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'Cohn and Edsall': Physical chemistry conclusively supports a protein model

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Abstract

The Harvard laboratory of Edwin J. Cohn and J.T. Edsall and the treatise on proteins that emerged from their work exerted a dominant effect from the 1920s to the 1950s. Its most important achievement was to solidify a molecular picture of proteins, especially the picture of a globular protein. This model representation did not originate with Cohn and Edsall, nor were they directly crusading for it. Their support for the model evolved simply from the sheer volume and diversity of their work. Everything they did or wrote about depended on a molecular picture. All of it was in the realm of physical chemistry and inexorably linked by rigorous theoretical equations to parameters of size, shape and ionic charge.

Keywords: History of proteins; Molecular size; Ionic charges; Harvard university

1. Introduction

The names 'Cohn and Edsall' are associated with what was in its time the most prominent laboratory in the field of protein chemistry. The same names are also associated with a memorable treatise, *Proteins*, *Amino Acids* and *Peptides*, of which they were co-authors. The guiding principle of both the laboratory and the book was expressed in the goal Edwin J. Cohn set himself while still a graduate student: he would devote himself unswervingly to the study of proteins, in particular to the use of physical chemistry in such a study. Physical chemistry provided a kind of certainty; with its mathematically rigorous equations, that often eliminated the need for speculation. In Cohn's own words, from a 1925 review [1]: 'The composition of proteins is revealed by analytical chemistry, and the behaviour of proteins by physical chemistry.' 'The physical chemistry of the proteins must in the last analysis depend on their structure.'

It is suggested in this paper that Cohn and J.T. Edsall's most influential achievement was to firmly establish a working model for what a typical protein molecule looked like, physically and chemically. This model was central to everything being done in the Cohn/Edsall laboratory and, by virtue of its success, came to dominate all thinking at the molecular level in the laboratory; a similar consensus emerged from the book and inspired the

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multitudes who did not have direct contact with the laboratory. It was the dominant model for many decades, until it was supplanted by the far more precise detailed structures obtained by X-ray crystallography. 'Supplanted' should by no means be equated with 'displaced', for the later structures at atomic resolution were found to be in full accord with the main features set forth by the earlier working model.

The essential features of this model were the following:

(1) Proteins are built entirely from α-amino acids, joined to each other by peptide bonds. It was of course known that some proteins had additional constituents, like the iron–porphyrin complex in haemoglobin or the covalently-linked sugars in glyco-proteins, but such *conjugated* proteins were clearly in a minority at the time and the extra chemical entities in them were seen to contribute special functional abilities (e.g. reversible binding of oxygen in the case of haemoglobin) but were not needed to define the intrinsic protein fabric itself.

(2) Proteins are macromolecules, molecular weights ranging from approximately 10 000 to more than a million. When insulin proved to have a molecular weight of approximately 6000, the question of whether it merited the name of 'protein' was seriously raised.

(3) Two categories of protein macromolecules were known to exist: the fibrous proteins and the so-called 'globular' proteins. Both categories were being investigated, but I shall concentrate in this account on the globular proteins. They were more interesting at the time because most enzymes, antibodies and other proteins with biochemical activity fell into that category. And they have remained more interesting well into the modern era because they have been the only proteins that could be readily crystallised and subjected to X-ray analysis. (Technical advances are only just now beginning to overcome this limitation.)

(4) As for specifics of the model for globular proteins, the latter were known at the time to be compact particles of astonishingly small dimensions. Typical particle radii for proteins of moderate size were 20–30 Å. Given the high molecular weight—equivalent to hundreds of amino acids per molecule—this can only mean extremely tight packing, with virtual exclusion of solvent water from the interior. Fibrous proteins, as their name implies, were in a quite different category, comprised of long, extended molecules. They too were being studied by the Cohn/Edsall group—in fact, myosin and its relation to muscle fibres were a special interest of Edsall when he first joined the group—but their categorical difference was never in doubt and in this short essay I shall say very little about them.

(5) Apart from the small particle size, the most striking aspect of the molecular image of globular proteins was that they are electrolytes, bristling with ionic charges: the charges are derived from the side chains of acidic and basic amino acids and the charged groups tend to behave approximately as they would have behaved when not attached to the protein, i.e. acidic side chains are uncharged at low pH and negatively charged at higher pH, while basic side chains are positively charged at low pH and lose their charge (by H^+ dissociation) at high pH. In the neutral region positive and negative charges must co-exist: given the compact particle size, they must often be in close proximity, not more than 5 or 6 Å apart.

