# Protein and Nucleic Acid Conference

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A GORDON RESEARCH CONFERENCE on "Physical Methods in Nucleic Acid and Protein Research" was held at New Hampton, New Hampshire, August 28 to September 1, 1950. The conference had a marked international character, largely in consequence of support from the Rockefeller Foundation. Ten European scientists were numbered among the seventy participants.

Broadly speaking, the major theme was the interaction of radiation with proteins and nucleoproteins *in situ* and *in vitro.* The development included electron microscopy; x-ray diffraction; ultraviolet and infrared spectroscopy, microspectroscopy, and microscopy; and photochemistry. The minor theme was developed in a symposium on the molecular size and shape of desoxypentose nucleic acid and several papers on recent ultracentrifugal research.

Two papers on electron microscopy demonstrated the striking progress that is being made in the study of viruses in the host and in the photography of protein molecules. R. W. G. Wyckoff (USPHS), in a brilliant series of photographs, showed the various stages of bacterial cells undergoing attack by bacteriophage (Figs. 1 and 2). With the aid of thin sectioning, it was quite readily demonstrated that bacteriophage proliferation takes place throughout the cell, not on the surface. On the other hand, the proliferation of influenza virus in infected tissue occurs at the edge, with filaments growing out. Considerable evidence of the linear aggregation of the spherical influenza virus was demonstrated. Finally, from the work of Dr. Wyckoff and Kenneth Smith, photographs were shown of the polyhedral bodies which form in such large numbers in virus infections of insects. These bodies are shown to be crystalline inclusions with virus particles readily discernible on the surface. In some cases a membrane is found surrounding the polyhedral bodies, and a marked irregularity occurs near the center of the body. These polyhedral bodies develop in the nucleus, not in the cytoplasm.

C. E. Hall (MIT) stressed the difficulty of identifying macromolecules that are readily discernible with a resolution of 30 A. Ambiguities are most readily eliminated by restricting examination to crystals, where the periodicity is a certain check on the macromolecule being viewed. By means of the shadow transfer replica technique, photographs slightly out of focus reveal the individual molecules in the crystal faces of many protein crystals. Edestin is found to be face-centered cubic, with a unit cell dimension of 114 A and a molecular weight of 300,000. Two of the three unit cell dimensions for canavalin are found to be 104 A and 68.5 A. Catalase is essentially orthorhombic, with unit cell dimensions of $64 \times 64 \times 159$ A. When these objects are brought further into focus, the periodicity becomes less discernible, but fine structure on the surface of individual molecules then appears. It is not yet clear whether these are characteristic of the native molecule or are the result of mild denaturation occurring during specimen preparation.

The dominant role of the α-keratin configuration in the molecular architecture of globular proteins gains further support as more structural information becomes available. W. T. Astbury (Leeds) described recent work on the x-ray diagrams from bacterial flagella prepared according to Sven Gard. These flagella, which are rodlike, with dimensions of about 120 A × 20,000 A, are among the simplest systems concerned primarily with producing movement and may be the most elementary manifestation of muscular activity. It is most interesting to find that films produced from these flagella show fine α-keratin reflections and, upon squeezing, this goes over into the β-keratin pattern. With respect to x-ray studies of nucleic acid, it appears that no photographs show any more reflections or detail than those taken in 1937. The original idea still prevails that details revealed by these photographs, together with the extraordinary high density of nucleic acid, point to a structure for the molecule in which the nucleosides have a parallel platelike arrangement perpendicular to the phosphate ester chain. I. Fankuchen (Polytechnic Institute of Brooklyn) gave a critical review of the interpretation of single crystal photographs.

The second day of the conference was devoted almost entirely to ultraviolet microscopy and microspectroscopy. The session was opened by T. Caspersson (Stockholm), who presented a masterful account of the problems inherent in quantitative ultraviolet microspectroscopy of tissues. Professor Caspersson pointed out that for quantitative work all the light from the object should be gathered by the microscope objective and that, therefore, it is necessary to use microscope objectives having numerical apertures of at least 0.85 and to take into due account the pos-

sible scattering losses caused by the variations in refractive index of the material being examined. He also stated that a polarizing prism has been in use in his ultraviolet microspectroscopic apparatus since 1940, and that the effects that have been observed were of the order of 0.1–1.0% in tissues. Such effects are very hard to measure accurately, although some attempts, particularly in connection with the protein absorptions, are now being made in that direction. A promising new development seems to be the use of x-ray absorption procedures as developed by Engstrom, to determine the mass distribution in tissues.

