Nucleic Acid and Protein Conference

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THE second Gordon Research Conference on "Nucleic Acids and Proteins" was held at New Hampton, New Hampshire, August 27–31, 1951.[2] Among the eighty-one scientists participating in the conference, twenty-three came from countries outside the United States—namely, England (11), Canada (5), Sweden (3), Denmark, Holland, Italy, and Israel. The international character of the meeting was, in large measure, due to the continued support of the Rockefeller Foundation.

The main topics under discussion dealt with nucleic acid structure, physical studies on viruses and virus metabolism, effect of ionizing radiations on biological systems, and protein studies. Twenty-two papers were presented at nine sessions.

The first two sessions were devoted to a symposium on the structure of nucleic acids and certain nucleotides. Erwin Chargaff (Columbia University), Maurice Stacey (University of Birmingham), Gerhard Schmidt (Boston Dispensary), Waldo Cohn (Oak Ridge National Laboratory), and Hubert S. Loring (Stanford University) discussed the subject from various points of view. Dr. Chargaff first reviewed previous work of his laboratory on the structure of desoxypentose nucleic acids, which had led to the conclusion not only that the compositions of these compounds deviate in most cases very considerably from that postulated by the classical tetranucleotide hypothesis, but also that they differ from each other in a manner characteristic for the species (but not the tissue) from which they have been isolated. On the other hand, certain remarkable regularities in the relative proportions of the nitrogenous constituents were observed. Thus, all desoxypentose nucleic acids so far examined possess a purine-to-pyrimidine ratio of unity, and the same holds for the ratio of guanine to cytosine. The AT-type contains adenine and thymine, whereas the GC-type contains guanine and cytosine as major components. It was found that all the purines can be removed, yielding polymers composed of pyrimidine nucleotides ("apurinic acids"). In the instance of pentose nucleic acids (PNA), a branched structure with a polyguanylic acid chain as the backbone was considered. In the subsequent discussion, R. G. Wyatt confirmed Chargaff's general conclusions on the basis of his own analyses. He found that desoxypentose nucleic acids (DNA) from various animal tissues contain a small amount of 5-methylcytosine, the amount being characteristic of the species serving as the source. In contrast, the DNAs of several insect viruses were found to be free of methylcytosine.

The nature of the linkages present in desoxypentose nucleic acids was discussed by M. Stacey, who pointed out that polynucleotide chains may be either straight or branched. They may be ruptured by various means, including ultrasonic waves and ion exchange resins. Upon chain rupture, RCHO-groups are set free, resulting in a positive Feulgen reaction, and the release of purine residues may be followed by the Dische reaction. Steric effects may arise from interaction between base (purine, pyrimidine) groups, leading to a partial stripping of the side branches from the main chain. The possibility of cross-linking by amino and enolic hydroxyl groups was considered. In the discussion, Chargaff pointed out that nucleic acids may also be considered as a form of polysaccharide. DNA, in contrast to PNA, does not appear to be highly branched, and it is possible that links other than phosphoric acid ester bonds are involved in the polymer DNA-structure. Stacey mentioned that D. O. Jordan's work indicates some degree of branching in DNA.

The enzymatic hydrolysis of ribonucleic acids, with particular reference to its bearing on the concepts of their structure, was discussed by G. Schmidt, who employed prostate phosphatase, an enzyme of high activity toward PNA. The hypothesis that PNA consists of a purine polynucleotide and a pyrimidine polynucleotide, which are linked together only at one point, was considered untenable on the basis of the results obtained by the enzymatic hydrolysis of PNA by ribonuclease and phosphatase. The action of ribonuclease leads to the conclusion that the core of PNA consists largely of purine nucleotides. PNA represents a highly branched structure, from which the ribonuclease removes terminal pyrimidine nucleotide residues.

