BIOLOGICAL SYSTEMS AT THE MOLECULAR LEVEL

A SYMPOSIUM was organized by the Commission of Molecular Biophysics of the International Union of Pure and Applied Biophysics during September 8–11, under the auspices of the International Laboratory of Genetics and Biophysics, Naples—the local arrangements being made by Prof. A. Buzatti-Traverso.

The first day's discussions, under the chairmanship of Prof. H. A. Scheraga (Cornell), were concerned fundamentally with the problem of predicting the conformations of proteins from their primary structure.

In the first paper Dr. S. Lifson (Rehovoth) gave an account of his recent statistical-mechanical investigations of conformational changes in polypeptides. His method consisted in defining sequences, sequence partition functions and sequence-generating functions and in using them to derive an equation for the contribution of each chain element to the partition function of the whole molecule. The results make possible, for example, an assessment of the relative importance of hydrogen bonding and hydrophobic interactions in determining the conformation of poly-L-alanine at different temperatures.

The remaining papers were more closely concerned with the investigation of specific conformations and the limitations imposed on them by interactions between non-bonded atoms. Given that the conformation of the peptide group is generally *trans* and planar, the most important variables determining the conformation of a polypeptide chain are the angles ψ and φ for rotation about the N—C_α and C_α—C^1 bonds respectively. Prof. G. N. Ramachandran (Madras) reviewed the earlier work in which he and his colleagues had studied the values of ψ and φ that are allowed when fixed minimum distances of approach are set for the atoms of a dipeptide unit comprising the two peptide groups and the β-carbon joined to an α-carbon atom. This had shown that only certain regions in a ψ, φ-diagram are sterically allowed and that these regions enclose, or nearly enclose, most of the conformations that have been proposed for polypeptides or observed in analyses of relevant structures. He then described an investigation of the effect of allowing the angle $NC_\alpha C^1$ to vary by $\pm 5°$ from the tetrahedral value (while the deviations from 110° of the other angles at C_α were minimized), which showed that the allowed regions in the ψ, φ-diagram are increased, by the introduction of this flexibility, to embrace most of the observed conformations that were previously just forbidden.

Prof. Ramachandran went on to consider the properties of helical polypeptide chains, in which the angles ψ and φ are kept constant at each α-carbon, with particular reference to the main chain —NH . . . O hydrogen bonding. He showed that families of helices of the right- and left-handed $3{\cdot}6_{13}$ or α-type and of the $3{\cdot}0_{10}$ type (with hydrogen bonds from peptides 1 to 4, and 1 to 3, respectively) are sterically allowed, with hydrogen bonds of acceptable length that depart from colinearity by less than 30°, provided that the bond angle at C_α is close to 110°. On the other hand, right- and left-handed π-helices (1–5 hydrogen bonded) are allowed only when the angle at C_α is 115°, and the $2{\cdot}2_7$ ribbon structure (1–2 bonded) is permitted only with hydrogen-bond-angles greater than 20°. The triple helix structure for collagen with two inter-chain hydrogen bonds (angles of 27° and 30°) for every three residues, as proposed in Madras, falls within a fully allowed region in the ψ, φ-diagram, and a new suggestion was put forward that this structure also includes additional CH . . . O hydrogen bonds between neighbouring chains. When collagen has the sequence gly-pro-hyp in all three chains, however, it appears that only one hydrogen bond can occur for every three residues and the preferred structural parameters closely follow those given by Rich and Crick.

Finally, Prof. Ramachandran discussed the conformations of amino-acid side-chains that have been observed in crystal structures, showing (in keeping with earlier reviews) that they usually adopt fully staggered conformations, but that any one of the possible variants may be adopted in response to a particular environment.