Following the above presentation, a symposium on ultraviolet microscopy was held. Almost all the participants described their work in using one or another kind of reflecting microscope objective. There seemed to be no question about the general utility of this type of microscope objective for microspectroscopic work. R. Barer (Oxford) described the work he has been doing with E. R. Holiday, using a Burch reflecting objective (N.A. 0.65) that has the large mirror aspherized. The arrangement for microspectroscopic studies makes use of a logarithmic cam to advance the plate holder. Dr. Barer also described a scheme he has been working on for the cathode-ray oscilloscope presentation of the ultraviolet absorption spectrum of a single cell. R. C. Mellors (Sloan-Kettering Institute) described various ultraviolet microspectroscopic arrangements that make use of the spherical reflecting and reflecting-refracting microscope objectives now available in this country. He outlined a possible application of such techniques to problems connected with cancer diagnosis. J. T. Randall (Kings College, London) described two-mirror nonconcentric spherical microscope systems now being developed in his laboratories by Wilkins and Seeds. If the two mirrors are kept nonconcentric, it seems possible to construct a series of microscope objectives of different numerical aperture which appear to have utility for certain spectroscopic applications. By the addition of an Amici component the system is no longer achromatic. but numerical apertures as high as 1.4 have been realized.

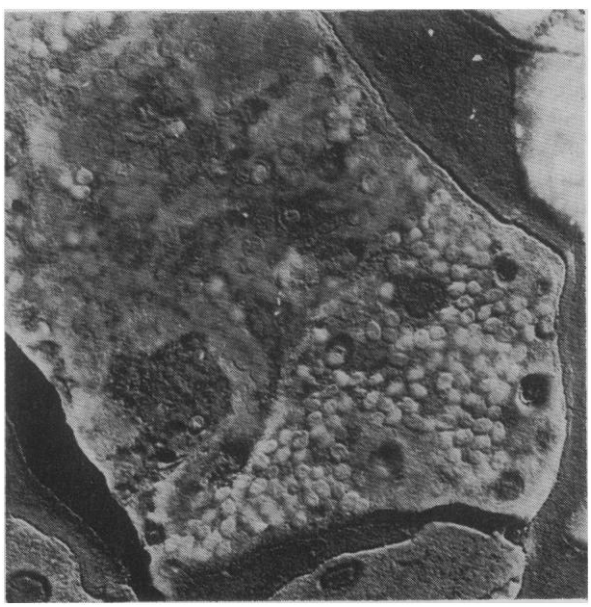

FIG. 1. Electron micrograph of a section through part of a single epithelial cell of chorioallantoic membrane of chick embryo diseased with vaccine virus. At bottom of cell the newly formed elementary bodies of the virus (about 5 mm in diameter each) stand out clearly from the remains of the cytoplasm in which they have developed. At the top of the cell the elementary bodies are more faintly seen embedded in the less altered protoplasm. ×8600 (approx.).

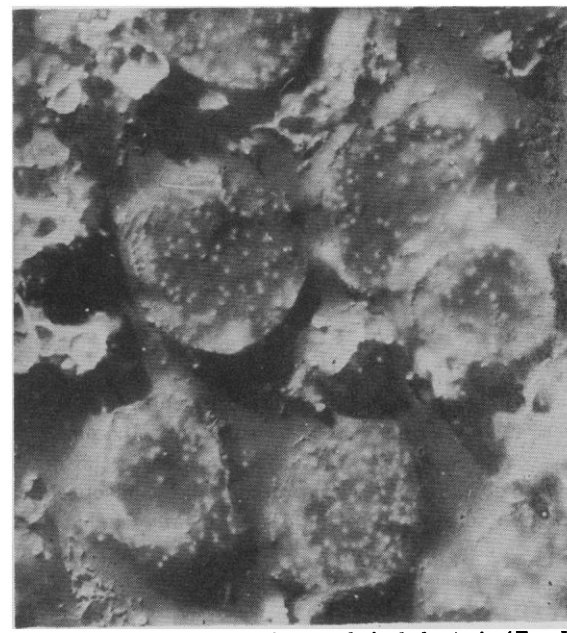

FIG. 2. A section through several single bacteria (*E. coli*) that have been infected with the $T_2$ strain of bacteriophage. The new particles of bacteriophage developing throughout the bodies of the bacteria are clearly visible as dots slightly more than 1 mm in diameter. ×9000 (approx.).

