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# John T. Edsall: His key role in the determination of the structure of proteins

Barbara W. Low*

*Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York, NY 10032, USA*

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**Abstract**

A letter, written in 1947 by John Edsall, outlined a declared intent to set up an X-ray crystallographic laboratory devoted to the study of crystalline heavy atom derivatives of proteins in an attempt to learn more about their structure. The fundamental idea, to the recipient (B.W.L.) totally new, revolutionary, and wholly contrary to all learned certainties, led to a correspondence, presented here in excerpt. Detailed plans were made for the laboratory to be built in the Department of Physical Chemistry at the Harvard Medical School. The proteins to be studied were reviewed and debated. The work of the laboratory is briefly described. Lack of success, the fatal consequence of a then unknown feature of the protein first chosen for study, is now only recently understood. The history of the Edsall idea and initiative is explored, from its beginnings to its acceptance and exploitation. John Edsall is here recognized as prime proponent and developer of the fundamental idea behind the most powerful and, for more than three decades, the only successful approach to the determination of protein structure.


John Edsall, pioneer and frontiersman in a decades-long pursuit of precise information about protein molecules came to his position of eminence not through serendipity but as the appropriate triumph of relentless explorations. He was among the first to recognize that proteins could be defined in molecular terms and was always bold and resolute in efforts to learn about their properties and detailed molecular structures.

## 1. A proposal—X-ray crystallographic studies of protein crystals

For me this story began with a letter he wrote on February 10, 1947 to a young visiting Fellow in the laboratory of Linus Pauling at the California Institute of Technology. The letter, sent from the Department of Physical Chemistry at the Harvard Medical School began and read in part:

> 'During the last two months, we have begun to consider very seriously the possibility of undertaking X-ray diffraction studies on proteins in this laboratory. We have never attempted work of this sort in the past, but we are strongly inclined to think that the time may be ripe for doing it now. This is in part because of general advances in the field, which are well known to you, and other workers, but also largely because we are beginning to have available here certain protein molecules (highly purified serum albumin and others) which can be brought into rather specific combination with certain heavy metals and with organic compounds containing heavy atoms. The possibility of

*Tel.: +1-212-305-8688; fax: +1-212-305-7932.
*E-mail address:* bwl3@columbia.edu (B.W. Low).

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carrying out such studies in a systematic way, utilizing such chemical modifications, appears to show a good deal of promise of obtaining certain results from X-ray diffraction studies on proteins, which definitely could not be obtained otherwise.

In considering the possibility of doing such work, we have been in frequent consultation with Dr Fankuchen[1], and he has suggested you as a person very well qualified to undertake X-ray work of this sort in conjunction with other members of the laboratory, who are expert in protein chemistry. We have reached no final decision about embarking on this undertaking, but I am writing to tell you of it now, because I thought that you might be interested. If you are interested in this possibility, we should like very much to have a talk with you about the whole situation….'

That letter and the implications of the proposed studies formed my introduction to a great scientific explorer: one of the quietest scientific revolutionaries on record. The operative and most provocative word in the whole letter was 'heavy' as it occurred in the phrase '…we are beginning to have available certain protein molecules…which can be brought into specific combination with certain *heavy* metals and…containing *heavy* atoms' (my emphasis): This apparent denial of the then received wisdom all recounted here in the most reserved terms! Accustomed by nurture to coded communication this was to me a spectacularly well-disguised account of a proposed attempt to do the undoable, the impossible. For it was considered at that time that known heavy atoms would be totally inadequate for phase determination in any attempt at X-ray crystal structure analysis of protein crystals. I had been taught (assured) as a student that atoms with atomic numbers of at least 200 (i.e. the scattering power of 200 electrons) would be needed even with the smallest proteins. Thus, in response I not only expressed interest (truly an understatement!) but wrote 'I wonder whether, as your letter indicates, your primary interest is in the long-term possibility of determining further details of protein structure by the investigation of heavy atom derivatives.'

In his reply of March 28th John Edsall broadened the field considerably. His letter began:

'I have delayed for some time in answering your letter of February 17, primarily because our own plans relating to the X-ray diffraction work are still unsettled. This is partly for financial reasons, and partly because we are still debating the question of what the most promising method of approach is. If we get into this problem, we shall certainly want to make it a long-term venture, with the aim of making a pretty thoroughgoing attack on at least some aspects of protein structure. It seems quite clear that any such approach would soon begin to involve computational problems of a very formidable character. I am going down to Princeton next week to talk over this aspect of the matter with Dr von Neumann and Dr Booth, who, as you doubtless know, has come over to spend some time with von Neumann, in order to consider these problems. Dr Fankuchen is very eager to help get such a program under way…. Drs Warren and Buerger, at M.I.T., would also be ready to give help and advice, though both of them proclaim quite frankly that they have no interest in proteins as such.

