the years went by at Cornell, Wilder Bancroft followed his own paths of interest. Although guided by an exalted concept of what he thought constituted the mission of physical chemistry, his ability to deal with mathematics was never strong. For this reason, his research focused on the more qualitative aspects of the field. He felt that the phase rule was a central feature of the science, and he resisted the growing influence of physics on chemistry. Wilder Bancroft also became infected with the notion of colloid chemistry as a new science with rules of its own.

As Bancroft's influence in chemistry declined, he became more obstreperous and outspoken. He must have been an embarrassment to his up-and-coming successors in the Cornell department. As a last straw, he became embroiled with the medical profession when he claimed that diseases of the nervous system (such as drug addiction, alcoholism, sleeplessness, depression, and the various forms of insanity) resulted from coagulation of proteins in nerve cells. He asserted that these diseases could be

treated by introducing peptizing agents, of which sodium thiocyanate was among the most powerful. (Sodium thiocyanate was also known as sodium rhodinate, and Bancroft preferred this designation so as not to frighten people with the idea that it was somehow derived from the familiar poison, sodium cyanide.) Bancroft and a group of co-workers went on to clinical trials of their ideas and came to the conclusion that sodium thiocyanate was a cure-all for mental derangements. For about a year he rode the crest of a wave of favorable publicity. In 1933, the Awards Committee of the New York Section of the American Chemical Society proposed that Bancroft be awarded the section's Nichols Medal for his work on the colloid chemistry of the nervous system. But in the end his work became totally discredited, though he never gave up his theories of the diseases of the nervous system. He continued to glory in the role of an iconoclastic prophet.

The Nichols Medal was never awarded to Bancroft. As the date for the award approached, the Awards Committee panicked because of the increasingly hostile criticism that Bancroft's activities were generating, especially among the responsible elements of the medical profession (who were justifiably outraged by the sloppy clinical tests reported by Bancroft). The chairman of the committee approached Bancroft with the suggestion that the wording of the basis for the award be changed, the work on the nervous system being replaced by work on the phase rule. Bancroft, angered by the proposed change, refused to accept the award under these conditions. The awards committee then announced that no award would be made in 1933. See Chapter 7 of Servos's book [1] for further details, including extensive references, of this remarkable controversy.

There are long articles in the *Journal of Physical Chemistry* describing Bancroft's ideas on the role of colloids in the nervous system [2]. In a curious paper related to the above [3], it is claimed by Bancroft that "As people grow older they usually sleep less, even though they need more sleep because they recuperate more slowly than their juniors. In many cases this inability to sleep well is due to irritated nerves and consequently we have been very successful by giving such people small amounts of sodium rhodinate in a glass of water every week or two. I take it myself and now sleep about seven hours a night instead of about four." He also claimed that he could counteract the effect of coffee by taking double the usual dose of sodium rhodinate in a glass of water after dinner.

The last gasp in print on this controversy appears to be a short paper by W.D. Bancroft, E.C. Farnham, and J.E. Rutzler, Jr. [4], in which it is asserted that the length of human life could be increased by at least two years if "every human being of 45 or over, for whom sodium rhodinate is not contraindicated, would take sodium rhodinate for the rest of his life." Efforts to raise money to prove this were, however, thwarted by members of the medical profession. "Our answer to the medical profession is sim-

ple. It is up to them to clean house. From now on it is a fight to the finish between the medical profession and ourselves. There can be only one outcome to this contest. The medical profession will lose."

One wonders how Bancroft's reputation would have fared if this episode had happened today, when the notions of "alternative medicine" are rampant. I have little doubt that Bancroft would have been hailed as a martyr to the repressive forces of the Establishment. Indeed, I am sure that if the supporters of "alternative medicine" were to become aware of this episode, a school of "sodium rhodinate practitioners" would arise and flourish!

A much more serious situation was developing in connection with Bancroft's tight control of the *Journal of Physical Chemistry*. As editor, he continually refused to accept purely theoretical papers in such areas as the exploding new field of quantum chemistry. This led to the founding of the *Journal of Chemical Physics* under the sponsorship of the American Physical Society. These editorial misjudgments almost led to the demise of Bancroft's most important contribution to American physical chemistry, but the *Journal of Physical Chemistry* eventually came under the sponsorship of the American Chemical Society. It struggled for years to overcome the consequences of Bancroft's misjudgments.

Indeed, Bancroft's distrust of physics and mathematical theory was widely shared in the 1920s and 1930s by many of the administrators of the American Chemical Society (the "power structure" of the time, as the current lingo goes). One still finds pockets of resentment toward the ACS among chemical physicists of a certain age. This attitude, so incredible today, is illustrated by the following.

In the 1920s and early 1930s, Bancroft took advantage of his "ownership" of the *Journal of Physical Chemistry* to write long reviews of recent books. In some of these he expresses his views of the direction then being taken by prominent physical chemists. Typical is his review of the textbook, *Elementary Physical Chemistry*, by H.S. Taylor [5].

After giving a long quotation from the preface, in which Taylor emphasizes the importance of the calculus, Bancroft states, "This book came as a disappointment and a shock to the reviewer: a disappointment because it was not better; a shock because it may be better than the reviewer thinks it is. . . . If this presentation means real progress, the reviewer is very much a back number—which may very well be true. There is no alternative, because there is no possibility of reconciling the author's general point of view with that of the reviewer. It may be that both will go under; but both cannot survive. There is nothing personal in this. The author's point of view is, unfortunately, that of the majority of physical chemists and illustrates the demoralizing effect of the theory of activity [of strong electrolytes—i.e., the Debye-Hückel theory] as at present expounded. The question at issue is whether we are going to study concentrated solutions and

to develop an exact theory, or whether we are going to stick to ultra-dilute solutions and to play with an approximation theory? Do we believe in one hundred percent dissociation of all electrolytes in all aqueous solutions, or don't we, and why?" Further along in the review, after a quotation in which Taylor justifies the introduction of the activity concept by G.N. Lewis, Bancroft asserts, "There is not a word to show that there are any objections to this theory, which means that no criticisms have been made which seem to the author worth mentioning. Evidently the walls of Jericho did not fall down at the first blast of the trumpet."

Bancroft's review is concluded with the suggestion that "it would be an excellent thing if the physical chemists of the world would get together in a co-operative effort to develop a satisfactory theory of concentrated solutions, perhaps under the auspices of the National Research Council; but that time seems still far distant. It is hoped, however, that the tide has turned and that this book represents the high-water mark for the theory of activity which has threatened to engulf us."

Appeals of this sort to a "co-operative effort" to solve a difficult scientific question will be mentioned later in connection with Bernal's approach to the problem of obtaining protein structures from single crystal X-ray diffraction patterns of proteins.

I have spent a lot of time on Wilder Bancroft because he was an important, though indirect influence on my development. Furthermore, he represented an important phase in the history of protein chemistry that was dying at the time. I have also chosen to discuss Wilder Bancroft—and his grandfather before him—at some length because they are excellent examples of the dangers of believing what we *want* to believe rather than what we *ought* to believe. Of course, we all believe what we want to believe, and it is sometimes not easy to decide what we ought to believe. But the example of Wilder Bancroft teaches us that we must be careful in this or we shall end up as one of those ridiculous figures that I have taken so much time to describe. There are today a number of scientists who (in my opinion) mistakenly believe what they want to believe—but I would not want to name them.

There were many others of the colloid chemical school who still had influence in the 1920s and into the 1930s. The German organic chemist and Nobel Prize winner, Richard Willstätter, spent an enormous effort in trying to get a handhold on how enzymes work. He had the idea that the active principle of an enzyme was a small organic molecule adsorbed onto an inert protein colloidal particle. The people working in his laboratory in Munich kept trying to separate these active principles from the proteins and, in the course of these separations, the activity would invariably disappear. But they kept trying, supported by Willstätter's reputation and his incredible and unshakable belief in the colloid chemical approach. A massive two volume monograph exists describing the efforts that were

made in Willstätter's laboratory and some very notable people appear as co-authors of papers [6].

The father of the famous theoretical physicist, Wolfgang Pauli, discoverer of the Pauli Principle, was another important founder of this misguided school. Still another was Wilhelm Ostwald's son, Wolfgang. Both Pauli and Wolfgang Ostwald were strong influences on Bancroft [7].¹

I entered Cornell as a freshman in the fall of 1933 knowing that I wanted to major in chemistry, and also with an interest in biology. My interest in chemistry arose at first from a chemistry set that had been given me when I was in grade school. It is a shame that chemistry sets nowadays do not contain potassium nitrate, so that one cannot make gunpowder!

I was also given a microscope (100–250×) when I was 13 years old. Observing the many species of protozoa that develop when grass clippings are allowed to stand in water for a few days (do they still call it a "hay infusion"?) brought endless fascination.

And I was impressed by the book *Microbe Hunters*, by Paul de Kruif, which is still in print and which describes in scientifically accurate detail, easily understandable to the layman, the early development of bacteriology. Furthermore, the book gives vivid portraits of Pasteur, Koch, and other famous scientists, which describe the warts as well as the greatness; it therefore manages to capture the human interest that is so important in scientific writing for the layman.

And the novel *Arrowsmith*, by Sinclair Lewis, made a great impression, romanticizing medical research as it does in a highly effective fashion. Lewis collaborated with Paul de Kruif in the writing of this novel. De Kruif had been on the staff of the Rockefeller Institute and was able to give quite realistic and amusing insights into the operation of a fictional McGurk Institute, modeled on the Rockefeller Institute of the day. Certain of the characters were said to be based on real people—Arrowsmith, for instance, might have been John Northrop, and the old doctor was either Loeb or Michaelis. The film made from this novel, with Helen Hayes and Ronald Coleman, was also smashing.

I really wanted to be a bacteriologist, but was told that this would require an M.D. Fortunately, there was not enough money available for this path to be taken. So while still in high school, I decided to become a chemist.

I should add that, while in the New Rochelle High School, I was lucky to have had a fantastically good phys-

ics teacher, Mr. Arthur Hussey. He had taught physics to my oldest brother, who became a radio engineer with RCA, and took an interest in me. Because of his excellent teaching, I was able to go into sophomore physics at Cornell.

Organic and inorganic chemistry were strong at Cornell in 1933, but Bancroft's influence on the teaching of physical chemistry remained, and I had an extensive exposure to the phase rule, which did me no harm. I was, however, puzzled by the rather extensive exposure to the notions of colloid chemistry and had difficulty understanding why the emphasis on surfaces and interfaces made it somehow different from the chemistry of small molecules. But being young, I absorbed the concepts of peptidization (which had something to do with bringing precipitates into the "colloid state"), emulsification, lyophobic and lyophilic colloids, gels and sols, the lyotropic (Hofmeister) series, the Freundlich isotherm, and the rest. The general intellectual level of the physical chemistry course was also rather low. It was taught by one Professor Briggs, a Bancroft protégé who had some difficulty getting across the ideas of the collision theory of the rates of bimolecular reactions.

At some time during my stay at Cornell, I had a revealing exposure to the late Wilder Bancroft. There had been a number of developments in the understanding of photosynthesis in the mid-1930s. The structure of chlorophyll had been determined by Conant, and a quantum yield had been measured by Warburg. The Society of Sigma Xi accordingly held a symposium on the subject. A physics professor described the concept of the quantum yield and showed how it came from the Einstein concept that each absorbed photon generated a potentially reactive molecule. Professor John Johnson, the organic chemist, described the structure of chlorophyll. A botanist then put all of this together, describing the structure of a leaf and the overall machinery as known at the time.

Finally, Wilder Bancroft spoke. A large man with a powerful voice and domineering presence, he declared that all we had just heard was nonsense. The Einstein "law" was not true because one rarely, if ever, obtained quantum yields of unity. Furthermore, there were papers in the literature that claimed to show that carbon dioxide in solution in the presence of sodium bisulfite, when exposed to visible light, produced the bisulfite adduct of formaldehyde. Carbohydrates could easily be built up from formaldehyde. These studies had never been followed up. "Give me a graduate student and two years and we'll solve the whole problem!" Even I, a callow undergraduate, was shocked by this performance. I believe that Wilder Bancroft was given the needed support and some poor fellow wasted two years on the project, from which, of course, nothing further was heard.

