RECENT WORK ON NUCLEIC ACIDS

AN informal discussion on nucleic acids, held under the auspices of the Colloid and Biophysics Committee of the Faraday Society, was held during October 12 and 13 in Manson House, London (Royal Society of Tropical Medicine and Hygiene). The chair at the opening session was taken by Prof. J. A. V. Butler, who arranged the meeting. At subsequent sessions Sir Alexander Todd, Prof. E. Chargaff and Prof. R. M. Herriott took the chair. Twenty-six papers were presented.

In the first session, Dr. J. Pouyet, J. Hermans and Dr. R. Vendrely discussed preparations of deoxyribonucleic acid of the two categories described by Sadron at the Brussels Congress in 1955. Further preparations of deoxyribonucleic acid from calf thymus have been made which are of the more compact configuration of category 2, and it has been shown that the absorption at 260 m μ is lower for these preparations than for those of category 1. Experiments suggest that the treatment of the nucleoprotein by salt and alcohol may affect the size and configuration of the deoxyribonucleic acid prepared from it. In a later paper, Dr. R. A. Welsh showed that if nuclei from calf thymus are extracted with 0.14 *M* sodium chloride, the deoxyribonucleoprotein can be dissolved in molar sodium chloride and precipitated in a non-fibrous form, in contrast to the fibrous precipitate which is obtained if the nuclei are extracted in water. To some extent the structural changes involved can be reversed, and a fibrous form can be prepared from the non-fibrous nucleoprotein. A further problem in the preparation of deoxyribonucleic acid from calf thymus was raised by Dr. K. V. Shooter, who showed that the small amounts of residual protein (less than 1 per cent) usually found in preparations may markedly influence the physical properties. Treatment with chymotrypsin suggested that 26 per cent by weight of the deoxyribonucleic acid may be cross-linked by the residual protein. A method of preparing deoxyribonucleic acid, involving the partition of a tissue homogenate between phenol and water in the presence of sodium chloride and the chelating agent *p*-amino-salicylic acid, was described by Dr. K. S. Kirby. Using this method, a high yield of deoxyribonucleic acid free from protein has been produced. The various techniques available for the isolation of deoxyribonucleic acid from the bacteriophage *D*₄ of *Salmonella enteritidis* were discussed by Dr. R. Wahl, Dr. M. Joly and Dr. E. Barbu. Viscosity measurements in salt solutions showed that the reduced viscosity of the deoxyribonucleic acid from this phage decreases as the solution is diluted and increases if the solution is concentrated again. To account for these results, it was suggested that the particles consist of short rods (400 Å.) associated parallel to each other to form much longer aggregates, the number of rods in each aggregate being dependent on the concentration.

A paper on the properties of deoxyribonucleic acid prepared from the leucocytes of patients with lymphatic or myeloid leukaemia was read by Dr. E. E. Polli. Extensive investigations have shown that many of the physical properties of these preparations

are similar to those of deoxyribonucleic acid from calf thymus but that the deoxyribonucleic acid from the leucocytes of myeloid leukaemia has an extra band in the infra-red spectrum and also shows some differences in behaviour on titration to acid pH. In addition, the deoxyribonucleoprotein from the myeloid leukaemia leucocytes was found to be 95 per cent dissociated in salt concentrations of 0.4–0.6 *M*, in contrast to the behaviour of calf thymus nucleoprotein and nucleoprotein from lymphatic leukaemia leucocytes which are only 5 per cent dissociated at these salt concentrations.

