GANDHI MEMORIAL LECTURE SERIES

TWENTYFIVE YEARS OF MOLECULAR BIOPHYSICS

PROF. G. N. RAMACHANDRAN
Institute Professor of Biophysics
Indian Institute of Science
Bangalore 560012

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I feel greatly honoured by the invitation to deliver the Gandhi Memorial Lecture at the Raman Research Institute this year. Mahatma Gandhi and Professor Raman were the leaders of culture in our country. Our revered Mahatma, as we all know, was the builder of modern India and the one who led us to political independence. However, it cannot be said of the bulk of the scientists of our country, that they think independently and feel equal to the rest of the western world. This was the attitude that Raman had and which he tried to instil into his younger colleagues. I am proud of the fact that, in the formative years of my scientific career, I had the privilege and pleasure of working with the great master of Indian Science and learning from him how to think and work out new ideas in the realm of science. Although Raman was mainly a physicist, his interests ranged over the whole field of natural sciences from mathematics to biology. He showed us that it is not necessary to have detailed background knowledge of all that has been done in a given field in order to put forth new and original ideas which are worth serious consideration, but what matter most are the methods of logical thinking and appreciation of theory. It is well-known that experiment and observation form the bedrock on which the citadel of science is built up, and that the building can grow to a monumental height and take a beautiful shape only when both theory and experiment go hand in hand—factual data leading to the enunciation of theoretical ideas, which are again tested and verified by further experimental observations.

Once we understand this close relationship between theory and experiment in science, we can also see why it is necessary to have adequately equipped laboratories for scientific research of good value to be produced in our country. We often hear the story of how Raman discovered the famous effect known by his name, using a spectrograph of the kind that could be found in every laboratory. But at the same time, it is true that it required the perception of a genius like him to see the consequences of the few stray lines which he found in his spectra. Not everybody is a Raman, and what we need is to make it possible for capable scientists to be able to work competitively with the best in other advanced countries. For this, we need good facilities, and in fact, this goes without saying. However, it is not possible to provide first-rate facilities in all the subjects in every big university or institute in the country. What should be done, therefore, is that different centres should be selected for different specialities (of course, based on their competence in each field), and these should be very well equipped with both men and materials and also given the necessary encouragement and support. All the above criteria together make for what may be termed 'the right atmosphere'. From my experience, I can say without any hesitation that the Indian scientist is not inferior in any way to those abroad but that either the atmosphere, or the facilities, do not come up to the required level and he is therefore not able to put forth the best of

his capabilities. I hope suitable attention will be paid to the question of making our scientific output commensurate with the large amount of money that we spend for this purpose. I do not wish to analyse this aspect further, as I would like to turn to the scientific aspects of my talk.

I have termed my talk "Twentyfive years of Molecular Biophysics". In fact, this subject is just about that old and its foundations were laid in the early 50's. What I propose to do is to highlight a few important aspects of the development of the subject and, in the course of this, tell you also about some of the salient contributions from our Laboratory. Obviously, it will not be possible to give a full history of the subject, but it can be said that starting from ideas of molecular structure in chemistry and mineralogy, they have been widely expanded to include the whole domain of bioorganic chemistry and have provided considerable information about the mechanism of action of biological systems.

Before I go into the details of all these, I would like to say a few words about how I happened to enter this field and what background I had for our group to contribute effectively to this subject. As I have already said, I learnt physics from Professor Raman and in doing so, I specialised myself in optics and x-rays. Professor Raman introduced me into the mysteries of diffraction theory and Fourier transforms, a field which has its applications not only in optics and x-ray diffraction, but also in other modern fields such as quantum mechanics and particle scattering. Following this early training in Bangalore, I went to Cambridge to the Cavendish Laboratory which was then directed by the eminent discoverer of x-ray diffraction, Professor Sir Lawrence Bragg. Although I worked on a rather recondite topic, namely, what is known as diffuse scattering of x-rays by crystals, I had the opportunity in Cambridge to get in touch with all that was known at that time on x-ray crystallography, and the use of x-ray diffraction for the solution of crystal structures, and determination of molecular structure.