At present, I cannot give you any final information about our plans…. I do want you to know that we are still deeply interested in these matters, and are very seriously contemplating an intensive and prolonged X-ray investigation of this sort….'

He added a postscript, which spoke strongly of firm intent.

'I gather from Dr Fankuchen and the manufacturers of the X-ray equipment that we should probably be able to get the equipment, at any rate by the latter part of the summer, if we do decide to go into the problem.'

The letter was certainly impressive evidence that a large-scale, long-range commitment of time, space and funds was to be made after, but only after, the broadest and most in depth investigation. Furthermore, with respect to methods the net was clearly being cast very wide indeed. The final principal decision makers (we) in the endeavor were Edwin J. Cohn, who dominated the laboratory

[1] *Dr I. Fankuchen.* Professor of Chemistry at the Polytechnic Institute of Brooklyn had worked in England with both Bernal and Dorothy Crowfoot Hodgkin before 1939 and had spent the years 1939–1940 in the same Department laboratories as John Edsall. He generously adopted all newcomers who arrived from the Bernal-Crowfoot network as protégées. Indeed he had written to me on January 20th before I heard from John Edsall. 'I have been promoting a job for you in the United States. Professor Edwin Cohn of Harvard Medical School is interested in doing some work with protein crystals….' In response to my reply he continued on February 20th. 'I am glad to hear that you are interested…. I think that the Harvard possibility is made to order for you. They make some of the purest and the best protein crystals in the world, and are probably the outstanding all around protein group in the United States.' Significantly he went on 'Dr Edsall has consulted with me regarding equipment…He is already receiving quotations on this equipment.'

dedicated primarily to the study of proteins, that he has established in 1924 and John Edsall who had joined him there in 1926. Professor Cohn then Chairman of the Department was named University Professor in 1948 and in 1950 became Director of the University Laboratory of Physical Chemistry related to Medicine and Public Health. It is not too fanciful to invent possible discussions between John Edsall and Edwin Cohn concerning the need for definite information about protein structure, not then available nor likely to become available in the foreseeable future. It is equally easy to suppose that, Edwin Cohn, dissatisfied with the progress of work in other laboratories, simply asked John Edsall to organize an assault on the problem and thereafter assumed that the problem was in the best possible hands. Which it was.

For at that time and for decades after, John Edsall was, as a 1951 article [2] in Colliers magazine said of him: '…[the person] generally conceded to know more about proteins than any other living man.'

To return to the proposed study, John Edsall wrote a third letter to me on April 11th with news of the final decision.

> 'During the last week, I have had a series of discussions with Drs Bernal, Fankuchen, Booth and von Neumann. As a result of all this, Dr Cohn and I are greatly encouraged and feel that we are definitely justified in going ahead on the contemplated program of X-ray diffraction work. Bernal and Fankuchen have both recommended you very highly…. On the basis of their recommendation, we are prepared to nominate you for an appointment…. No doubt you may have further questions…before reaching a definite decision concerning this offer…. It would of course be very much better if we could talk this over personally, and if you could actually pay a visit to our laboratory and see what the situation is here: However…I hope very much that you would like to accept this offer and to embark on the X-ray protein studies, which we are now planning to undertake.'

That letter ended with a hand-written postscript, which read in part.

> 'P.S. The crystalline proteins which interest us particularly at present are (1) Serum Albumin, especially in a new crystalline form obtained recently by Dr W.L. Hughes Jr. in this laboratory. The crystals contain mercury: one Hg to two albumin molecules, and we have various techniques on trial for introducing other heavy atoms. (2) A metal binding protein of plasma recently crystallized here. This binds Fe, Au and probably Zn. All these metals apparently combine at the same point in the protein…. Naturally we shall also explore other crystalline proteins to see which may be most promising for a deeper study using X-ray methods and chemical modification.'

Bernal wrote to me on that same day 'I have been talking things over here and I think the opportunity here for setting up a new and powerful X-ray protein school are very good.'

