

RECOLLECTIONS

Solvent water and protein behavior: View through a retroscope

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A half-century is a long period in modern science, for the corpus of knowledge and understanding of natural phenomena swells on an ever-increasing scale. If Rip Van Winkle, Ph.D. 1940, in the natural sciences, had fallen into a deep sleep at his degree convocation and been awakened 50 years later at an annual meeting of the Protein Society or of the American Society for Biochemistry and Molecular Biology, he would have been astonished and perplexed by the different talks he would have heard. On one hand, descriptions of current work in gene structure, replication, and regulation would have convinced him that he had been transported to the land of Uqbar [1], for they would contradict what was “well known” to him, that genetic information must be encoded within proteins. On the other hand, he would have found recent elucidations in intermediary metabolism eminently credible and impressive, for isotopic labeling was familiar to him from his graduate work.

Progress in our understanding of protein structure and behavior falls somewhere between that for molecular biology and intermediary metabolism. Nevertheless, it is difficult for us today to appreciate how remarkable the advances in some areas have been [2]. Some problems ap-

peared to be so formidable 50 years ago that very eminent protein scientists felt they would never be solved. For example, my retroscope has revealed two relevant quotations. Felix Haurowitz in 1950 wrote, “While the sequence of amino acids in the shorter peptide chains can be determined, it would be hopeless to endeavor to fully characterize peptide chains containing 100 or more amino acids.” Linus Pauling in 1939 (in a paper with Carl Niemann) said, “. . . the great complexity of proteins makes it unlikely that a complete structure determination for a protein will ever be made by X-ray methods alone.”

The vast majority of protein investigators at that time would have agreed whole-heartedly with these prognostications. As Niels Bohr once remarked [3], “Prediction is very uncertain, especially of the future.”

Not all areas of protein science have made such spectacular giant leaps forward as has the elucidation of primary, secondary, tertiary, and quaternary structures. Our molecular concepts of the basis of the effects of solvent water on protein behavior have gone through several fashions in the past half century and are still malleable and equivocal. I will present one possible guided tour through this region.

A cardinal property of the H_2O molecule is its ability to form hydrogen bonds. Simultaneously, in the context of the present commentary, we note that the CONH group is a prime hydrogen-bonding entity. Let us start our tour, therefore, with a visit to this area.

The concept of the hydrogen bond was discovered or invented by M.L. Huggins while he was still a student at Berkeley in 1919 [4]. Almost 20 years later, in a classic paper with A.E. Mirsky in 1936 on the structure of proteins, Pauling described the role that peptide hydrogen bonds could play in establishing specific conformations [5] and assigned a value of 5–8 kcal to a hydrogen bond energy, with the $\text{N}-\text{H}\cdots\text{O}=\text{C}$ bond being placed near the lower boundary.

No reference is given by Pauling to the origin of the 5–8 kcal figure for the hydrogen bond energy. Nevertheless, it was universally accepted [6] for $\text{N}-\text{H}\cdots\text{O}=\text{C}$ bonds

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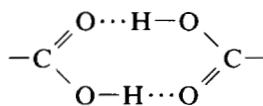
Irving M. Klotz received his undergraduate and Ph.D. degrees (Chemistry) from the University of Chicago. He then joined the faculty of Northwestern University, where he rose through the ranks from Assistant Professor of Chemistry to Professor of Chemistry and Biochemistry. At present, he is Morrison Professor of Chemistry and Biochemistry, Emeritus. In addition to his long-term interest in solvent effects on protein structure and behavior, Dr. Klotz has devoted substantial efforts to investigations of ligand-receptor interactions, structure and function of nonheme oxygen-carrying proteins, chemical modifications of proteins, and the construction of polymers with enzymelike properties. At present, he is also trying to understand some of the interpenetrations of science with the humanities.