Aside from these residual by-products of the Wilder Bancroft epoch in the Cornell Chemistry Department, there were some excellent strengths. John Kirkwood was

¹ Professor Schutt has called my attention to a most interesting paper by John Edsall ("Proteins as macromolecules: An essay on the development of the macromolecule concept and some of its vicissitudes," *Arch. Biochem. Biophys. Suppl.* 1, 12–20 [1962]). Written 30 years ago by one of the participants in the final stages of the controversy between the "colloid school" and those who regarded proteins as large molecules, it provides a nice counterpart to my impressions as a student at the time of the dénouement.

there at the start of his career. He taught the course in chemical thermodynamics (out of Lewis and Randall—which went over my head). I also audited a part of Kirkwood's graduate statistical mechanics course, which was excellent. I took notes, but the notion of a Gibbs ensemble bewildered me and I stopped attending the course—a bad mistake. My notes, which I still have, show that it was a superb course, and I have always been sorry that I did not stick with it.

Furthermore, the training at Cornell in organic and inorganic chemistry was outstanding. Laubengayer's inorganic chemistry course was exceptionally good and up-to-date.

During one semester the English stereochemist William H. Mills gave the Baker lectures. He covered all of the then-current ideas on stereochemistry in an up-to-date fashion. In particular, the concept of *molecular conformation* was thoroughly treated (although, of course, the tools by which conformation could be studied at that time were feeble by today's standards). I was as well grounded in the concepts of stereochemistry as it was possible to be at the time. It is unfortunate that Mills's Baker lectures were never published in book form, as the Baker Lectures were supposed to be.

So, while an undergraduate, I decided that I wanted to be an organic chemist.

In my senior year, the instructor in a course in advanced organic chemistry [8] happened to mention the fact that some molecules had something called a dipole moment—the centers of gravity of the positive and negative charges were not in the same place. This was my first exposure to a concept from the area of chemical physics (from which I had previously been protected by the influence of Wilder Bancroft), and I was astonished that people could think in such terms. After the lecture I went to the instructor and asked him to tell me more. He replied that I really didn't have to know about that and that he was sorry he had brought it up.

So I went to my advisor, John Johnson, and asked *him* where I could find more about dipole moments. Johnson referred me to the excellent volume by N. Sidgwick based on his recent Baker lectures at Cornell. This text set forth the understanding of the chemical bond as it existed at the time [9]. I devoured the book, which happened to be written at just the right level for me under the circumstances. And for the first time in my life, I realized that one can learn something important by reading independently, without taking a course. (Nowadays, of course, we recognize that the most important thing we ought to be teaching in college is how to learn without taking a course. We now tell our freshmen this!)

In my senior year I also discovered the current periodical shelf in the Chemistry Library. One of the journals there was the *Journal of Chemical Physics*, and I was impressed by the titles of the papers (few of which I even began to understand). In particular I noticed that there

were a lot of papers with fancy titles [10] by one Henry Eyring of Princeton University's Chemistry Department. Although I knew that Eyring was not an organic chemist, I thought it would be great to be in the same place as he was. So I applied to the Princeton Graduate School. John Johnson told me to apply also to Illinois, which I did. I was interviewed (in Ithaca) by Carl Marvell, who happened to be passing through, but I don't think he was much impressed. On the other hand, I did get a number of encouraging letters from Princeton, indicating interest, so that in April, when I received a formal offer of admission to the Princeton Graduate School, I immediately accepted. (I don't remember whether I ever received an offer from Illinois.)

I went to Princeton in the fall of 1937. What a change in atmosphere I experienced—and the headiness in the air continued for the next three years! At the heart of this was Hugh Taylor, a remarkable scientist and human being who was in touch with all of the current developments in physical chemistry and its close cousin, chemical physics. There were no course requirements—we were expected to take what we thought might be useful to us. And we were encouraged to take graduate courses in physics. The General Examinations, normally given over a period of three days at the end of the second year, were not particularly onerous, though they covered the entire field of chemistry. (One did not worry about them because everyone passed them.) For me, at any rate, the Graduate School in the Princeton Chemistry Department at the time was everything that education at that level ought to be.

Hugh Taylor also had a deep interest in developments that were taking place in the understanding of proteins. And an understanding of polymers was developing—the new science of macromolecular chemistry was replacing the vagaries of the colloid school. The landmark papers by Mark, Meyer, Guth, and Flory had recently appeared and continued to appear. The notion of a random coil was clearly stated and dealt with mathematically. One spoke of Markov chains. These developments had an obvious bearing on our understanding of proteins.

The state of protein chemistry in the late 1930s was not nearly as bad as the picture I have painted in describing the influences of Bancroft, Wilstätter, and the colloidal school. For many years a number of more sensible people had been working with the idea that proteins are simply large molecules that could be studied using the normal concepts of organic and physical chemistry. There was Emil Fischer, who, before World War I, had proposed the idea that proteins were polypeptide chains. Although this idea was, of course, correct, it was not easy to prove. Because Fischer was unable to protect reactive side chain groups, the peptides that he synthesized were all very hydrophobic and became increasingly insoluble as the chain was lengthened. (I have read somewhere, though, that he once prepared a meal for the Kaiser in which the meat course was one of his synthetic polypeptides!) Neverthe-

less, other ideas on protein structure were still floating around in the 1930s—such as Abderhalden's notion that proteins were agglomerations of diketopiperazines.

One of the earliest of those seeking to make physical chemical measurements on proteins was Sørensen at the Carlsberg Laboratory in Copenhagen, and of course, the Carlsberg Laboratory was built upon this program and continued to operate on it under Sørensen's successor, Kai Linderstrøm-Lang—of whom more later. Another leading figure in this area during the early 20th century was Jacques Loeb. Still another was Leonore Michaelis (both of these ended their days at the Rockefeller Institute). The laboratory of E.J. Cohn at Harvard was another center of this point of view. It began operating in the 1930s and the great monograph by Cohn and Edsall, appearing in the early 1940s, summarized the magnificent work of this group. Svedberg in Sweden was using the ultracentrifuge to demonstrate that proteins were entities of a uniform size and shape, with definite molecular weights. Northrop's group at the Princeton Division of the Rockefeller Institute was crystallizing enzymes and proving that they were true chemical individuals, as was J.B. Sumner who, at Cornell, had crystallized urease. (Willstätter refused to take this seriously; I am told that when Willstätter came to Cornell in the 1930s to give a talk he did not even deign to notice Sumner, who was in the audience. I am also told by Professor Neurath that the *Journal of Biological Chemistry* at first refused to publish Sumner's paper.) When I was at Cornell, I was dimly aware of the work of Sumner. I can recall seeing him at seminars—and that he had only one arm. He was in the Medical School, and unfortunately I did not take his course in biochemistry—perhaps because it was restricted to medical students.

An interesting controversy had already developed before 1930 between the colloid chemist Pauli and the biochemist H. Weber. The notion of an amphoteric ion had been proposed by that time (the idea that for instance glycine existed as $\text{NH}_3^+\text{-CH}_2\text{-COO}^-$, not as $\text{NH}_2\text{-CH}_2\text{-COOH}$). Pauli had stated that a protein at its isoelectric point was uncharged and therefore contained no charged groups, whereas Weber maintained that it contained many charged groups, the numbers of positive and negative groups being equal. To prove that isoelectric proteins contained -NH_3^+ and -COO^- groups, Weber pointed out [11] that there is a large volume change when protons are removed from or added to these charged groups. Thus the reaction $\text{R-COO}^- + \text{H}^+ \rightarrow \text{R-COOH}$ gives a volume increase of about 10–15 mL/mol and the reaction $\text{R-NH}_3^+ + \text{OH}^- \rightarrow \text{R-NH}_2 + \text{H}_2\text{O}$ gives a volume increase of the order of 20 mL/mol (R being a simple aliphatic group such as methyl or ethyl). Weber demonstrated that when a given number of moles of a strong acid are added to an isoelectric protein at around neutral pH, so that carboxyl groups are titrated, the expected volume increase, per mole of acid, is observed. And similarly, when a strong

base is added, the volume increase expected for the titration of an NH_3^+ is observed. One could hardly find a more convincing clarification of the confusion of the ideas of the colloid chemists [12].

I continue to feel that the study of the volume changes in protein reactions is sorely neglected. They may be determined by dilatometry and by the effects of pressure on protein equilibrium constants. The results complement the results of the determination of enthalpy changes as measured by calorimetry and the effects of temperature on equilibrium constants. Much useful insight at the molecular level can be obtained from a knowledge of volume changes.

It was exciting to be a graduate student at Princeton in the late 1930s. Many things were happening in chemistry generally, but for one interested, as I was, in the biological side of chemistry, it was particularly satisfying to be in at the final breakup of the old fashioned ideas personified by Wilder Bancroft. Hugh Taylor saw to it that many people on the forefront of the new developments came to speak at Princeton, and he was especially interested in the growing understanding of proteins, so there were a lot of seminars on protein chemistry.

One of the important developments at the time was Pauling's discussion of the experimental observations of Landsteiner on the effects of molecular shape of haptens in the antibody-antigen reaction [13]. The idea of the complementarity in shape of the hapten and a cavity in the protein is, of course, central to much of present day protein research in enzymes and protein recognition in general. These ideas, so clearly stated by Pauling, made a real impression at the time.

Another challenging set of observations arose from the application of X-ray diffraction to proteins.

In the early 1930s, Astbury [14] had made the striking observation that when moist hair was stretched by 100% there was a drastic change in the X-ray fiber diffraction diagram. He suggested that the polypeptide chain underwent a conformation change from a coiled form (which he called the α -form) to an extended form (which he called the β -form). We now know that the coiled form is the α -helix and the β -form is one of the β -pleated sheets suggested by Pauling and Corey in their classic paper. Although the structures suggested by Astbury at the time are known to be incorrect, the (α -, β -) terminology that we now use was suggested by Astbury, and his was probably the first demonstration that protein chains could have definite and regular structures which are interconvertible under the proper circumstances.

The impossibility of the structure of α -keratin suggested by Astbury was pointed out by Neurath [15]. Neurath tried to construct models of Astbury's suggested fold using atomic models built according to the specifications of E. Mack, with correct atomic bond radii and van der Waals radii. He found that there were steric interactions

that rendered the Astbury fold impossible. (This, incidentally, must have been the first use of space filling models to evaluate protein structures.)

It is interesting that Hugh Taylor, on reading Neurath's paper, was challenged by the problem of determining the folding pattern that might lie behind Astbury's diffraction pattern of α -keratin. He had an undergraduate, Bernard Becker, toy with the recently developed Fisher-Hirschfelder space filling models (made according to atomic covalent radii and van der Waals radii known at the time) to see whether a structure could be found that was consistent with Astbury's findings. In this way they came upon a structure resembling the α -helix. This was published in an article [16] based on Taylor's Franklin Medal address, given 24 April, 1941. Here was surely one of the first examples of the use of model-building to arrive at a reasonably correct structure in a protein molecule. But of course this "discovery" was premature, and Taylor did not realize the importance of what he had accomplished.

I first encountered the Fisher-Hirschfelder models as a graduate student. It was fascinating to work with them instead of with the ball-and-stick models that every organic chemist owned because they demonstrated the difficulties that molecules could generate with the steric interferences of groups packed around a given atom. The models as originally sold had the disconcerting property of falling apart very readily because of the instability of the conical pins used to join the atoms together.

The Fisher-Hirschfelder models became known as Fisher-Hirschfelder-Taylor models when Taylor suggested that the conical pins be replaced with snap fasteners, and the suggestion was taken up by the Fisher Scientific Company. I believe that Taylor had a patent taken out on this, with assignment to Princeton University, and that a small annual income came to the department for a while from it.

For many years after World War II, Astbury was considered a sort of "grand old man" authority on protein structure. He attended all of the Gordon Research Conferences on proteins and would give talks on his latest studies in which he attempted to classify proteins into various groups, according to their fibre diffraction diagrams.

Astbury also may have had a certain role to play in the funding of British research after the war. I understand that at one point he recommended that Perutz's research support at the Cavendish Laboratory be terminated because it was getting nowhere and there was no reason to expect that it ever would get anywhere! It was the strong support that Bragg gave which saved the project. I believe that Bragg stated that the most important thing that he did in his lifetime was his support of Perutz—which is quite a statement by a man who had accomplished so many other important things!

This is perhaps also the place to mention that Bernal

had stated in a lecture in 1939 (quoted by I. Fankuchen, *Adv. Protein Chem.* 2, 387–405 [1945]):

The problem of the protein structure is now a definite and not unattainable goal, but for the success it requires a degree of collaboration between research workers which has not yet been reached.