Developments of the limiting-contact approach to the analysis of polypeptide conformations were also described by Dr. G. Némethy. In these studies the influence of various amino-acid side-chains on the conformation of the dipeptide unit was investigated, the results being presented again in terms of the allowed regions in the ψ, φ-diagram. It was shown that the addition of a γ-carbon atom reduces the percentage of allowed conformations from 16 per cent for C_β alone to 14 per cent, even though there are three rotational positions giving staggered conformations of the side-chain from which to choose. The addition of atoms beyond C_γ in an unbranched side-chain does not limit the range of allowed conformations any more, but the presence of more than one δ-atom, as in leu, reduces the allowed conformations to 11 per cent of the possible, and branching at C_β, as in val, ile or thr, reduces this total to only 4·5 per cent.

From all these analyses by the limiting-contact method it is clear, therefore, that local steric restrictions, quite apart from the interaction of groups widely separated in the primary structure, severely restrict the number of conformations accessible to a polypeptide chain and go some way (though not yet far enough) towards making practicable the calculation of conformation from amino-acid sequence. A general method for handling such calculations has been pioneered by Prof. A. M. Liquori (Naples), and his colleagues, who described his preliminary attempts to calculate the helical conformations of minimum potential energy, taking into account the forces between non-covalently-bonded atoms. These calculations have led to diagrams of the potential energy as functions of ψ and φ which indicate, for example, that the right-handed α-helix with the original Pauling and Corey parameters is a very stable conformation even without consideration of the hydrogen-bonding that stabilizes it still further. There are four other potential-energy minima, including one corresponding to the left-handed α-helix which is shown, encouragingly, to be less stable than its right-handed counterpart.

Analysis of the structure of myoglobin has shown that these stable helical values of ψ and φ also occur frequently in non-helical regions of the molecule. Prof. Liquori suggested, therefore, that it might be interesting to investigate an idealized structure for myoglobin in which all the dihedral angles were constrained to take the values at the closest of these favoured pairs. Here again, of course, the aim was to reduce the number of conformations that has to be considered to manageable proportions.

Unfortunately there are very few reliable data from which the conformational potential energy of a polypeptide can be calculated. Prof. Liquori presented some evidence to suggest that the general features of the ψ, φ potential energy diagram for helices do not depend very sensitively on the exact shape of the interaction potential curves used in the calculations. He emphasized his belief that while precise energy values are not yet available, proper use of the relatively reliable van der Waals's radii may enable progress to be made towards the prediction of conformations.

In a final short paper in this session, Dr. D. C. Phillips (London) described the structure of lysozyme, recently determined at the Royal Institution, remarking particularly on the occurrence in it of some residues forming an anti-parallel pleated sheet and of some others in the conformation of the $3{\cdot}0_{10}$ helix with corresponding hydro-

gen bonding. Nearly all the helices in the molecule appear to be distorted to some extent from the standard α-structure and there is evidence that the hydrogen bonds in them sometimes depart from linearity by 20° or more. He described how comparison of the main features of the structure with the varying hydrophobicity of the amino-acid residues in the primary structure had suggested that the polypeptide chain folds itself from the terminal amino-end, forming first a compact unit with a hydrophobic core, then an extended arm of hydrophilic residues, partly in the β-conformation, and finally a coil that nearly closes the gap between the two parts, leaving a cleft that appears to be the active region, before winding itself around the terminal amino-end. Following ideas developed in collaboration with Dr. P. Dunnill, he noted that analysis of the distribution of hydrophobicity along a polypeptide chain might be useful in predicting conformations if enough guiding principles could be established.

A preliminary calculation of the dihedral angles ψ, φ in lysozyme had shown them to be nearly all in allowed regions of a potential energy diagram calculated for a peptide unit by the use of Lennard–Jones type interaction potentials. This diagram, which was similar to that calculated by Brant and Flory, differed from the original limiting-contact diagram mainly in allowing a greater range of conformations near those appropriate to the $3{\cdot}0_{10}$ and the left-handed α-helices. This was presumably because in these regions the occurrence of marginally short contacts, which are forbidden in the limiting-contact analysis, are outweighed, in the calculation of potential energies, by the presence of a large number of favourable contacts. The effect on the ψ, φ-diagram is very similar to that reported by Prof. Ramachandran to result from allowing the $NC_{\alpha}C^{1}$ angle to depart from 110°.