Studies on the structure of various nucleotides isolated from nucleic acids were described by W. Cohn and H. S. Loring. Dr. Cohn reported the discovery of new nucleotides from previously known nucleic acids from the work of his group on ion exchange adsorption columns. In addition to the separation of isomeric forms of adenylic, guanylic, cytodylic, and uridylic acids from yeast PNA, a fifth desoxypentose

[1] Publication No. 8 of the Department of Biophysics.

[2] For a report on the first Nucleic Acid and Protein Conference see E. R. Blout and P. M. Doty, *Science*, **112**, 639 (1950).

nucleotide (5-methylcytosine) was isolated from DNA. The new ribose compounds consist of two sets of isomers of the well-known 3′-nucleotides, one set being esterified at the 5′-position and arising from enzymatic hydrolysis, the other set being derived by chemical hydrolysis of PNA. In addition, diphosphates of cytidylic and uridylic acids have been isolated from enzymatic digests. The nearly quantitative yields of 5′-nucleotides from such systems support Gulland's earlier postulate that this linkage is of major importance in intact PNA.

Dr. Loring described improved methods for the isolation of pure cytidylic acid isomers from alkaline yeast PNA-hydrolysates, involving the use of ion exchange resin (Dowex 2) columns or the selective precipitation of the isomer of lower optical activity in the form of a crystalline monoammonium salt. From their chemical behavior it was concluded that the isomerism involves the location of the phosphate grouping rather than an α, β-isomerism of the ribose residues. The comparison of the two compounds indicates that the isomer of lower optical activity ($[\alpha]_D = -8°$) represents the 2′-phosphate ester, whereas that of higher activity ($[\alpha]_D = +46°$) is the 3′-ester. In the discussion, K. Linderstrøm-Lang (Carlsberg Laboratories, Copenhagen) stated that the splitting of PNA by ribonuclease is accompanied by a marked volume contraction, which is indicative of the formation of charged groups, such as amino groups.

Stig Claesson (University of Upsala) discussed recent advances in adsorption analysis, with particular reference to the optical methods developed by Arne Tiselius and himself for the study of "flowing chromatograms." He discussed the method of carrier displacement analysis and the use of interferometry, polarography, and radioisotopes in the study of the separation of complex systems; e.g., protein and fatty acid mixtures. In certain phases of this work—e.g., in the separation of polymers by fractional extraction according to Desreux—automatic arrangements consisting of packed columns, syphon systems capable of removing samples with an accuracy of 0.5 per cent, and a series of collecting tubes mounted on revolving turntables were employed with satisfactory results.

The following two sessions were utilized for the discussion of work involving protein systems of different types. Dr. Linderstrøm-Lang spoke on the volume changes taking place during proteolysis. When peptide bonds are broken at pH 6–7 by the action of enzymes, two ions appear, a carboxylate and a substituted ammonium ion. The compression of the water dipoles surrounding these ions results in a considerable volume decrease (Δv), which may be determined accurately by dilatometric methods. Experiments with simple amino acids and peptides have shown that this "electrostriction" effect reaches a maximum when a peptide bond near the center of a long peptide chain is split ($\Delta v = -20$ ml/mole peptide bond). In the case of many globular proteins, however, the much larger Δv values that have been observed (over -100) are explained by assuming that the collapse of the protein molecule caused by the instability resulting from the enzyme action is accompanied by a volume decrease, which is additive to the direct electrostriction effect.

It is unlikely that the breaking of salt-linkages is responsible for this extra volume decrease. The phenomena observed may be tentatively interpreted in terms of Linus Pauling's recent theory of protein structure (*Proc. Natl. Acad. Sci. U. S.*, 37, 205, 235, 241, 251, 256, 261, 272, 282 [1951]) employing the postulate of helical configurations. Such a structure might allow for two different types of protein denaturation; namely, a reversible type involving the stretching of the helices in the direction of their long axis and an irreversible type consisting in the separation of helices.