Bernal was here referring to the vast amount of information present in the small-angle X-ray scattering that had been obtained in his laboratory. In fact the solution of the problem was not obtained by the vast "collaboration" proposed by Bernal. Rather the problem was solved by a very small group of people, taking a chance by grappling with a difficult problem surrounded by darkness and doubts.

In this connection it is interesting to read the remarks on "The new Marxism," by Max Perutz [17]. Perutz here warns against the dangers of "organized science" that were so close to Bernal's heart.

One is reminded of the suggestion by Wilder Bancroft in 1927, that the future development of a theory of concentrated ionic solutions be decided by a committee under the guidance of the National Research Council!

It should also be mentioned that Taylor, whose main research area was in heterogeneous catalysis and therefore in surfaces, was at that time very much interested in the recent developments in Langmuir's laboratory—in particular the Langmuir-Blodgett-Pockels trough and the built-up surface layer technique that Blodgett had worked out. We had detailed lectures on these matters, and we also learned all about soap micelles and lipid membranes. So already as a graduate student some of the basic concepts of the hydrophobic bond had been seeded in my mind [18].

Another important development at that time was the paper by Bernal and Fowler on the structure of water—and an understanding of water was one of those things that eventually had to fold itself into the solution of the problems presented by proteins [19]. This classic paper was familiar to all of us and served as an important basis from which to start on anything having to do with water.

There were, of course, some slip-ups in the progress of our understanding of proteins in terms of the concepts of macromolecular chemistry. Some were based on erroneous observations and some on erroneous conceptions. It is instructive to recall a few of them because they had great appeal at the time and seemed to indicate the existence of simple principles of immense significance. They were consequently the subject of much discussion at the time I was a graduate student.

One of these was the belief expressed by Svedberg in the early 1930s that the molecular weights of all proteins appeared to fall into a relatively few classes [20]. Many

of these classes seemed to have molecular weights of multiples of 34,500—a fact that of course appeared to have profound significance and led to much speculation, especially among us students. Svedberg even suggested that, “At sufficiently high alkalinity all proteins have the same molecular weight, viz., 34,500.”

Astbury and Woods [21] speculated at some length on this and suggested that the maximum length of a polypeptide chain must be limited by the “vibrational instability of peptide chains when their length exceeds a certain value.” X-ray data seemed to suggest a “periodic repetition of comparatively simple units.” If this were so, “then the probability of disruptive resonance occurring among the constituents of the peptide chain will continually increase with the length, so that excessively long chains would be liable to spontaneous decomposition into shorter chains. We may imagine some such process taking place in the laboratory of the living cell as the amino acids are lain down in long chains at a surface and consolidated by crystallographic groupings in the manner suggested [earlier in the paper], or by intra-molecular folding such as has been demonstrated in the case of wool and hair.”

This quotation is given to show the kind of gibberish that was circulating at that time. Of course, neither the arguments proposed nor the facts they are supposed to explain were correct.

Bergmann and Niemann [22] point out that if the above arguments were correct, the basic molecular weight of 34,500 would have to depend on the temperature.

Based in part on this “fact” of the existence of molecular weight classes, Dorothy Wrinch, an English mathematician, came up with the extremely clever idea that the polypeptide chain could fold into various patterns that she called “cyclols.” These were rings formed in polypeptide chains in which an N-H of an amide would add to a carbonyl, closing into a six-membered ring with an O-H group on the former carbonyl carbon (whence the name cyclol). Wrinch further showed that these cyclol rings could be folded into various large polyhedra that had molecular weights corresponding to those found by Svedberg [23]. Wrinch made the bold statement that, “The present theory suggests that the explanation [of Svedberg’s molecular weight classes] is to be found—not in chemical or physiological considerations—but in the abstract geometry of polymers of amino- and imino-acid residues.”

Irving Langmuir was among those who were strongly attracted to the idea [24]. Dorothy Wrinch presented her ideas at a symposium on proteins organized by Hugh Taylor at Princeton at which, as I recall, Langmuir was present.

It might have been at this symposium that Pauling attacked the idea as worthless. Neurath [25] had already shown that there were difficulties in constructing models with the cyclol structure, and Pauling and Niemann had written a devastating paper [26] criticizing the cyclol theory on energetic and many other grounds. Among these

other reasons was the fact that if the cyclol theory were correct, there would be many fewer carbonyl groups in proteins than in protein hydrolysates—whereas there is essentially no change in the carbonyl ultraviolet absorption band on protein hydrolysis [27].

It was Hans Neurath [28] who showed that the idea of molecular weight classes was quite without foundation when the most accurate recent molecular weights were considered.

Thus by the early 1940s there was little left in support of the cyclol idea. Nevertheless, Dorothy Wrinch held onto her idea and even wrote a book about it [29]. She was embittered by the vehemence with which her ideas were cast aside by the establishment.

Dorothy Wrinch (1896–1976), after an unhappy first marriage, married O.C. Glaser, a well-known biologist at Amherst College, and used to summer at Woods Hole, where she would complain about her treatment to anyone who would listen. (My wife took a course on X-ray diffraction from her at Woods Hole.) A talented young lady, Wrinch had a fascinating but, in the end, a miserable life [30]. Some of the current feminists who write books about women in science might be tempted to hold her up as an example of the mistreatment of women in the old days by a male-dominated scientific elite, and to a considerable extent her earlier career did suffer from the prejudices of the time against women in science. But I would judge her treatment in the matter of the cyclol hypothesis as just another example of the dangers of believing what one wants to believe, carried to an extreme. Women are just as capable of doing this as men.

Another exciting idea that was in the air at the time came from the amino acid analyses of proteins that Max Bergmann and Carl Niemann were carrying out at the Rockefeller Institute [31]. These analyses were carried out with all possible care, but this was before the days of chromatography and the precision was marginal. Based on their results, Bergmann and Niemann thought that the frequency of occurrence of any amino acid in a protein could be fit into the formula $2^m 3^n$, where m and n are small integers. We were all excited about this observation and wondered what it might mean. Of course, it turned out to be nonsense. But how were we to know this at the time?

Although I began working as a research assistant for the carbohydrate chemist, Eugene Pacsu, he soon came to the correct conclusion that “Walter Kauzmann is not cut out to be an organic chemist,” and reported this to Hugh Taylor, who was Department Chairman. I was called in by Taylor, who told me of Pacsu’s evaluation. Of course, I was crushed at the news. But dismay turned to amazement when Taylor suggested that I go to work with Henry Eyring. Taylor seems to have based his recommendation on my performance in the placement examinations that were given to all entering graduate students in chemistry. He also mentioned my showing on the

Graduate Record Examination. The Educational Testing Service had just set up this examination and needed to give it to samples of the graduate student population in order to be able to establish its norms. Since ETS was (and still is) located in Princeton, the examination was given to all Princeton graduate students. I believe that I might even have taken it twice, in two different forms. Taylor was impressed by my score in the more mathematical portions. I suppose that it was mainly for these reasons that he suggested that I work for Eyring. In any case, nothing could have been closer to what I really wanted to do (but had not the confidence to suggest to myself).

And that is how I came to have Henry Eyring as my thesis advisor. This was only one of many lucky breaks that I have had in my life. It also illustrates Hugh Taylor's kindness and his willingness to take a chance.

Henry Eyring was a remarkable individual. He could be challenged by any problem that had even the slightest scientific content. He served as the first Director of the Textile Research Institute when it moved to Princeton. Among his more than 600 scientific publications are papers on such subjects as the reason for the propensity of nylon to resist release of gray adsorbents on laundering [32].

He also had a deep interest in biological problems. This interest, and a long collaboration with Professor Frank Johnson of the Princeton Biology Department, resulted in a long series of publications and two monographs [33] that had an important influence on my own development. Frank Johnson was a student of Newton Harvey and carried out many important studies on bioluminescence. A remarkable series of studies of the effect of pressure on the luminescence of bacteria led Johnson and Eyring to intriguing ideas about pressure effects on protein unfolding. Their ideas were later to have an important bearing on my own research.

Among Johnson's many discoveries was the observation that narcosis in bacteria and also in vertebrates is reversed by hydrostatic pressure—an effect that turned out to have important medical consequences in the determination of the dosage of anesthetics required in the treatment of deep sea divers (especially in the North Sea) injured at depth and operated on in high pressure chambers. The basic paper in the field [34], by F.H. Johnson and E.A. Flagler, is of interest to me because Elizabeth A. Flagler became my wife in 1951. I met her through her work in Frank Johnson's laboratory, where she conducted the experiment (made famous by Eyring in his lectures) in which tadpoles, rendered immobile by alcohol, regained their mobility when a few hundred atmospheres of pressure were applied.

On several occasions when Elizabeth was in the audience, Eyring would, in the course of describing the spectacular effects of pressure on anesthetized (or "tipsy") pollywogs, point her out as the person who had actually performed these experiments. My pride in this accom-

plishment was always surpassed by her mortification at receiving this notice.

While I was a graduate student Eyring and Allen E. Stearn published a paper, "The application of the theory of absolute reaction rates to proteins" [35], which included an extensive discussion of protein denaturation. It was from this paper that I learned about the large positive entropies of activation that invariably accompany protein unfolding—another of the observations that aroused my interest in this phenomenon.

Henry Eyring was also a devout Mormon. He had been ordained a High Priest in the Mormon Church during a stay in Berkeley, and he proceeded to form an active congregation of Mormons whom he had encountered after he came to Princeton. He was instrumental in building this group up and eventually became President of the New Jersey District of the Church.

When he went to Utah, he wrote a column on science for a Church periodical and became known as the leading Mormon scientist and very influential in church affairs. His older sister had married Spencer W. Kimball, who was for some time President of the Mormon Church. According to Heath [36], Eyring, through his influence with the church authorities, and also through his tact, played an important role in heading off the acceptance as church doctrine positions based on a literal reading of the Bible that are inconsistent with the findings of science.

Because of my contacts with Henry, and because of the friendly treatment I have received on several visits to Salt Lake City, I have always had a warm spot in my heart for the Mormon Church. It astonishes me that there exist very strong anti-Mormon prejudices among some people in the west. But apparently some Mormons are capable of harsh actions, particularly against those who leave the church. Such actions do not tend to be directed against people like me, who were never properly exposed to the Mormon doctrines and therefore cannot be expected to know any better. Henry once told me that my wife and I were destined to occupy the second level in heaven—reserved for good people who are not Mormons.

At the time I began working with Eyring, he had just published a paper with E.U. Condon on a new theory of optical activity. I decided to continue work in this area by applying the theory to whatever data I could find in the literature—or to make measurements myself. There was a superb polarimeter in the Frick Laboratory that could measure rotations across the visible spectrum, and I was fully aware of the importance of trying to evaluate contributions by individual absorption bands to the rotations.

Only one instrument for measuring optical rotations in the UV existed in the scientific universe of the day (circular dichroism could be extracted from such measurements). It was in P.A. Levene's laboratory at the Rockefeller Institute. I visited Levene in the spring of 1940 to see if I could work with this instrument. I can remember a small,

thin, feeble figure, sitting on a stool, shaking his head and telling me that Alexandre Rothen, who was in charge of this instrument, had dismantled it in order to measure film thicknesses in a new area of research that he was undertaking. Levene died in September of that year.

There is another story here: Rothen believed that enzymes might act through distances of tens of Ångströms. To test this idea, he coated a plate with an enzyme, managed to cover this with a polymer film of controlled thickness, stuck the plate into a solution containing the enzyme's substrate and found that the substrate was digested by the enzyme! The film (whose thickness was measured using the quartz prisms that had made up the UV polarimeter that I so badly wanted to use) could be as thick as 50 Å or more! This work appeared just after the war, as I recall, and created a great stir—until it was shown that Rothen's films had holes in them. Another example of the dangers of *wanting* to believe something that was not there. Poor Rothen was desperate to make a major scientific discovery.

Eyring's main interest at the time I began working with him was in developing a general theory of liquids. So he left me alone. My thesis was largely based on evaluation of data in the literature, as well as conducting a calculation of the optical rotation arising from the weak near-UV band of ketones. I also did some laboratory work on Hudson's rules for the optical rotations of carbohydrates under Pacsu's direction, and in this way redeemed myself with him.

It was fortunate for me that optical activity is the most conformation-dependent physical property that existed at the time because proteins are optically active. They are always levorotatory, and when they denature the levorotation always increases. (Another curious bit of information that I ran across was that the optical rotation of gelatin increases markedly when gelatin gels.) So I was again lucky, this time in my choice of a thesis topic. I determined in my mind that if I ever had the chance to do research in my own lab, I would use optical rotation to study proteins.

