has an improved RCA-EMU microscope, installed in mid-1950, and all facilities for auxiliary techniques and maintenance. The development of techniques for biological material includes the culture of tissues, especially nerve, on parlodion films, ultramicrotomy, a special technique for leukocytes and platelets, and a technique for macromolecules dispersed as aerosols and electrostatically precipitated. Biological, medical, and veterinary problems studied include nerve structure, elastic fibers, bacterial flagella, foot-and-mouth disease virus, anaplasmosis, myxomatosis, and the ciliary and neuromotor apparatus of *Tetrahymena geleii*. Studies are now being made of the virus-cell relation in myxoma of the rabbit, of the ultrastructure of cilia and neuromotor apparatus of infusoria, and of plasma particles associated with mouse leukemia. Nineteen publications have been prepared.

Conclusion

Considering the number of instruments, about 25, the results obtained so far are disappointingly few. Only a few laboratories are working in full, and producing original results. Contributory factors seem to include the following: a lack of scientific personnel specially trained for ultrastructural studies; a lack of technical personnel for the repair and maintenance of the instruments; poor choice of instruments (about half are of a model of limited usefulness); a general lack of supplementary instruments and facilities for auxiliary techniques; the lack of replacements, for maintenance; customs regulations that seriously impede electron microscopy in Latin America.

In line with the interest of UNESCO in forwarding this new discipline and scientific progress in Latin America, the following steps are recommended: fellowships to specialized centers for ultrastructure in the United States, Europe, or Latin American countries; improved interchange of technical information through periodical meetings of specialists in the field, and perhaps the formation of a society for studies of fine-structural problems in Latin America; modification of customs regulations to expedite the import of replacement parts; improved facilities for maintenance of electron microscopes; and an intensive theoretical and practical course in electron microscopy at a Latin American center.

Pasadena Conference on the Structure of Proteins

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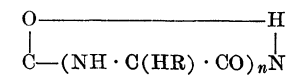
A SMALL GROUP OF INVESTIGATORS, active in the study of protein structure by x-ray diffraction and other physical methods, met at the California Institute of Technology, Pasadena, on September 21–26, 1953, in a conference organized by Linus Pauling. In many respects the conference could be regarded as a direct continuation of a discussion held by the Royal Society in London on May 1, 1952 [*Proc. Roy. Soc. London* **141B**, 1–103 (1953)]. Indeed, many of the participants were the same in both meetings. However, while the meeting in London had occupied only a single day, five days were fortunately available for discussion in Pasadena, so that an opportunity for constant and repeated interchange of ideas between the participants was possible over a relatively prolonged period. The conference was outstanding for the number of distinguished British participants involved, including Sir Lawrence Bragg, W. T. Astbury, J. T. Randall, M. F. Perutz, J. C. Kendrew, D. P. Riley, A. Elliott, F. H. C. Crick, I. MacArthur, M. H. F. Wilkins, and I. F. Trotter. The conference was made possible by the support of the Rockefeller Foundation, the National Foundation for Infantile Paralysis, and the American Institute of Biological Sciences in conjunction with the Office of Naval Research.

At the beginning the discussion was centered on recently determined structures of amino acids and peptides, and their implications for protein structure. R. Pasternak discussed the structure of glycyl-asparagine, which further confirms the planarity of the amide group and the adjoining carbon atoms in the fundamental repeating unit (—C · CO · NH · C—), and other features of peptide structure already established. E. W. Hughes reported his work with H. Yakel on N,N′-diglycylcystine, establishing accurate values for the distances sulfur-sulfur (2.04 A) and sulfur-carbon (1.87 A) and for the S—S—C angle (103°); the whole structure is of importance in the consideration of cross-linkages between polypeptide chains which involve cystine disulfide groups.

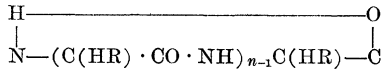
The discussion then turned to possible helical configurations of polypeptide chains. Barbara W. Low discussed the configuration of the π helix, in which each CO group is hydrogen-bonded to an NH group four residues beyond it along the polypeptide chain. She described some of the relationships within and between the two different families of polypeptide

chain helices which may be called the "α" and "γ" series. The helix belongs to the "α" series, that is, it corresponds to the chemical sequence



where $n=4$, and there are 16 $(3n+4)$ atoms in the hydrogen-bonded loops so formed. Four helices of the "α" class have thus been described: "α"$_{n=1}$ corresponds to the 2_7 or α_{II} configuration; "α"$_{n=2}$, to the 3.0_{10} helix; "α"$_{n=3}$, to the α helix of Pauling and Corey; and "α"$_{n=4}$ is the π helix. It is readily seen by examination of models that all the helices of the "α" series can be interconverted by relaxing or tightening the "wrap up" without complete unwinding.