Further work on the physical properties of calf thymus deoxyribonucleic acid was reported by Prof. P. Doty. The examination of a series of samples of molecular weights from 8×10^6 to 3×10^5 , produced by sonic degradation, has shown that $[\eta] \propto M^{1.08}$ and $S_0 \propto M^{0.37}$. Heat-denaturation studies in the presence of salt have shown that on the addition of hydrogen-bond-breaking agents such as urea or guanidine hydrochloride, the temperature at which a marked fall in viscosity occurs is reduced. The increase in the absorption at 260 m μ increases by as much as 32 per cent if the measurements are made at the temperature of the heating experiments; but on cooling the solution, the absorption falls to only 14 per cent greater than before heating. This change was attributed to the random formation of hydrogen bonds when the solution was cooled. It was also found that the denaturation process is not a first-order reaction, since not only does the rate of denaturation increase with temperature (above the denaturation temperature) but also the extent of the reaction increases. The correspondence between this process and the progressive melting of crystallites in a polymer was discussed. Dr. P. Alexander and Dr. K. A. Stacey dealt with the denaturation of herring sperm deoxyribonucleic acid by urea. At concentrations less than 0.003 per cent, the molecular weight is reduced by about one-half. Electron microscope photographs showing the disappearance of fibrils after treatment with urea at room temperature lend support to their suggestion that the twin strands of the helix are separated under these conditions. The effect of denaturation has been studied by Dr. A. R. Peacocke using titration techniques. These experiments showed that heating or sonic degradation disrupts the hydrogen bonds between the two pairs of bases at random, whereas degradation by γ -rays or by acid treatment produces a non-random rupture of the hydrogen bonds.

Further work on the analysis of the structure of deoxyribonucleic acid and nucleoprotein was described by Dr. M. F. H. Wilkins. Another contribution was made by Dr. H. Wyckoff, who showed that the reversible change from the *A*- to the *B*-form can be produced by varying the alcohol concentration of a solution between 75 and 90 per cent, and that in 90 per cent alcohol the *B*-form can be obtained from the *A*-form by stretching the filaments. X-ray diffraction studies on the synthetic ribonucleotides, polyadenylic and polyuridylic acid, were described by Dr. A. Rich, who showed that on mixing solutions

of these two polymers the molecules combine to give a twin-stranded helix. Further work on ribonucleic acid was reported by Dr. R. Franklin, who has compared the X-ray diffraction pattern of tobacco mosaic virus with that from the reconstituted protein. The results show that the ribonucleic acid of the virus lies 40 Å. from the axis.

Dr. G. D. Hunter showed that the nucleic acids of *B. megaterium* can be obtained by treatment of the protoplasts with dodecyl sulphate and the ribo- and deoxyribo-nucleic acids separated by sedimentation. Some nucleic acid, mainly deoxyribonucleic acid, which is precipitated with the protein during treatment with the detergent, has been isolated by treatment with chloroform. This deoxyribonucleic acid differs in composition from that which dissolves on treatment with the detergent. Ribonucleic acid from a strain of the organism trained to grow on lactose has the power to induce β -galactosidase activity in an untrained strain in the absence of any other inducer. Dr. A. S. Jones and Dr. A. R. Peacocke reported the isolation of ribonucleic acid from *Aerobacter aerogenes* using sodium cholate as an enzyme inhibitor. Titration studies have shown significant differences between the forward and back titration curves in the acid and alkaline regions. This hysteresis implies the existence of hydrogen bonds on both the 6-amino and the 6-keto groups. Investigations of the composition of the ribonucleic acid in nucleoprotein preparations from a wide variety of organisms were described by Prof. E. Chargaff. In all cases the number of 6-amino groups has been found to be equal to the number of 6-keto groups to within a few per cent, although the ratio of purines to pyrimidines is in general significantly different from unity. Work on the proteins of the nucleoprotein complex was also reported.

New methods of fractionating deoxyribonucleic acid in nucleoprotein using polysulphates were described by Dr. M. Hickens, Dr. P. W. Kent and Dr. P. V. F. Ward. Dr. G. A. Gilbert commented on the use of electrophoresis measurements for studying the interaction between deoxyribonucleic acid and proteins, and showed how it is possible to obtain three peaks in the ascending boundary with these systems. The interaction of herring sperm deoxyribonucleic acid with amino-acids and peptides has been studied by Dr. R. Zahn, who has found that the reaction occurs in two stages: a relatively rapid reaction leading to an increase in solubility in alcohol, followed by a slower reaction in which the solubility is reduced.