It must be appreciated that Cohn and Edsall did not create this model, nor even contribute compelling original work to its creation. It was the product of the efforts of many scientists: the high molecular weight was established by chemical means in the earliest days of protein studies and then confirmed by all conceivable physical methods; the compact folding was most convincingly demonstrated by Svedberg's measurements of sedimentation rate with the ultracentrifuge; the ionic charges and their dependence on pH had been established both by electrophoresis and by more specific electrometric analysis. Details of the progress towards understanding and description of the critical experiments along the way (with pertinent original references) are given in a recent book on the subject [2].

What Cohn and Edsall did was to *promote* the multi-ionic globular protein in their research program, which was firmly based on the model I have outlined and they *verified* it repeatedly with experimental studies in their laboratory. They did not actually trumpet the model directly; but all the

results they accumulated were dependent on it. They trained more than a hundred students and postdoctoral fellows, not by indoctrination, but by what may be called common consent—it was a simple fact that no alternative model existed that could conceivably have accounted for the wealth of diverse experimental data that was emerging from the laboratory.

2. Historical beginnings

Harvard University had the good fortune, over a span of more than 80 years, to have three successive outstanding presidents, who were committed to advancing higher education, ever ready for innovations and reforms, and who set the stage for much of the image of universities in American culture today. They were Charles William Eliot (president from 1869 to 1909), Abbott Lawrence Lowell (president from 1909 to 1933; brother of the poet Amy Russel Lowell) and James Bryant Conant (president from 1933 to 1953). They fostered such now familiar features of American universities as the elective courses, which allow each student to fit his study program to his own unique needs, and equal education for women—Harvard's sister college, Radcliffe, was developed during Eliot's presidency. As well as affecting the educational process per se, these men took the lead in founding a tradition for the participation of universities in national affairs—Lowell passionately advocated American membership in the League of Nations; Conant resigned from the Harvard presidency to become the US Ambassador to Germany in the stormiest days of the cold war.

In 1920, during Lowell's term of office, the university established a novel entity, a department of physical chemistry *in the medical school.* The juxtaposition of physics and chemistry with medicine was unheard of in modern times, probably since the days of Islamic science in Spain in the middle ages. Even more unprecedented was the concept that the new department was to be entirely free of direct teaching duties, with no obligation to participate in the elementary instruction of medical students [3].

The nominal head of this new venture was Lawrence J. Henderson, a man who today would be considered a maverick. He was an M.D. who never practised medicine, had broad interest in almost every aspect of biological science and its history, and was the author of a widely acclaimed book entitled *The Fitness of the Environment,* seen in retrospect today to have been rather less admirable than at the time. He recommended that Cohn, who was his former student and at this point a young member of the faculty, should be appointed as de facto leader of the new venture, while Henderson himself wandered off into other subjects, such as sociology, asking questions such as: could there be a parallel between human interactions in society and molecular interactions in solution?

Cohn had made the decision early in life, even before completing his Ph.D., that he would devote himself from that point onward to the study of proteins—a decision which of course sounds reasonable today, but may well have seemed absurd at the time. There were hardly any people as yet who would classify themselves as 'protein chemist'; few could possibly have imagined the potential importance of protein molecules in the chemistry of life. Among Americans the closest to being an acknowledged authority in the field was Thomas B. Osborne: he was a splendid chemist, but specialised in vegetable proteins, which lacked the glamour of human or animal proteins and their often close relationship to what goes on in our own bodies. Osborne was moreover employed for his entire career by the Connecticut Agricultural Research Station—not seen at the time as being in the same class as the Rockefeller Institute or a major university. The Svedberg, who a decade later would emerge as the leader of protein physical chemists by virtue of his invention and use of the analytical ultracentrifuge, was still a colloid chemist in the early 1920s, still trying to discover the 'peculiar forces' that might be responsible for the large particle size of proteins and other 'colloids' in solution, convinced that the forces were not ordinary chemical bonds, convinced that colloidal particles were not true 'molecules'. (As everyone knows today, it was Svedberg himself who provided the strongest evidence that proteins were, in fact, true macromolecules.)