I received my Ph.D. in June of 1940. World War II had begun, but the US was at peace, though also still in the grips of the Great Depression. Academic jobs were scarce. E.U. Condon had left Princeton to lead a program of fundamental research at Westinghouse Electric Company in East Pittsburgh. One feature of his program was to attract promising young Ph.D.s to Westinghouse with two-year appointments as "Westinghouse Research Fellows," with freedom to do research on anything that was mutually agreeable. The stipend was \$2,000—a magnificent sum that equalled the stipend of the prestigious NRC fellowships that financed so many of our outstanding young people before WWII.

Eyring had received one of these NRC postdoctoral fellowships and had gone to Berlin where he and Polanyi

had calculated the first potential energy surface for a chemical reaction—the $H + H_2 \rightarrow H_2 + H$ reaction.

Michael Polanyi was one of the many Hungarian-trained scientists (Wigner, Teller, von Neumann, Szilard, etc.) who migrated to Germany in the 1920s and 1930s and made immense contributions, mainly in physics, but also in chemistry. After Hitler came to power Polanyi moved to England, where he became interested in the philosophy of science and made many important contributions (he enjoyed the advantage over most philosophers of science of that time of having had a productive career as a scientist, so that he knew how the endeavour actually worked).

His book, *Personal Knowledge* [37] concerns basic attitudes towards scientific theories with which I find myself in agreement, though they are quite at odds with much current thinking on such matters. It also has several chapters on the subject of "Passion and controversy in science," which are particularly pertinent. But this is not the place to discuss these matters, important though they may be. I do commend the book to the reader who is interested in pondering such things.

Michael Polanyi's son, John Polanyi, is a physical chemist at the University of Toronto, where he achieved the unusual feat of excelling his brilliant father in a field—reaction kinetics—in which his father had made perhaps his most important contributions. John Polanyi won the Nobel Prize in Chemistry in 1986. He had spent two years, 1954–1956, at Princeton working under Hugh Taylor's auspices—a delightful person as well as an outstanding scientist.

When Eyring had returned to the USA, he was having a hard time finding an academic job because few chemists had any idea of what his paper with Polanyi meant. It was Hugh Taylor who, hearing a talk by Eyring at a AAAS meeting, saw the significance of Eyring's work and brought him to Princeton. Taylor later claimed that Henry Eyring was his greatest scientific discovery—mimicking the famous remark by Humphry Davy that Michael Faraday was his greatest scientific discovery.

I accepted an appointment to a Westinghouse Research Fellowship and spent two extremely useful years doing my thing at Westinghouse. Of course, this did not include work on the optical rotation of proteins. I came to know that fabulous scientist and public figure, Ed Condon—but this is not the place to discuss that great experience. (He is one of many gods that I have brushed up against in my lifetime.)

Ideas derived from work done at Westinghouse led to a later paper, "The nature of the glassy state and the behavior of liquids at low temperatures," [38] which pointed out the "Kauzmann paradox"—so named by my friend C.A. Angell, now at Arizona State University. Of this paper, Robert W. Cahn has recently written [39], "It is rare indeed for a scientific paper to remain central to current

concerns several decades after its publication. . . . The increasing number of physicists who are struggling to come to grips with that most familiar and most recalcitrant of processes, melting, keep coming back to Kauzmann and his eponymous paradox. . . .”

Modesty should have dictated that I not mention this, but as my former student, Howard Schachman, once said, “False modesty is better than no modesty at all,” and I have therefore chosen to assume a cloak of false modesty in the matter. I might add that I have never met, nor do I know, the said Robert W. Cahn. Nor can I recollect that he has ever sought favors from me. But of course, if I were ever in a position to grant him a favor, I should be delighted to do so.

At Westinghouse, even before December 7, 1941, the rumblings of World War II were heard clearly. We were also generally aware of the possibility of utilizing uranium fission for making a bomb, but that this would require the separation of the scarce ^{235}U isotope from the abundant ^{238}U .

I had an adventure with an idea for separating isotopes that, if it had worked, would have made me famous. The idea ran like this: the normal mode lattice frequencies of a heavy atom like uranium surrounded by light atoms would, one might think, be much higher than those of the same heavy atom surrounded by other heavy atoms. Consider, then, the equilibrium of mercury ions dissolved in water with mercury atoms in liquid mercury. Mercury has stable isotopes of mass numbers and abundances 198 (10.1%), 199 (17.0%), 200 (23.3%), 201 (13.2%), 202 (29.6%), and 204 (6.7%). According to my reasoning (which turned out to be basically faulty), one would expect a fractionation to occur with the heavy isotopes favoring the metal phase.

As I recall it, I attempted to test the idea by placing some mercury and a solution of mercurous nitrate into a test tube, shaking them for some period of time, removing the solution, equilibrating it with a fresh batch of liquid mercury, and continuing this process for something like seven or eight times. The solution would in this way have been depleted in the heavier isotopes. The metal was then recovered from the solution and given to the people running the mass spectrometer—which happened to be in an adjoining laboratory.

Excitement ran high when they reported that a definite enrichment in the lighter isotopes had been observed! There was a caution, however. The instrument had been giving trouble recently and was about to be shut down for an overhaul. The operators could not vouch for the validity of the result. The remaining sample of mercury was therefore sent to A.O. Nier’s laboratory in Minnesota for a better analysis.

Pending that result, Harold Urey was contacted in his laboratory at Columbia University. He asked that I come to see him, which I did—I believe that I was sent there by

plane, which would have been my first trip by air. I also filled out my first and only patent disclosure—in preliminary form.

Urey told me that he found it hard to believe that I had achieved the result I had expected because the theoretical basis of my idea was faulty. Teller had shown by most transparent reasoning that, using classical statistical mechanics, there was no way (short of making the *potential energy* depend on the masses—as it would in a centrifugal field) in which a chemical equilibrium constant could depend on the masses of the atoms involved. Only when one used quantum statistics (and there mainly through the zero point energies of the various modes) could one expect to find the dependence that I was seeking. My arguments had depended only on classical mechanics so they could not possibly be valid.

Nevertheless, Urey admitted that the facts appeared to show that I had accomplished an enrichment, so he encouraged me to go ahead with further efforts—and especially to try to figure out how I might apply my thoughts to the problem of separating uranium isotopes.

Fortunately, shortly after my return to Pittsburgh, Nier reported that he could not detect any enrichment. There was thus no point in doing anything further with the idea. If this approach had worked, the separation of uranium isotopes might have been much simpler to accomplish, and it might have been much easier to have made an atomic bomb based on ^{235}U than by the gas diffusion method that was ultimately used. All kinds of small countries might have been tempted to make uranium bombs (whose assembly is also much easier). Furthermore, I might have spent the rest of the war at Oak Ridge instead of at Los Alamos.

I did not like living in Pittsburgh. When the two-year term of my fellowship was finished in August 1942, I decided to leave Westinghouse.

I then joined an NDRC laboratory that had been set up at Bruceton, south of Pittsburgh, to study high explosives. The head of this laboratory was George Kistiakowsky, and my direct boss was Louis Hammett. Hammett’s lieutenant was Frank Long. One of my colleagues was Rufus Lumry—just out of his Harvard B.A. The Bruceton laboratory was loaded with outstanding people.

After the war, Rufus returned to Harvard to do a thesis under Kisty which investigated one of the well-known fables of the time—a “transition” that many biological reactions (in vitro as well as in vivo) showed when you plotted the log of the rate against the temperature. There was supposed to be an abrupt change in slope, almost always at the midpoint of the data, generally around 30 °C. Kisty realized that this must be nonsense because molecules the size of proteins are simply not large enough to show what amounts to a phase change over a very narrow temperature range. He suggested that Rufus measure a very large

number of rates for urease at closely spaced temperatures over a range of temperatures. As expected, Rufus found in this way that the data fell on a smooth curve, not on two straight lines [40]. Thus the intriguing and mysterious “transition,” that had seemed to many of us to contain such deep significance for our understanding of living systems, vanished into thin air.

A year and a half at Bruceton was followed by two years (1944–1946) at Los Alamos—a fabulous experience, but having nothing to do with the subject at hand. Nevertheless, I’ll take this opportunity to mention a matter that may be of interest to younger people to whom World War II is ancient history.

Many people today, especially younger people, wonder how it could be that anyone would agree to work on such a dreadful weapon as the atomic bomb.

One reply to this concern was that a terrible war was going on in the early 1940s and it was far from clear, especially before 1944, who was going to win. (It should not be necessary to point out that a victory by Hitler and the Japanese militarists would surely have led to a world totally inconsistent with all that the USA, let alone the enlightened forces of the Western—not to mention the Eastern—world, stand for.) If it could be made to work, the bomb would be a tremendous factor leading to victory. And indeed, before 1944, it was not at all clear that the Germans would not get it to work before we did.

But there was another, even stronger reason that many of us felt justified our working as hard as we could on the bomb. It was felt that if it were possible to make atomic bombs, somewhere, someday, someone would figure out how to do it and some country would proceed to make them. If this were done after World War II was over, the bomb would very likely be made as a secret weapon. This would inevitably lead to World War III. Therefore, there were strong reasons for trying to make the bomb—and use it—before the end of World War II. The people of the world would then know the terrible thing that could be unleashed if there were a World War III. And the politicians could then be encouraged to try to do something about preventing World War III.

Of course, this was exactly what happened—though something like 40 years of terrible fears for the future of humanity had to be endured before the world finally reached the present (1992) state of affairs. At long last it seems to be assured that the superpowers will not, after all, clobber each other (and undoubtedly ordinary powers and even innocent countries as well) with hundreds of bombs and superbombs, most likely ending civilization as we know it.

Under the circumstances that existed in the early 1940s, I therefore feel that it is inconceivable that any reasonable person should have refused to work on a project such as that which existed at Los Alamos.

Is it necessary to point out, especially to young people, that the problem of adjusting to evil in this world of ours

is not a simple one? It is not enough to take the easy step of simply refusing to do an obviously bad thing—there are often complications that make these decisions difficult. It is not enough to grasp the halo and place it on one’s head, where everyone can see it and where it makes you “feel good inside.” The problems of the world must be *solved*, not merely *disregarded*.

At the end of January 1946, I left Los Alamos and drove to Colorado, where I rented a cabin in the mountains a few miles below Long’s Peak, about 50 miles from Boulder. The cabin belonged to Professor Lubovich of the University of Colorado Physics Department. It was located in Peaceful Valley, above Raymonds, at the head of the South St. Vrain Canyon west of Lyons.

Professor Lubovich and his wife were delightful people. Between February and June 1946, I had lunch with them every Friday when I made my weekly trip to Boulder to buy groceries and to take out more books and journals from the University Library. I learned to drink tea, sweetened with jam, from a glass, and to eat salmon roe as a cheap substitute for caviar, thickly spread on a slice of bread.

The Luboviches had left St. Petersburg with the retreating German Army in 1918 after the take-over by the Soviets, believing that the country would soon fall apart. He was a spectroscopist and they eventually came to the United States, where he joined the faculty of the University of Colorado. They had many wonderful stories to tell—a typical Russian couple with a pungent sense of humor and strong liberal opinions, also proud of the role that Russia had played in the defeat of Hitler.

One of their stories had to do with the notion of freedom. They pointed out that there are two kinds of freedom: political freedom and social freedom. In Russia there was no political freedom under the czars. But there was a much more liberal social freedom in czarist Russia than existed in Boulder in 1946. By this they meant the following: if a villager—let’s call him Vladimir—decided to walk the streets barefooted in czarist Russia, everyone would have noticed it and talked about it; but it would have been accepted and all would have remained friends with Vladimir. But if Professor Lubovich had decided that the thing to do in Boulder was to walk the streets barefooted, he would have been socially ostracized. Of course, this was in 1946; Boulder after 1970 could hardly have been a more socially liberated city!

When I left Colorado in June of 1946, Professor Lubovich offered to sell me their cabin, and I was tempted. It was, however, fortunate that this did not happen. I have recently tried several times to find this cabin without success. That stretch of Peaceful Valley is now crowded with small vacation cottages. The road has been relocated and it is possible that the cabin is no longer in existence. (Professor Lubovich told me that the cabin had been built by a prizefighter—I believe he was from Missouri—who had left his wife and fled to the mountains with another

woman. The prizefighter had built it himself in something of a hurry and it was not built to last.)