Papers on the protein components of deoxyribonucleoproteins were read by Dr. P. F. Davison and Prof. K. Felix. Prof. Felix dealt with the fractionation of the protamines of herring sperm and reported results on the composition and molecular weight of the fractions. Further work on the composition of nucleoprotein of the testes of *Trutta fontinalis* at different stages of maturity has shown that nucleoprotamine appears about the mid-point of the ripening period and that its composition does not change after this stage. At all stages a second basic protein, probably a histone, is also present. These results suggest that the protamine is synthesized by a special process similar to that by which the proteohormones of the glands are formed. Dr. Davison discussed the histones obtained from nucleoprotein of ascites tumour cells. Of these preparations, 25 per cent or less is precipitated at pH 6.5; the properties of the remainder are found to be similar to those of histones from normal tissues. Fractionation and

analysis of the material precipitated at pH 6.5 suggests that it is an aggregate formed from histones and a non-basic protein. There is some doubt whether this non-basic protein is a constituent of the nucleus. Photographs of the isolated nuclei show the presence of cytoplasmic contamination.

Recent work on the transforming principle of *Haemophilus influenzae* was reported by Prof. R. M. Herriott. Sedimentation and diffusion studies have given a preliminary molecular weight of 15×10^6 . Investigation of the absorption mechanism using tracer techniques has shown that some of the absorbed deoxyribonucleic acid can be removed by washing or by the action of deoxyribonuclease. For every 1,000 molecules of deoxyribonucleic acid which are irreversibly absorbed, about eight cells are transformed. There is no evidence that the absorption process fractionates the transforming activity. Studies on *Haemophilus* inactivated by ultra-violet irradiation show no reactivation on exposure to visible light. If, however, a cell-free extract of *E. coli* B is added, 30–50 per cent of the original activity of the *Haemophilus* is restored.

Incorporation studies in *in vitro* systems were discussed by Mme. Y. Khouvine and by Prof. J. N. Davidson and Dr. R. Y. Thomson. Mme. Khouvine has investigated the incorporation of phosphorus-32 into the nucleic acids of cell fractions of an epithelioma of the rat under a variety of conditions, and has adduced evidence that the enzyme systems involved in the incorporation differ among the various cell fractions. Preferential incorporation into ribonucleic acid rather than deoxyribonucleic acid is found. Prof. Davidson demonstrated that the incorporation of carbon-14 formate into the nucleic acids of bone-marrow and ascites cells and a strain of mouse sarcoma cells growing *in vitro* occurs to a much greater extent into thymine than into the purines, suggesting that the nucleic acids are synthesized from preformed purines. The addition of a particle-free extract of mouse or pigeon liver results in an increase in the incorporation of carbon-14 formate into the purines, presumably by providing the enzyme system for purine synthesis. Studies on the incorporation of phosphorus-32 into the ribonucleic acid of rat liver were described by Dr. H. N. Munro. The incorporation is considerably lower in rats fasted overnight after a diet containing adequate protein than in rats maintained on protein-free diet. It was suggested that breakdown of ribonucleic acid occurs when the supply of amino-acids is stopped, and that the products dilute the isotopically labelled precursor pool. Feeding protein at the time of injection increases the uptake to that of the rats fed on the protein-free diet.

Investigations made by Dr. D. B. Dunn of the incorporation of some thymine analogues into the nucleic acids of a strain of *B. coli* suggest that there are possibly two routes for the incorporation into deoxyribonucleic acid. The growth inhibition of these analogues is ascribed to the formation of deoxyribonucleic acid in which many breaks occur in one strand of the helix, thus blocking further duplication. Further metabolism studies involving 8-azaguanine were reported by Dr. H. G. Mandel and Dr. R. Markham. The incorporation of the analogue into the ribonucleic acid of *Bacillus cereus* was demonstrated. Addition of guanylic acid to a culture inhibited by the analogue leads to an increase in growth-rate, the analogue being ejected from the bacterial cells as the nucleoside. K. V. SHOOTER