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PROTEIN RESEARCH IN CAMBRIGDE IN THE POSTWAR YEARS

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Ladies and gentlemen, it is a great pleasure to be back again in Barcelona. Professor Guerrero mentioned that I came in 1967. Actually, the first time I came was in 1956 for another reason, so I have been to Barcelona a number of times, and it is a great pleasure to have the opportunity of talking to the Catalan Society of Biology.

Needless to say I did not understand everything that Professor Guerrero said because my knowledge of Catalan is zero, so I think they were rather kind words but I am not absolutely sure. Well, you might expect from a visiting biologist to hear about some of his own results in the field (you had a little of that from Professor Guerrero) but you will not get it from me because I need to talk to you about what is already ancient history. I ought to apologize for this, but I really have no choice because I am myself a bit old, what is now old history. I have long retired from any work of my own in the laboratory. My own research was all done in the 1950's and the early 1960's. This is just to warn you because I know that generally, active scientists in any field never read papers more than about three years old. But this is about things that happened forty years ago. Of course, these days biochemistry, molecular biology and microbiology have become really the same subject. So, I am going to talk about some early biochemical research in Cambridge and about the beginnings of molecular biology.

In spite of my title I should make at least a passing reference to DNA as well as to proteins. Actually, at the very beginning I had to move for a short time away from Cambridge to the Royal Institution in London in the 1920's. The Royal Institution is famous for its friday-evening discourses intended for the general public and they began with Humphry Davy and Faraday. Traditionally the directors of the Royal Institution have always been experts in giving popular lectures, specially lectures to children. In the 1920's the director was William Bragg. You may know that there were two

Braggs, father and son, and the younger Bragg, Lawrence Bragg, really thundered the subject of the X-ray crystallography by determining the substructure of a crystal containing a molecule with only two atoms in it, that of sodium chloride. The Braggs shared the Nobel Prize. The younger Bragg was really very young when he got his. In fact he told me that he got the telegram telling him he had won the prize when he was in the trenches in the First World War, so he was quite surprised, I think.

Well, we will come back to the younger Bragg later, but now for the older Bragg. One day Bragg, as director of the Royal Institution, was going to give a popular lecture with the title "On the imperfect crystallization of common things". He went round the young people in the lab, in the research group, and told them to take X-ray pictures of anything they could think of. One of the younger students there was Astbury. He took his first X-ray photograph of human hair and found that it did give an X-ray pattern. Furthermore, he found that if he stretched the hair, the pattern changed. This of course became known as the alfa- and beta-carotene patterns and I believe that they really were the first experiments in molecular biology. Later, by the way, Astbury also took the first X-ray pictures of DNA, but he never solved the structure. Bragg had another brilliant young student there in the Royal Institution, Desmond Bernal. We will come back to him later. Well, that was the Royal Institution in the twenties, so now I think we can go back to Cambridge. So, how did biochemistry begin in Cambridge? It grew out of the older department of Physiology, and Gowland Hopkins had been brought there in 1898 to work on what was then called "chemical aspects of physiology". In 1914 the University decided to create a separate department, called the department of Biochemistry and Hopkins was the first professor.

The emergence of biochemistry from physiology was not without battles and bitterness. The old against the new. There was a similar history in the emergence of molecular biology from biochemistry. Just to give you two examples of this, my close colleague, Max Perutz, was not invited by the professor of biochemistry to give any lectures in the department of Biochemistry and so he had to give his lectures in Professor Keilin's laboratory which was called parasitology. I remember too that my old friend -and he really was a friend of mine in spite of what I am just about to say-, Ernst Chain, who of course won a Nobel Prize himself, was once my Chairman at a meeting on the history of biochemistry. I had to give a talk about the history of molecular biology, so Chain introduced me by saying that I was about to talk on a subject that did not exist because he said molecular biology is simply a branch of natural products chemistry. So you see, there were problems concerning new names those days. But those battles are really over now and, as I have already said, there is really no difference today between physiological chemistry, biochemistry, molecular biology and some other biological subjects.