Albert Wassermann (University College, London) discussed the influence of cation exchange on properties of muscle proteins on the basis of joint experiments with M. L. R. Harkness. Myosin, as extracted from muscle with salt solutions at 0° C, will adsorb a considerably smaller number of inorganic cations than would correspond to the side chain carboxyl groups of the protein preparations. However, when rabbit muscle is cooled to $-79°$ C prior to mincing, "K^+-protein" gels are obtained in which the side chain carboxyl groups are largely neutralized by potassium counter ions. Conversion into the potassium-free "H^+-protein" form may be accomplished by treatment with the hydrogen form of a commercial cation exchange resin (Amberlite). This stoichiometrically well-defined, reversible potassium-hydrogen ion exchange is accompanied by a marked, reversible decrease of the viscosity and flow birefringence of the myosin gel. The effect can be explained by a change of the molecular shape of the main valency chains to which the side chain carboxyl groupings are attached.

Recent experiments on the mechanism of reaction of hemoglobin with oxygen and carbon monoxide were discussed by F. J. W. Roughton (Cambridge University), with special reference to the problem of protein individuality. According to the intermediate compound hypothesis of Adair, the hemoglobin molecule combines with oxygen (or carbon monoxide) in four successive stages. The resultant equation contains four equilibrium constants corresponding to the four successive reactions postulated. Recent improvements in technical accuracy, together with the introduction of new mathematical methods of analyzing the results, now make it possible to determine the numerical values of the four constants with fair accuracy. The new data, obtained on ram hemoglobin, indicate that the combination of the first three oxygen molecules facilitates greatly the combination of the fourth molecule with hemoglobin, probably in consequence of a steric effect.

The structure of hemoglobin and myoglobin was discussed by J. C. Kendrew, also of Cambridge. His approach was chiefly based on x-ray diffraction studies carried out in collaboration with Perutz and Bragg. The Patterson projections of the diffraction

patterns of several crystalline proteins (two species of hemoglobin and of myoglobin, ribonuclease) indicate rodlike structures that may be interpreted as the vector translations of more or less parallel, straight polypeptide chains, about 10 A apart, often in close hexagonal packing. All conclusions going beyond this point must be regarded as tentative. In the case of horse hemoglobin, Perutz' views have lately undergone some modifications. The stepwise shrinkage of the crystals is now attributed to the jerky movements of irregularly shaped molecules past one another, rather than to the withdrawal of successive layers of water molecules from ordered liquid layers in the crystal.

With regard to the helical configuration of the α-chain proposed by Pauling and Corey, the recent discovery by Perutz of 1.5 A reflections from synthetic polyamino acids and from α-keratin and muscle lends strong support to the suggestion that these materials are built up essentially of such chains. In globular proteins the situation is still very confused since, although Perutz had obtained a faint 1.5 A reflection from hemoglobin, an examination by Carlisle of his very complete three-dimensional data for ribonuclease has failed to reveal any such reflection. There exist grave objections to the hypothesis that hemoglobin or similar proteins are made up of any simple arrangement of chains of the configurations proposed by Pauling or by any other workers: When the Patterson projections are put on an absolute scale, the heights of the peaks attributed to polypeptide chains are found to be very much smaller than those calculated. The structure of hemoglobin seems to be more complicated than has heretofore been supposed. More three-dimensional data are needed, but once they are available it is not unreasonable to hope that the analytical methods now available will yield unequivocal results. Kendrew pointed out that Pauling's helical chains are very rigid. Any distortion would lead to the breakage of hydrogen bonds. The chains might be short, however, and sharp turns may occur only at the proline links.

David Harker (Polytechnic Institute of Brooklyn) discussed the interpretation of x-ray data obtained on proteins. Instead of individual atoms, one might consider groups of atoms ("globs") as scattering units. Projections perpendicular to those of Bragg and Perutz have been studied. One of the difficulties is that Patterson diagrams yield distances and intensities, whereas an elucidation of the molecular structure requires a knowledge of atomic positions and amplitudes. Dr. Harker has recently found that, in certain instances, intensities can be converted directly into amplitudes.