For those of you who know something of x-ray crystallography of the present day, with all its sophisticated diffractometers with complicated electronic circuits and computers capable of millions of operations a second, the methods of approach adopted in those days would indeed appear very primitive. They were, in fact, so primitive that when I returned to Bangalore in 1949 from Cambridge, I had no difficulty at all in starting work on x-ray crystallography and guiding a student for the Ph.D. degree. Using the elementary techniques available in the laboratory, my student, Gopinath Kartha worked out the structures of two or three crystals, which were considered commendable by the foreign examiners to whom they were sent. I say this to point out that equipment and facilities were not difficult to obtain in India at that time on a level comparable to those being used by persons abroad—except, perhaps, in the field of nuclear physics which had its cyclotrons and such high energy particle generators.

I was at the Indian Institute of Science, Bangalore, for only three years from 1949 to 1952 and then I joined the University of Madras as Professor and Head of the Department of Physics which had just then been established in that University, as a purely research Laboratory. This was indeed a unique opportunity, and it was due, in no small measure, to the strong recommendation which Professor Raman sent to Dr. Lakshmanaswamy Mudaliar, the then Vice-Chancellor of the Madras University.

Dr. Mudaliar gave me a free hand in the choice of my subject and in the equipping of our laboratory. Naturally, I chose x-rays, being well experienced in that subject both at Bangalore and in Cambridge, and this was indeed a very lucky thing, as you will see presently. There was no difficulty at all in importing the necessary equipment, including two or three x-ray tubes for starting work in this field. To start with, I took up some interesting problems on x-rays and on crystal physics and I looked around for a really good research programme to pursue. Here again—call it luck or chance—I had the good fortune to become aware of the very exciting developments that had taken place during the previous couple of years in Cambridge and in Pasadena. The structure of biological fibres, particularly fibrous proteins such as hair, muscle, silk and tendon, was eagerly sought to be solved during the previous decade. In fact, x-ray diffraction photographs of these, the so-called fibre patterns, had been recorded as early as the late 30's and early 40's but no satisfactory molecular arrangement could be obtained even though several attempts were made. In these studies, Astbury in England, and Meyer and Mark in Switzerland, made substantial contributions and the structure of silk could be readily solved. This structure consists of fully extended peptide chains going alternately up and down and joined together by periodic hydrogen bonds. Many of you are aware of what is known as the covalent bond between two atoms by which atoms join together to form molecules. These bonds have relatively high energies when compared to hydrogen bonds. But, although the energy of a single hydrogen bond is rather small, the total energy of a molecular chain becomes very much lowered, leading to stabilization of the structure, if a series of systematic hydrogen bonds can be produced. This happens in fact in the structure of silk and of what are known as the β-types of keratin and myosin, the proteins in hair and muscle respectively. As a consequence, the resultant structure is highly stabilized and forms the material for making up a good strong fibre.

Astbury and the other pioneers in this field well realised that the solution to the structure of all fibrous proteins, and in fact, of all biological fibres, is to be looked for in the existence of such a series of stable hydrogen bonds. In 1950, Bragg, Kendrew and Perutz made a systematic attempt at finding out all the types of helical structures which a polypeptide chain can have. I should mention that strong evidence from various sources had indicated that both in α-keratin and in collagen, the protein, or polypeptide, chain is probably coiled around and takes up the shape of a helix. Although Bragg and others obtained

some very interesting possibilities regarding the types of helices that could be built up, in which there occurred a systematic set of hydrogen bonds, they were too steeped in basic crystallography to make a break from tradition. As a result, they only tried for helices having 2, 3 and 4 units per turn—the integral values 2, 3 and 4 being the only ones that are allowed for the symmetry axis of a crystalline structure. However, almost simultaneously, in 1951, there came out a series of brilliant papers by Linus Pauling and his collaborators in which also, they sought for the regular helical structures of polypeptide chains, but made a definite break-away from traditional crystallography and postulated entirely new structures. I should also mention that Pauling had, in the previous few years, proposed the nature of the so-called peptide unit which forms the building block of all protein and polypeptide structures. In addition to finding the best values for the dimensions of the peptide unit, Pauling and Corey also drew a conclusion from theoretical chemistry that the peptide unit would be highly planar.