My own connection with biochemistry began when I was reading some natural sciences at Cambridge. In 1937 I took a subject, biochemistry, and I was surpervised by somebody you may know the name of, namely, Robinson. He was a lecturer in physiology at the time and he told me that he always taught people physiology previously but he wanted to change so he was going to try to teach biochemistry now, but he was going to take the same course of lectures as I was to learn the subject and he thought that he probably knew very little more than I did, so he said: *why don't you*

come to the lab and I'll show you what I'm doing on the oxygen dissociation curve of haemoglobin?. That was my first acquaintance with heme proteins. Well, of course, the big break in Cambridge, as in other countries, was the war and most of the staff of the biochemistry department and others went to do war service. Academical research throughout Cambridge was at a very low ebb.

Things began again of course after the war but, even before the war ended, protein research in Cambridge had its real beginning with the appointment of Chiminal to the professorship of biochemistry. He succeeded Gowland Hopkins, who had retired. Chiminal came from the Imperial College in London where he had been professor of biochemistry. Already when he was there he began with the study of the amino acid composition of proteins. Modestly, when he came to Cambridge he did not eject the retired professor Gowland Hopkins from his office. He thought he would simply build a hut outside the main building and that was where Chiminal took place and it was always known as the "Protein hut". He went on working there until he retired in the mid-fifties. That was really the place where protein studies in Cambridge took off.

But what was really known about proteins in those early days?. In the beginning they were thought to be almost colloids, namely aggregates of not very defined composition. Then came the notion, the discovery, that proteins did have a definite molecular weight. That was due to Svedberg in Sweden, using an osmotic pressure, who did the first proper measurement of the molecular weight of haemoglobins (67000 Da). Well, the next stage perphaps was to realize that proteins had a definite, reproduceable amino acid composition. That was really due to Chiminal. Then came the development of purification methods, quite a bit later. I mean, I myself in my work used to fraccionate myoglobin with ammonium sulfate and, when I go back on it, it is a very crude method and I am really quite surprised that we ever got any crystals at all. And indeed that was no good unless you had a rather concentrated protein solution to work with. Of course, as you probably know, Martin and Synge, who worked in Cambridge earlier than that, in the thirties, developed a chromatography, but certainly when I began my work we had no knowledge of or made no use of chromatography. Well, I think the next step was to realize that proteins from different species were different and, in other words, there was a species-specificity. I remember a conference in Cambridge in memory of Barcroft, where Cavannaught came from Helsinki and talked about the differences between the haemoglobin of adult sheep and fetal sheep. In fact I myself worked on adult sheep and fetal sheep haemoglobins at one time.

When I came onto myoglobin by then it was clear that every species had its own kind of myoglobin. They all had the same molecular weight, they all had very similar amino acid compositions but they were different and produced different kinds of crystal. I think we got about fifteen different kinds of crystal from different animals. That clear recognition of the species-specificity was really the beginning of my work on myoglobin. Of course, crystallizing proteins has always been a kind of black art, it has never become a science. My advice to anybody tackling a new protein is always to say to them: *look at this protein through every species you can find and sure you will find one which has good crystals.*

Well, I think the next stage was the determination of the end-groups, the amino acid end-groups of proteins. That was done by Sanger and Porter. They showed that the

same proteins from different species might have different end-groups, another example of species-specificity. Then came Sanger's great work on the sequence, the complete amino acid sequence of insulin, which of course was a very small protein, but it was the first whose sequence had been determined. After that of course came the work on the three-dimensional structure of proteins. This was the kind of development of ideas about proteins which had really only grown at the time I am speaking of. Chiminal's group in biochemistry in Cambridge played a really important role in those developments. Besides his own work on the amino acid composition of proteins with people like Perry and Porter, he had in his group, as I have already mentioned him, Fred Sanger. You may know that he got himself two Nobel Prizes, one for the complete sequence of a protein, insulin, and the other for his work on developing methods of sequencing nucleic acids. Another one that I would like to mention was Kenneth Bailey. He worked first of all on the denaturation of proteins. He worked on myosin, on actomyosin and he discovered tropomyosin.