3. Cohn

Cohn (1892–1953) [4] was the son of a rich New York merchant. He first went to Amherst College for his undergraduate education, but transferred to the University of Chicago to get better training in physics and chemistry. He came to Harvard to do postgraduate work with Henderson, which led to papers on acid/base equilibria in seawater and the physical chemistry of bread making. Then back to Chicago for his Ph.D., which dealt with spermatazoa. It was here that he decided that the kind of biological systems he had been studying were by themselves too large for direct investigation and that one had to break the systems down to smaller components—proteins being the obvious choice—to learn about specificity and organisation. No sooner said than done, and off he went to Connecticut to study for a few months with Thomas Osborne. After the end of the war, he was off to Europe: to study with S.P.L. Sørensen in Copenhagen, Svante Arrhenius in Stockholm, and William Hardy and Joseph Barcroft in Cambridge. Cohn was a driven man, who generally knew what he wanted to accomplish. He was ever restless; flitted about from lab to lab; never stayed longer in one place than was needed to achieve his objective.

Cohn's stint with Sørensen in Copenhagen was probably the most influential one for his career, for Sørensen's studies of the osmotic pressure of egg albumin solutions are generally acknowledged to have been a landmark in the history of protein chemistry [2,5]. They established the molecular weight of the protein in solution, but, more importantly, demonstrated that the laws of physical chemistry were being strictly obeyed, something which colloid chemists had been trying to claim was not the case. The pivotal criterion was derived from solubility studies: solubility of proteins was shown to be independent of the amount of the saturating phase as required by thermodynamics—a corollary of the famous 'phase rule' of J. Willard Gibbs. The theoretical conclusions of Sørensen were completely convincing to Cohn, and they led Cohn inexorably towards his own studies of solubility and its relation to protein purification and fractionation, and to effects of pH, salt concentration, temperature, etc. on solubility and on other molecular properties. These topics would provide the core of the Cohn and Edsall agenda for many years to come.

Had it been 5 or 6 years later, Cohn might well have had the insight to pick Svedberg as a teacher—in fact he visited him in Uppsala in 1921 at the end of his European tour and *predicted* that Svedberg would find proteins to be true macromolecules at a time when Svedberg himself had not yet put proteins into his ultracentrifuge and still believed they would be colloidal aggregates.

In 1925 Cohn wrote a classic review of what was then known about the physical chemistry of proteins [1], which in effect laid out the plan which his new laboratory would follow—I have already quoted directly from the review's introductory paragraph: 'The composition of proteins is revealed by analytical chemistry, and the behaviour of proteins by physical chemistry'; 'the physical chemistry of the proteins must in the last analysis depend on their structure.' From this review, Cohn's commitment to the working model outlined at the beginning of this paper is manifestly clear. The review devoted more than 50 pages to the accounting of molecular charges in terms of amino acid compositions, to the correlation of measured ionic mobilities (electrophoresis) with charges determined by thermodynamic titration, and other experimental data that verify the multipolar molecular model. Viscosity data are cited: Cohn unerringly understands and accepts Einstein's viscosity equation and affirms its unambiguous indication of small particle volumes. (The review is a mature work, being preceded by a series of shorter papers beginning in 1921, with the serial title 'A physicochemical method of characterising proteins'.)

Most significant as an illustration of commitment was a 1926 paper with Conant, who was a chemistry professor then, not yet president of the university. It had to do with the colloid/macromolecule dispute. Some German chemists had found that the freezing point depression of proteins dissolved in phenol was unexpectedly large, corresponding to molecular weights of only a few hundred. It was suggested that this was strong evidence for the colloidal association theory, phenol breaking up the large particles seen in aqueous

solution. Conant was impressed, but Cohn knew otherwise, on the basis of more convincing data than the depression of freezing point. Nevertheless, the result *was a threat to the working model* and needed to be challenged. Cohn and Conant worked on the problem together [6] and showed that phenol tended to contain water as an impurity (some of it in this case may have been water of hydration displaced from the protein particles), leading to the appearance of low molecular weight molecules in the phenol solution which were not protein fragments, but which were thermodynamically indistinguishable from them in the procedure that was used. When the freezing point depression measurement was repeated at controlled constant water activity, the result obtained was conclusive: proteins had the same high molecular weight in phenol as in aqueous solution.