By these means, Pauling, Corey and Branson proposed the now famous α-helix for the structure of the molecular chain in α-keratin. As one can see from a model, every NH group in the helical chain is hydrogen bonded to the oxygen of a CO group, four steps behind. Based on the conditions for best stability of such a structure, Pauling and co-workers deduced that the stablest structure would correspond to 3·6 residues per turn, a non-integral value whose postulation led to a new era in fibre crystallography.

Very soon after Pauling thus proposed a non-integral helix (which, after its proposal, appeared to be such a natural choice), Cochran, Crick and Vand in Cambridge came out with the theory of x-ray diffraction by such helices, and it was immediately obvious that the x-ray pattern of α-keratin, which had defied analysis all the previous years, could be very naturally explained by Pauling's structure. The theory also predicted that a meridional reflection would be observed for this protein at 1·5 Å and Perutz, in Cambridge, verified this by experiment. So, within a year, Pauling's hypothesis, if one may call it so, was completely confirmed.

Along with his studies on the α-helix, Pauling also worked out a possible structure for collagen and various other fibrous proteins. All these appeared in the Proceedings of the National Academy of Sciences of the United States in the year 1951, and they reached Madras soon after. I remember with delight the thrill that I had in reading through these articles of Pauling. Even before this time, I had read the beautiful book by Pauling entitled "The Nature of the Chemical Bond" and had, in fact, taken him as my third *Guru,* in addition to Raman and Bragg.

Pauling's papers made me take a decision to work in this new and exciting field of biomolecular structure, but I did not know where to begin and what to

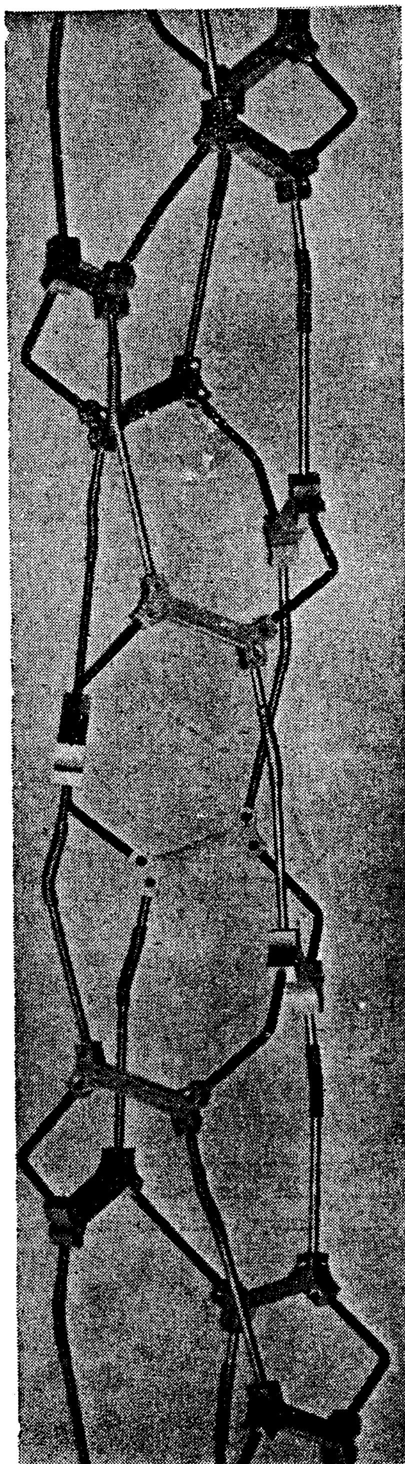

Figure 1. Photograph of a model of the α-helix, which was presented to the author by Prof. Pauling. Note the hydrogen bonds (long rod and spring) connecting each unit with the third unit away from it.