Professor Klotz is a member of the National Academy of Sciences, Fellow of the American Academy of Arts and Sciences, Fellow of the Royal Society of Medicine, and recipient of the 1949 Eli Lilly Award of the American Chemical Society and the 1993 William C. Rose Award of the American Society for Biochemistry and Molecular Biology.

in polypeptides and used to interpret stability and conformational changes in proteins. Thus if one measured an enthalpy of denaturation of perhaps 50 kcal/mol, one proposed that 8–10 hydrogen bonds had been broken in going from native to the denatured state. Likewise, in the 1950s, when Linderstrom-Lang and his coworkers found that the slow class of hydrogen-deuterium exchanges in protein N–H groups has an activation energy of 20 kcal/mol, they ascribed it to the need to open three adjacent N–H···O=C bonds (each presumably requiring 6–8 kcal) in order to unfold a helical segment [7].

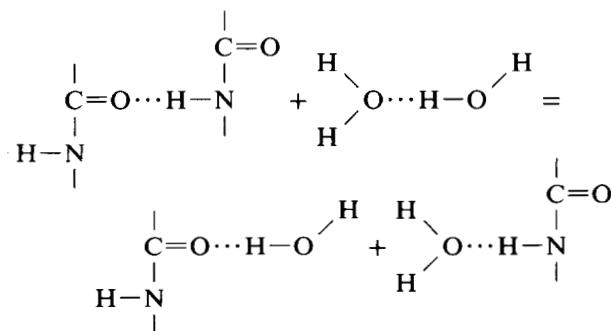
In a similar vein, Pauling and Mirsky ascribed the denaturing effects of urea to its ability to disrupt hydrogen bonding in proteins because of its “well-known hydrogen bonding” properties. This explanation of urea effects persists widely to the present day, although more recently styles have shifted to an explanation in terms of interference with hydrophobic bonding [8].

Returning to the 5–8-kcal hydrogen bond energy, I do not wish to imply that Pauling had no basis for his suggested values; quite the contrary. Since the early part of this century, apparent molecular weights of compounds such as acetic acid had been measured in the vapor phase and had clearly shown the presence of dimeric molecules. Once hydrogen-bonding was appreciated, it became apparent that head-to-head



bonds must be present. Studies of the temperature dependence of the vapor phase equilibrium then led to the energy (ΔE^0 or ΔH^0) of the hydrogen bond, around 7 kcal/mol.

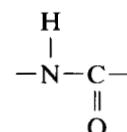
However, if we are looking at protein C=O···H–N bonds, this figure is really not pertinent. Strictly speaking, a bond energy refers to the dissociation energy in the gas phase. But that is not what one needs to know for a peptide hydrogen bond that might be involved in stabilizing a protein conformation. Because the protein is in solution in water, the interpeptide hydrogen bond is exposed to competing bonds with water. So the following interchange can occur:



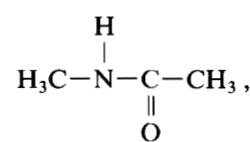
The net energy change, or ΔH^0 , would be expected to be much smaller for the opening of the N–H···O=C bond in aqueous solution than it is in the gas phase or in a non-polar, non-hydrogen-bonding solvent.

This conclusion had been reached by a number of protein scientists, at least by the 1950s. In fact, John Schellman and Walter Kauzmann, independently, had estimated the N–H···O=C bond strength by assuming that deviations from ideal behavior in very concentrated aqueous solutions of urea are due entirely to the formation of aggregated species of urea. However, deviations from ideality, especially in concentrated solutions, are an unreliable basis for measuring aggregation of solute molecules because other important interactions may be occurring between molecules in the nonaggregated state.

It seemed to me then that the probe of the N–H···O=C bond should be a vibrational spectroscopic one, which reflected directly the state of the constituent groups. James Franzen and I found that overtone infrared spectroscopy in the 1.5- μm range could be adapted for this purpose with aqueous solutions. However, we were unable to locate a supplier of naked



groups. Because in a protein each of the open bonds in this entity is attached to a carbon atom, we settled upon *N*-methylacetamide,



as our prototype group. The energy (ΔH^0) of N–H···O=C formation was found to be –4.2, –0.8, and 0.0 in the solvents carbon tetrachloride, dioxane, and water, respectively. This trend from very nonpolar to polar to aqueous solvent, as well as the direct result in water, shows clearly that the amide hydrogen bond energy in an aqueous environment is near zero.