4. Members of the team

Cohn and Edsall and their associates were working uniquely on proteins: their fractionation and purification; their individual sizes and shapes and other molecular properties. In their laboratory every experiment result, every day of the week, could hardly be described without reference to a mental picture of protein molecules. Their single-minded concentration on proteins was unparalleled, with no diversion to metabolic pathways or enzyme kinetics, or other aspects of biochemistry that would have been obligatory concerns had they been part of a typical medical school Biochemistry Department. That their group should turn out to have become the centre of wisdom for the totality of protein chemistry should not be considered surprising, for hardly an hour could go by without some aspect of what was known (or assumed to be known) being subjected to critical testing.

Within this framework, the precise backgrounds of other members of the Harvard team are relatively unimportant. Most of them had little or no previous knowledge of proteins, but then, who did?

Edsall joined the laboratory in 1926, while still a third year medical student at Harvard, under a program designed to give those who were so inclined a research experience to relieve the tedium of purely clinical studies. He had spent the previous 2 years at Cambridge University in England, and had acquired an interest in the chemistry of muscle. It was understood that the principal muscle protein, myosin, had to belong to the category of fibrous proteins and Edsall (together with the Swiss physiologist A. von Muralt, who was a visiting fellow at Harvard), would soon leap across disciplinary gulfs to investigate the shape of the myosin molecule by use of the method of flow birefringence, a method ideally suited to provide information about the orientation of long asymmetric particles as a function of flow velocity—with comprehension of rather esoteric theoretical and mathematical laws a prerequisite [7].

In the spirit of the Cohn/Edsall philosophy the appropriate control experiment was done: globular proteins did not produce birefringence in aqueous solution, did not have sufficient particle asymmetry to lead to molecular orientation in a flowing stream. But the control experiment in this case went beyond what was strictly necessary. The theory for flow-induced orientation predicted that birefringence could be observed for solute particles with relatively weak asymmetry if solvents of sufficiently high viscosity were used. This was confirmed experimentally [8]. Glycerol was added to increase solvent viscosity: serum albumin, the very epitome of a globular protein, gave no detectable birefringence even in 70% glycerol, but a measurable degree of molecular orientation set in at 90% glycerol. Semi-quantitatively the result was consistent with data using other physical tools, indicating that serum albumin and most other globular proteins were not perfectly spherical, but could be represented mathematically as ellipsoids with axial ratios of approximately 2:1.

Several years later Edsall would use a quite different esoteric tool, Raman spectroscopy, to provide unambiguous proof for another essential prop for the protein model and its underlying physical chemistry: namely the assertion that amino acids must be zwitterions in their neutral (uncharged) state [9]. Edsall could be counted on at all times to understand complex equations and concepts and even to retain in his mind exact references to the original publications. He was the major contributor to the jointly authored book that

was written later (see below) and probably to other writings as well.

Subsequent permanent appointments to the laboratory staff were J.L. Oncley in 1935 and W.L. (Pete) Hughes in 1940. Oncley was an instrument man, in charge of the most powerful hardware in the department: an analytical ultracentrifuge that was built in the laboratory to the Pickles/Beams design and installed in 1939 and a Tiselius electrophoresis set-up installed the same year. (The Pickles/Beams ultracentrifuge in those days was still a serious rival to the Svedberg instrument.) Hughes was unusual for this physically oriented laboratory in that he was a 'natural' chemist, a test-tube chemist, who often did not need expensive equipment to interpret what was going on in an experiment—it is amazing how much information one can get from quite simple observations if one's brain is engaged. In addition to the permanent staff, there were more temporary investigators, who stayed for a year or two or sometimes even longer. They included many whose names became well-known, e.g. Jesse Greenstein, John D. Ferry, Jacinto Steinhardt, John Bateman, I. Fankuchen, I.H. Scheinberg, etc.

A remarkable feature of the lab was that major figures outside the lab's formal structure became voluntary associates and became as committed to the lab's mission as the formal members. The most notable example is George Scatchard. He and Cohn had been contemporaries at Amherst, but Scatchard did not like Cohn then. Cohn was aggressive, impatient, sometimes offensive. Scatchard was a quiet man who played the cello for relaxation, a deep thinker. The association between the two men began after 1923, when Scatchard joined the MIT faculty as a physical chemist. Scatchard came to know Cohn after some social occasion and was surprised to find that many of Cohn's ideas and scientific principles were like his own. Cohn subsequently stimulated the direction of much of Scatchard's research, though they never published a paper together. Papers with other lab members, however, testify to Scatchard's central role: his work on osmotic pressure [10], for example, is one of the great classics in the literature of the physical chemistry of proteins—but it should be noted [11] that his contributions were by no means limited to his own special field of solution thermodynamics.