These papers provoked a lively discussion in the course of which Dr. F. H. C. Crick (Cambridge) remarked that the study of allowed helical conformations has really progressed very little beyond the results obtained by Donohue from the careful measurement of models. He urged strongly that rigorous attempts should be made to distinguish the important conformational variables, by comparison of the relative energies involved, from among covalent bond-lengths and bond angles, hydrogen bond lengths and angles, van der Waals's contact distances and rotations about bonds, suggesting that calculations based on simple crystal structures might provide necessary criteria for the establishment of valid energy parameters. Dr. J. C. Kendrew (Cambridge) described the helices found in myoglobin, noting that they are less regular than was first supposed and that some residues, particularly at the carboxyl ends of α-helices, are in the $3{\cdot}0_{10}$ conformation. There was general agreement that models based only on α-helices and 'random coils' must be considered inadequate. In view of the present very large number of investigations in which peptide conformations are described in terms of ψ, φ-diagrams, a plea was made for the general adoption of the standard conventions for labelling rotations that have been drawn up by Dr. G. Némethy. These are shortly to be published in the leading journals.

The second day, under the chairmanship of Dr. G. M. Edelman (New York), was devoted to the structural basis of the immune response. Dr. A. Nisonoff (Urbana) gave an extensive review of work done on structure of immunoglobulins over many years, leading up to the present-day concept of a divalent multi-chain structure consisting of two heavy chains, and two light chains with two antigen-binding sites per molecule. Even specific antibody directed to a single antigen is highly heterogeneous. Four classes of immunoglobulins are known, which share light chains but differ in heavy chains. Although the main antigen-binding site is on the heavy chain, a consensus of opinion is that both light and heavy chains contribute to the configuration of the antigen-binding site. Recent studies suggested that the two heavy chains are held together by only one S—S bond. In closing, Dr. Nisonoff discussed recent work by Drs. Hilschmann and Craig and Dr. Putnam and his colleagues, who have determined a partial amino-acid sequence of three Bence Jones proteins of type I (equivalent or similar to light chains of type I immunoglobulins). The C-terminal half of the molecule is constant in the three Bence Jones proteins, while the N-terminal half shows wide variation. Dr. Milstein (Cambridge) gave further data on the structures of several antigen proteins and suggested that the results were incompatible with the concept that the variations are due to single point mutations. We all await further sequence studies to throw light on the constant and variable regions of the antibody molecules.

Next, Prof. Jerne (Pittsburg) discussed the cellular kinetics of the antibody response when antigen is injected into the whole animal. Studying haemolytic antibody formation by individual spleen cells, using his plaque assay technique, he observed the events following the injection of sheep red blood cells. There is a rapid rise and fall in the number of antibody-forming cells. Subsequent to the injection of sheep red blood cells one is dealing with a cell population multiplying for a few days, after which time the cells reach an end-stage and do not divide into further antibody-forming cells. Dosage of sheep red blood cells affects the slope of the increase in antibody-forming cells as well as the time (in days) taken to reach a peak number of antibody-forming cells. At low doses of sheep red blood cells only two-thirds of the animals tend to respond and the slope of the increase of antibody-forming cells is much flatter. This is not easily explained by a population of reactive cells multiplying at a certain rate. Prof. Jerne discussed various possibilities. He suggested that perhaps the most likely explanation would be that lymph gland has several compartments, and that contact of antigen with reactive cells can only occur in certain parts of the lymph gland, perhaps the germinal follicles.