In the decades since then there have been periodic studies with other amides, all of them more complicated in structure. Even today there is much activity in this area, utilizing up-to-date techniques and molecular theoretical deconstructions. Thus in this very year (1993), a review has been published by Dudley Williams in the *Proceedings of the National Academy of Sciences* describing the work of his group on the binding of a series of small peptides to the antibiotic ristocetin in aqueous solution. To evaluate the amide–amide hydrogen bond energies in these relatively complicated complexes, one must make allow-

ances for a host of other free energy contributions: van der Waals interactions, conformational rearrangements, translational and rotational restrictions, polar group interactions, hydrophobic effects. After such corrections, the net result for the free energy of $\text{N}-\text{H}\cdots\text{O}=\text{C}$ bond formation is given as about $-0.5 \text{ kcal mol}^{-1}$ with an uncertainty near $\pm 0.4 \text{ kcal}$. When I was a graduate student in a seminar run by James Franck, he once remarked, "always double an experimenter's estimate of his error." Thus, despite the astronomically greater use of computer calculations for the current evaluations, the intrinsic stability of the amide hydrogen bond in aqueous solution is still near zero.

Let us now turn our retroscope to aspects of the influence of water on protein behavior aside from its involvement in amide hydrogen bonding. A great deal more is now known about the structure of water and its participation in protein structure and conformation. Very many individuals have contributed to these advances in the past half century.

In view of current public concerns about ethical behavior in science, it behooves us to raise our consciousness about potential improprieties in scientific practice. One of these is "failure to cite work of others" [9], or as stated in constructive form, "respect for priority." With some trepidation, therefore, and somewhat arbitrarily, I shall start this part of our tour by pointing to a very influential paper by J.D. Bernal (Fig. 1) and R.H. Fowler published [10] in the founding volume of the *Journal of Chemical Physics* in 1933.

This paper on a "Theory of Water" presented a detailed picture of the structure of liquid water, based on theoretical analyses and extensive experimental information. It proposed that in the liquid state, irregular four-coordinated, tridymite-like, quartz-like, and ammonia-like molecular arrangements exist. Most important for our story were the theoretical calculations of ion hydration that led to the suggestion that ions could increase, or decrease, the intermolecular "coherence" of water. Out of this came the concept of a "structural temperature" for this liquid, increased by large ions, decreased by small ones.

A major further step was that taken in 1937 by J.A.V. Butler, who proposed that nonpolar solutes in water restricted the orientations of the solvent molecules and therefore lowered the entropy of the system. This concept was elaborated upon by a number of others, including D.D. Eley, D.H. Everett, and R.W. Gurney, and was extended in several respects by Henry Frank. In 1949, Jean Urquhart and I completed a study of temperature effects on the binding of organic molecules by proteins. This demonstrated clearly that the favorable free energies of binding were due to positive entropy changes, which we ascribed to release of restrained water molecules.

In the protein field these concepts were assembled into a coherent, broad picture by Walter Kauzmann in his very influential review in 1959, which also denominated clearly