Scatchard was an active member of the laboratory from 1923 onwards. No-one else in America had a deeper understanding of the physical chemistry of solutions; he was a perpetual watchdog—probably every paper published from the group had to pass his scrutiny—assuring that laws of thermodynamics and all other principles of physical chemistry were strictly obeyed. All the chemical complexities of proteins, amino acids and peptides—multiple charges interacting at close range being the most crucial—were re-examined over and over again as successive papers passed the test of his approval.

It was probably at Scatchard's instigation that an interruption in the continuity of the original master plan of the laboratory occurred in the 1930s. Cohn had been attracted to proteins because they were simpler than complete biological systems, but sufficiently complex to express functional specificities. But physical interpretation of measurements on proteins—acid/base titration, solubility as a function of pH, volume measurements reflecting molecular electrostriction, dielectric properties, etc.—were increasingly being hampered by a gap in available data and relevant theory. Systematic literature that was intermediate between the protein literature and that for the simplest electrolytes (NaCl, KCl, etc.) did not exist; it became apparent that an expansion of the laboratory's work to molecules simpler than proteins—amino acids and peptides—was essential to achieve full understanding. Some of the most revealing papers from the 1930s accordingly dealt with these simpler 'models' for protein behaviour. Edsall's conclusive demonstration of the zwitterionic nature of amino acid molecules was in this category [9].

It was probably also through Scatchard that John Kirkwood, one of the greatest theoretical physical chemists of the time, was drawn into the Cohn/Edsall circle. Kirkwood and Scatchard had been contemporary postdoctoral fellows in Germany, partly in the laboratory of Peter Debye, and had published a paper together (in German) in the *Physikalische Zeitschrift*, which extended the Debye–Hückel theory of ionic interactions to mul-

tipolar ions [12]. Kirkwood was now a young research associate at MIT and he further extended this work into a classic paper that dealt specifically with amino acids and proteins [13]. Kirkwood left MIT in 1934, but always retained his interest in proteins and other macromolecules.

With the advent of World War II, Cohn and Edsall returned from model compounds to real proteins. They acquired government contracts related to blood fractionation (see below) and one of the consequences was that George Scatchard became officially associated with the lab and a salaried member of it for the period 1941–1946.

5. The treatise

Proteins, Amino Acids and Peptides as Ions and Dipolar Ions was published in 1943 [14], having taken approximately 15 years from first conception to completion. The authors' aim is stated explicitly as being to examine the evidence concerning the size and shape of protein molecules and their fragments, and the number and distribution of the electric charges which they bear. Above all, 'we shall consider the implications of their charged structure for their physical properties, and their physico-chemical interaction with other molecules.' The limitation in these goals is emphasised: the book is not intended as a systematic treatise on the chemistry of proteins or even on their overall physical chemistry—size and shape and charge were the narrowly defined focus.

It was a collective effort, echoing the already-mentioned spirit of the 'think tank' in the laboratory. George Scatchard contributed a 50-page chapter on thermodynamics and simple electrostatic theory, a model of exposition à la Willard Gibbs—the chemical potential is singled out as the most important property. Kirkwood added a more sophisticated mathematical theoretical chapter on solutions of dipolar ions. Hans Mueller from MIT has an exposition of the theory of electrophoretic migration. The 'home team' (Cohn, Edsall and Oncley), with additional collaboration from Ferry, J.W. Mehl and H.B. Vickery, contributed authoritative chapters on an extraordinary variety of topics, often theoretical, often conceptually advanced, at the very cutting edge of progress in the entire field being treated. Raman spectra, acid/base equilibria, dielectric constants and dipole moments, molal volume and heat capacity, solubility, sedimentation and diffusion, dielectric relaxation, viscosity, X-ray diffraction—these were typical of topics given a lucid presentation.