The authorities at the University of Colorado very kindly permitted me to borrow books and journals from their excellent library. I proceeded to learn all that was known at the time about muscle, thinking that I ought to be able to devise a model for the direct conversion of chemical energy into mechanical energy. My notion was that the muscle protein, actomyosin, would exist in two states. (These two states might possibly be a random coil and a more regular folded form. Surely many others must have had the same idea at the time.) The stimulation of the muscle would convert the one form into the other, the transition being fueled by ATP. On the return to the stable form, the difference in energy could appear as either mechanical work or as heat.

The program that I had in mind was to fit this idea into the observed facts. What derailed the program was the well-known Fenn effect, and the thermochemical measurements of A.V. Hill [41], which showed that the amount of heat produced when a muscle shortens on stimulation is essentially the same, regardless of whether the muscle does work or not as it shortens. It was clear from this that any model of a real muscle would have to include complex kinetic effects, the biological purpose of which presumably had to do with the requirements for control of the muscle. I dropped the matter, though I have maintained an interest in the subject throughout the years. Of course, a great deal more has been learned about the structure of muscle since 1946, and it is now obvious that at that time there would have been no way of constructing a satisfactory model. For one thing, the structure of the muscle as determined by electron microscopy revealed a complexity that no one would or could have imagined in 1946.

I have continued to watch developments in the muscle field ever since. A fairly recent account of the current state in the field will be found in a book by T.A. McMahon [42]. Another exceptionally clear account is given by Sir Andrew Huxley [43]. The now widely accepted Huxley sliding filament model has, however, in my opinion a weak point in that the force-generating element is supposed to reside in some way in a hinge connecting the myosin "heads" to the body of the "thick filaments." (Huxley himself [44] raises this difficulty as one of those that remains to be explained by his model.) The amplitude of the movement of the heads must be quite large. It is difficult to see how the range of the normal forces that interact between atoms and between molecules (over no more than a tenth of a nanometer at most) could be made to produce the required movements of several nanometers between the actin filaments and the myosin rods.

Recently my Princeton colleague C. Schutt, along with U. Lindberg of the University of Stockholm, has proposed a model for the action of muscle based on the discovery of two interconvertible forms of actin (both of

whose structures are under investigation by X-ray diffraction). In this model, the force generator is the actin filament, not the myosin. The conversion of the one form into the other results in a length change of the actin filament. It presumably results from changes in the relative positions of actin monomers, and can occur by small changes in the positions of one monomer at a time. In this way large movements can occur that involve only molecular interactions between the monomers. This model, which places the force-generating element in the actin rather than in the cross-bridges, thus avoids the above-mentioned difficulty [45].

So it is possible that the goal of understanding the mechanism of the direct conversion of chemical energy into mechanical energy in muscle that I sought in 1946 may have been achieved in part in the Department of Chemistry at Princeton!

In 1946 Henry Eyring decided to leave Princeton for the University of Utah. The motivation for this move was his wife's unhappiness at the prospect of raising three sons in the non-Mormon atmosphere that existed in Princeton. Henry would have liked to remain in Princeton. Hugh Taylor strove mightily to change Mrs. Eyring's mind in this, but to no avail. Many years later I heard Henry give a talk at some festive occasion. He outlined a number of rules which he had found made his life easier. One of these was to "always do what your wife tells you to do; it simplifies things immeasurably." I must say that I, too, have found this to be a generally useful piece of advice.

Henry evidently recommended me for the position that he was leaving—though I believe that at least one other person was offered the job and turned it down because the salary was too low. In any case, Hugh Taylor, after some trouble, located me in the cabin in the Rockies and I was offered the position. I have always been grateful to the Mormon Church for its role in this situation.

I was now in a position to follow my interest in applying physical chemistry to proteins. I began by using optical rotation and other conformation-dependent properties to follow the conformation changes of a number of readily available proteins on denaturation. A series of able graduate students produced a number of papers which were centered more on the kinetics of the process than on the thermodynamics (although Eyring's rate theory could be used to relate these two, and indeed, the great mass of subsequent studies in many laboratories have shown that the effects of temperature, pressure, salts, solvents, etc. on the rate of denaturation parallel the effects on the thermodynamics).

The students mainly involved in this work were Richard B. Simpson, John Schellman, H. Karl Frensdorff, Marshall T. Watson, and, somewhat later, Joseph P. Simko, Jr. I might add that Charles Tanford was a graduate student in the Princeton Chemistry Department when I came there in 1946. His thesis was concerned with the velocity of the flame front in a combustible mixture

of hydrocarbons with oxygen, which at the time was thought to be determined entirely by the rate of conduction of heat across the front. He suggested a possible role of the diffusion of hydrogen atoms in determining the flame speed—a remarkably ingenious idea. Tanford attended my informal lectures on proteins and we talked a lot about the subject. He decided that his future lay with proteins rather than with flames, and he went on to a postdoctoral position in the Cohn group at Harvard, and of course, to a very distinguished career in protein chemistry. So perhaps I can claim him as one of my most important discoveries.

Naturally this work, and the reading of the literature, made one wonder about the molecular factors that governed protein behavior. Much discussion of these matters was taking place at the time. An important local source of information on this and related subjects resulted from the location, at that time, of the Princeton Division of the Rockefeller Institute, where the research groups of John Northrop and Wendell Stanley were located. I soon came to know Roger Herriott and Moses Kunitz, of Northrop's group, and more especially, Max Lauffer, Gerry Oster, and Howard Schachman, of Stanley's group.

Howard was enrolled as a graduate student in the Princeton Chemistry Department, and although he worked nominally under Lauffer's direction and officially under Taylor as thesis advisor, he began to come to see me quite regularly and we discussed what might be going on in his TMV problem. I certainly learned a lot more from Howard than he ever learned from me—though he claims that he was my first graduate student.

One of Howard's interesting observations came from a study of the dependence of the sedimentation constant of TMV on the density of the medium. If one plotted the sedimentation constant against the density, a straight line was obtained, and if this line was extrapolated to find the density, d_p , at which the TMV no longer sedimented, one would in this way be able to determine the overall apparent density of the TMV particle. Howard found that when the density of the medium was varied by adding sucrose, d_p was quite a bit larger than when the density was varied by adding bovine serum albumin. This could be explained if the TMV were enclosed in a semipermeable membrane—but this would be extremely difficult to accept. I was able to show that this result could be explained solely on the basis of the different sizes of the sucrose and serum albumin molecules—a result of the notions I had absorbed in following the simple hydrodynamic basis of fluid flow around large molecules that went back to Einstein's early work on the viscosity of suspensions of rigid spheres [46].

The phenomenon just discussed brings up a subject that has always bothered me: the hydration of proteins. The notion that proteins are "hydrated" goes back at least to the early 1930s. In order to make sense of the data on sed-

imentation velocity, diffusion constant, and viscosity of proteins in solution, models were developed based on the hydrodynamic theory of the flow of a continuous fluid around rigid spheres and ellipsoids. This was a very reasonable approach, as a first approximation, and gave the result that proteins generally behaved, hydrodynamically, as if about 0.3 g of water was immobilized per gram of protein. This might be a rigid layer of water approximately one molecule thick attached to the outside of the protein, or it could equally well be that this amount of water was tied up inside the protein molecule and moved along with it.

But before taking this result too seriously, one has to ask what, on the scale of protein molecules in water, might be happening in the layer of water with a thickness comparable with the size of a water molecule (approximately 3 Å) immediately adjacent to the surface of the protein. If the water is to be treated as a continuum, there should be a negligible gradient in the shear rate as one passes through this first layer. In fact, if one looks at the situation in detail [47] one finds that there would be large shear gradients within the first water layer. It therefore becomes difficult for me to take this "hydration" value of 0.3 g per gram of protein seriously. Similarly, the relatively large hydration values determined by sedimentation equilibrium in cesium chloride gradients can be explained very easily if one realizes that the ions of cesium chloride are repelled as they move from the high dielectric constant of water toward the low dielectric constant of the protein [48].

As a result of considerations such as these, I have become more and more disillusioned with the fuzziness of the concept of "protein hydration." I will not go into this any further at this point, however. It is a big subject, and there continues to be a great deal of interest in it—but in my opinion "hydration" is something of a buzzword, and I wish that people would find some other way of talking about it—or at least recognize the shortcomings of the concept.

I had known Gerald Oster from my days at Westinghouse, he having had a summer job there while a graduate student at Cornell. His thesis advisor was Peter Debye, who had come to Cornell after I had graduated; I never had the benefit of his remarkable perceptions, but had to put up with those of Bancroft, as we have seen. At the Rockefeller Institute, Oster had been studying light scattering by TMV solutions. One day he came into my lab with great excitement. It was known that concentrated solutions of TMV separated into two clear, liquid phases. When Gerry had observed the scattering of monochromatic light by the dense phase after it had been standing for a long time, he was astonished to find very strong scattering at a single angle. This turned out to be a Bragg reflection. The rodlike TMV molecules oriented themselves in a parallel arrangement with astonishingly large inter-

molecular distances—hundreds of Ångstrom units. At the time it was thought that this must be the result of exceptionally long range forces, and a lot of papers appeared speculating over how this could be. There was a feeling that some extension of the Debye–Hückel theory might reveal a small minimum in the distance vs. free energy curve for long charged rods in dilute ionic solutions; this was typical of some of the ideas that were bandied about at the time.

Finally Onsager [49] showed that the phase separation had an absurdly simple geometrical explanation: if one tries to cram more and more pencils into a box, one will reach a point at which a new “phase” will appear in which the pencils are packed in a parallel arrangement. I was present at the New York Academy meeting at which Onsager presented this explanation. But he, like Niels Bohr, was famous for giving unintelligible talks. (He spoke with a strong Norwegian accent in a soft tone of voice, hardly audible, and he expressed his ideas almost exclusively in terms of equations.) Few in the audience understood him. After the talk, Peter Debye, who was chairing the session, explained what Onsager had said in the exceptionally clear fashion for which he was famous. It was always a good idea to have Debye around when a speaker presented a paper that no one could understand.

There were other advantages in having the Rockefeller Institute group present in the Princeton neighborhood. There were frequent small group meetings at which people from the New York branch of the Institute came down to tell the Princeton people what was going on in New York, and I found that I was welcome at these sessions.

I can remember hearing in this way about the epoch-making work of the Avery group in identifying DNA as the genetic factor in the transformations of the various forms of pneumococcus. It is worth remembering that Avery met with a great deal of skepticism at the time over his claim that genetic information might reside in the nucleic acids. Many people felt that genetic information had to reside in proteins, nucleic acids being felt to be too simple. (After all, there are only four different nucleotides in a nucleic acid molecule, but 20 different amino acids in a protein molecule!)

Unfortunately the powers that were responsible for the well-being of the Rockefeller Institute felt it necessary to close down the Princeton Division in the late 1940s, so these interactions came to a halt. Most of the Princeton Rockefeller people dispersed across the country—Northrop and Stanley to Berkeley, Herriott to Baltimore, Lauffer to Pittsburgh, Schachman to Berkeley. Gerry Oster spent a few years in England and France before settling at Brooklyn Poly (where, incidentally, I gave course lectures for a period around 1950), and later at Mount Sinai. Kunitz was one of the few who went to New York.

I did, however, resume an interaction with the Institute when for several years I gave a course in quantum me-

chanics in New York to the Rockefeller University graduate students. In this way I came to know Longworth, Gerty Perlmann, Edelman, Stein, Moore, Shedlovsky, and many others.

The Gordon Research Conference on Proteins, located then as now at the New Hampton School, had begun shortly after the end of the war, and I attended nearly all of them for many years from the very start and got to know many of those working in the protein field.

In June of 1949, I attended a Cold Spring Harbor Symposium on protein structure [50] at which Linderstrøm-Lang spoke. A sabbatical term was coming up, and I asked him if I might spend some time at the Carlsberg Laboratory. He graciously agreed.

In July of 1949 I flew to Copenhagen, where I spent a few days soaking in the glorious summer atmosphere of that delightful city and made arrangements for a three month's stay at the Carlsberg Laboratory beginning in October. Then after an exhausting but memorable month of walking in the Alps, I spent six weeks in Cambridge, where the first International Congress on Biochemistry was taking place. All of the great names in the field were there, and I learned a great deal from the talks.