A. W. Pollister (Columbia University) emphasized the revolution in cytology that has been initiated by the introduction of the reflecting microscope, together with the ultraviolet and infrared investigations that it makes possible. With this in mind he described the work he has done on estimating nucleic acids by a combination of ultraviolet microscopy and quantitative staining reaction. B. Commoner (Washington University) described the measurement of ultraviolet spectra of structures in intact living plant cells. By means of a microspectrophotometer employing a Grey reflecting objective, ultraviolet (and visible) absorption spectra of areas and pigments in living plant hair cells have been obtained. Such spectra are difficult to interpret because they represent the added

absorption of the vacuole fluid and the surrounding protoplast layer. By measuring a second absorption spectrum through another thickness of the same cell, however, it is possible to calculate the separate absorption spectra of vacuole and protoplast. The protoplast spectrum shows a sharp maximum at 280 mμ and one at 340 mμ. The separate ultraviolet absorption spectra of living plant cell nuclei have been obtained by similar measurements. Dr. Commoner emphasized that the absorption spectra of intact cells cannot be properly interpreted from measurements made at a single point in the cell.

The session was concluded with fine papers by J. F. Scott (MIT and Massachusetts General Hospital) and A. D. McLaren (Brooklyn Polytechnic). Dr. Scott pointed out that certain purines and pyrimidines, as well as some simple amino acids, show a considerable sharpening of their absorption spectra, and an increase in the fine structure in the ultraviolet region, when films of such materials are cooled to very low temperatures. Unfortunately, the application of such techniques to intact biological systems such as tissues appears to be limited, since the more complicated molecules such as nucleotides, nucleic acids, and proteins generally show very little sharpening or increase in fine structure of their ultraviolet absorption bands when cooled. Correlation of such data, however, with that from infrared measurements for simple molecules may lead to interesting structural information. This work was done in collaboration with J. R. Loofbourow (MIT).

Dr. McLaren discussed his recent work on the photochemistry of proteins, enzymes, and viruses. Of potential interest to cellular biologists investigating the structure of living tissues with ultraviolet light microscopy is the influence of ultraviolet light on cellular constituents. In view of the inactivation-denaturation of enzymes and related substances, it is obvious that, in the act of observing living tissue with ultraviolet light, the tissue undergoes degradation as a result of photolysis. The quantum yields for inactivation are low and are lower the greater the molecular weights of the substances involved. Whether the quantum yields are sufficiently large seriously to affect observations on cellular constituents with ultraviolet light remains to be determined.

In model experiments performed with phenyl-substituted alkyl amides, an increase in the photochemical effect was noted when there were three carbon atoms between the phenyl group and the amide group, as compared with the effect noted when there were two, one, or four carbon atoms intermediate between these two groups.

The third day of the conference was devoted entirely to infrared studies. G. B. B. M. Sutherland (University of Michigan) started the session with a lucid talk describing the principles governing the infrared method of structural analysis. The key frequencies for the $COO^-$ and the $NH_3^+$ groups in amino acids were discussed, and certain anomalies in the spectra of the amino acids (especially those containing sulfur atoms) were noted. Dr. Sutherland pointed out that, in the infrared spectra of synthetic polypeptides made from one or two amino acids, key frequencies are sometimes available for the identification of certain hydrocarbon groups in the amino acid residues. Recent work on the spectra of proteins using polarized infrared radiation to determine the orientation of NH and CO bonds was critically reviewed.

R. S. Halford (Columbia University) presented a careful and instructive discussion of the possible utility of infrared polarization data in assisting in the determination of the unit cells of certain organic compounds. He described his work with ammonium nitrate, and pointed out the various pitfalls that exist if inadequate crystallographic precautions are taken in the preparation of the samples. Dr. Halford emphasized the importance in infrred polarization studies of working with crystals cut along at least one of the major crystal axes. He then presented data showing infrared polarization obtainable with several types of oriented protein fibers.

The infrared spectra of nucleic acids, nucleosides, nucleotides, and certain purine and pyrimidine components were presented and discussed by E. R. Blout (Polaroid Corporation and Children's Medical Center). It was shown that certain of the infrared absorption bands of adenine and guanine could be used for analyzing mixtures of these two compounds in the solid state. A considerable discussion was evoked by the statement that oriented films of sodium desoxyribonucleate showed no apparent infrared dichroism in any of its absorption bands. Infrared polarization data for single crystals of cytosine and thymine were presented, but the data were not interpreted other than to point out that practically each absorption band of these compounds is highly polarized. It is evident that further fundamental work must be done in the field of infrared polarization of simple organic compounds before adequate theoretical interpretations can be made from the data that are beginning to accumulate in many laboratories.

The day's discussions were concluded with a presentation by Dr. Barer in which he emphasized some of the difficulties inherent in infrared microspectroscopic studies on tissues. In spite of the fact that resolution is low because of the long wavelength radiation used, it is hoped that interesting data will be obtained from tissues containing relatively homogeneous groups

of cells or, in certain special cases, single cells.