## 2. Design of an X-ray laboratory. Studies to be undertaken

I initially hesitated to accept his invitation. After further correspondence characterized by John Edsall's generous understanding of both my spoken and unspoken concerns, a short visit to the laboratory was arranged as he had proposed earlier: Those were memorable June days. I seized upon my good fortune with delight and accepted Dr Cohn's nomination to a 3 year appointment.

The next few months were spent in long range planning, most of it carried out by correspondence.[2] Once the planning began the overall decisions to be made were major and far-reaching. Essentially three main principal features of the whole broad proposal remained to be resolved. (a) Development of complete plans for a new X-ray crystallography laboratory. (b) Rethinking any final decision about the best possible initial approach to the question. Was the proposed study of heavy atom derivatives the most direct way to proceed? and (c) What crystalline proteins should be chosen for the initial study.

During this period I knew that here was a grand master of scientific enquiry and design. John Edsall, a meticulous and demanding experimentalist, was as tireless in pursuit of the best solutions to experimental and instrument problems as he was relentless in analysis of the most recent ideas and findings of other scientists in the field. His letters, a joy to read, provide a first hand and detailed account of that period: Originator, scientist

[2] Before I left the United States to return to England for a few months, John Edsall and I did spent one whole ('strenuous' J.T.E.) week in November working together in the laboratory. Most of the major decisions had been made by that time, but much remained to be worked through.

and colleague John Edsall was the staunchest of allies in times of need. The experience was extraordinary, unforgettable.

By chance the question (b) concerning the best approach was dealt with first:

(I) This involved consideration of the two theoretical approaches to phase determination then being proposed. Here the decision was essentially his alone. His advisors were the best in the field and, as an experimentalist I knew my own skepticism to be based solely on the weak scattering power of protein crystals and perhaps therefore on shaky ground. On July 3rd John Edsall had written:

> 'I talked to Dr Fankuchen on the telephone two nights ago, and he was quite excited about the paper which Harker gave at the conference in Canada on the possibility of determining the phases of at least some of the X-ray reflections. Do you think that this shows real promise of permitting a deeper attack on the protein problem in the relatively near future? At present, I know nothing of the details of Harker's method. I expect to learn more about it from Fankuchen, when I see him next week.
>
> We also had a very enthusiastic description from Dr Booth, when he stopped here 2 weeks ago, of the power of his new method of calculation by steepest descent. Here again, I have not the detailed evidence to go on; but I have the impression that for molecules as complicated as proteins, there may be difficulties of which Booth is not fully aware. Even so, however, it might be quite a big advance.'

Later in another letter dated July 23 he wrote:

> 'Naturally, I am very much interested in the possibilities of applying the Harker method to proteins. However, I can assure you that I am not indulging in any excessive optimism at the present time…. You may feel quite assured that we are not expecting you to produce miracles on your arrival here.' (Fankuchen had been told that I thought he had given John Edsall an overly optimistic report about Harker's methods).
>
> I gathered from Fankuchen, by the way, that Harker now thinks his method, in its original form is likely to be inapplicable to proteins, but that with a modified form of the mathematical analysis, it could still be made applicable. I gathered at least some of the argument on which this statement was based but since you are much more fully informed [sic] than I am about the whole thing, there is no need to elaborate on the matter now.'

Thus, the idea of an initial approach to be based on theoretical arguments and relationships was to be set aside at least *pro tem*.

(II) *Plans for a new X-ray laboratory*. The most compelling need was for the means to maintain the albumin crystals constantly at a temperature of $\sim -5$ °C. They were unstable at room temperature. In June, at Harvard, when I had first learnt of this problem I had proposed that all necessary equipment, the whole laboratory, except the X-ray generator, be housed in a cold room. This provoked a dismayed response. Arguments revolved around the possible merit of an alternative, the design of a series of inter-related cold boxes. Was the major expense to be expected in building such a cold room absolutely necessary? (The diffraction data were to be recorded on film wrapped round the inner surface of a cylindrical UNICAM camera.)

Our correspondence after July 1st led to a letter from John Edsall on October 6th which opened 'Everyone has definitely accepted the idea that a cold room is necessary for the X-ray work.' On September 13th John Edsall had already written. 'I am thoroughly convinced on the subject, myself.' Before that we had both independently sought the best possible advice and consulted widely. There had been lengthy conferences between John Edsall, Dr Cohn, the engineers and advisors at Harvard. So it was that a cold room was built, the first of its kind designed for X-ray studies. Thanks to the advice of Dr R.W.G. Wyckoff the design included a vestibule kept at $\sim -35$ °C built to trap moisture in the air. Dr. J.L. Oncley wrote that the design was later copied elsewhere. Cold room clothes were army-surplus arctic issue! I had earlier sent John Edsall a report outlining the expected total needs of an X-ray laboratory. Once the major cold room hurdle was overcome, there were as the planning progressed only relatively minor adjustments in design requirements.