The residue unit $(-C_1 \cdot C_1'O \cdot N_1H \cdot C_2-)$ configuration in the "γ" series is markedly different from that in the "α" series, both the CO and NH groups being sharply inclined to the helical axis with the C_1-C_1' and N_1-C_2 bonds practically perpendicular to this axis. The members of this series correspond to the chemical sequence



with $3(n-1)+5$ atoms in the hydrogen bonded loop so formed. For the "γ" helix, $n=5$. Transition between helices of the "γ" series corresponds to relaxation or tightening of the "wrap up." Interconversion between helices of different series is not possible without complete unwinding and rewinding. As yet there is no evidence that a helix of the "γ" series exists in any natural or synthetic protein or polypeptide. There is strong evidence for the reality of the α helix of Pauling and Corey, but as yet no clear evidence for other helices of the "α" series has been produced. These relations are further discussed by J. Donohue, *Proc. Natl. Acad. Sci. U.S.* 39, 470 (1953) and by B. W. Low and H. J. Grenville-Wells, *ibid.*, 785.

J. Donohue discussed the energy relationships and relative stabilities for six helical structures, four of the "α" series including 2_7 or α_{II}, 3.0_{10}, α, and π, and two of the "γ" series 4.3_{14} and the γ helix itself. He concluded that the stabilities of the five structures in which the hydrogen-bond angle is less than 20° are in the order

α, (3.6_{13}); π, (4.4_{16}); 2_7("α"$_{n=1}$); 3.0_{10}("α"$_{n=2}$); γ(5.1_{17}); 4.3_{14}("γ"$_{n=4}$).

The numerical subscripts denote the number of atoms in the hydrogen bonded loops; the numbers to which the subscripts are attached denote the number of residues in a complete turn of the helix. The coordinates of the β-carbon atom are different in the left-handed and right-handed forms of the same helix, and the radial distribution functions are, therefore, also different. Donohue reported that, for the π helix, the radial distribution functions of both left- and right-handed structures are similar to each other and to the α helical function. There was a very active discussion of the significance of radial distribution functions derived from x-ray scattering in powdered preparations in discriminating between left- and right-handed helices and between the α helix and other configurations. D. P. Riley took a leading part in this discussion and presented extensive data that he had obtained on many proteins, which could be interpreted in terms of the α helix in one of the other of the two possible configurations α_1 (left-handed screw with β carbon in Pauling's position 2) and α_2 (right-handed screw with β carbon in Pauling's position 1). Pauling discussed the problem of bending the α helix, especially in terms of the effect of proline and of the introduction into the sequence of one or more loops with 16 atoms in the hydrogen bonded loop (π configuration). Bending must be considered, since the evidence for many corpuscular proteins appears decisive in indicating that a number of bends must occur in the chains in order to pack them into the dimensions indicated from our general knowledge of the sizes and shapes of protein molecules. The insertion of a proline residue in a peptide chain eliminates one possible hydrogen bond and disturbs the repeating pattern of the helix. Models can be constructed in which bending around a corner can be achieved if the existence of hydrogen bonded loops with 16 atoms in the loop (as in the π helix) is assumed in the immediate neighborhood of the proline residue. To permit the helix to bend around a corner and reverse its direction, at least two intrachain hydrogen bonds must be broken which would normally be present in the straight helix.

The second day began with an active discussion of the structure of muscle and other α proteins. W. T. Astbury reviewed the natural α structures with particular stress on recent work on bacterial flagella. R. S. Bear discussed characteristic features of the diffraction patterns of paramyosin of molluscan muscles, and of another component (probably actin) which is observed in all muscles. These diffract at small angles according to a well-defined "selection rule" which has been known for some time: $n=(k-mG)/T$. Here all symbols are integers; k is the layer-line index, n expresses the distance of the observed intensity maxima at a given layer line from the pattern meridian, G and T are numbers characteristic of the fiber involved, and m is any positive or negative integer yielding the observed range of $n=0$ to ± 2. Two possible interpretations of these findings are available. One interprets them in terms of a large-scale net structure, which is also suggested by the electron microscope studies of Schmitt, Hall, and Jakus on paramyosin. The alternative interpretation is in terms of the transform of a helical structure as suggested by Cochran, Crick, and Vand; T and G could be alternatively the respective number of turns and the number of equivalent scattering centers, both per axial period of a discontinuous helix. For the "actin" pattern of muscle, $G=15$, $T=7$, and the diameter of the corresponding helix would be 50 A. The ambiguity of interpretation as between the proposed net or helical structures must be resolved by further work. H. E. Huxley then discussed his recent remarkable studies in the electron microscopy of

muscle, which reveal different types of parallel rod-shaped structures, some of diameter about 110 A, others near 40 A. Evidence was given that the former are probably bundles of myosin and the latter of actin.