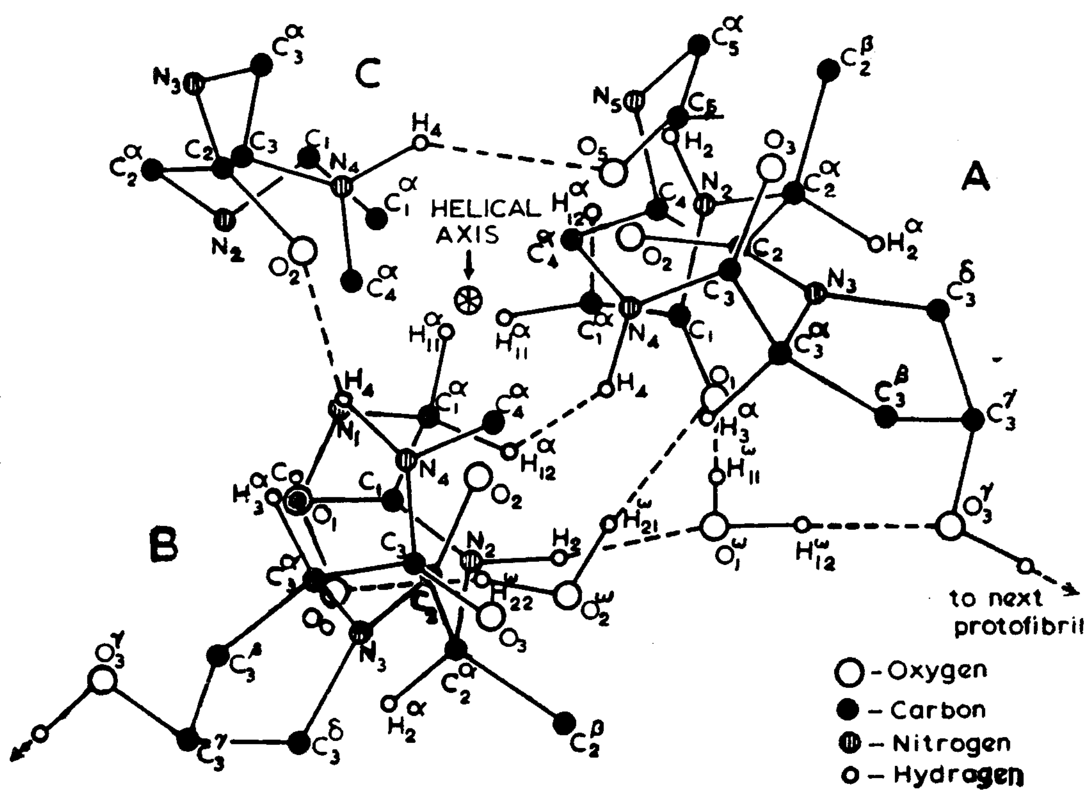

Figure 2. Projection down the helical axis of the atoms in the three helical chains A, B, C of the collagen triple helix. The interchain hydrogen bonds are indicated by broken lines. Note the two water molecules firmly bound to the chains A and B.

start upon. It was the visit of the eminent crystallographer, Professor J. D. Bernal of London to Madras that helped me resolve this difficulty. Although himself not a fibre crystallographer, Bernal, being in touch with the latest developments in Europe and USA, gave me the friendly advice that, although Pauling's structure for α-keratin was well established, the structures proposed by him, and by others, for collagen did not fully explain all the known facts about this fibre, and that, perhaps, I could study its x-ray diffraction pattern. Also, I had the good fortune of having with me Dr. Gopinath Kartha, who had joined us just then as a post-doctoral fellow, after completing his doctorate in Bangalore. Kartha and I took the diffraction pattern of kangaroo tail tendon obtained with the assistance of the Central Leather Research Institute, just next door to our Laboratory in the Guindy Campus of the Madras University. Of course, this did not show anything new for us, other than what had been reported in the literature. However, having a pattern obtained first-hand in our own laboratory made it possible for us to remeasure the various spots and to check them in different ways with any tentative ideas that we had regarding the possible ways in which the collagen chain might be built up. Before going further with a brief account of how we did this, it is necessary to have a clear picture regarding the elements of protein molecular structure and the special nature of the protein composition that is displayed by collagen.