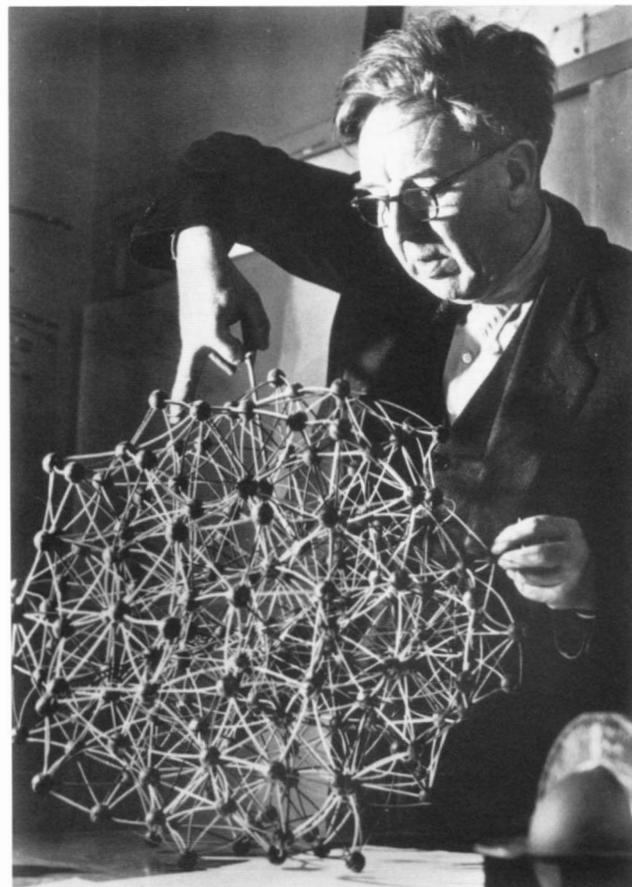


Fig. 1. John Desmond Bernal (1902–1970), one of the many gifted sons of Ireland who became famous individuals in British arts and sciences. He played a pioneering role in elucidating the molecular structure of molecules from water to viruses. He was also an avowed Marxist and staunch friend of the Soviet Union. Despite his far-left politics, he was asked to join the British military operations analysis group during World War II, where he held a senior position and played an important role in the defeat of the Nazis in North Africa and in the preparations for the Allied landings in Normandy. Although his Catholic ancestry can be traced back for at least 12 generations, he was often referred to in England as "the Irish Jew." Whether this was intended as a commendation or a depreciation I do not know.

the solvent–water entropic contributions by the term "hydrophobic" effects. Kauzmann's paper prompted a number of groups to investigate experimental model systems that could provide estimates of hydrophobic transfer free energies of amino acid residue side chains. For two decades, and independent of one another, Charles Tanford, J.H. Fendler, and Richard Wolfenden made extensive measurements in different phase systems. Their results are not entirely concordant with each other but they are in agreement in broad respects.

Concurrent with the development of these ideas were several lines of speculation that water molecules form lattice-ordered structures at the surfaces of biological macromolecules. In the 1950s, B. Jacobson proposed such hydration shells on nucleic acids based on NMR and other

studies. (Nowadays one would place these shells in the major and minor grooves of the double helix.) Albert Szent-Gyorgyi proposed similar pictures to interpret his energy-transfer experiments in biological systems (see Fig. 2 [11]). In the late 1950s it had struck me as intriguing that apolar molecules (analogous to protein side chains) form clathrate hydrates with water. X-ray crystallography, particularly from von Stackelberg's and Pauling's laboratories, had revealed the polyhedral surfaces in these clathrates, which can be quite varied in molecular geometry (hexagonal, pentagonal, quadrilateral), clearly adapting to the structure of the enclosed entity. These suggested to me that similar types of interactions should occur with apolar groups at the surfaces of proteins, leading to polyhedral arrangements of surface water molecules and contributing to the stabilization of protein structure.

It seemed pertinent, therefore, to obtain some information on the distribution of apolar side chains between the interior and surface of a globular protein. By the late 1960s, several crystallographic structures had been published. From this information I prepared a chart in 1970 that assigned apolar side chains to one of two groups: sur-

face or internal. This binary distribution was based on their accessibility to solvent water molecules. Some arbitrary decisions had to be made when side chains were only partially exposed to solvent. In any event, for the four proteins examined, papain, ribonuclease, subtilisin, and lysozyme, it became abundantly evident that a very large fraction of apolar residues in a protein molecule is accessible to solvent.

A more precise assessment of exposure became possible when the restraint of a binary distribution was replaced (by B. Lee and F.M. Richards in 1971), with one of finer resolution, based on rolling a sphere the size of a water molecule over the surface of a protein molecule. Ultimately, it was found by Richards and by Martha Teeter that, at the protein surface, more hydrophobic than hydrophilic area is presented to the solvent. For carboxypeptidase A and myoglobin, the ratio is near 55:45. In the plant protein crambin it is 68:32.