It is important to emphasize that the Cohn and Edsall decision to initiate X-ray studies was prime and independent of the identity of the X-ray crystallographer ultimately chosen. True, Edsall sought, as he told me, references with regard to my possible appointment from Fankuchen, Bernal and comments on the decision from Warren, and also when he later visited the laboratory from Linus Pauling. But, throughout the early decision-making I believe, I was, at once possible nominee, then nominee but always in one sense specialist

X-ray crystallographic consultant. It was an exhilarating challenge.

(III) *Choice of Proteins for the proposed study*. In a letter sent on September 10 concerned largely with instrumental questions, I had written.

> 'I have never really told you what I should like to do when I come to Harvard. I would like to examine as many protein crystals as possible of those which have never been examined by X-ray methods, and determine molecular weights and keep a general lookout for common features. And especially of course the metal containing crystals and if possible a very small molecule, e.g. secretin.
>
> Whether we shall get any worthwhile correlation or not I don't know. The investigations may suggest one molecule as worthwhile to pursue (e.g. Bernal, Crowfoot, and Fankuchen on Sterols).'

John Edsall's reply of October 18 was a masterly survey of the field. It began:

> 'We have done a good deal of discussing about the question of suitable small molecules for X-ray work that is, substances in the molecular range between 3 and 4000 and 18 000. Unfortunately, the number of such molecules that are really suitable is quite small. Furthermore, if they are really to be exploited to the fullest possible extent in their possibilities for X-ray work, they should be available in moderately large amounts, so that suitably modified chemical derivatives can be prepared from them. Preferably, they should be worked on simultaneously and quite intensively by some of the chemical group here, so that there will be at least one or two people in the laboratory who are thoroughly familiar with the chemical characteristics of the molecule…. It is very hard to meet all these conditions adequately. I have thought of seven more or less well-known substances in this connection and will list them one by one.'

There followed a survey of seven proteins. I shall cite here only three. They illustrate both the scientific and collegial aspects of his approach to decision making.

(1) Secretin (molecular weight ~5000). This offers an exceedingly attractive prospect in many ways, but to my knowledge, it has never been prepared in a high degree of purity outside of Hammersten's laboratory in Sweden, and the amounts prepared even there are not large. The criteria of purity, even for the best preparations, are still to my knowledge inadequate. Secretin is certainly a possibility, but it would require large-scale preparative work to make it available in satisfactory quantities for both chemical and X-ray studies.

(3) Ribonuclease (molecular weight ~13 000). This has of course been studied by Fankuchen in a preliminary way and he feels very hopeful about the prospects of further study. I do not think he has any intention at present of making such further studies himself, and probably would be willing to turn the whole thing over to this laboratory if we wanted to take it up. As with the trypsin inhibitor, the yields are very small. The Armour laboratories are at present producing ribonuclease; the price is $250 per g, but it may come down in the future. However, the amounts required for chemical modification studies would probably be 'prohibitively' expensive at the present time.

(6) Lysozyme (molecular weight ~18 000). This is apparently superb material for X-ray studies, but since Palmer is already working on it, I think we should leave it alone.

He continued:

> 'This is an attempt to evaluate the pros and cons of what seem to me the most likely substances for consideration in the low molecular weight group. Have you any others to suggest? It will certainly be worthwhile to make observations on various molecules of this class, and some of them might look particularly promising for further investigation, but the difficulties about obtaining enough material may make such further investigation quite difficult and one would have to be very strongly convinced of the promising character of the lead given by the X-ray data before attempting an intensive chemical study…. We must be on the lookout for further molecules of this type, which are very likely to turn up within the next few years. In spite of its larger molecular weight, I think that serum albumin is still the best bet for intensive X-ray work in this laboratory, since there are so many ways of modifying it chemically without denaturing it and further approaches are being developed in the laboratory all the time…. I think that serum albumin…should be given a very thorough and careful investigation and all possible leads explored. This of course will not be incompatible with less intensive studies of other crystals….'