There was a lively discussion, principally by F. H. C. Crick and Pauling, regarding the bending of α helices; model structures can readily be constructed involving triple ropes or seven stranded cables, and such models may account for some of the discrepancies between the x-ray patterns of the natural α proteins and the simple long chain polyamino acids, some of which at least appear to be simple α helix structures. Crick proposed that the very nature of the side chain packing in closely adjacent α helices would tend to make them fit together with an angle of approximately 20° between the axes of the individual helices. Astbury, Low, and others pointed out the difficulties of effecting the dynamic transformation from the α helix to an extended β form; it was agreed that the stretching of a coiled coil would probably be a very difficult matter even if the disulfide cross-linkages were broken and almost impossible if these were maintained intact. However, the evidence for such structures as coiled coils or seven stranded cables appears to be plausible enough to require serious consideration. R. E. Marsh discussed the structure of silk; the x-ray patterns fit in well with the assumption of repeating glycyl-alanyl residues in the crystalline portion of the structure, and the repeat distance of 7 A is compatible with the anti-parallel pleated sheet structure of Pauling and Corey.

In the afternoon there was some discussion of infra-red data with particular emphasis on their use in discriminating between the α and β configurations of polypeptide chains. G. B. B. M. Sutherland presented numerous infra-red spectra of proteins and synthetic polyamino acids. A. Elliott discussed the evidence for the correlation of absorption bands at 1660 and 4600 cm^{-1} with the α, and at 1630 and 4530 cm^{-1} with the β configuration. Questions were raised by Sutherland and others as to the extent to which the infra-red and the x-ray data on α and β configurations could be correlated. Elliott emphasized that one should not expect high quantitative accuracy in correlating the intensities of the bands with the percentage of the α or β form in any particular structure. I. F. Trotter presented excellent and detailed x-ray data on poly-L-alanine that were in general in close accord with the α helix structure with 3.615 residues per turn. Poly-alanine in the β form gave an x-ray diagram in good agreement with what was to be expected for the pleated sheet structure of Pauling and Corey; interesting relations to the structure of silk were also indicated.

There was a very active discussion on collagen. Bear concluded that the wide angle diffraction pattern of collagen is best accounted for by assuming an axial period of 28.6 A which would contain 10 equivalent scattering groups arranged in three turns of a discontinuous helix. It had been suggested earlier that each equivalent scattering group contains three amino acid residues. Careful consideration of the density of collagen, which is probably near 1.41 rather than 1.35 as usually assumed, together with other considerations, suggest that there may be as many as four residues per equivalent scattering group. This would give $7 \times 4 = 28$ residues along three turns of the chain helix in a period of 28.6 A. R. Pasternak also discussed recent data obtained in Pasadena which are generally in satisfactory agreement with those of Bear as far as the experimental findings are concerned, although some differences of interpretation remain. Astbury reported on new studies of the stretching of collagen by K. M. Rudall which may indicate transformation of collagen to the β configuration. Pauling emphasized that he had given up his own earlier views on the structure of collagen but was not yet ready to propose a new one. F. O. Schmitt presented his recent work with J. H. Highberger and others on the formation of two types of structures with spacings greater than 2000 A, denoted respectively as segment long spacing and fibrous long spacing structures. He pointed out the reversible interconversions possible between normal collagen, with its period of 640 A, and the two types of long spacing structures. The presence of ATP was found to be important for the formation of the segment long spacing structures. J. T. Randall discussed the developmental changes of collagen in the embyo.

On the third day the discussion was centered on the globular proteins. Sir Lawrence Bragg discussed the analysis of the x-ray diffraction patterns obtained with protein crystals, in particular the pattern due to the '*b*' projection of horse hemoglobin. [The basis of this work is presented in the Royal Society discussions already quoted and in an article by Bragg and Perutz, *Proc. Roy. Soc.* **A213**, 425 (1952).] It is a very complicated matter to determine the phases corresponding to the spots in the pattern, which must be known before a Fourier picture of the molecule can be made. By a study of hemoglobin crystals at various stages of swelling and shrinkage, nodes and loops along the layer lines of the transform can be outlined and many sign relationships established. (Since the '*b*' projection is centrosymmetrical, the determination of phases reduces to the problem of assigning a positive or negative sign to each term.) M. F. Perutz continued the discussion and showed how all remaining uncertainties about the signs had been cleared up. Rapid progress has been made recently by the preparation of crystals in which *para*chloromercuribenzoate is combined with the two sulfhydryl groups of the hemoglobin molecule. These crystals are isomorphous with the normal form, and the heavy mercury atoms produce a measurable change in the intensities of the x-ray reflections. In conjunction with the earlier work, these changes permit signs to be determined and make it possible to calculate the position of the mercury atoms and to draw a projection of the hemoglobin structure on the *b*-plane. The analysis has so far only been carried out for the inner part of the diffraction pattern, so that detail finer than 7 A is not shown in the Fourier. However,