In the third paper in this section, Dr. M. Cohn (La Jolla) gave a lucid discussion of the potentiality of single cells. Since it has not been possible to clone antibody-forming cells in tissue culture, three approaches have been used. Different investigators have studied: (1) antibodies formed by single cells; (2) immunoglobulins formed by clones of transplantable murine plasma cell tumours; (3) the use of fluorescent staining techniques to detect different types of immunoglobulins in individual cells. The various studies agreed in finding that at least 90–95 per cent of the cells make only one antibody, one class of immunoglobulin and one type of light or heavy chain. In an investigation testing for genetic markers on heavy chains less than 5 per cent of the cells had a potential to express both alleles. Therefore, only one structural gene appears to be expressed by the majority of cells. The significance of the low percentage of apparently multipotential cells is not clear at present. No two myeloma proteins have been found to be alike. Therefore, the number of possible light and heavy chains must be very large.

In closing, Dr. Cohn discussed theories of antibody formation, germ-line versus soma. Soma would rely on mutation during the life of the cell and be less useful than the germ-line, which would follow Mendelian genetics.

Dr. B. Askonas (London) discussed the processing of antigens and the role of information at macromolecules in the immune response. She stated that the problem of control of antibody synthesis by antigen runs far behind the other problems. The fate of antigen was discussed at the cellular and biochemical level. Radioactive antigen is taken up by phagocytic cells throughout the lymph gland; in the secondary response it is particularly concentrated in the dendritic cells in the germinal centre. Although a major part of the antigen taken up is degraded very rapidly, the remaining antigen persists for weeks and is lost only gradually from the cells. The failure to detect radioactively labelled antigen in antibody-forming cells by G. J. V. Nossall and J. H. Humphrey and their

collaborators has shown that there can be only very few antigenic determinants present in the antibody-forming cell.

The question of how antigen stimulates the potential antibody-forming cell is still a vital problem. Whether it does so by direct interaction with the reactive cell or has to go through an intermediary cell needs further clarification. Since the phagocytic cells, the macrophages, take up the antigen they have been implicated as possible intermediary cells. RNA preparations containing antigen can be extracted from macrophages and they are highly active in inducing antibody, but whether this is an essential step in the induction of antibody is not clear. Suggestions that antigen-free informational molecules are transferred from macrophages to the reactive cells have also been made, but this has not been shown convincingly. Further experimentation is required to throw light on this problem.

On the third day a discussion on allosteric enzymes was held with Dr. F. Jacob (Paris) as chairman. The fact that a combination of one molecule of ligand with a macromolecule can influence the combination of another, the same or different, has been known for a long time. The term 'allosteric' proteins was introduced by Monod and Jacob to describe proteins in which such interactions occur. Although it is to be expected that many proteins are in some degree allosteric, the introduction of this term has been especially useful in directing attention to a particularly important class of phenomena involving enzymes which, potentially at least, provide an explanation of the regulation of metabolic processes in the organism.

The original observations of such interactions were on haemoglobin which may be considered as a type case of an allosteric protein. This was the subject of Dr. Wyman's talk. Dr. Wyman discussed mainly the haem–haem interaction, considered as a model of interaction between sites for the same ligand.

The second speaker, Dr. J. Monod (Paris), described a model which aims at explaining both the interactions between similar and between different ligands, in terms of quaternary structures of proteins. In this model an allosteric protein is considered to be a polymer with an axis of symmetry, which can exist under at least two different states which are assumed to differ between them by the degree of association between the sub-units. The two states are supposed to differ in their affinity for the ligands which the protein can bind so that the presence of a given ligand can push the equilibrium towards a given state.

The third speaker was Dr. H. K. Schachman (Berkeley, California), who gave a physico-chemical description of the enzyme aspartyltranscarbamylase of *E. coli*. Dr. Schachman showed that the enzyme is made of different sub-units, some of which possess a site specific for one of the substrates, aspartate, and others a site specific for CTP which inhibits the reaction catalysed by the enzyme. Isolated sub-units still exhibit affinity for their respective ligand but without co-operative effects, those being restricted to the complex polymer.

