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NUCLEIC ACIDS, THEIR STRUCTURE AND FUNCTION*

D.O. JORDAN

The Royal Society of New South Wales

Denis Oswald Jordan

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DENIS OSWALD JORDAN 1914-1982

Denis Oswald Jordan was born on September 23, 1914 in Southgate, London. He was educated at Michenden Grammar School, Southgate. His parents were unable to fund him in tertiary education, so when he left school in 1933 he became a full-time laboratory assistant at the British Launderers' Research Association that was linked to DSIR. By attending night classes at the Sir John Cass College he obtained a B.Sc. degree in 1936 from the University of London. For the next three years he continued working at DSIR and his first paper, in collaboration with J. Powney, was on the application of the glass electrode to the measurement of the pH of alkaline solutions. As a result of this, and related work, he was awarded an M.Sc. degree from the University of London, and in 1939 was appointed as Assistant Lecturer in Applied Chemistry at University College, Nottingham which at that time was a college of the University of London. In 1941, acting on advice from Professor J.M. Gulland, FRS, he embarked on research on the physical chemistry of nucleic acids. He was promoted to Lecturer in 1942, graduated Ph.D. (University of London) in 1944, and was awarded a D.Sc. degree from the same university in 1952. He served as a Member of the Senate of the University of Nottingham (1945-53), and on the Council of that Institution (1951-3). He was promoted to Reader in Physical Chemistry in 1953. Soon after that he applied for the newly-created Chair of Inorganic and Physical Chemistry at the University of Adelaide: he was successful, and arrived in Adelaide in 1954. After the retirement of Professor A.K. Macbeth in 1955, the Chemistry Department of the University of Adelaide was split into Organic Chemistry with G.M. Badger as Head of Department, and Physical and Inorganic Chemistry with Jordan (popularly known as 'DOJ') as Angas Professor and Head of Department. He continued research on the physical chemistry of nucleic acids, and in 1960 he published the first full-length monograph on this topic 'The Chemistry of the Nucleic Acids'. He worked on synthetic as well as natural polymers, and the first Australian National Conference on Polymer Chemistry was organised by him in Adelaide in 1957. This was a forerunner to the establishment of the Polymer Division of the Royal Australian Chemical Institute (RACI) in November, 1964. Denis Jordan was the first recipient of the RACI Polymer Medal, which since his death (in February 1982) has been named the Jordan Medal.

Denis Jordan was Dean of the Faculty of Science in 1958-9, and served on several committees and on the Council of the University of Adelaide; he was Pro Vice-Chancellor of that Institution during 1974-5. He was President of the Council of the Australian Institute of Nuclear Science and Engineering during 1958-9, and 1961-2. For the period 1976-9 he was a member of the Council of the Australian Academy of Science. In 1979 he was President of the Polymer Division of the RACI, and Chairman of the inaugural session of the first Joint Scientific Meeting of the Pacific Area Chemical Societies.

Honours and Awards

1954 FRACI (Fellow of the Royal Australian Chemical Institute)

1963 Liversidge Research Lecture, Royal Society of New South Wales

1970 FAA

1974 Medal, Polymer Division, RACI

1980 Officer of the Order of Australia

1981 Leighton Medal, RACI

1981 The Physical and Inorganic Chemistry Building, University of Adelaide, named the Jordan Laboratory

Biographical Sources

1) Bruce, M.I., 'Denis Oswald Jordan 1914-1982', obituary in *Chemistry in Australia*, 1982, **49**, 255-256.

2) Coates, J.H., 'Denis Oswald Jordan 1914-82', *Historical Records of Australian Science*, 1985, **6**, 237-246.

3) Best, R.J., 'Discoveries by Chemists: a History of the Chemistry Departments of the University of Adelaide 1885-1984', The University of Adelaide Foundation, 1987.

Scientific Publications by D.O. Jordan

The 100 scientific publications of D.O. Jordan and his collaborators are listed as an appendix in reference (2), Biographical Sources.