One of the outstanding contributions to the conference was a paper by F. Sanger (Cambridge University) on the structure of insulin. His experiments, carried out with H. Tuppy, led to the elucidation of the amino acid sequence in the phenylalanyl chain of insulin. Fraction B, which was derived from this chain by oxidation, was subjected to partial hydrolysis with acid and alkali, and the resulting mixture of peptides was fractionated by paper chromatography. From the structure of these small peptide fragments five amino acid sequences were deduced as being present in insulin, but it was not possible to work out the whole sequence. Hydrolysates were obtained by the action of proteolytic enzymes, and from the peptides identified by various methods (paper chromatography, adsorption of aromatic amino acids on charcoal and of peptides of cysteic acid on basic ion exchange resins) the following structure of the phenylalanyl chain was established: Phenylalanine-valine-aspartic acid-glutamic acid-histidine-leucine-cystine-glycine-serine-histidine-leucine-valine-glutamic acid-alanine-leucine-tyrosine-leucine-valine-cystine-glycine-glutamic acid-arginine - glycine-phenylalanine-phenylalanine-tyrosine-threonine-proline-lysine-alanine. Fraction A, which possesses a terminal glycine group, is joined to Fraction B by disulfide bridges of cystine residues. The results obtained agree with a molecular weight value of 6000 for insulin obtained by other methods. The construction of a helical chain model of insulin of the type postulated by Pauling shows that the amino acid side chains project from the peptide backbone without steric hindrance.

An important section of the conference was devoted to a symposium on virus metabolism. F. W. Putnam (University of Chicago), S. J. Singer (California Institute of Technology), C. G. Hedén (Caroline Institute, Stockholm), and L. W. Labaw (National Institutes of Health, Bethesda) presented papers dealing with the formation of nucleic acid in bacteriophages of the T system as they multiply in *Escherichia coli* organisms. Tracer studies provided most of the information.

Investigations of the origin of bacteriophage nitrogen, carbon, and phosphorus, with emphasis on bacterial precursors, were described by Dr. Putnam. *E. coli* cells were labeled by growth in synthetic lactate media containing N^{15}, P^{32}, and carboxyl C^{14} lysine, or by growth in a medium containing C^{14} purines. They were then infected with $T6r^+$ or T7 bacteriophages. It was found that host nitrogen, phosphorus, and purines were used independently for synthesis of virus nucleoproteins. Host purine and host lysine were incorporated intact into virus protein. Preformed bacterial acid-soluble components were excluded as a major source of virus nucleic acid phosphorus.

In the case of T6 bacteriophage, only 20–30% of the phosphorus and nitrogen originates in preformed constituents of the host. However, multiple infection of N^{15}- and P^{32}-labeled cells with T7 bacteriophage revealed that most of the nitrogen and phosphorus of T7 is derived from the bacteria. These observations were interpreted in terms of synthesis of bacteriophage nucleic acid from a pool of virus precursors derived initially in large part by degradation of host desoxyribosenucleic acid, and subsequently from precursors synthesized after the assimilation of phosphorus from the growth medium.

Dr. Labaw described recent experiments in which

radioactive phosphorus was used to study the phosphorus uptake of bacteriophages of the T system. His studies were interpreted to indicate that all the bacteriophages obtained substantially the same absolute amount of phosphorus per infectious unit from the host, *E. coli*. The phosphorus is derived for most bacteriophages from end products of the phosphorus anabolism, but at least in one instance it is derived from phosphorus in transit. Labaw concluded that there seems to be a similar unit of desoxypentose nucleic acid in all the *E. coli* bacteriophages.

Dr. Hedén discussed the possibilities of obtaining information about the preconditions for phage multiplication by studying the metabolism of the host cells under different physiological conditions. He stressed particularly that the cell in the lag phase should be studied, because in a very short period of time the cell changes from a poor to a good substrate for virus production. In the case of *E. coli* infected with T2r$^+$ bacteriophage, the yield of phage calculated both per cell and per unit of volume was greater in the later lag phase than in the earlier lag phase. However, the latent period for phage multiplication was constant. As a result of studies involving both ultraviolet absorption and tracer data, Hedén concluded that phage nucleic acid is synthesized much more rapidly than bacterial desoxyribosenucleic acid in the lag phase cell, especially at low bacterial densities.