It was not a book about protein chemistry in general, but almost entirely confined to physical chemistry. Amino acid composition was the only non-physical topic included and could hardly be avoided, given the overall emphasis on the charged groups of proteins, most of them derived from the side chains of lysine, arginine, aspartic and glutamic acids, etc. One had to know how many of each there were per molecule. About half the book was devoted to amino acids and peptides, obvious model compounds that would serve as testing grounds for the behaviour of proteins, echoing the direction that was being taken in the laboratory work at the time the book was written.

It is instructive to list a few actual chapter titles, as is done in Table 1. Such broad coverage of theory (often mathematically complicated) in a book intended exclusively for use by protein chemists was unparalleled. Even textbooks of physical chemistry intended for students of 'pure' chemistry did not normally cover many of these topics.

6. The laboratory in wartime

Unlike many other academic science centres, the Cohn and Edsall laboratory flourished during the war years. The laboratory had a vital military function, the mobilisation of expertise in relation to blood transfusions, the need for which had been foreseen soon after the war began in Europe in 1939, before the United States itself became directly involved in the war. Blood transfusions were a life saver and every resource of the latest scientific advances needed to be mobilised for clinical use: not just the collection and preservation of whole blood, but its separation into its individual protein components and an understanding of their potential clinical applications would be put to use by the medical services of the armed forces. It was all a rare opportunity to be patriotic and humanitarian at the same time, and to serve the interests of scientific progress as well.

Table 1

Part I: amino acids and peptides
Spectroscopy and dipolar ionic structure
Thermodynamics and simple electrostatic theory
Dielectric constants and dipole moments of dipolar ions
(Kirkwood) Theoretical interpretation of the properties of solutions of dipolar ions
Part II: proteins
X-ray diffraction studies and protein structure
Osmotic pressure and molecular weights
Translational diffusion
Sedimentation and diffusion in centrifugal fields: molecular weights
Proteins as acids and bases
Rotary Brownian movement. Shape as determined from viscosity and double refraction of flow
Electric moments and relaxation times of proteins as measured from their influence upon the dielectric constants of solutions
Theory of electrophoretic migration
Elementary and amino acid composition of proteins

Generous public support was granted, blood was donated to the project by the American Red Cross, financial support came from National Research Council. When the Office of Scientific Research was formed Cohn became the director of a nationwide organisation for studies of blood and blood derivatives, supported by a formal government contract from August 1941 until June 1946. Parts of the overall project were assigned to other laboratories, e.g. Erwin Brand at Columbia University, who was in charge of amino acid analysis of proteins. But the core of the project resided at Harvard and the most vital contribution of the work done there was the development of methods of fractionation, based primarily on solubility differences. At first the traditional use of salts (especially ammonium sulphate) as precipitating agent was continued, but later the main reliance shifted to low temperature fractionation in alcohol/water mixtures. A series of *scientific* papers was published under the overall heading 'Chemical, Clinical, and Immunological Studies of the Products of Human Plasma Fractionation' and these were supplemented by progress reports, papers in clinical journals, etc. Cohn and Edsall are the authors of separate reviews, which provide details [15,16].

There was a strange mixture of chains of command reflecting the difference in goals between different facets of the project. One was the scientific one, already mentioned, with its exacting standards of chemical reproducibility, thermodynamic rigor, etc. The needs of production on what was essentially a commercial scale posed quite different problems—scaling up from laboratory bench to pilot plant and then to the equivalent of a factory. Finally, safety and sterility of the mass-produced product had to be given an overriding priority, even if the safety officers were not always familiar with the details of the pathway by which a production routine had grown from the earliest chemical specification.

This led in 1941 to a baffling crisis: serum albumin, made by a commercial manufacturer along a properly tested pathway, ended up with a propensity to kill animals into which it was injected for clinical testing. This turned out to be the result of an extra safety precaution: a mercurial preservative had been added to whole blood serum at the behest of a medical officer, to protect against possible bacterial contamination. It turned out that mercurials had a strong affinity for serum albumin and were thus *concentrated* in purified serum albumin, to a level to where the protein preparation itself became poisonous! A few years later, Hughes identified a single cysteine residue as the site on serum albumin with high affinity for mercury. With this knowledge it became possible to remove bound mercury and thereby to salvage old preparations of dried blood plasma. At the strictly scientific level it led to understanding of dimer formation by serum albumin, including its crystallisation in the dimeric state [17].