NUCLEIC ACIDS, THEIR STRUCTURE AND FUNCTION*

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Introduction

Mr. President, Ladies and Gentlemen,

I would like to thank the Council of the Royal Society of New South Wales for the honour they have conferred on me by inviting me to give the Liversidge Lecture for 1962. In his will, Archibald Liversidge refers to the "vastness of the subject", the subject being chemistry, and in my lecture this evening I have chosen a subject which well illustrates the vast scope of modern chemistry. The great progress in our knowledge of nucleic acids has only come during the last twenty years through the application of a great number of techniques, chemical, physical, biochemical and biological. These techniques have all been aimed at determining the structure and behaviour of nucleic acids at the molecular level, or in other words, at their *chemical* structure and behaviour. In another part of his will, Liversidge directs the lecturers not to give "popular lectures dealing with generalities and giving mere reviews of their subjects", but rather that they should "primarily encourage research and stimulate the lecturer and the public". Although part of my lecture might almost be classified as a review, I trust that this will not be contrary to the directions of Liversidge. The development of our knowledge of the nucleic acids over the last two decades affords almost a perfect example of how the parallel contributions of organic chemistry, physical chemistry, biochemistry, biology and genetics can contribute to the solution of a single problem.

It is just over twenty years ago since the late Professor J.M. Gulland, F.R.S., persuaded me to investigate the physical chemistry of nucleic acids, and I would like to pay tribute to his encouragement at a time when our ideas on the macromolecular structure of nucleic acids were just developing. My own contribution to nucleic acid chemistry has been through the application of the methods of physical chemistry to the problems of structure and behaviour and I trust that if I emphasize this aspect of nucleic acid chemistry tonight I will be acting in conformity with the wishes of Liversidge.

The discovery of nucleic acids was a result of work by Miescher in 1868 in Hoppe-Seyler's laboratory. It created particular interest because it was only the second organic compound known at that time containing phosphorus: the other being lecithin. The preparations of Miescher were undoubtedly of high molecular weight, although most probably not free of protein, and the early workers were fully aware of the care needed in preparation to preserve the macromolecular properties. However, at that time chemists were not prepared to accept high molecular weight substances as worthy of study and in 1899 Neumann described a preparation which involved the use of concentrated sodium

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hydroxide which was to set the pattern for preparations for the next thirty or forty years. There thus followed an intensive study of the chemistry of the breakdown products of nucleic acids and the study of the macromolecular structure had to await the development of new techniques which were initiated by the study of protein chemistry and the development of synthetic high polymer chemistry.

The Structure of the Nucleotides

The nucleic acids are copolymers of the nucleotides, which in many respects are analogous to the role played by amino acids in the proteins. The nucleotides are phosphoric esters of the nucleosides which are *N*-glycosides of purines and pyrimidines. All the main structural features of the nucleotides have been determined by the classical methods of organic chemistry through the work of Levene, Gulland, and more particularly Todd, who has achieved the chemical synthesis of all the main nucleotides.

The nucleic acids fall into two main groups which differ in their chemical composition, their macromolecular structure and in biological function. These are the deoxyribonuclcic acids (DNA) and the ribonucleic acids (RNA). These are named after the sugar moiety which occurs in the two groups, 2-deoxy-D-ribose in one (DNA) and D-ribose in the other (RNA). In both nucleic acids it is usual to find at least four nucleotides, these being the phosphorylated *N*-D-ribosides of guanine, adenine, cytosine and uracil in RNA, and the phosphorylated *N*-2-deoxy-D-ribosides of guanine, adenine, cytosine and thymine (5-methyl uracil) in DNA. From the synthetic work of Todd and his collaborators (1944, 1947, 1951) and from the X-ray work of Furberg (1950) and Huber (1957) we know that the point of attachment of the sugar is at N_3 [*sic*, N3] in the pyrimidines and at N_9 [sic, N9] in the purines. The configuration of the glycosidic linkage is always β and the sugar is always in the furanose form. Other nucleotides have been isolated, generally in small amounts, from some nucleic acids, the incidence of these will have biological significance.

The Internucleotide Bond

Various possibilities exist for the linkage between the nucleotides, but from titration evidence all possibilities except that of a phosphoester linkage can be eliminated. This is confirmed by the isolation of the three isomeric 2', 3' and 5' phosphates of the ribonucleosides on chemical or enzymic hydrolysis of RNA and on the isolation of the 3' and 5' phosphates of the deoxyribonucleosides from DNA. In DNA the linkage is thus clearly a 3'-5' phosphoester linkage, whereas in RNA it could be a linkage between 2' or 3' and the 5' position, present evidence favours the 3'-5' linkage as in DNA. We thus see that the polymeric "backbone" of the molecules of RNA and DNA consists of a repeating unit of the following atoms

$$.C_3'\text{-}C_4'\text{-}C_5'O\text{-}P\text{-}O.$$

We may call this the primary structure of the nucleic acids.