Dr. Singer described experiments in which T2r$^+$ bacteriophage desoxyribosenucleic acid was released by grinding the bacteriophage particles in neutral 0.1 ionic strength buffer in a colloid mill. The nucleic acid released was purified by centrifugation. It was shown to have an extrapolated sedimentation constant of 13.1 Svedbergs, a value quite similar to that obtained for thymus desoxyribosenucleic acid. Singer also described preliminary electrophoresis and ultracentrifuge studies of high molecular weight components of normal *E. coli* cells and of T2r$^+$ bacteriophage infected cells. Several components were found in each case. In infected bacteria, desoxyribosenucleic acid was attached to components of low electrophoretic mobility. These Singer regards as possibly being nucleoproteins that are related to the bacteriophage particles ultimately produced. In the discussion, H. K. Schachman (University of California) presented somewhat similar findings relative to degradation products of bacterial cells.

Singer also presented studies on cytoplasmic constituents of normal plant tissue. A constituent, probably nucleoprotein in nature, composing from one third to one half the total cytoplasmic protein, was obtained. This material was apparently homogeneous by the criteria of ultracentrifugation and electrophoresis. The sedimentation constant was found to be 18 Svedbergs; a molecular weight of about 600,000 was reported.

In the discussion, Max A. Lauffer (University of Pittsburgh) pointed out that the findings of Stanley, Knight, and others, that different strains of tobacco mosaic virus are associated with proteins of distinct but different amino acid composition regardless of the host, exclude the possibility that high molecular weight constituents found in high concentration could be incorporated per se in virus synthesis. Barry Commoner (Washington University, St. Louis) described electrophoresis studies on plant tissue extracts obtained from both healthy tobacco plants and tobacco plants infected with tobacco mosaic virus. Commoner presented evidence that virus synthesis does not take place at the expense of the major normal constituent; on the contrary, tobacco mosaic virus formation is associated with a general stimulation of the synthesis of other proteins.

At a session devoted to physical studies on viruses Lauffer considered the problem of hydration of virus characteristic particles. Estimates of the hydrodynamic volume of particles of any shape can be obtained from the limiting volume of pellets centrifuged from suspension and from the backward displacement of an indicator substance in an ultracentrifuge experiment.

In principle, hydration can also be evaluated from knowledge of the partial specific volume and the density of the hydrodynamic unit in solution, as determined by sedimenting the material in media of different densities. In spite of the limitations of the various methods, all yield values of hydration of virus-characteristic particles in the range of 50–80% by volume on a wet basis for tomato bushy stunt, Southern bean mosaic, influenza, and rabbit papilloma virus particles.

Schachman showed what happens to tobacco mosaic virus nucleoprotein particles when they are broken down in alkaline media. Ultracentrifuge experiments carried out in collaboration with W. F. Harrington showed that at pH 10 the virus protein particles, which have a sedimentation rate of around 190 Svedbergs, are transformed into particles with much lower sedimentation rates. One fragment with a sedimentation rate of 4 Svedbergs accumulated more rapidly at 0° than at 25° C because it aggregates into a material with a sedimentation constant of about 50 Svedbergs, and aggregation takes place more rapidly at 25° C than at 0° C. The significance of this observation is the demonstration that medium-sized particles obtained as a result of disintegration of virus protein particles are not necessarily fragments of the original material. This renders invalid many of the attempts that have been made to deduce the structure of virus protein molecules from degradation studies.

M. F. H. Wilkins (Kings College, University of London) showed how studies with polarized light could be used to elucidate the nature of the inclusion bodies frequently found in cells of plants diseased with tobacco mosaic virus. The inclusion bodies are generally considered to be composed of the virus nucleoprotein. Wilkins observed a grating effect or a Bragg reflection effect indicative of a uniform spacing in these inclusion bodies of around 4000 A. From other studies the characteristic nucleoprotein particles of tobacco mosaic virus are known to be

rodlike bodies about 3000 A in length. Thus, the 4000-A spacing observed by Wilkins might be related in some way to the lengths of the individual virus protein particles in the crystalline inclusion body.