At this point, I simply cannot resist another short diversion from Cambridge. In fact is a diversion to China and about Kenneth Bailey's Chinese assistant, who was called Chao Tse Ching. He was brought to Cambridge in 1946 by Joseph Needham, who was a distinguished embryologist in the biochemistry department before the war. During the war he switched to the history of Chinese science and technology, which must be something like 16 volumes now. It is a great work and the Chinese would always tell you that it is the standard work on the history of Chinese science and, by the way, Needham spoke fluent Chinese himself. This came about because during the war he went to Chong-Qing, where the British Embassy was attached to the headquarters of Jiang Jie-Shi (*Chiang Kai-Shek*). That was before the comunist revolution in China. That is why he learnt Chinese and why he began his studies of history and collecting books. He had one or two young Chinese as his assistants, so one of them was Chao Tse Ching. Chao arrived in Cambridge in 1946 and took a B.A. degree in 1948. Then he became a graduate student of Kenneth Bailey, took his PhD, I think in 1951, and became a fellow of King's College which was Needham's college and I believe he was the first Chinese scientist to become a fellow of the Cambridge college. Well, his work with Bailey was really very important. It was on the fragmentation of myosin and the subunits of myosin, which later really provided the basis for the later discovery of the myosin light chain. He also worked on the size and shape of tropomyosin and a number of other related topics. He continued this work when he returned to China.

He played a leading part in initiating one of the great successes of Chinese biochemistry during the World War which was the total synthesis of insulin which nobody had even attempted before that. But it was a tragedy for him, I think due to his western contacts, that, when the cultural revolution came, there by then both he and his wife were members –not very enthusiastic members– but they were members of the Communist Party, he was put into some of those chicken farms for four years and this rid his health. After the revolution, he re-emerged and became deputy director of this institute. His health really wasn't good and in fact he died only about 18 months ago. I tell the story because he was really a rather friend of mine and in fact I went to China this spring to participate in a symposium which China arranged in the memory of Chao Tse Ching. That was in fact an important element in the development of protein research in Cam-

bridge. The insulin synthesis part of this actually happened in China.

Shortly before the war another man that I have mentioned, namely Desmond Bernal, who had been in the Royal Institution, came to Cambridge to work as a crystallographer under the younger Bragg. The younger Bragg had now become Cavendish Professor, that is, professor at the Cavendish Laboratory, which was the home of nuclear physics in Cambridge, and he succeeded Rutherford and J. J. Thomson. Bernal students were Max Perutz and Dorothy Hodgkin. Dorothy Hodgkin moved to Oxford and also got a Nobel Prize. Max Perutz stayed in Cambridge. Of course the seminal discovery by Bernal was that, although if you just put a protein crystal on a pin and took pictures of it, you got almost no reflections at all, but if you kept the crystal wet by closing it in a thin glass capillary tube, sealed the ends so it kept wet, then you got a beautiful pattern with thousands of reflections on it. He first demonstrated this with pepsin. From that beginning originated Max Perutz's work on haemoglobin which proved really to be his life's work.

The Medical Research Council Laboratory in Cambridge began when I joined Max after the war and later we were joined by Francis Crick. We were supported at the beginning by the Rockefeller Foundation and then by the Medical Research Council. Really, it was the enthusiasm of Keilin and Bragg which persuaded the Medical Research Council to take us on. By the way, I think Bragg was the only crystallographer in Cambridge, and probably the only crystallographer anywhere, who thought it was possible to determine the three dimensional structure of a molecule as complicated as a protein. I remember myself when I came back to Cambridge after the war. I did a PhD late because I had been away in the war and my research supervisor was not Max, it was W.H. Taylor, who had worked on the X-ray analysis of silicates with the younger Bragg when he was a magister and his opening remark to me was: *I think you take on a project which has absolutely no chance of success, nevertheless I will do my best to help.* So that was what the professionals thought about protein crystallography. I think Bragg himself was the only one who really believed it. I should say that Bragg did not know anything much about biology when this started. He was really a pure crystallographer and he was fascinated by the idea of studying proteins because he liked all the complicated puzzles, and this was just about the most complicated puzzle he could think of in the field. Well, all the early work I learnt was in fact on proteins. Max Perutz was working on horse haemoglobin. After a little work I have already mentioned about fetal sheep haemoglobin, I picked myoglobin because it was a nice, small protein and had fewer atomes in it than haemoglobin did. People think of Crick as a DNA man of course, but in fact he came to our lab to work on proteins. His first work in the lab was on the structure of lysozyme but he never got crystals of it. I remember Francis Crick coming into the laboratory in the morning with an onion. He needed to rub the onion on his eye to make himself weep. Human tears contain quite a lot of lysozyme, so that was the source of his material but it never crystallized.