The Secondary Structure of DNA

Our present ideas on the secondary structure of DNA stem largely from the structure suggested by Watson and Crick (1953, 1954). In the nine years that have elapsed since the introduction of this formula, only minor modifications have been made to the structure and it is now widely accepted as explaining the transfer of genetical characteristics at cell

division although the full story is not yet known. The main features of this structure are : (1) the DNA molecule consists of a double helix consisting of two polynucleotide helices wound round a common axis; (2) the double helix is regular; (3) the two polynucleotide molecules are held together by hydrogen bonds, which by virtue of the regular nature of the double helix must be specific in that adenine is always bonded to thymine and guanine to cytosine (Fig. 1). Watson and Crick suggested only two hydrogen bonds in the guanine-cytosine base pair, but Pauling and Corey (1956) later pointed out that three bonds are possible.

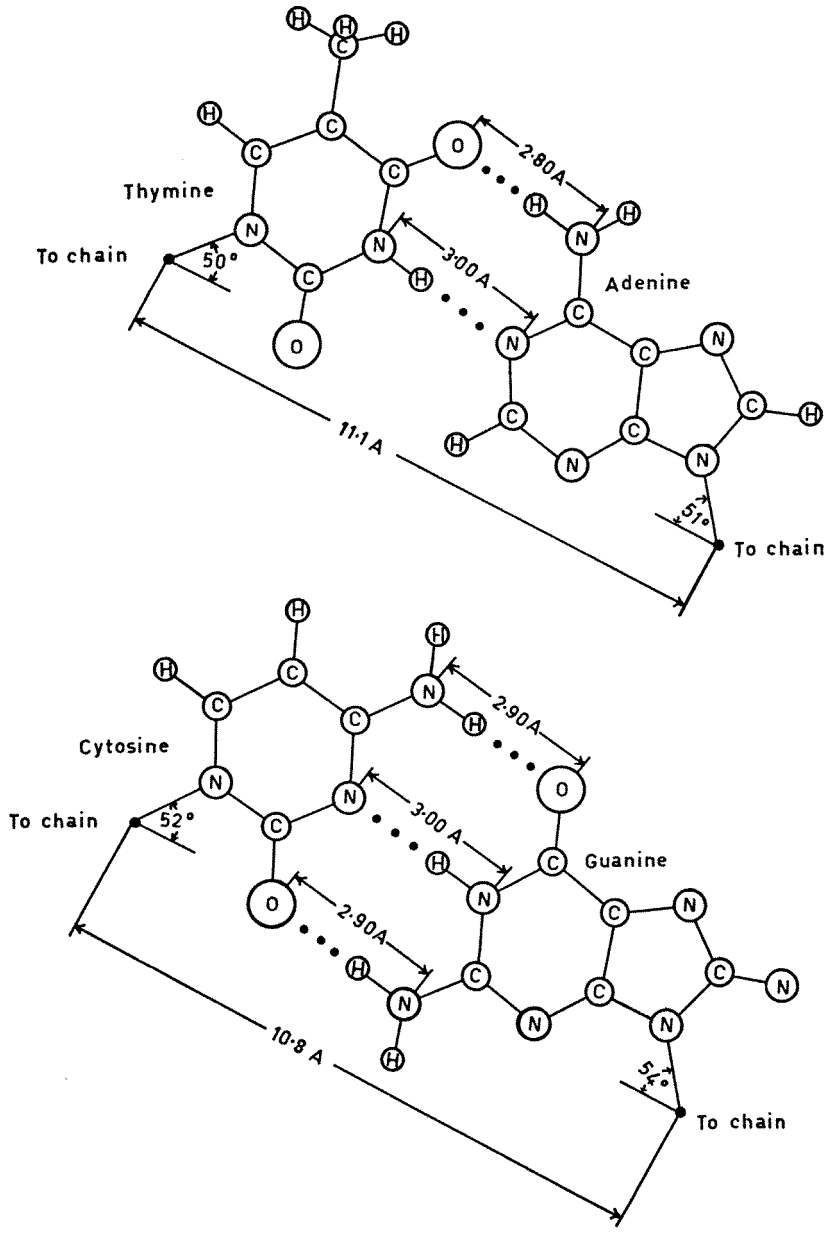

Figure 1

In view of the importance of this structure, let us examine the experimental evidence on which it rests. The presence of hydrogen bonds between the bases was first suggested by Professor Gulland, Dr. Taylor and myself (1947) to explain the anomalous titration curve we observed with calf thymus DNA. When solutions of the sodium salt of DNA originally at pH 6 - 7 are first titrated to pH 2.5 or to pH 12, the titration curves differ markedly from the back titration curves. The addition of acid or alkali to the deoxyribonucleate does not at first bring about the ionization of acid and base groups and

the unbuffered region in the initial titration curves extends from pH 4.5 to 11.0 compared with pH 6 to 9 in the back titration curve. Outside the pH range 4.5 - 11.0 a rapid liberation of groups occurs in the pH range 2.0 to 6.0 and 9.0 to 12.0. The same back titration curve is obtained whether the back-titration is commenced at pH 12 or 2.5. These observations were originally interpreted in the belief that the amino groups of guanine, adenine and cytosine were titrated in the range pH 2.0 to 6.0 whilst in the pH range 9.0 to 12.0 the -NH-CO- groups of guanine and thymine were dissociated. Whilst the latter assumption is still regarded as correct, it is almost certain that the ring nitrogens of the purines and pyrimidines are more basic than the amino groups. In the protonated form of adenine it has been established by X-ray and nuclear magnetic resonance studies that the proton is located at N_1 and not on the amino group, and the same result is reached from theoretical calculations (Broomhead, 1951; Cochran, 1951; Jardetzky and Jardetzky, 1960). The location of the proton on cytosine will also be at N_1, but in guanine since N_1 