I joined the laboratory in March 1948. The decision to concentrate first on studies of human serum albumin, so carefully formulated and one with which I, of course, agreed completely, was unhappily fatal to our hopes of the successful exploration of this wholly new idea and our intended goal. But the dominant aspect of this

celebration of John Edsall's proposal lies in the introduction of a revolutionary idea which was to be the key to success—elsewhere.

## 3. History of an idea

(I) *Background and development*. John Edsall's key contribution to the history of the determination of protein structure can be briefly summed up. At a time when the prospect of determining the detailed structure of a protein looked bleak he established a new school of protein structure research founded on the premise that heavy atoms could be used in phase determination. This would permit the measured intensities and thus the amplitudes of the diffracted X-ray beams (reflections) from protein crystals to be used in the calculation of the electron density distribution in the crystal. It was indeed a bold move.

Questions pile up. True, John Edsall was the protein expert's protein expert but whence his rigorous background in diffraction theory and X-ray crystallography? Why was *He* the author of so revolutionary a proposal? Certainly he had followed the published studies of Astbury and knew of the work by Perutz on hemoglobin. The presence of Fankuchen in the laboratory (1939–1940) may have provided a fundamental and rigorous introduction to both the theory and practice of X-ray diffraction and X-ray crystal structure analysis. In 1940 Fankuchen gave two lectures and distributed two sets of notes as part of a Department course 'Methods and Procedures employed in the Preparation and Characterization of Proteins.' His notes entitled 'The use of X-rays in the Study of Biological Problems with Emphasis on the Use of Diffraction Methods in the Study of Structure of Biological Interest' were brief but excellent textbook—style expositions. Fankuchen also discussed possible future work on proteins. He particularly emphasized the hazards and limitations involved in comparisons of a model structure of molecules with the data.

Fankuchen wrote in his notes:

> 'In the case of protein crystals…. The Patterson diagrams can test only gross features of the proposed structure…. It would appear desirable, from this discussion, to work if possible from X-ray data to a structure instead of attempting to use the X-ray data to test structures. The rate of progress may then be painfully slow but to compensate, such a procedure reduces the danger of believing that more has been revealed than is actually the case.'

There must have been many early occasions when the problems associated with protein structure determination were explored by Edsall alone, or together with Fankuchen as well as in open debate with Edwin Cohn. In the monumental 1943 treatise 'Cohn and Edsall' [1] John Edsall wrote a chapter entitled 'X-ray Diffraction Studies and Protein Structure' about the results and models proposed from studies on both fibrous and globular proteins. It attests to his understanding of and complete familiarity with the results of early work. The chapter could have been written by an X-ray crystallographer. I do not know whether he ever carried out experimental work but he wrote and talked always as though he knew precisely what one did with one's hands.

Edsall clearly had an excellent background but this still leaves open the question of the proposed use of heavy atoms. Here I move from documentation to anecdote and speculations, all certainly consistent with the then known circumstances and my own acquaintance with all but one, John von Neumann, of the major cast of characters. I shall follow the process I had personally employed in response to John Edsall's proposal.

My own acceptance of the idea that heavy atoms could be employed in protein crystal structure studies was a two-stage process. First came, swiftly, the early certainty that Edsall would not embark on such a project unless absolutely convinced that it was scientifically sound. My early 'received truth' must be abandoned. This was followed, faith giving way to reason, by a belated consideration of the true significance of the well known and off retold experimental observation that *protein crystals are weakly diffracting*. I imagine that John Edsall, unhampered by prior beliefs, first considered the recent successful use of heavy atoms alone and in isomorphous replacement methods, in the determination of the structures of small molecules of biological interest. He must then have asked himself why such methods could not be employed with protein crystals. In further read-

ing and discussions with Fankuchen he would have seized immediately on the significance of the reported 'weakly diffracting' feature of protein crystals as observed in X-ray diffraction patterns. There are no outstandingly strong X-ray diffraction maxima (reflections). The vector sum of the scattering contributions from all the atoms in a bulky three-dimensional protein molecule cannot, for any one set of planes be very large: The atomic (electron) distribution is nowhere markedly anisotropic neither in the molecule nor in the unit cell. The absolute magnitude of the largest $|F_{hkl}|$ value is in consequence small in spite of the very large number of atoms in one protein molecule. Ergo, heavy atoms, located at specific sites of a molecule, should make discrete, observable and measurable intensity differences in the diffraction pattern. At no time did John Edsall ever discuss this question with me. At no time did I ever tell him of my initial and amazed response to his first letter. He clearly regarded all the very serious future problems to be those directly associated with the preparation of protein crystals appropriate for the proposed studies.