The next thing was that Jim Watson came to our lab. He too came as many people know. He came to the lab to work with me on proteins but he did not really get on with proteins. He brought a very exciting piece of news to our lab which was that DNA was the genetic material, and not proteins. This work had been demonstrated in the States a year or two earlier, but the work was not generally known and was certainly totally unknown to any of us, even to Francis Crick.

So that is how Francis Crick got converted from proteins to DNA and of course was joined by Watson, who thought it would be less hard work than working with proteins. But I am not going any further with DNA, after all this is not my topic today.

On the protein site, we benefited very greatly from being in the Cavendish Laboratory, which was in the physics department and therefore had very extensive workshops. Really everything in the development of protein crystallography depended on new methods and new techniques. Apart from the X-ray cameras we used, everything was home-made, starting with the X-ray tubes. Conventional X-ray tubes were not powerful enough. Protein crystals are at least half water and therefore give very weak diffraction patterns. So we had to develop very powerful X-ray tubes. On that thing the first rotating anode X-ray tube which really worked was developed in the Cavendish laboratories.

Then, measuring up the spots on the picture. It all began with an accident through a discovery of my own. I was visiting King's College in London one day. There was a cell biologist there who was very ingenious with his hands and had made with his own hands a home-made equipment: a densitometer for measuring the optical density of thin sections of cells. I was watching him doing this work and suddenly it occurred to me we might be able to use this machine for measuring the optical density of spots on an X-ray film and that was how densitometry began. You see, it was clear that even if you could solve the structure, the amount of computing you would have to do would be not only difficult but impossible to do by manual methods or using those old-fashioned machines where you turn a handle. There was just too much of it. Mistakes would occur and it just would have never been possible to work them through.

Well, it so happened that in Cambridge at this time, not in the Cavendish but in what was then known as the Mathematical laboratory, there was the first working electronic computer in England. It was called Ersatz Mark I and it was home-made, using old fashioned semionic belts which the director had brought up from the army. They were ex-army belts from the war. It had a total store of 512 words and I remember the great day when they doubled it and we got 1024 words. Of course it was by normal standards extremely slow. Well, of course, the PC I have on my desk at home has a store of 450 megabites and it is much much faster, but this was the first working electronic computer which you could program to solve your own problems. Two of us working with a graduate student in Max's lab developed a programme which could calculate for a synthesis which you need for doing the calculations for protein structure.

You see, it is interesting that this computer came on stream, just about the time when we were getting some results, and I am perfectly sure if this computer had only arrived ten years later, the structures would not have come out until ten years later. So that was a happy accident, if it was an accident. I think it was, but some people may think our own providence was at work. Well, of course, the whole thing became possible because of a proposal made by Bernal himself before the war. He maintained correspondence with Dorothy Hodgkin, who was working on a molecule which had a zinc atom in it. He suggested to Dorothy that if they could substitute a cadmium atom for the zinc atom, that might slightly change the spots in the X-ray pattern and then you could determine the basis of the reflections as well as their intensities, and then the problem would be soluble.