One incident at this conference sticks in my mind with special force. Fred Sanger gave a talk on how he proposed to determine the sequence of the amino acids in insulin, using his reagent, fluorodinitrobenzene, to tag the ends of the chains of partially hydrolyzed protein at various degrees of hydrolysis. It was a beautiful idea, but as I remember it, a number of people sprang to their feet after the talk and explained why the method might not work; it seemed likely to them that during the acid hydrolysis one might expect peptide–peptide interchange reactions to take place, resulting in sequence rearrangements. It would be hard to tell whether or not this had happened except after a great deal of work. Sanger just stood there with a slight smile and a distant look on his face and said nothing. I continue to admire his guts in not letting the pessimists discourage him—or so it seemed to me.

While at Cambridge I also met Max Perutz, who was struggling with the problem of deciphering the X-ray diffraction pattern of hemoglobin.

An interesting observation had been made in our laboratory in the course of adding crystallized bovine serum albumin to concentrated urea. A cloudiness developed which produced a curious sheen on stirring. Examination under the microscope revealed tiny needle-like crystals. Max jumped at the possibility that we had here a crystalline form of a denatured protein—which would have been a major discovery. A small quantity of the precipitate was prepared, the needles were oriented in a capillary, and a diffraction photograph was taken. It did not, however, reveal any long spacings, so the matter was dropped.

In October 1949, I went to the Carlsberg Laboratory for my first exposure to the wonderful atmosphere gen-

erated there by Linderstrøm-Lang. I mentioned the curious precipitate that we had observed when serum albumin was exposed to concentrated urea. It might have been Lang who remarked that people in the lab were suspicious of urea because they had observed that when kerosene and strong urea solutions were mixed and stored in the refrigerator, the contents of the flask solidified, giving a white solid. I confirmed that observation and proceeded to expose samples of every organic liquid in the Carlsberg storeroom to strong urea. I found that every straight chain organic liquid (including, for instance, diethyl ether—the atoms in the chain did not all have to be carbon) produced a crystalline solid, but that if the chain was branched, no solid was formed. It was thus evident that our precipitate was the urea-decanol clathrate formed by the release on denaturation of the decanol that was used at that time to induce the crystallization of serum albumin.

At this point Lang's son-in-law, Max Møller, mentioned that he recalled seeing an article on this subject in the recent German literature. Sure enough, a long paper by Schlenk [51] had just appeared which not only described the phenomenon, but gave X-ray structures and many details. It turns out that urea is able to crystallize in a form that produces tubular channels which are of the correct dimensions to hold straight chain hydrocarbons—a clathrate or inclusion compound.

The article by Schlenk notes that, in 1940, Friederich Bengen had made a German Patent application for the separation of straight chain hydrocarbons from mixtures based on similar observations. It appears that this patent application had been noted during the war by American and British authorities. I understand that it was responsible for an extensive development program with the aim of improving the octane rating of gasoline by removing the straight chains. (Branched chain hydrocarbons have considerably higher octane ratings than straight chain hydrocarbons.) This would permit higher compression ratios with improved aircraft engine performance. The discovery of catalysts which converted *n*-hexane and *n*-heptane to benzene and toluene (which have markedly higher octane ratings), however, rendered the urea process unnecessary. (H.S. Taylor played a role in developing this catalytic process.)

According to J.E. Davies [52], the use of urea to separate straight chain from branched chain hydrocarbons was patented in the USA in 1950 [53]. The crystal structure of the urea-dodecane complex was described by A.E. Smith [54].

In any case, if any of several other groups had not, over the previous nine years, discovered these urea-straight chain clathrates, I would have been the discoverer.

This observation made a strong impression, although it was not my introduction to the notion that nonpolar molecules can generate clathrate structures in hydrogen bonded compounds; people were well aware of the exis-

tence of such structures by that time. (The term clathrate had been introduced by H.M. Powell in 1948 [55].)

This episode, which occurred in the fall of 1949, served to impress upon my mind the concept of a molecule's ability to generate structures in its surroundings—which was to be an important element eventually in understanding the thermodynamics of the hydrophobic interaction, as applied to protein folding.

It is now time to turn to the history of my thoughts on the so-called “hydrophobic bond” or as some prefer to call it, the “hydrophobic interaction” or the “apolar bond.” Wu [56] had been perhaps the first to point out in clear terms that in native proteins the polypeptide chain must have a definite conformation. Before 1940 many people had thought about the forces that must be operating to hold proteins in their “native conformations.” Mirsky and Pauling and Huggins [57] had suggested that hydrogen bonds must be an important factor in maintaining this conformation. Others had suggested that “salt bridges” between positive and negative side chains might be of some importance.

Finally, there had been a general realization that the nonpolar side chains would tend to exist in the interior of the protein, and out of contact with water. This last “interaction” was often called at the time the “van der Waals Interaction.” It was based on the same kinds of interactions as those responsible for the formation of micelles in detergent solutions; they were lucidly discussed by Linderstrøm-Lang in his Lane Lectures at Stanford [58]. I vividly recall a paper by Palmer [59] in which a structure is proposed for ovalbumin with the polypeptide chain folded in zigzags in flat layers, the layers being held together by “van der Waals bonds” and the overall shape of the molecule being more or less a cube. (Back in those days, when one thought about what the structure of a protein might be, this is the sort of thing that one came up with! Note that the actual structure of ovalbumin is now being worked out by Toni Wright—who spent many years in an adjoining laboratory in Princeton. And how satisfying it is to learn that ovalbumin is not the dead, biologically uninteresting substance that most of us thought it was! It turns out to be a member of an important class of proteins known as the serpins—the serine protease inhibitors [60].)

It seemed to me that, in view of the fact that the driving force for the sequestering of the nonpolar groups in the interior was their desire to get away from the aqueous environment, it would be more appropriate to call these bonds “hydrophobic bonds.” I coined the term in a paper entitled “Denaturation of proteins and enzymes,” given at a conference at The Johns Hopkins University in June of 1953 and published in 1954 [61]. The rationale given was that “the forces ordinarily called van der Waals forces probably contribute only a small fraction to the total energy of hydrophobic bonds.” Furthermore the re-

mark was made that “in a sense, hydrophobic bonds also represent a kind of hydrogen bond (or ‘anti-hydrogen bond’) because they arise by being unable to participate in the strong hydrogen bonding of water molecules; that is, they are stabilized by the rather strong hydrogen bonds of water.” In my mind at that time I had the picture that a nonpolar molecule in water actually *disrupted* the water structure by breaking hydrogen bonds, although I did not actually say this.

Of course, this is quite different from what I now believe is the actual driving force; it took me much longer to realize this, however, than it should have!

The light finally went on when I visited the Carlsberg Laboratory again for a seven month stay in 1957. When I first made my appearance, Lang was not feeling well, and I can still see him stretched out on a couch. We were discussing the hydrophobic bond, and I guess I said something about the reason for the reluctance of nonpolar groups to expose themselves to water must be that they break or disrupt the hydrogen bonding in the water. Lang’s response to this was, “That’s strange. When you mix alcohol with water, heat is given off!”

It was this remark that set me to thinking more carefully and to reading further in the literature. Several people had become aware of the presence of strange entropy effects when nonpolar molecules mixed with water [62]. It was, however, the paper of Frank and Evans [63] that most clearly set the stage for what I believe is the correct picture of the hydrophobic bond. Frank and Evans considered the process in which a nonpolar gas phase atom or molecule (such as argon or methane or benzene) is introduced into a liquid solvent. They found that the entropy decrease expected from an extended form of Trouton’s Rule (known as the Barclay–Butler plot [64]) is observed when the solvent is almost anything but water. For the transfer into water, however, there is a considerably larger entropy decrease. Frank and Evans concluded that this was caused by the formation of what they called “icebergs” (which can be considered a synonym for the term “structure”) in the liquid around the solute molecules. They were careful to mention that the “iceberg” need not have the structure of ice; it was merely a more rigid and specific arrangement of water molecules than that found in normal liquid water.

The existence of such structured water is now supported by a whole range of crystalline hydrates of molecules such as argon, methane and chlorine, many of which have similar structures, and many more complex structures into which molecules and ions can sculpture the water in their vicinity [65]. Molecular simulations of water containing a molecule of methane or an atom of neon also support the existence of such structures [66] in aqueous solutions.

All of this is set forth in the paper that I wrote in 1959 [67]. But it was the small yet highly significant remark made by Linderstrøm–Lang while he lay resting on his

couch that set me off, belatedly, in the right direction. I am sure that I must have read the Frank and Evans paper long before this, but somehow its message did not set in as it should have until 12 years later.

I am, however, always perplexed by the reluctance of some workers to accept this picture of the hydrophobic bond, and similarly with proposals to redefine the reference state to a high temperature [68]. I could go on at length about my reasons for rejecting these objections, but in the end we have here no more than another example of someone who believes what he wants to believe. Let us leave it at that for the present.

I will close these reminiscences with the recollection of another event, important to me, which took place nearly 25 years ago—the *Study Program in Biophysical Science* organized mainly by F.O. Schmidt in Boulder, Colorado, July 20–August 16, 1958 [69]. It was here that I heard for the first time the description by John Kendrew of the myoglobin structure at 2 Å resolution. This was one of the most thrilling moments of my life! It indeed announced the opening a new era in the study of proteins!

The conference was intended to interest physicists and chemists in the many challenging problems that exist in biological systems, and to provide background for further study. The general areas covered, usually by some of the foremost workers in the fields, were:

- Cellular biology
- Physical and chemical characteristics of macromolecules
- Energy transfer and biochemical synthesis
- Genetics and replication of proteins and nucleic acids
- Biological effects of radiation
- Molecular organization and function
- Connective tissue and muscle
- Nerve
- Sensory reception and signal processing
- Specificity in the chemical control of biological systems

Anyone scanning the papers presented at this “study program” can see most of the currents flowing or beginning to flow into presently active areas of research in proteins and molecular biology.

A large number of well-known physicists and chemists attended portions of the conference, among them Astbury, Norman Davidson, David Davies, D.F. Hornig, P. Morrison, John Platt, R. Podolsky, S.J. Singer, L. Szilard, and C.H. Townes.

Leo Szilard gave a talk, unfortunately not included in the proceedings, probably on his invention, with A. Novick, of the chemostat. After the talk there were some questions. In response to one of these, having to do with some experiment performed by a biologist, Szilard remarked, “Biologists simply do not know how to do the right experiments!” I have never forgotten the look of contempt on his face when he made this remark.

The Boulder Conference resulted in a development important to the Kauzmann family that is perhaps of interest.

Elizabeth and I drove out to Boulder in a station wagon with three children (ages 1, 3, and 5) plus a 17-year-old Danish au pair girl, Hedda Wassard-Jørgensen, who had stayed with us in the previous year in Denmark. We camped (in two tents) on the way out and occupied two rooms in a University of Colorado dormitory for the entire conference, taking our meals in the dining room with the rest of the conferees. On weekends we went off camping—just to see if it were possible (and we found out that it *was* possible). After the conference we toured the northern Rockies—Jackson Hole, Yellowstone, the Devils Tower in the Black Hills—camping the whole way.

As a result of this experiment, I expected that in the future our vacations would consist of trips to the Rockies, packing in to some remote lake, hiking, fishing, and enjoying timberline living.

But this was not to be. As the summer of 1959 approached, Elizabeth suggested that we defer the 2,000-mile drive across the Midwest until the children were a bit older. I was agreeable, so we went off on a leisurely camping trip to New Hampshire, Maine, New Brunswick, and Nova Scotia.

We ended up at the northern tip of Cape Breton Island. Here we were lucky to find and purchase an abandoned house close to the ocean, with a view of mountains rising straight out of the sea, a fantastically beautiful beach (with water temperatures in the 65–70° range), enormous areas of trackless wilderness, stream and ocean fishing, and wonderful people. We have been going back there summers ever since, at first for a month or so, and now for as long as 3½ months.

Experiments in life as well as in science lead to unexpected results. And the best experiments are those that lead to the least expected results.

Going back to, say, 1940, I can state with no hesitation that no one at that time could have believed that 50 years later we would be asking the questions about proteins that are now being asked.

And I have little doubt that 50 years from now the same will be said of us today. People will continue to believe what they want to believe and the criteria for Truth will be debated in more or less the same way as we debate them today. Man will still be driven by Schopenhauer's Will (which, after all, is all that is behind believing what one wants to believe). But can we not hope that Reason will have gained a bit more control over The Will than it now has?

Acknowledgments

I should like to thank Jacques Fresco, John Edsall, Charles Tanford, Bruce Martin, and C. Peter Kauzmann for pointing out various errors and obscurities in the original manuscript.