With this information it became even more tantalizing to try to see the organization of water molecules at the surface of a protein molecule. NMR studies proved to be inconclusive. There seemed too little prospect that



Fig. 2. Convocation in 1959 at Four Winds in Woods Hole to discuss energy transfer and water structure. From left to right, top row: Zoltan Bay, Koluman Laki, Andrew Szent-Gyorgyi, Irwin Isenberg, Guy Williams-Ashman, T.(?) Fujimori, William McElroy, Sidney Velick; middle row: Irving Klotz, William Arnold, (?), Bernard Pullman, Michael Kasha; bottom row: Richard Steele, Henry Linschitz, Marta Szent-Gyorgyi, T. Förster (?), Alberte Pullman, Albert Szent-Gyorgyi, Hugo Theorell.

X-ray crystallography would be revealing. In fact, in 1960 an eminent protein crystallographer stated to me categorically that there is no ordered water in proteins. Nevertheless, some 20 years later, diffraction resolutions had improved remarkably, in some proteins to 1.4 Å or better. In crambin in 1984 a resolution of 0.95 Å was attained, and it disclosed an array of conjoint pentagonal water rings forming a clathrate hydrate structure in a region of the protein surface with a strong presence of apolar residues. Pentagonal rings of water have also been revealed in insulin and cytochrome c. It seems reasonable to expect, therefore, that as better resolutions are attained with other crystals, clathrate-like water structures will become evident in other protein molecules also.

Periodically in the sciences someone takes a giant conceptual or experimental leap forward that, in time, turns out to have catapulted him over a precipice. Most recently, in physics one might cite the "fifth force," in chemistry, "cold fusion." In a reminiscence involving water, I am reminded of "polywater" or "water II" and its biological ramifications. This revolutionary discovery was brought to the attention of European scientists about two decades ago by a respected Russian surface chemist, B.V. Deryaguin. His new phase of liquid water had remarkable properties, including a freezing point tens of degrees below zero and a boiling point somewhere in the hundreds of degrees centigrade. Its structure was supposed to be more ordered than that of the normal liquid [12]. I do not wish to describe other astonishing properties or the fate [13] of polywater, but I do want to mention some ramifications in the life sciences.

Polywater stimulated the discovery and disclosure of a number of surprising biological observations. A French group, J.R. Beaumont et al., discovered and patented a form of water from melted snow that possessed amazing analgesic and healing effects in the treatment of burns. The Information Office of the Soviet Embassy also disclosed some years ago that Soviet scientists I. and V. Zelepuklin had long recognized that water from melted snow can stimulate a variety of biological processes. As recently as 1987, these investigators reported that such activated water has a long-lived "structural memory." At the molecular level, this conceptual trend reached an apex in 1988 in the publication by J. Benveniste and his associates in *Nature*, that anti-IgE at very high dilution (amounts as small as harpo- or groucho-moles [14], i.e., 10^{-24} or 10^{-30} moles, respectively) degranulates basophils. At grouchomolar concentrations, no antibody molecule can even be present in the solution, yet its influence is purportedly manifested because at higher initial concentrations, the solute protein imprinted itself into the solvent water structure, and this aqueous structural form presumably maintained itself throughout successive dilutions with fresh, unexposed solvent. As one wit has said, there will always be individuals with a penchant for tailoring irrelevant facts to suit distorted theories [15].

Returning to my main theme, I want to mention still another path toward understanding protein conformations and interactions with solvent that was opened several decades ago, the theoretical one using statistical- and quantum-mechanical principles. This approach was pioneered by Harold Scheraga, starting at a Brookhaven symposium in 1960. These modes of analysis have flourished expansively during the succeeding decades as more and more theoreticians have been attracted to the field and the capabilities of computers have increased astronomically. Although I had taken courses in statistical mechanics and in quantum mechanics when a graduate student, the problems treated were largely limited to diatomic molecules in the gas phase or isotope effects, and "computers" were Marchand hand calculators whose gears were rotated by a hand crank. It took me about 3 months during the preparation of my thesis to calculate (and check) the data assembled in an array of a dozen long tables; my guess is that a current desktop computer could complete the same calculations in seconds. It is hard to believe that one can now buy programs, such as CHARMM and BIOSYM, that, given a primary structure, will compute the three-dimensional conformation of the purportedly [16] thermodynamically most stable species of a protein, and that even interactions with water molecules can be encompassed. Still more impressive visually are the molecular graphics presentations, in color, displaying three-dimensional views of biomacromolecules and their interactions with docking ligands.