Before giving the final go ahead to the proposed project Edwin Cohn is said to have consulted John von Neumann. My version of this anecdote was I believe, told to me by Edwin Cohn himself. They were at dinner together, and having been told of the proposed study, von Neumann made a few rapid calculations on the cuff of his shirt and assured Edwin Cohn that the proposal was theoretically sound. (There is, I am told, another version where a menu is substituted for the shirt cuff.) It would be good to find documentation. Without documentation it all makes perfect sense even down to the starched shirt cuff. Here Oncley agrees completely. Edwin Cohn would certainly have debated the merits of the proposal, listened carefully to all the arguments and essentially accepted the conclusions. But, once all aspects of the proposal had been seriously considered, he would characteristically have sought outside advice before making the final, truly major, decision to go ahead. Edwin Cohn's whole empire was crucially involved. He was proposing to commit not only space and funds but his reputation to the endeavor.

(II) *Experimental work in the Harvard X-ray laboratory. The Edsall initiative 1948–1953.* The fatal error in the decision to begin work on human serum albumin crystals was based on the assumption that the protein was pure; that the crystalline 'heavy' atom derivatives of Lewin [2] were homogeneous crystalline preparations of salts or complexes and that the mercaptalbumin mercury dimer of Hughes [3] would serve as prototype for the successful preparation of a series of crystallizable chemically-modified albumin derivatives. None of these assumptions were valid. As Oncley has discussed here in his paper 'Dielectric Behavior and Atomic Structure of Serum Albumin' the 'pure' preparation we both studied were heterogeneous mixtures of protein–fatty acid complexes. In the case of the crystalline 'heavy atom preparations' the fatty acids may well have blocked potential heavy atom binding sites. The structure of fatty acid free human serum albumin was established elsewhere by He and Carter in 1992 [4] in crystals grown in the presence of polyethylene glycol. Their finding of the molecular shape poses an unresolved problem. In crystals of the Hughes mercaptalbumin mercury dimer [5] the mercury atom is in a special position: on a twofold axis. The main features of the intensity distribution were compatible with a molecular shape similar to the contemporary model then proposed by Oncley (personal communication). See also his later findings [6]. X-ray and optical studies [7] appeared to impose limitations on one molecular dimension.[3]

In the beginning when we were learning to handle protein crystals, Jacob E. Berger, an undergraduate honors student, Frederic M. Richards, my graduate student, and I spent some time studying the permeability of protein crystals, the swelling and shrinkage stages of protein crystals on controlled drying. At some point an Oncley graduate student, Howard Dintzis, became interested in X-ray crystallographic studies of proteins. He sug-

[3] Most remarkably these crystals, which alone were totally inadequate for phase determination, showed perfect cleavage parallel to one face. It was so easy to split the crystals, that even, with careful handling, many single crystals proved in fact to be multiple crystalline lamellae. Such cleavage also appeared to indicate one limiting molecular dimension. I hope that this dilemma may one day be solved.

gested to me that heavy atoms could be introduced directly into protein crystals by immersing them in heavy-atom containing solutions. I was skeptical. Our permeability studies had led on occasion to marked disorder effects. Furthermore chemical modification studies were, as Edsall had asserted earlier, being carried out simultaneously by expert protein chemists in efforts to prepare covalently bound heavy atom derivatives of human serum albumin. Hughes' work had established a promising starting point. Studies of other crystalline proteins included work on a new crystalline orthorhombic form of insulin. Attempts to prepare heavy atom derivates were unsuccessful.

The possible usefulness of Patterson structure studies was not overlooked. In retrospect I believe we should have neglected them entirely. Here again John Edsall's insights were uncanny. It was he who met, with critical reserve, my enthusiasm for the four-layer hemoglobin model structure, proposed by Perutz from interpretation of Patterson structure studies.

In 1953 John Edsall left the laboratory. It was a great blow. We had not even made promising first steps towards fulfillment of his idea and initiative.