A lively symposium on the physical-chemical investigation of desoxypentose nucleic acid revealed many areas of agreement from different types of measurements, but emphasized the extraordinary difficulty of the molecular characterization of nucleic acids and the need for more definitive work.

The behavior of nucleic acids as polymeric electrolytes was discussed by D. O. Jordan (Nottingham). The nucleic acid polymer contains both strong and weak electrolytic groups, and the presence of the former and the ionization of the latter affect the viscosity of its solutions and the shape of the titration curve. The viscosity of desoxypentose nucleic acid shows considerable structural character, and the viscosity at low pressures is high. The addition of electrolyte reduces the viscosity considerably, as has also been described for simpler systems—for example, polymethacrylic acid and poly-*N*-*n*-butyl-4-vinyl pyridonium bromide. This process is explained by the repulsion of the charged groups ($PO^-$ at pH 7) maintaining a stretched polymer molecule in aqueous solution. The addition of electrolyte reduces this repulsion, thereby permitting the molecule to coil, thus making it more symmetrical and reducing the viscosity, the structural character of the viscosity, and the streaming birefringence. In the absence of added salt, the reduced specific viscosity does not rise at low concentrations as that of a typical polyelectrolyte should. This is probably because of the incompleteness of removal of foreign ions from solutions of sodium thymonucleate.

The same sample of nucleic acid that had been used by Drs. Jordan and Ogston had been examined by light-scattering techniques by B. Bunce and P. M. Doty (Harvard). These measurements, which extended down to concentrations of 0.005 per cent, indicated a molecular weight of 4 million and a moderately coiled structure, the chain stiffness being comparable to that of cellulose derivatives. When the salt concentration is diminished below 0.2 *M*, uncoiling of the chain is noted.

The problems involved in the determination of the molecular weight of nucleic acids by means of the ultracentrifuge were considered by A. G. Ogston (Oxford). Marked molecular interaction makes it necessary to extrapolate the sedimentation and diffusion constants to zero concentration. The sedimentation constant $S$ may be satisfactorily extrapolated as $1/S$ against concentration to give a limiting value for the desoxypentose nucleic acid of calf thymus of $13.2 \times 10^{-13}$. The extrapolation of the diffusion constant is performed with the aid of Flory's theory of the entropy of solutions of large particles to give a value of $0.80 \times 10^{-7}$. The molecular weight was then found to be 890,000, and the axial ratio is 120, which is to be compared with the value of 190 obtained from viscosity measurements when a high rate of shear is used. A possible reservation is made with respect to the diffusion measurements, since the concentration dependence is not certain. A more likely explanation of the disagreement, however, may be found in the possible heterogeneity of the nucleic acid preparation, since the light-scattering method gives a higher average value than the sedimentation and diffusion data. Evidence for the polydispersity was presented by K. G. Stern (Brooklyn Polytechnic) in the marked spreading of the sedimentating boundary at low concentrations. His sedimentation measurements on a Signer preparation in 0.2 *M* NaCl were in very good agreement with those of Dr. Ogston on a Gulland-Jordan preparation. In the absence of added salt the concentration dependence is so marked that extrapolation to zero concentration is not possible. H. Kahler (USPHS, Bethesda) reviewed his work on a nucleic acid preparation of Greenstein. Sedimentation and diffusion measurements led to a molecular weight of 1.5 million. M. Goldstein (Brooklyn Polytechnic) reported on preliminary streaming birefringence measurements on desoxyribose nucleic acid. The problem of concentration dependence of the rotary diffusion constant has not yet been solved, and here, as in the other kinetic methods of investigation, the interpretation of the data was seriously hampered by the necessity of relying on the prolate ellipsoid as the only model.

In separate papers Dr. Ogston described his recent work on the theoretical basis of extrapolating sedimentation constants to zero concentration, and J. L. Oncley (Harvard) summarized the work of his group of the β-lipoprotein of human sera. Dr. Ogston pointed out that, inasmuch as both sedimentation and diffusion constants of asymmetrical particles vary strongly with concentration, extrapolation to zero concentration is necessary if reliable values for molecular weights and shapes are to be obtained. In the case of sedimentation, only the frictional coefficient varies. This is not adequately accounted for by variation of the macroscopic viscosity. A theoretical treatment was outlined, based on the equations of flow through a porous plug, which predicts correctly the linear variations of $1/S$ with $c$. This may be used to estimate the effective volume and asymmetry of the particles. In the case of diffusion, the frictional coefficient may not be the same as in the sedimentation case and may be best measured by the macroscopic viscosity at zero velocity gradient. In addition, the diffusion coefficient varies with the entropy contribution to the chemical potential.