All proteins consist of a long chain of 20 different amino acid residues. In the actual molecular structure of this chain, the building blocks are what are known as peptide units, which are rigid planar group as postulated by Pauling, who also assumed that these could be of two varieties, namely, *cis* and *trans* units. But we assumed that only *trans* peptide units occur in collagen, a supposition that was supported by the infrared data available then. Since then, various other evidences have strongly confirmed this assumption of ours.

Of the 20 amino acid residues, only one, called glycine, has a very short side chain consisting of just one hydrogen, while all the others contain at least a carbon, if not more atoms. The amino acid composition of collagen from widely different sources indicated that .glycine occurs as 33 per cent of the residues. We made the bold supposition from this that glycine occurs at every third position in the chain and that there was a stereochemical reason for this to occur. When we looked for this reason, we could deduce that a triple helical structure would best fit the data and that each helix would also have approximately three units per turn, every third glycine coming in the interior of each chain of the triple helix, leading to close packing of the chains. When such a structure was built in 1954, we found that it also had the hydrogen bonds nearly at right angles to the fibre axis, as suggested again by infrared studies. Also, the chains could accommodate the rigid side chain rings of proline and hydroxyproline which occur very abundantly (25 per cent) in collagen.

On examining in detail the x-ray photograph, it indicated that the number of units per turn should be approximately 3 1/3 rather than 3. From this, we

deduced that collagen had a coiled-coil structure, a suggestion which differed very much from all the previously proposed structures. In this structure, which we published in 1955, there were two hydrogen bonds for every three residues, which is the maximum that is possible. This was considered to be not reasonable by Rich and Crick of Cambridge, who suggested that only one hydrogen bond of this type could occur.

The controversy regarding hydrogen bonds could not be solved by any experiment, although the studies by von Hippel and others in the early 60's indicated that two of the NH groups for every three residues were involved in hydrogen bonding. The solution of the dilemma was given in 1968 by our proposal that the second of the two hydrogen bonds was occurring *via* a water molecule, and that there was also one more interhelical hydrogen bond *via* a water molecule. Very soon, the evidence for two waters per three residues was supported by the nuclear magnetic resonance data of Berendsen. Also, the postulation of glycine at every third position was fully supported by recent studies made in the 70's of amino acid sequence by various laboratories and pioneered by Karl Piez. One more feature of the collagen structure is the value of 30° for the superhelical twist, which was indisputably indicated by our measurements of the x-ray diffraction pattern. Yet, for the last 20 years, a value of 36°, corresponding to the rational ratio 10/3 for the number of units per turn, was the only one that had been quoted in the literature. I am happy to say that recent work by Cunningham and others from Tennessee has shown that this angle is in fact, extremely close to 30°. Thus, all the available evidence now supports the structure of collagen given by us in the 50's and revised and improved during the succeeding two decades. The structure could also explain the unique occurrence of the amino acid residue hydroxyproline in collagen (but in no other protein) and the role of stabilization of the structure played by the hydroxyl group of this amino acid.

I shall now deal with some aspects of the molecular structure of globular, or crystalline, proteins. Crystalline proteins like haemoglobin and insulin were studied even as early as the 30's in Bernal's laboratory ; the molecular weight was very high and the problem looked almost insoluble, but in the 40's, Perutz took it on and obtained x-ray diffraction photographs of haemoglobin ; the so-called isomorphous replacement method was adopted by him steadily for 10 to 15 years. In the late 40's, another oxygen-carrying protein occurring in muscle, namely, myoglobin, was taken for study in the same laboratory (Cavendish Laboratory, Cambridge) by John Kendrew. Myoglobin has only one-fourth the molecular weight of haemoglobin. Therefore, Kendrew was able to make much better progress than Perutz could with haemoglobin, but the two studies went side by side. In 1958-60, Kendrew published the structure of myoglobin with reasonable accuracy. It was found to contain a number of α-helices, which was very lucky because the α-helices are rod-like and fairly large to be detected against the background. The location of the iron-containing