The conformations and dynamics of protein molecules must be affected by interactions with water molecules. Theoretical calculations incorporate such considerations in treatments of proteins in hydrated crystals and in aqueous solution. The results of these theoretical energy minimization and molecular dynamics computations are being put to more stringent tests [17] by the remarkable improvements in X-ray diffraction resolution.

In the past dozen or so years, resolutions beyond 1.4 Å have made it possible to establish definitively the positions of water (oxygen atoms) in a protein framework. For example, in crambin at low temperatures, all the water molecules (about 91) in the crystal have been located. Almost all of the 283 water molecules in insulin have been placed in position. Internal water molecules also have been located unequivocally within many other protein molecules, often appearing in clusters. These, together with water molecules lodged at the surface, must contribute to the stabilization of protein conformation. Furthermore, in solution there is no sharp boundary between solute molecules and solvent molecules. J.W. Gibbs and P. Duhem a century ago established on thermodynamic grounds that a perturbation of the solvent must affect the free energy of the solute also. These couplings will need to be accounted for in molecular mechanics approaches if the functional properties of proteins are to be understood fully in terms of their structures.



Fig. 3. Portion of group photograph at Gordon Research Conference on Proteins at New Hampton, New Hampshire in the 1960s. From left to right, top row: Rufus Lumry, Walter Kauzmann, John Rupley; bottom row: John Kendrew, John Edsall, Jeffries Wyman, Paul Flory, Frederic Richards, Mildred Cohn, Howard Schachman, Irving Klotz, Harold Scheraga. Distributed in other positions are J.W. Williams, Edmond Fischer, Felix Haurowitz, Lyman Craig, Gertrude Perlmann, and other faces that readers may recognize.

Until very recently, the major communal convocation of protein scientists was the annual Gordon Conference held in June in New Hampshire. For as long as I can remember, the management of this enterprise always took a group photograph of the attendees (somewhat over a hundred in number). These photographs provide a treasure trove for a historian of protein science because, from the faces and names, one can tell what were the major topics at the forefront of the field at that time. Furthermore, from the sequence of photographs one can follow trends and new directions. I have in my files only a few of these photographs and I have selected one from June 1965 (Fig. 3) to insert in this recollection. Only a portion of the photograph has been submitted because it would have been difficult to distinguish faces in the full picture at the magnification limited by a journal page size. From the faces identified, one can guess reliably what was discussed at the meeting that summer.

Glancing backward through the survey provided in this account, we can discern the following trends during the past half century. Some problems, such as those of the primary, secondary, tertiary, and quaternary structures of proteins, have reached definitive solutions. Some have vanished [18]. For example, there was a time when one could consider seriously whether a pure protein really was a widely heterogeneous assemblage of different polypeptides. Other problems have been rephrased. The molecular nature of protein denaturation was a subject of wide speculation even 50 years ago. Now the question has been

flipped 180° and is formulated as the problem of protein folding. Some issues, such as the relative importance of various forces in stabilizing proteins, seem to be immortal. Perhaps they can never be resolved because in a protein macromolecule they are all intimately coupled. The problem may have to be rephrased in different terms. Finally, there is also a category of techniques and concepts that appeared essentially *de novo* during past decades; for example, high-resolution X-ray crystallography, molecular mechanics and molecular dynamics theoretical calculations, and NMR multidimensional spectroscopy. These are being progressively refined and will continue to provide new insights and perspectives that would have been undreamed of 50 years ago.

I would not dare to hazard any guesses as to what might appear in a Recollection that may be written by Dr. Rip Van Winkle's grandson or granddaughter 50 years from now. Nevertheless, I suspect that the trends will fall into the same general categories described above. Despite dramatic changes with time, there is an inescapable continuity in science.

Endnotes

1. In his famous book *Labyrinths*, the Argentinian writer Jorge Luis Borges describes his discovery of the land of Uqbar, where the intellectual establishment is immersed in modes of thought and concepts in science, mathematics, etc., that are completely antithetical to ours.