Endnotes

1. The history of the introduction of physical chemistry to the United States, mainly by Bancroft, A.A. Noyes, and G.N. Lewis, is told in the fascinating book, *Physical Chemistry from Ostwald to Pauling. The Making of a Science in America*, by John W. Servos (Princeton University Press, Princeton, New Jersey, 1990). Wilder Bancroft plays an important role in this story—which Servos describes in great detail. For biographies of George Bancroft see *George Bancroft, Brahmin Rebel*, by Russel B. Nye (Alfred B. Knopf, New York, 1945), and *George Bancroft, the Intellectual as Democrat*, by Lilian Handlin (Harper & Row, New York, 1984).

The Bancroft here mentioned is not to be confused with Hubert Howe Bancroft (born 1832), who wrote extensively on the history of Western America and who created the Bancroft Library at the University of California, Berkeley.

2. W.D. Bancroft and G.H. Richter, "The chemistry of anesthesia," *J. Phys. Chem.* 35, 215–268 (1931); W.D. Bancroft and J.E. Rutzler, Jr., "The colloid chemistry of nervous systems," Part I, *J. Phys. Chem.* 35, 1185–1211 (1931); Part II, *J. Phys. Chem.* 35, 3037–3057 (1931); Part III, *J. Phys. Chem.* 35, 3189–3206 (1931); Part IV (with R.S. Gutsell), *J. Phys. Chem.* 36, 1521–1548 (1932); Part V (with R.S. Gutsell), *J. Phys. Chem.* 36, 2011–2082 (1932); Part VI, *J. Phys. Chem.* 36, 3162–3174 (1932).

See also a long series of articles in the *Proceedings of the National Academy of Sciences*: W.D. Bancroft and G.H. Richter, "Claude Bernard's theory of narcosis," *Proc. Natl. Acad. Sci. USA* 16, 573–577 (1930); W.D. Bancroft and J.E. Rutzler, Jr., "Reversible coagulation in living tissue," *Proc. Natl. Acad. Sci. USA* 17, 482–484 (1931).

These are followed by a series of papers entitled "Reversible coagulation in living tissue": W.D. Bancroft and J.E. Rutzler, Jr., *Proc. Natl. Acad. Sci. USA* 17, Parts I, pp. 105ff.; II, pp. 186ff.; V, pp. 482ff.; VI, pp. 570ff.; VII, pp. 575ff.; VIII, pp. 597ff. (1931); W.D. Bancroft and G.H. Richter, *Proc. Natl. Acad. Sci. USA* 17, Parts III, pp. 294ff.; IV, pp. 410ff. (1931); W.D. Bancroft, R.S. Gutsell, and J.E. Rutzler, Jr., *Proc. Natl. Acad. Sci. USA* 17, Part IX, pp. 637ff. (1931); *Proc. Natl. Acad. Sci. USA* 18, Part X, pp. 8–15 (1932); *Proc. Natl. Acad. Sci. USA* 19, Part XI, pp. 85–91 (1933); W.D. Bancroft and J.E. Rutzler, Jr., *Proc. Natl. Acad. Sci. USA* 20, Part XII, pp. 501–509 (1934).

3. W.D. Bancroft and J.E. Rutzler, Jr., "The agglomeration theory of sleep," *Proc. Natl. Acad. Sci. USA* 19, 73–78 (1933).

4. W.D. Bancroft, "One aspect of the longevity problem," *Science* 81, 152–153 (1935).

5. W.D. Bancroft, Review of *Elementary Physical Chemistry*, by H.S. Taylor, *J. Phys. Chem.* 31, 1584–1588 (1927).

6. Richard M. Willstätter, *Untersuchungen über Enzyme*, 2 vols., J. Springer (1928). It is interesting that Willstätter, in his autobiography, *Aus meinem Leben* (translated by L. Hornig: *From My Life*, W.A. Benjamin, Inc., New York, 1965), makes relatively little mention of the tremendous effort he expended on this problem.

7. Strangely enough, the physicist W. Pauli's godfather was Ernst Mach, the influential Austrian physicist of the late nineteenth century, famous for his refusal to believe in the existence

of atoms. Mach's ideas had an important influence on Wilhelm Ostwald, and through him on Bancroft.

8. I believe that this was M.G. van Campen. In the elementary organic laboratory my teaching assistant was H.S. Snyder, later to become Professor of Chemistry at the University of Illinois. Because Professor Johnson was away on leave, the lecturer in introductory organic chemistry was Ralph Connor, who went into industry and became a dominant figure in the Rohm and Haas Chemical Company during and after the 1940s.

9. Nevil V. Sidgwick (1873–1952), *Some Physical Properties of the Covalent Link in Chemistry* (Cornell University Press, Ithaca, New York, 1933).

10. A few of Eyring's papers that attracted my attention were:

"The quantum mechanics of seven and eight electrons with spin degeneracy," *J. Chem. Phys.* 1, 239 (1933).

"Molecular symmetry and the reduction of the secular equation," *J. Chem. Phys.* 2, 410 (1933).

"Resolution of crossed bond eigenfunctions in terms of the uncrossed set," *J. Chem. Phys.* 2, 299 (1934).

"The resolution of bond eigenfunctions in terms of a linearly independent set," *J. Chem. Phys.* 3, 224 (1935).

"Calculation of the energy of the H₃ molecule," *J. Chem. Phys.* 4, 121 (1936).

11. H.H. Weber and D. Nachmannsohn, "The independence of its ionization of the hydration of protein," *Biochem. Z.* 204, 215–252 (1929); H.H. Weber, "Bjerrum's theory of 'two ions' and the hydration of proteins," *Biochem. Z.* 218, 1–35 (1930). The volume increase is caused by the removal of the electrostriction of the water surrounding the electrically charged ionic groups, as pointed out in the seminal paper of P. Drude and W. Nernst, "On electrostriction by free ions," *Z. Phys. Chem.* 15, 79–85 (1894).

12. An interesting further study of these volume changes, revealing some puzzling and still unexplained problems, will be found in studies by J. Rasper, A. Bodanszky, and W. Kauzmann, "Volume changes in protein reactions. I and II," *J. Am. Chem. Soc.* 84, 1771–1777, 1777–1788 (1962).

13. K. Landsteiner, *The Specificity of Serological Reactions* (Harvard University Press, Cambridge, Massachusetts, 1945). See, however, L. Pauling, "Theory of the structure and process of formation of antibodies," *J. Am. Chem. Soc.* 62, 2643–2657 (1944). Although Pauling's ideas on the importance of shape complementarity in protein interactions are of the utmost significance, his theory of the formation of antibodies in living organisms—so attractive to us at the time—turned out to be quite wrong and illustrates the dangers of approaching biological problems with overly simplistic physical theories. As will be described further on in this paper, I was to stumble in a similar fashion (but not in print!) some years later in my efforts to understand how muscles convert chemical energy into mechanical energy.

Twenty years later I had occasion to summarize some of these ideas of complementarity in a review paper, "Chemical specificity in biological systems," *Rev. Mod. Phys.* 31, 549–556 (1959).

14. W.T. Astbury and A. Street, "X-ray studies of the structure of hair, wool and related fibres. I. General," *Trans. R. Soc. Lond. A230*, 75–101 (1931); W.T. Astbury and H.J. Woods,

"X-ray studies of the structure of hair, wool and related fibres. II. The molecular structure and elastic properties of hair keratin," *Trans. R. Soc. Lond. A232*, 333–394 (1934); W.T. Astbury and W.A. Sisson, "X-ray studies of the structure of hair, wool and related fibres. III. The configuration of the keratin molecule and its orientation in the biological cell," *Proc. R. Soc. Lond. A150*, 533–551 (1935).

15. H. Neurath, "Intramolecular folding of polypeptide chains in relation to protein structure," *J. Phys. Chem.* 44, 296–305 (1940).

16. H.S. Taylor, "Large molecules through atomic spectacles," *Proc. Am. Philos. Soc.* 85, 1–12 (1942). At about the same time Huggins also came upon a structure resembling the α -helix by manipulating space-filling models. See M. Huggins, "The structure of fibrous proteins," *Chem. Rev.* 32, 195–218 (1943).

17. M. Perutz, "The new Marxism," *New Scientist*, July 15, 1989, pp. 72–73.

18. The scandalous story of the German woman scientist, Agnes Pockels (1862–1935) should be better known than it is. Deeply interested in physics but denied entry into any German university for advanced work, she developed the trough method of measuring surface tensions in her kitchen. The story is well told in the book, *Ben Franklin Stills the Waves*, by Charles Tanford (Duke University Press, Durham, North Carolina, 1989). See also C.H. Giles and S.D. Forrester, "The origins of the surface film balance," *Chemistry and Industry*, January 9, 1971, pp. 43–53.

19. J.D. Bernal and R.H. Fowler, "A theory of water and ionic solutions, with particular reference to hydrogen and hydroxyl ions," *J. Chem. Phys.* 1, 515–548 (1933). As far as I am concerned, the final word on the structure and thermodynamic behavior of water was presented in the paper "Equation of state of a random network, continuum model of liquid water," by A.R. Henn and W. Kauzmann, *J. Phys. Chem.* 93, 3770–3783 (1989). The ideas in this paper have their beginning in the Bernal–Fowler paper, as well as those of Stuart Rice and M.G. Scats.

20. T. Svedberg, "Mass and size of protein molecules," *Nature* 123, 781 (1929); "Ultracentrifugal dispersion determinations of protein solutions," *Kolloid Z.* 51, 10–24 (1930); "The pH stability regions of proteins," *Trans. Faraday Soc.* 1930, 741–744 (1930); "Sedimentation of molecules in centrifugal fields," *Chem. Rev.* 14, 1–15 (1933).

21. W.T. Astbury and H.J. Woods, "The molecular weights of proteins," *Nature* 127, 663–665 (1931).

22. M. Bergmann and C. Niemann, "The structure of proteins: Cattle hemoglobin, egg albumin, cattle fibrin and gelatin," *J. Biol. Chem.* 118, 301–314 [esp. p. 305] (1937).

23. D.M. Wrinch, "On the pattern of proteins," *Proc. R. Soc. Lond. A169*, 59–86 (1937). The quotation will be found on page 75. See also D.M. Wrinch, "The cyclol hypothesis and the 'globular' proteins," *Proc. R. Soc. Lond. A161*, 505–524 (1937).

24. Irving Langmuir and Dorothy Wrinch, "Nature of the cyclol bond," *Nature* 143, 49–52 (1939). Also Irving Langmuir, "The structure of proteins," *Proc. Phys. Soc. Lond.* 51, 592–612 (1939); Irving Langmuir and Dorothy Wrinch, "A note on the structure of insulin," *Proc. Phys. Soc. Lond.* 51, 613–618 (1939); these two papers were followed by active discussion in which the ideas were criticized by Bernal (*Proc. Phys. Soc. Lond.* 51, 618–620) and others.

25. See note 15.

26. See L. Pauling and C. Niemann, "The structure of proteins," *J. Am. Chem. Soc.* **61**, 1860–1867 (1939), where a great many powerful arguments against the cyclol theory are presented.

27. F. Haurowitz and T. Astrup, "Ultraviolet absorption of genuine and hydrolysed protein," *Nature* **143**, 118–119 (1939).

28. H. Neurath, "Some physical and chemical properties of the proteins," *Cold Spring Harbor Symp. Quant. Biol.* **8**, 80–91 (1940). Note especially pp. 82–83.

29. D. Wrinch, *Chemical Aspects of the Structures of Small Peptides: An Introduction* (Munksgaard, Copenhagen, 1960).

30. See *Uneasy Careers and Intimate Lives. Women in Science, 1789–1979*, Pnina G. Abir-am and Dorinda Outram, Eds. (Rutgers University Press, New Brunswick, New Jersey, 1987), pp. 239–280.

31. M. Bergmann, "Proteins and proteolytic enzymes," *Harvey Lect.* **31**, 37–56 (1935–1936); M. Bergmann and C. Niemann, "Blood fibrin. A contribution to the problem of protein structure," *J. Biol. Chem.* **115**, 77–85 (1936); "The structure of proteins: Cattle hemoglobin, egg albumin, cattle fibrin and gelatin," *J. Biol. Chem.* **118**, 301–314 (1937); "Newer biological aspects of protein chemistry," *Science* **86**, 187–190 (1937).