(III) *Acceptance*. The failure of the Harvard studies in no way diminishes the magnitude of the key influence the Edsall idea and initiative had on the subsequent history of the determination of protein structure. Echoing however, legendary past histories of other seminal ideas and discoveries his ideas were, initially rejected, ignored and, when finally accepted, his key role went completely unacknowledged: the basic idea attributed to others elsewhere. True, John Edsall was the least assertive of scholars and scientists. At the time of his proposal, however the little world of protein X-ray crystallographers was very small indeed. The world of X-ray crystallographers working on structures of biological interests was not much larger. After the war vigorous efforts were made to open up communications: Ideas traveled rapidly back and forth. Everyone with the slightest possible interest, knew, must have known, that an X-ray laboratory had been established at Harvard University in the Medical School, largely on John Edsall's initiative, with the expressed purpose of exploring the use of crystalline heavy atom derivatives in studies directed towards the determination of protein structure. Strong proponents and supporters of the idea, named here earlier, were leading scientists of the time.[4]

Edwin Cohn, in whose laboratory these studies were developed, was an early (1948) outspoken and repeated advocate of the heavy atom method of protein structure determination. One of the earliest occasions was his Theodore William Richards Medal Address [8].

In writing this account of the stages to the final acceptance and exploitation of Edsall's ideas and initiative it is appropriate to reconsider Edwin Cohn's role in this history. It was, as I suggested earlier, most probably he who initially expressed dissatisfaction with the progress of studies in other laboratories. Equally it is certain that it was John Edsall who thought through the problem and provided the key to its solution. Throughout this period it was Edwin Cohn, he, and he alone who spoke and wrote publicly about the whole idea. Always, whenever he did so, John Edsall's key role was acknowledged and made unmistakably plain. John Edsall had never himself written about his ideas and initiatives.

In my chapter [9] entitled 'The Structure and Configuration of Amino Acids, Peptides and Proteins' in the 1953 treatise 'The Proteins' I cited the 1948 Cohn reference. Because I knew that, even in 1952, the idea was still being rejected in some quarters, I attempted to show, with examples, that consideration of the absolute magnitudes of the intensities of the strongest reflections in different structures (small, medium, and large) could provide a persuasive quasi-quantitative demonstration of the validity of this approach. From the beginning, after I had carefully thought through the Edsall proposal to my own 'But of course,' I assumed that this reaction would be, had been,

[4] Following the long-standing Harvard laboratory tradition: tell colleagues openly about your ideas and the state of work in progress, Dintzis was later to take to Cambridge (UK) his knowledge and understanding of the work in the X-ray laboratory and also, his own suggestion that protein heavy atom derivatives could be prepared by the immersion (soaking) of protein crystals in solutions containing heavy atoms. That suggestion proved immensely valuable there in the first determination of the structure of a protein.

echoed by all those who subsequently learnt of his idea, accompanied, on occasion perhaps, by 'Why didn't I think…?'

In my article [9], I neglected to discuss there the background history of the Edsall heavy atom initiative, as well as the development and consequent work then in progress in the Harvard laboratory[5]. John Edsall's name did not appear in that context. It was an omission that I cannot now understand: I find it inexcusable. Furthermore it was an omission in conflict with the Harvard laboratory tradition of an openness in conversation with colleagues allied to scrupulous attribution in formal lectures and publications.

There is, of course, one other fate of a seminal idea: Those most interested in its significance may never have learned of it. This is the case with J.M. Robertson's 1939 published prediction [10] that the structure of insulin would be solved by the use of heavy atom or isomorphous replacement methods. I did not learn of it in 1942 when I began X-ray crystallographic studies. I certainly believe that John Edsall had not done so.

Neither Monteath Robertson's insights, nor the timely and well known initiative of John Edsall, nor the subsequent published and outspoken advocacy of Edwin Cohn in any way changed the history of the idea as it was written. The wheel was to be totally reinvented.

Thus, nowhere has John Edsall's key contribution been appropriately acknowledged. The approach he proposed certainty led directly to the first determinations of protein structures. It should be recognized. It is the only one that for nearly four decades, before the triumphs of NMR, proved successful.

[5] John Edsall read drafts of my 1952 article with critical care. He, of course, made no suggested changes in the presentation. We both, he and I, co-authored a 1956 article on protein structure studies and there we made no reference to the idea or to the work of the Harvard laboratory.

Our early years as colleagues and collaborators and all the years after abundantly confirm my view of John Edsall as the true pioneer. Beyond the magnitude of his scientific contributions, I celebrate also John Edsall the man, his scientific integrity, his steadfast and resolute stand for the public good and last of course with great personal pleasure the warmth of his friendship.

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