2. For example, a half-century ago, the triplet of letters NMR would have been assumed to be the acronym of a Federal agency.

3. Although the scientific literature attributes this aphorism to Niels Bohr, it is uncertain whether he ever uttered it, and if he did it was as a quotation from the famous Danish cartoonist Storm P. who wrote, "DET ER VANSKELIGT AT SPÅ, ISÆR OM FREMITEN!"

4. Huggins named this interaction a "hydrogen bridge" and was unhappy with possible implications of the word "bond." The British chemist H.E. Armstrong, well-known for his sardonic wit, coined the term "bigamous hydrogen." Armstrong, in a related context and in a derogatory manner, also referred to the great American thermodynamicist G.N. Lewis (after misspelling his name) as a "California thermodynamiter."

5. A similar proposal had been made earlier (in 1933) by W.T. Astbury and H.J. Woods in a graphical presentation of bonding between protein chains, but they did not use the term "hydrogen bond."

6. When a very prestigious scientist states a "fact," it is likely to be accepted as gospel truth. I recall in this connection an incident at which I was present shortly after World War II. At an American Chemical Society meeting, a small group of young scientists was clustered around Peter Debye, who told us, among other things, the following story. Some decades earlier he had calculated theoretically what the dipole moment of HCl (I think) is, and obtained a value of (let us say) 1.85 (Debye units). The then available experimental value was markedly lower (let us say 1.40). New experimental studies were undertaken, therefore, by a succession of physicists, and these yielded dipole moments progressively closer to 1.85. At this point, for reasons that I have forgotten, Debye was prompted to reexamine his theoretical calculation. Lo and behold he discovered he had made an error in his earlier theoretical computation. When corrected, the theory now led to a dipole moment of 1.40, in agreement with the first experiments, which were then widely confirmed.

7. Even at that time, I doubted this explanation. My skepticism was confirmed a few years later when Bruce Frank and I showed that *N*-methylacetamide, $\text{CH}_3(\text{C}=\text{O})(\text{N}-\text{H})\text{CH}_3$, in deuterated water exchanges its amide N—H with an activation energy of about 20 kcal/mol. In water, *N*-methylacetamide is a monomer, not a constituent of a helix.

8. In the 1950s because it was "well-known" that urea breaks hydrogen bonds, Allison added urea to sickled hemoglobin and indeed found that aggregation could be diminished. In the 1970s, when it was becoming "well-known" that urea interferes with hydrophobic bonding, Nalbandian used it for the clinical treatment of sickle-cell disease and reported favorable outcomes. His results could not be reproduced by others. Ironically, nevertheless, on the assumption that they might be true, A. Cerami and J. Manning were prompted to look at a hydrolysis product of urea in water, cyanate. This does indeed inhibit sickling, by reacting with amino groups of hemoglobin. That is how science sometimes progresses.

9. Except when there has been a failure to cite my publications, I find the issue of "priority" a bottomless morass. To appreciate this, one needs only to be aware of a few famous historical examples—Galileo/Grassi, Newton/Hooke, Newton/Leibniz, Bernoulli/Bernoulli. Furthermore, even when one thinks one has reached the ultimate source, surprises lie in wait. A few years ago I was astonished to learn (from a book by the sociol-

ogist-historian R.K. Merton) that the very famous statement "if I have seen farther, it is by standing on the shoulder of giants" did not originate with Newton; it had been expressed by, among others, Bernard of Chartres, who lived five centuries before Newton.

10. This paper was born because weather prediction in the 1930s, as now, was not an exact science. Bernal and Fowler, concluding a visit to Moscow, had gone to the airport to board a plane to return to England. However, a thick fog set down on the airport and stayed for days. During this interval, Bernal and Fowler created their famous paper on the structure of aqueous solutions.

During the summer of one year in the late 1950s, I had the accidental good fortune of spending a full day alone in an automobile with Bernal as passenger. At the request of Albert Szent-Gyorgyi, I was driving Bernal from a Gordon Conference in New Hampshire to Woods Hole, Massachusetts, where he was to be the house guest of Szent-Gyorgyi. Because Bernal loved to talk about many subjects, and I was an awestruck, avid listener, I acquired a treasure of anecdotal history.