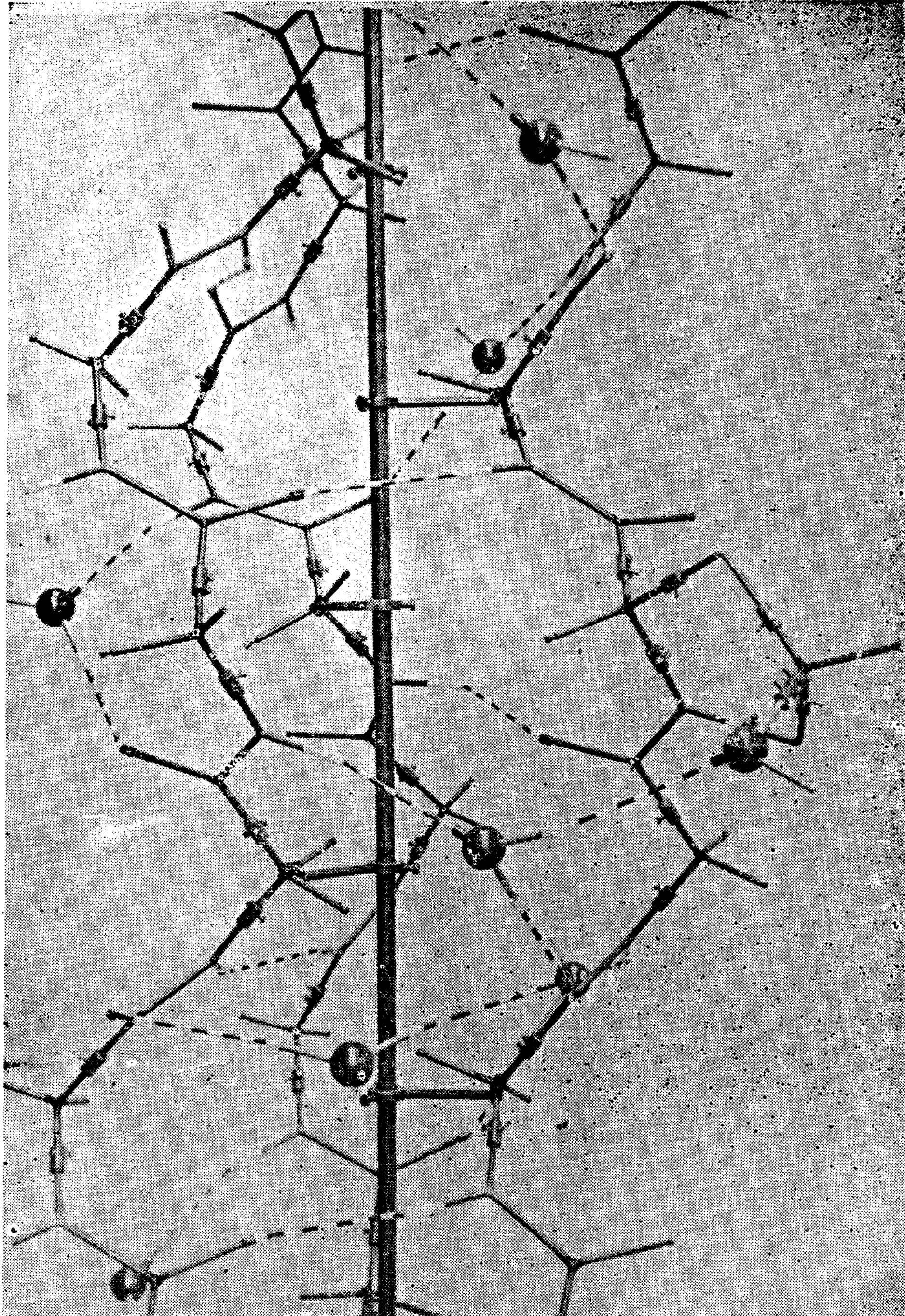

Figure 3. Photograph of the triple helical collagen structure, built in the author's laboratory corresponding to the calculated atomic positions shown in figure 2.