32. Some titles to be found in the list of Eyring's publications will illustrate this extreme breadth of interest:

"The mechanical properties of textiles" (a series of 11 papers).

"Stability of detonation."

"A theory of creep of ceramic bodies under constant load."

"Some applications of modern rate theory to physiological systems."

"Chemical alterations produced in tissues by irradiation."

"Molecular mechanisms in inflammation and stress."

"Sodium transport in isolated frog skin."

"A theory of thixotropy and its application to grease."

"Flow properties of lubricating oils under pressure."

"Scientific creativity."

"The physical chemistry of nerve action."

"The mechanical properties of rat tail tendon."

"The electrochemical theory of smelting and related reactions."

"Formation of color centers in sapphire by solar radiation."

"Strain electrometry and passivation of aluminum electrodes."

"Biological electron transport."

"Experimental study of the effects of temperature and ultra-high pressure on the coalification of bituminous coal."

"Mechanism for the plastic deformation of yule marble."

"The dynamics of life. I. Death from internal irradiation by ^{239}Pu and ^{226}Ra , aging, cancer and other diseases."

"Magnetic circular dichroism in molecules in dense media: Benzene."

"Applications of the survival theory to ecology."

"Interferon induction: A conformational hypothesis."

"Mechanisms of triboluminescence."

33. F.H. Johnson, H. Eyring, and M.J. Polissar, *The Kinetic Basis of Molecular Biology* (John Wiley & Sons, New York, 1954); F.H. Johnson, H. Eyring, and B.J. Stover, *The Theory of Rate Processes in Biology and Medicine* (John Wiley & Sons, New York, 1974).

34. F.H. Johnson and E.A. Flagler, "Hydrostatic pressure reversal of narcosis in tadpoles," *Science* **112**, 91–92 (1951); "Activity of narcotized amphibious larvae under hydrostatic pressure," *J. Cell. Comp. Physiol.* **37**, 15–25 (1951).

35. H. Eyring and A.E. Stearn, "Application of the theory of absolute reaction rates to proteins," *Chem. Rev.* **24**, 253–270 (1939).

36. A detailed biography of Henry Eyring, including many details of his activities in the Mormon Church, is given in the master's thesis "Henry Eyring Mormon scientist," by Steven Harvey Heath (Department of History, University of Utah, Salt Lake City, Utah, 1980).

37. M. Polanyi, *Personal Knowledge. Towards a Post-Critical Philosophy* (University of Chicago Press, Chicago, Illinois, 1962).

38. W. Kauzmann, "The nature of the glassy state and the behavior of liquids at low temperatures," *Chem. Rev.* **43**, 219–256 (1948).

39. R.W. Cahn, "Missing atoms and melting," *Nature* **356**, 108–109 (1992).

40. G.B. Kistiakowsky and R. Lumry, "Anomalous temperature effects in the hydrolysis of urea by urease," *J. Am. Chem. Soc.* **71**, 2006–2013 (1949).

41. A.V. Hill, "The heat of shortening and the dynamic constants of muscle," *Proc. R. Soc. Lond. B126*, 136–195 (1935).

42. T.A. McMahon, *Muscles, Reflexes and Locomotion* (Princeton University Press, Princeton, New Jersey, 1984).

43. Sir Andrew Huxley, *Reflections on Muscle* (Princeton University Press, Princeton, New Jersey, 1980).

43. Sir Andrew Huxley, *Reflections on Muscle* (Princeton University Press, Princeton, New Jersey, 1980).

44. Sir Andrew Huxley, *Reflections on Muscle* (Princeton University Press, Princeton, New Jersey, 1980), pp. 41–42.

45. A preliminary description of the model will be found in C. Schutt and U. Lindberg, "Actin as the generator of tension during muscle contraction," *Proc. Natl. Acad. Sci.* **89**, 319–323 (1992).

46. H.K. Schachman and M.A. Lauffer, "The hydration, size and shape of tobacco mosaic virus," *J. Am. Chem. Soc.* **71**, 536–541 (1949).

47. I.D. Kuntz, Jr. and W. Kauzmann, "Hydration of proteins and polypeptides," *Adv. Protein Chem.* **28**, 239–345 (1974).

48. See M.F. Perutz, "Electrostatic effects in proteins," *Science* **201**, 1187–1191 (1978).

49. L. Onsager, "The effects of shapes on the interactions of colloidal particles," *Ann. N.Y. Acad. Sci.* **51**, 627–659 (1949).

50. See *Cold Spring Harbor Symposia on Quantitative Biology*, Volume XIV, where a list of attendees is given, along with several photographs of Linderström-Lang.

51. W. Schlenk, Jr., "Die Harnstoff-Addition der Aliphatischen Verbindungen," *Ann. Chem. Leipzig* **565**, 204–240 (1949). Schlenk's paper came from the ammonia laboratory of BASF in Oppau. See also W.J. Zimmerschied, R.A. Dinerstein, A.W. Weitkamp, and R.F. Marschner ("Complexes of urea with linear aliphatic compounds," *J. Am. Chem. Soc.* **71**, 2947 [1949]), who confirm the ability of urea to remove linear hydrocarbons from a mixture containing nonlinear hydrocarbons.

52. J. Eric D. Davies, "Species in layers, cavities and channels (or trapped species)," *J. Chem. Ed.* **54**, 536–539 (1977).

53. P.J. Garner, L.N. Goldsbrough, and T.B. Byers, "Separation of straight-chain hydrocarbons by means of urea complexes," U.S. Patent 2,518,677 (1950).
54. A.E. Smith, "The crystal structure of the urea-hydrocarbon complexes," *Acta Crystallogr.* **5**, 224 (1952).
55. H.M. Powell, "The structure of molecular compounds. IV. Clathrate compounds," *J. Chem. Soc.* **1948**, 61-73 (1948). The term "clathrate" is derived from *clathratus*, enclosed or protected by the cross bars of a grating.
56. H. Wu, "Studies of denaturation and coagulation of proteins. XIII. A theory of denaturation," *Chin. J. Physiol.* **5**, 321-344 (1931).
57. A.E. Mirsky and L. Pauling, "On the structure of native, denatured and coagulated proteins," *Proc. Natl. Acad. Sci. USA* **22**, 439-447 (1936); M. Huggins, "The structure of fibrous proteins," *Chem. Rev.* **32**, 195-218 (1943); this paper refers to ideas on protein structure that were reported as early as 1937.
58. K.U. Linderström-Lang, *Lane Memorial Lectures* (Stanford University Press, Stanford, California, 1952).
59. K.J. Palmer, "The structure of an egg albumin-detergent complex," *J. Phys. Chem.* **48**, 12-21 (1944).
60. See "Crystal structure of plakalbumin, a proteolytically nicked form of ovalbumin. Its relationship to the structure of cleaved α -1-proteinase inhibitor," by H. Toni Wright, Hua Xi Qian, and R. Huber, *J. Mol. Biol.* **213**, 513-528 (1990). After a brief struggle with my sense of modesty, I note with some pride, pleasure, and gratitude that this paper has been dedicated to me. (Again I quote Howard Schachman, that "it is better to have false modesty than no modesty at all," as justification for mentioning this fact here.)
61. W. Kauzmann, "Denaturation of proteins and enzymes," In *The Mechanism of Enzyme Action* (W.D. McElroy and B. Glass, Eds.), pp. 70-110 (The Johns Hopkins Press, Baltimore, Maryland, 1954).
62. J.A.V. Butler, "The energy and entropy of hydration of organic compounds," *Trans. Faraday Soc.* **33**, 229-238 (1937); D.D. Eley, "On the solubility of gases in water. I. The inert gases in water. II. A comparison of organic solvents with water," *Trans. Faraday Soc.* **35**, 1281-1293, 1421-1432 (1939); J.T. Edsall and G. Scatchard, In *Proteins, Amino Acids and Peptides as Dipolar Ions* (E.J. Cohen and J.T. Edsall, Eds.), p. 183 (Reinhold, New York, 1943).
63. H.S. Frank and M.W. Evans, "Free volume and entropy in condensed systems. III. Entropy in binary liquid mixtures; Partial molal entropy in dilute solutions; Structure and thermodynamics in aqueous solutions," *J. Chem. Phys.* **13**, 507-532 (1945). The concept of "icebergs" is introduced on p. 520.
64. I.M. Barclay and J.A.V. Butler, "The entropy of solutions," *Trans. Faraday Soc.* **34**, 1445-1454 (1938). In the Barclay-Butler plot one plots the entropy change on transferring a mole of solute from the vapor into the solvent (at some standard molality) against the enthalpy change for the same process at a given temperature. For nonpolar molecules in nonpolar solvents all points fall on a common line. When hydrogen bonding in the solvent is involved there are some deviations, but when the solvent is water the points fall far from the "Barclay-Butler line."
65. See the excellent review by D.W. Davidson, "Clathrate hydrates," In *Water: A Comprehensive Treatise* (F. Franks, Ed.), Volume 2, Chapter 3 (Plenum Press, New York, 1972).
66. See, for instance, S. Swaminathan, S.W. Harrison, and D.L. Beveridge, "Monte Carlo studies on the structure of a dilute aqueous solution of methane," *J. Am. Chem. Soc.* **100**, 5705-5712 (1978), in which there is noted a tendency for a methane molecule to be surrounded by 19 or 20 water molecules in relatively favorable hydrogen-bonded states—that is, a tendency of methane to induce "structure" in the surrounding water. This has also been noted by J.C. Owicki and H.A. Scheraga, "Monte Carlo calculations in the isothermal-isobaric ensemble. 1. Liquid water. 2. Dilute aqueous solutions of methane," *J. Am. Chem. Soc.* **99**, 7403-7412, 7413-7418 (1977), and by others.
- See also D.L. Beveridge, M. Mezei, G. Ravishanker, and B. Jayaram, "Free energy simulations: Applications to the study of liquid water, hydrophobic interactions and solvent effects on conformational stability," *Int. J. Quantum Chem.* **29**, 1513-1523 (1986).
67. W. Kauzmann, "Some factors in the interpretation of protein denaturation," *Adv. Protein Chem.* **14**, 1-63 (1959).
68. See, for example, J.H. Hildebrand, "A criticism of the term 'hydrophobic bond,'" *J. Phys. Chem.* **72**, 1841-1842 (1968); reply by G. Nemethy, H.A. Scheraga, and W. Kauzmann, *J. Phys. Chem.* **72**, 1842 (1968).
- Also I.M. Klotz, "Non-covalent bonds in protein structure," In *Protein Structure and Function. Report of Symposium Held June 6-8, 1960* (D.E. Koshland et al., Eds.) (Biology Department, Brookhaven National Laboratory, Upton, New York; issued November 1960 as BNL 608 [C-30] [Biology and Medicine], Office of Technical Services, Department of Commerce, Washington, D.C.).
- Also R.A. Pierotti, "The solubility of gases in liquids," *J. Phys. Chem.* **67**, 1840-1845 (1963); "Aqueous solutions of non-polar gases," *J. Phys. Chem.* **69**, 281-288 (1965).
- Also M.H. Klapper, "On the nature of the protein interior," *Biochem. Biophys. Acta* **229**, 557-566 (1971); "The apolar bond—A reevaluation," *Prog. Bioorg. Chem.* **2**, 55-132 (1973).
- Also P. Privalov and S.J. Gill, "Stability of protein structure and hydrophobic interaction," *Adv. Protein Chem.* **39**, 191-234 (1988); K.P. Murphy, S.J. Gill, and P. Privalov, "Common features of protein unfolding and dissolution of hydrophobic compounds," *Science* **247**, 559-561 (1990).
- Also A. Hvidt, "Interactions of water with nonpolar solutes," *Annu. Rev. Biophys. Bioeng.* **12**, 1-20 (1983); "The solubility of hydrocarbons in water," *Acta Chem. Scand.* **A37**, 99-103 (1983).
- Also (though here I am somewhat less inclined to be dubious than in the above) A.-S. Yang, K.A. Sharp, and B. Honig, "Analysis of the heat capacity dependence of protein folding," *J. Mol. Biol.* **227**, 889-900 (1992).
69. The papers given at this conference were published in the January and April 1959 issues of *Reviews of Modern Physics*, of which E.U. Condon was Editor. (They also appeared in book form under the title *Biophysical Science. A Study Program*, J.L. Oncley, Editor-in-Chief, with F.O. Schmidt, R.C. Williams, M.D. Rosenberg, and R.H. Bolt (John Wiley & Sons, New York, 1959). Condon was at the University of Colorado at the time.