The final subject considered at the conference was the effect of ionizing radiations on nucleic acids, proteins, and other molecules of biological interest. J. Weiss (University of Durham, England) described the chemical effects on some simple molecules and on nucleic acid. Large doses of x-radiation were applied to chemical substances in aqueous solutions. Conditions were such that the effect observed could be expected to be due largely to monovalent hydrogen and hydroxyl radicals formed by the absorption of radiation by water. It was shown that benzene, when treated with x-rays and γ-rays, is converted into phenol and some diphenyl. Doses of x-rays of the order of a million roentgens applied to nucleic acid led to the formation of ammonia, to an increase in van Slyke nitrogen, to an increase in titratable acid groups, and to the liberation of phosphate and of free adenine and guanine. When amino acids were irradiated, it was found that ammonia, carbon dioxide, acid aldehyde, and acetic acid would be recovered. In acetic acid solutions, cholesterol is converted quantitatively into triol.

J. A. V. Butler (Chester Beatty Research Institute, Royal Cancer Hospital, London) described experiments on the action of x-rays and some radiomimetic chemicals on desoxyribosenucleic acid. The x-ray doses used in Butler's experiments were considerably lower than those described by Weiss, and the effects on the nucleic acid were essentially physical. Prior to treatment, desoxyribosenucleic acid solutions are highly viscous and give strongly skewed diffusion curves. Irradiation and treatment with such radiomimetic chemicals as nitrogen mustards resulted in decrease in viscosity, decrease in the skewness of diffusion curves, and alteration in the slope of the electrophoretic mobility curves. These changes were interpreted in terms of the breaking of hydrogen bonds and the consequent decrease in the stiffness of the molecules.

Virgil L. Koenig (Los Alamos Scientific Laboratory) described experiments on the physiochemical changes in bovine fibrinogen which result from irradiation with 45 kv x-rays. The fibrinogen was irradiated in the dry state and also dissolved in 0.1 *M* NaCl and in 0.1 *M* disodium phosphate solutions. Changes in the sedimentation rate of the protein at various concentrations and in the viscosity of the protein solutions resulted. The fibrinogen was also irradiated in the presence of oxygen, nitrogen, and helium. The oxygen and nitrogen did not change appreciably the effect produced, but the helium increased it, presumably because of high-energy absorption by helium. Very large doses of radiation resulted in the formation of a heavier second component in the ultracentrifuge diagram. In the dissolved state, about 50,000 roentgens will produce an observable effect, but in the dry state at least a million are required.

Ernest Pollard, of Yale University, described research being carried out in the Biophysics Laboratory of that institution on the action of primary ionization on enzymes and viruses. Dry specimens of trypsin, pepsin, T1 bacteriophage, and other biologically active proteins were bombarded with deuterons and electrons. In general, the loss of biological activity decreased exponentially with the dose of irradiation. This result is consistent with the target theory of the action of ionizing radiation and permits the evaluation of a target cross section or a target volume. In general, the target sizes obtained in this manner for the biologically active unit agree well with the sizes of the protein molecules or characteristic particle associated with the active substance. This fact indicates that a single ionization anywhere within a structure is sufficient to lead to loss of biological activity. The results also provide additional evidence for the identity of the particles causing the biological effect—that is, the enzyme activity or the virus infectiousness, with the protein molecule or the virus characteristic particle.

R. W. G. Wyckoff was chairman and Kurt G. Stern was vice chairman of the conference. It was decided to hold the next Gordon Nucleic Acid and Protein Conference in 1953, with Stern as chairman and M. L. Anson vice chairman. The program committee includes, in addition to these officers, K. Linderstrøm-Lang, R. W. G. Wyckoff, Waldo Cohn, John L. Oncley, Gerhard Schmidt, and Seymour J. Singer.