the success of the method holds out promise that it can now be applied to get pictures with high resolution. Also heavy atoms may be attached to other key groups in the molecule and reveal their positions. J. C. Kendrew presented results on a series of crystals of myoglobin obtained from at least six different species of whales, from porpoises, and from penguins. Some of these crystals are considerably more suitable for x-ray study than those of horse myoglobin. Previous notions about the dimensions of the myoglobin molecule may require some revision. It now appears to be about 40 A long with a cross section of about 900 A^2. The data accumulated recently are so numerous that there has not been time to evaluate their full significance. D. Harker discussed the progress of the work on protein structure from x-ray analysis at the Polytechnic Institute of Brooklyn. Techniques have been devised for the rapid collection of intensity data using a Geiger counter. Five different forms of crystalline ribonuclease have been obtained, and preliminary data are available on them all. The program is of long range and most of the results are still to come. V. Luzzati gave a theoretical discussion of intensity distributions in systems containing large numbers of atoms with an assumed type of random distribution.

The morning discussion on the fourth day was entirely devoted to desoxyribonucleic acid (DNA) structure. Major emphasis was laid on the structure recently proposed by J. D. Watson and F. H. C. Crick [*Nature* **171**, 737 (1953)]. The evidence is clear from the x-ray data that the structure must be helical, and there appear to be compelling reasons for assuming a double helix involving two chains running in opposite directions and held together by hydrogen bond cross-links between adenine and thymine and between guanine and cystosine. This is in good accord with recent analytical findings of Chargaff and others indicating a 1:1 ratio of purines and pyrimidines in DNA. M. H. F. Wilkins discussed the evidence from the recent and beautifully detailed x-ray studies on crystalline DNA at King's College, London [Wilkins. Seeds, Stokes, and Wilson, *Nature* **172**, 759 (1953)]. The patterns from many different biological sources are indistinguishable, although the ratios of the bases differ markedly from one species to another. The crystalline like the paracrystalline form of DNA appears definitely to be a double helix; the former probably contains 11 nucleotides per turn of the helix. The nucleotides appear like a series of rods, inclined to the fiber axis at an angle of about 65°, in a sense opposite to the inclination of the helix. J. T. Randall discussed the recent work in the same laboratory demonstrating that DNA can be fractionated into different portions in which the ratio of adenine: guanine varies from 0.7: 1.3. M. Delbruck, F. O. Schmitt, and others discussed the biological significance of the structure proposed by Crick and Watson in relation to mitosis and to crossing over.

On the final day of the conference after a further discussion of the collagen studies, there was a lively discussion between D. P. Riley and Barbara W. Low regarding the structure of insulin. Riley from his radial distribution studies concludes that one chain of insulin (the B chain) is in the α_2 (right-handed) configuration, the A chain being presumably α_1. Low presented her studies on the structure of acid insulin sulfate and the clear-cut evidence for parallel rod-shaped structures from the three-dimensional Patterson diagram. Since the amino acid sequences in both A and B chains are known, the chief remaining chemical problem is the arrangement of the disulfide linkages. This would determine whether the molecule has four (2A and 2B) or two (A and B) chains. Model studies based on the α helix suggest certain possible interchain linked structures and show that the intrachain disulfide linkage required in the two-chain structure cannot be formed without very marked distortion.

Several major developments emerged from the discussion. The evidence for the reality of the α helix as the basis of structure, not only for synthetic polypeptides but for a number of proteins, appears increasingly strong. Generalized relations for the description of possible helical structures in polypeptide chains, together with criteria for their relative stabilities, have been formulated. The insertion of heavy atoms at defined points within the hemoglobin structure has opened a great realm for further investigation which promises to be extraordinarily fruitful. Knowledge of the structure of collagen has deepened and widened. The recent advances in the structure of DNA promise to be of almost revolutionary importance. However, the most important aspect of the conference was probably not in the explicit formulation of these established advances so much as in the constant interplay in the discussion involving the tentative presentation of half-formulated thoughts. Although much of the time had to be given to somewhat formal presentations, the greatest effect of the conference was undoubtedly in the opportunity which it provided for the germination of newer and more tentative ideas.