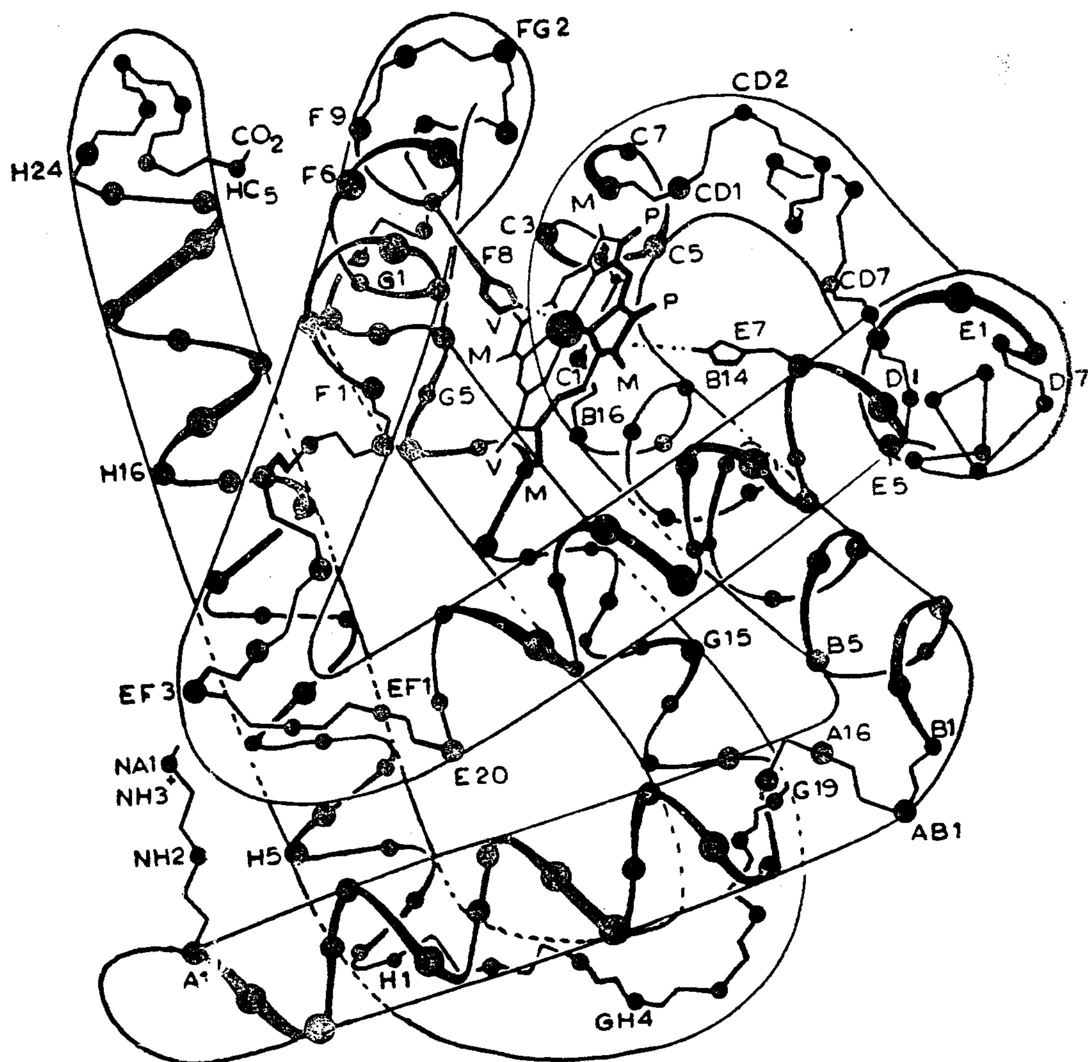

Figure 4. Diagram showing the molecular chain of one molecule of myoglobin. Note the α-helical segments indicated by the symbols A to H. The heme group containing an iron atom in the centre is also shown.