7. The postwar years

The momentum of the war project was maintained for a few years after the war. The number of scholars from abroad increased, for fractionation and purification of blood components had become priority items for all the more advanced countries in the world. Barbara Low arrived on the scene from England, a new permanent faculty member appointed to explore the new vista of exact protein structures promised by X-ray crystallography. More Americans came to the laboratory for their Ph.D. research or as postdoctoral fellows. I was one of the latter (from 1947 to 1949) and the project I worked on, assigned by Cohn, can serve as an illustration of the dominance still exerted by what I have called the 'working model' for globular proteins. The model had not changed: we were still in the era before the advent of X-ray crystallography; no α-helix had yet been proposed to lead us to think in terms of organised structures; even DNA existed only in a chemical sense, its relation to proteins unknown.

Ostensibly my project was a study of acid/base equilibria in serum albumin—each of the more than 200 individual acidic and basic groups per molecule were titrated sequentially and the corresponding equilibria (apparent p*K*s) established [18]. But the underlying working model for the particle was an intrinsic part of the project because the equation for the overall titration, including effects of ionic strength and temperature, was dependent on it. The equation commonly used at the time had originally been derived by K. Linderstrøm-Lang [19] and it represented the protein particle as an equivalent sphere. Electrostatic interaction between different ionic groups was formally incorporated, but only crudely, in terms of the total charge, growing and waning as the pH was changed. Conceptually, the interaction could be thought of as a kind of average over all ion pairs that a model with discrete charges would take into account individually.

Electrostatic interactions depend of course on how close charges are to each other and are thereby related to particle dimensions. The pertinent term in the Linderstrøm-Lang equation is the '*w*' factor (a measure of the electrostatic contribution to the free energy of protonation), given by Eq. (1)

$$w = \frac{N\varepsilon^2}{2\mathrm{DRT}}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) \quad (1)$$

in which b represents the radius of the equivalent sphere and a is the radial distance of closest approach of mobile ions to the sphere, a quantity only slightly larger than b. The term κ is the familiar parameter from the Debye–Hückel theory, which expresses dependence of interactions on ionic strength; likewise all remaining parameters in Eq. (1) have the same meaning as in the Debye–Hückel theory.

The interest of everyone in the laboratory was usually confined to the native state of any protein we studied. Cohn was very sensitive to the fragility of the native state (excessively so, it ultimately turned out) and the desire to keep all components in their native states was uppermost in his mind as fractionation and purification procedures were developed—part of the rationale for developing the ethanol fractionation method, which was carried out at *low temperature*. This scrupulously careful attitude was a constant factor in my own work and the dimensional parameters that emerged from my titration were therefore intended for the native state. As indeed they turned out to be: the electrostatic equivalent radius for serum albumin was essentially the same as its hydrodynamic counterpart, determined by diffusion or sedimentation.

A few years later, after I had left Harvard, I extended my acid/base titration study to denatured states of serum albumin. The '*w*' parameter used for the native protein of course no longer fitted the data; the new value that was needed required that charges be further apart, i.e. overall molecular dimensions had to be increased. In retrospect, it seems to me that nothing better illustrates the confidence we had in the working model, as a concept that made all experimental results hang together, than the fact that I never had any hesitation in accepting that this was actually happening. On the basis of my results I equated denaturation with molecular expansion—unfolding of the tight native structure. And my former

Harvard colleagues readily approved my interpretation. Using electrostatic interaction as prima-facie evidence for molecular dimensions was of course not by itself rigorous and we went on to confirm our conclusion by use of more conventional hydrodynamic tools [20]. But, in terms of our working model, what alternative explanation could there be?

8. Postscript

Cohn had for many years been in poor health and needed frequent injections of adrenalin to maintain the hectic pace of his professional life. His mind deteriorated in the last year or two and when he died in 1953 he was found to have a brain tumour. Harvard University wisely decided not to prolong the existence of the laboratory, for it could not have succeeded without Cohn's aggressive leadership. Edsall became a member of the Harvard College Biochemistry Department and other staff members dispersed to universities all over the country. The personnel directly involved in the manufacture of blood products formed a non-academic organisation in Boston, which still exists to make improvements and to market licensing rights.

The application of physical chemistry in the way I have here described it, based primarily on theoretical equations, using one's brain and selected critical experiments to derive structural information, has in the meantime gone out of fashion. Why reason from equations when you can get the actual molecular picture on a computer screen on the basis of X-ray analysis?

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