Well, it turned out that for technical rea-

sons Dorothy could not use the scheme. After the war Max Perutz and his collaborator, Ingram, got it to work with haemoglobin. Shortly afterwards, we got it to work with myoglobin. That was when the whole thing began to come out and, in fact, the results of my own protein. The first picture of it was obtained at low resolution in 1957 and at high resolution, that showing all the atoms separately, was in 1959. A little bit later, Max Perutz got haemoglobin out too. His protein, being four times as big, took longer. Of course one person who was very pleased with this was Linus Pauling, because Linus Pauling had shortly before proposed the α-helix as a structural component of proteins. Of course the α-helix had never been observed, so it was a purely theoretical proposal, but once we got the high resolution picture of myoglobin we could see the α-helix in there. Linus Pauling was very pleased to see this but rather wished that he got to solve the structure of proteins himself, just as he wished to solve the structure of DNA which, as you probably know, he failed to do because he went for a structure with three chained helixs but the real structure has only two chains.

That is really the story and I tell it briefly. Of course, since then protein crystallography has become a major activity worldwide. I mean, we took for operating work ten years. When I remember back on it, it is really an amazing thing that the Medical Research Council kept this going for ten years. They never refused giving us money. We were never assessed and we had no results! The only papers we published were ones which later were shown to be quite incorrect. So it was really a kind of golden age for basic experimental science. I cannot imagine this happening today. Of course, now the whole business in solving protein structures has become much more rapid, so that the research of a graduate student can often solve the structure as part of one PhD project. In the meantime, the Medical Research Council thought our laboratory required its own building, and we persuaded Fred Sanger to come to biochemistry and Sydney Brenner from South Africa and Aaron Klug came to join us. Aaron Klug himself won a Nobel prize and he is now President of our Royal Society.

Of course, another thing that has happened is that this useless subject -molecular biology- has become very important in the wider world. When I say useless I do not remember anybody in our lab thinking or suggesting that knowing the structure of proteins could ever be important except as a piece of pure knowledge. However, that knowledge of a protein structure together with the knowledge of the structure of the DNA has now become the foundation of this very important new industry of biotechnology. So useless subjects often do become useful later. That reminds me, by the way, one of Bragg's very distinguished critics in the Cavendish Laboratory was Rutherford. It was at the time when J. J. Thomson had discovered the electron and Chadwick had discovered the neutron and the Bohr atom was well established. Rutherford was interviewed by a press man who asked him if that work would ever be useful, and he said: *Of course not, it is just a piece of pure knowledge*!

Only five years after that came some good and some very bad things, nuclear engines came out as well as the atomic bomb. So, practical things do come out, non practical science. Indeed, I think now that the pendulum has swang too far in the direction of applied science. I do not know what is like here in Spain but certainly in my country most of the money goes into applied science and, of course, a lot of it is handled by industry. Well, it is very important because, certainly, in England we were not good at

applying science and industry; we did not do it properly. But I think the pendulum has now swung too far and really basic science –what I call useless science: learning knowledge for its own sake– is being very badly starved for money, not only in England, but in many other countries. The fact is that you could never have applied this new field, biothecnology. It could not have existed without this useless research on the structure of proteins and the structure of DNA in the 1950's. You got to have good basic science in order to get good applications. We keep telling this to the politicians but they never listen.

By the way, molecular biology has of course changed too, because at the time I am speaking of it was sort of a subject to do with proteins. Nowadays people rather forget the proteins and they think it is all molecular genetics and cloning and they think of DNA but forget proteins, but of course it is really both. Well, that really brings me up to modern times. I think the activities on protein research today are well known, but a lot of it did begin in Cambridge, and I must say I was myself extremely fortunate to have been in Cambridge at the time all this happened.

Like Rutherford, we cannot predict the future. Another example of this: I remember a refugee, Professor Frish, who was involved in the discovery of nuclear fission, came to Cambridge and worked in the Cavendish. Few years after the war he was giving a lecture which I attended about nuclear physics and somebody put his hand up on his back and said: *Professor Frish, what's going to happen next in nuclear physics*? and he simply said: *If I knew that I would be doing it*!. Well, we none of us know what is going to happen and really none know how it all will finish. I think, in fact, science is unpredictable. This is exactly what makes it the most exciting activity in the world. So, thank you very much.