In the discussion, many other enzymes were discussed which exhibit similar behaviour. This is the case, for example, of the enzyme *d*CMP amino hydrolase which has been extensively investigated by Dr. Scarano. In contrast, other complex enzymes appear to operate on a different scheme. This is the case, for example, of the enzyme glutamine synthetase of *E. coli*, investigated by Dr. E. Stadtman, an enzyme the activity of which is susceptible to partial inhibition by eight different compounds of widely different structures.

In the final session on "Molecular Aspects of Differentiation", with Prof. J. Brachet (Brussels) as chairman, the main topic discussed was the synthesis of nucleic acids and proteins during early development. Prof. Monroy (Palermo) described in detail the significance of the events following the process of fertilization in sea-urchin eggs. Ribosomes from unfertilized eggs are not capable of incorporating amino-acids, while those obtained from fertilized eggs are capable of doing so. Prof. Monroy presented the following simple experiment. When RNA from unfertilized eggs was added to liver ribosomes the latter incorporated amino-acids. But no incorporation was detected when RNA from unfertilized eggs was added to ribosomes from unfertilized eggs. He has therefore suggested that some inhibitor is present on the ribosomes which prevents them from synthesizing protein. Though the unfertilized egg has a store of messenger RNA, the ribosomes become active only after fertilization. Prof. Monroy further indicated that inhibition on the inactive ribosomes could be lifted considerably by treating them with trypsin and removing the trypsin by washing through a sucrose layer.

Dr. D. Brown (Baltimore) examined the synthesis of ribosomal, soluble and DNA-like RNA during development of *Xenopus*. The kind of RNA synthesized varies conspicuously as development proceeds. Dr. Brown has been able to show that DNA-like RNA and soluble RNA are synthesized during late cleavage phase and the synthesis continues after gastrulation. The synthesis of ribosomal RNA starts only at the onset of gastrulation and increases as development proceeds.

The final paper was one on cell interactions and carcinogenesis by Dr. L. Sachs (Rehovoth), who discussed *in vitro* studies on the mechanism of carcinogenesis by polyoma virus and by carcinogenic hydrocarbons. In the experiments with polyoma it was shown that virus infection can induce the synthesis of cellular DNA after normal cell DNA synthesis has been repressed by contact inhibition or by X-irradiation, that each cell is induced to synthesize about double its DNA content, and that this induction is not dependent on the replication of viral DNA, but is a function of the viral genome. It was suggested that all the known experimental findings on cell-virus interactions with the small DNA tumour viruses can be explained by the synthesis of a messenger RNA early after virus infection that mediates the induction of cellular enzymes required for DNA synthesis by way of alteration of the cell surface. In the experiments with carcinogenic hydrocarbons it was shown that these chemicals can directly and rapidly induce *in vitro* a high frequency of transformation of normal cells to tumour cells. Such *in vitro* investigations provide evidence on the similarities between the two types of carcinogenesis.

B. Askonas D. C. Phillips
J. A. V. Butler L. Sachs
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LIVINGSTONE'S CONTRIBUTIONS TO MEDICINE

DAVID LIVINGSTONE has usually been looked on as a missionary and explorer, yet his contributions to medicine were numerous and varied. A review of them is given by M. Gelfand in a recent issue of *The Central African Journal of Medicine* (**11**, No. 7, 192; July, 1965).

He qualified as a licentiate of the Royal Faculty of Physicians and Surgeons (Glasgow) in 1840, and throughout his life, so far as circumstances would permit, he did all he could to keep himself fully informed and up to date. For the greater part of his time in Africa he was tolerant of native practices in medicine, seeking to understand their materials and methods. Patients flocked to him in hundreds, and by the standards of the day he was particularly successful with eye complaints and obstetrics. He was probably the first to describe trypanosomiasis in