My mental image of Bernal at that Gordon Conference, which had some sessions on water and proteins, is that of Figure 1, even to the presence of the structural model. This photograph, however, was not taken at that meeting, but in England at about the same time. John Finney of Birkbeck College gave it to me.

11. In 1959, Szent-Gyorgyi invited a group of individuals to spend several weeks during the summer at Woods Hole, on Cape Cod in Massachusetts. Each afternoon this group met at his mansion, Four Winds, on scenic Penzeance Point, to discuss in a broad-ranging style, problems of energy transfer and water structure. Figure 2 is a photograph of the individuals present at one of these sessions.

12. A particularly excited theoretical chemist arrived at a structure of polywater on the basis of his quantum mechanical calculations. He was so convinced of his results that he wrote in his publication thereof, "We have presented arguments, supported by quantum mechanical calculations, which we believe establish [polywater's] existence and characterize its properties." Subsequently, when the polywater bubble burst, this theoretician wrote an interesting mea culpa analyzing the reasons why he had been enticed into this misstep.

13. In contrast to the discoverers of other nonexistent remarkable phenomena (such as N-rays, mitotic radiation, krebiozen, E-rays, cold fusion, electrical creation of living insects, etc.), Deryaguin had the intellectual honesty and emotional courage to ultimately concede, in print, that he had been wrong.

14. In a parody on units of measurement for doses in homeopathic medicine, P.F. Duggan has published, in the *Journal of Irreproducible Results*, an extension of the naming of extremely small amounts of a molecule. Currently established are the S.I. prefixes: pico = 10^{-12} , femto = 10^{-15} , ato = 10^{-18} , zepto = 10^{-21} . Newly recommended (starting with the presumption that zepto must arise from the name of Zeppo, the fourth of the Marx Brothers of movie fame) are harpo = 10^{-24} , chico = 10^{-27} , and groucho = 10^{-30} .

15. Discoveries such as polywater, N-rays, etc., are frequently labeled "pathological science," a term first used by Irving Langmuir in a "valedictory" talk given at the General Electric Company Laboratories. I feel this pejorative term is an unfortunate choice, for deviant science is only occasionally patholog-

ical. Nevertheless, this term has generated a cottage industry of writings on "pathological science." Many of these writers in their wisdom have even set up explicit criteria for detecting such science. A year ago one such article in the *American Scientist* proposed two such defining characteristics: (1) the effects are very weak; and (2) the proponent readily disregards prevailing concepts. When I read that, it occurred to me that Einstein's 1905 ideas on special relativity fulfill these criteria for pathological science. The difference between him and Newton for the time it takes light to travel 1 m (on the earth as it moves around the sun) is 0.00000003 s, surely a very weak effect. And Einstein certainly disregarded prevailing concepts.

It is my impression that at the time of announcement of an amazing discovery, it is often extremely difficult to distinguish a feather merchant from a diamond dealer.

16. I have inserted this adjective because I recently came across (once again) a remark of Einstein's. According to the eminent polymer chemist the late Herman Mark, Einstein once said to him "Do you know what is the difference between an experimentalist and a theorist? An experimentalist does something that everyone believes, except himself. A theorist does something

that nobody believes, except himself." Because Herman Mark was in residence at the Kaiser Wilhelm Institute in Berlin in the late 1920s, when Albert Einstein was its Director, this story should probably be assigned a higher than legendary status.

17. Pertinent to the current status of theoretical efforts is an anonymous verse that I once discovered:

Grant, oh God, Thy benedictions
On my theory's predictions
Lest the facts when verified
Show thy servant to have lied.

18. The same phenomenon has occurred in other fields of science also. With the advent of thermodynamics in the middle of the 19th century, efforts to unveil the properties of "caloric" stopped, and attempts to design perpetual motion machines declined drastically. (They have not vanished entirely; in 1985, a "perpetuum mobile" was described by S. Marinov in *Nature*.) In this century, with the advent of special relativity, experiments to reveal the characteristics of the "ether" have stopped.