molecule heme was also detected. In another four or five years, Perutz also succeeded in solving the haemoglobin structure, which has two so-called A molecules and two B molecules. Each of the A and B molecules closely resembles myoglobin and, therefore, the mechanism of action of myoglobin in muscle and haemoglobin in blood are closely related. As you probably know, Kendrew and Perutz were given the Nobel Prize for this memorable work. In fact, Perutz had been pursuing the structure of haemoglobin in great detail even since, both when it carries an oxygen molecule and when it is devoid of it and so on, and the series of studies made by him has given us now a reasonable picture of the mechanism of transmission of oxygen by blood corpuscles.

I remember very well learning about these studies of Kendrew and Perutz when I was a student in the Cavendish Laboratory in the late 40's. Perutz was already at it for 5 to 10 years and it took another ten years or more before he could get results worth study by others. There is no doubt that, but for the support and encouragement that these workers had from Professor Bragg, they would not have been able to continue so long. If we are to have similar outstanding work done in our country, it is absolutely necessary that not only should funds be provided to young scientists, taking on such challenging problems, but also encouragement by the senior scientists. The senior scientist should be able to evaluate and appreciate work of this type, and his word should be taken as law by the grant-giving authorities.

I am very proud to say that after the pioneer work of Kendrew and Perutz, the next structure of a globular protein to have been solved was achieved by an Indian scientist working in USA. This was the protein ribonuclease which is an enzyme, and it was solved by Gopinath Kartha, a product of Bangalore and Madras, in association with Professor David Harker. This was achieved within a couple of years of the Cambridge work. In fact, the third protein to be solved was that of another enzyme, lysozyme, which was achieved again in Professor Bragg's Laboratory in London by David Phillips. The studies made by Phillips by x-ray crystallography confirmed fully what the chemists had surmised about enzyme action, regarding molecular fit. Actually, this mechanism of molecular fit is a very general feature of most biological reactions. For example, an immunoglobulin produces immunity to a particular disease by shaping itself to engulf the relevant molecule.

So much for some typical examples of the contributions from x-ray crystallography. This method, which took the pioneers 20 years to work out, has now been applied to as many as fifty different proteins. The hope is that, by studying a large number of proteins and thus obtaining information about their shapes and modes of action, some unified theories of the mechanism of biological metabolism and control can be developed, which will help us very much not only in the basic understanding of biology, but even in such things like finding a cure for cancer and other diseases.

I feel that I should not end this lecture without some comments on biophysics in general. There is a strong feeling among scientists now-a-days that the phenomena of life are not mysterious, and that they can be understood ultimately in terms of ideas and knowledge available in the fields of physics and chemistry. As we understand it at present, the whole of biological metabolism is based on biochemical reactions and so are, in fact, even the phenomena of genetics. Almost all the functions that take place in the body can be explained if one goes deep enough into their ultimate chemistry, and even neurological science is nothing but the effects of electrical signals, which again are generated by means of chemical reactions in living cells. But the question may be asked whether this is all that matters, and if physical and chemical aspects can explain the entire domain of living systems. I have to say that the answer is 'No'. We come up against some difficulties when we try to examine the brain and the mind. Many of the functions of the brain cannot be understood only in terms of physico-chemical mechanisms. When it comes to the mind and reason, and the phenomena connected with understanding one's surroundings, the use of language in speech, and the methods of logical thinking and so on, one has to think beyond the physical sciences. Many eminent persons have written about these things which fall in the broad field of philosophy. Some pragmatists believe that the whole of Nature can be comprehended in terms of scientific ideas. But I doubt it. I feel that there are many aspects which makes one believe that there is certainly a super-organisation of Nature which man may perhaps perceive, but can certainly not comprehend in its fullness. Whether to call this by the word God or by any other name, I will leave to you.

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