The β-lipoprotein discussed by Professor Oncley has

a density of only 1.03: it is spherical, having a diameter of 185 A and a molecular weight of 1,300,000. Only 23 per cent of the molecule is composed of polypeptide components, the remainder being phopholipid and cholesterol. In another paper Mr. Baldwin and J. W. Williams (Wisconsin) presented a general method for the determination of sedimentation constant distributions. The distribution completely defines the heterogeneity of the preparation with respect to sedimentation behavior, and the method is applicable even when the diffusion constant varies widely among the molecules. The theory does not apply to concentration-dependent systems, and all quantities must be extrapolated to infinite dilution. The standard deviation of the sedimentation constant distribution, as well as the weight average diffusion coefficient, can be calculated independently.

The organization of this conference was carried out by E. R. Blout and K. G. Stern. The success has led to plans for a similar conference during the last week in August 1951. This conference will be under the chairmanship of R. W. G. Wyckoff.

# *Technical Papers*

## The Effect of X-Irradiation in Oxygen and in Hydrogen at Normal and Positive Pressures on Chromosome Aberration Frequency in *Tradescantia* Microspores[1]

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Investigations by Giles and Riley (*1*, *2*) demonstrated a marked effect of oxygen in increasing the frequency of x-ray-induced chromosomal aberrations in microspores of *Tradescantia paludosa*. In certain experiments (*2*), the relation between aberration frequency and percentage of oxygen present during irradiation was studied. Although there was an almost linear increase in aberration frequency between 2% and 21% oxygen (air), no significant difference was noted between 0% oxygen (pure helium) and 2% oxygen (plus 98% helium). Additional experiments have now been performed to reinvestigate the effect of low percentages of oxygen. In addition, studies have been made of the effect on aberration frequency of irradiation in various percentages of oxygen under pressure. Preliminary experiments have also been carried out on the effects of irradiation in hydrogen at normal and positive pressures. The general methods utilized for x-irradiation of inflorescences and subsequent cytological analysis have been described previously (*2*).

In the experiments designed to reinvestigate the effects of low percentages of oxygen, special attempts were made to remove as much oxygen as possible from the inflorescences before irradiation. The following procedure was utilized: (1) The inflorescences, after being placed in the gas-tight exposure chamber, were subjected to prolonged evacuation. (2) Helium, which had been especially freed of any residual oxygen, was then introduced into the evacuated exposure chamber. To remove any oxygen, the helium was passed slowly through a coil in liquid nitrogen and then over copper foil heated to 500° C before entering the exposure chamber. (3) Finally, the inflorescences were kept in the dark for an hour before irradiation to facilitate the removal by respiration of any further oxygen. In addition to the exposures in helium, irradiations were performed in atmospheres containing 2%, 5%, and 10% oxygen (plus helium). The percentages of oxygen in these gas mixtures were accurate (on the basis of analyses furnished by the vendor, the Ohio Chemical and Surgical Equipment Co.) to ±0.2%. All experiments were carried out at room temperature (approximately 25° C). To increase the statistical reliability of the determinations, a larger number of cells was scored than previously. The data are presented in Table 1. They are also plotted in Fig. 1, together with the averages of points at higher percentages of oxygen, which were obtained in previous experiments (*2*). These results indicate that there is still a substantial yield of aberrations even in the complete (or nearly complete) absence of oxygen. When oxygen is present during irradiation, there is a rapid rise in aberration frequency above this base level. This increase is linear between 0% and 10% oxygen, after which the rise is apparently more gradual, and shows a definite leveling off at around 20% oxygen.

TABLE 1

THE EFFECT OF IRRADIATION IN VARIOUS PERCENTAGES OF OXYGEN ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN *Tradescantia* MICROSPORES
(All X-Ray Exposures of 400 r at 50 r/min)

| Oxygen percentage | No. cells | No. interchanges | Interchanges /cell | Interstitial deletions | I.D./cell |
|---|---|---|---|---|---|
| 0 | 1,000 | 234 | 0.234 ± 0.015 | 287 | 0.287 ± 0.017 |
| 2 | 1,200 | 347 | 0.289 ± 0.016 | 394 | 0.328 ± 0.017 |
| 5 | 1,000 | 427 | 0.427 ± 0.021 | 501 | 0.501 ± 0.022 |
| 10 | 600 | 364 | 0.607 ± 0.032 | 453 | 0.755 ± 0.035 |

[1] Work performed under Contract No. W-7405-Eng-26 for the Atomic Energy Commission.