is already linked to hydrogen, the -NH-CO- group being in the keto form, the proton most probably is attached to N_7 or the amino group. In spite of the change of viewpoint concerning the groups titrated in the acid range, the interpretation that the groups titrated in the two ranges were linked by hydrogen bonds which thus weakened the dissociation of the groups is still valid. That the bonds were intermolecular and not intramolecular was determined by viscosity and streaming birefringence studies (Creeth, Gulland and Jordan, 1947; Mathieson and Matty, 1957). A very marked decrease in the viscosity and streaming bifringence was observed at those pH values where the ionization of the groups occurred. This result could be explained only by a breakdown of the hydrogen bonded structure to yield either smaller molecular units or a less asymmetric molecule.

More detailed information on the structure of DNA comes from the X-ray studies of the Randall and Wilkins group at King's College, London. Franklin and Gosling (1953), using oriented fibres of DNA, produced by withdrawing a fibre slowly from a concentrated solution at constant humidity, obtained good X-ray diffraction patterns for sodium deoxyribonucleate. More recently more detailed patterns have been obtained with the lithium salt (Wilkins, Langridge, Wilson, Hooper and Hamilton, 1960). From the analysis of the diffraction pattern, and in particular from the cylindrical Patterson function, it was evident that the model consisted of a regular double helix and the essential parameters could be deduced, the distance between the nucleotide residues is 3.4 Å, the repeat of the helix occurred every 34 Å or ten nucleotides, and the diameter is 17 Å. These dimensions are dependent on the water content of the fibre but are unlikely to be greatly different in solution.

The contribution made by Watson and Crick, which led to the well-known formula, arose from attempts to construct models of DNA. In order to do this it was necessary to know the structure and configuration of the nucleotides. Previous attempts to construct a model of a nucleotide by Astbury and others had been made on the assumption that the purine or pyrimidine ring lay in the same plane as the sugar ring. This was shown not to be so by Furberg (1950), who determined the structure of cytidine (cytosine-*N*-riboside) and showed that the angle between the rings was almost a right angle. On this basis Furberg (1952) had constructed models of nucleic acid which were single stranded and showed that the chain was twisted in the form of a helix. By extending this structure to the double helix and introducing the specific hydrogen bonds Watson and Crick were able to obtain their structure. That this structure is maintained in aqueous solution has been demonstrated by the small angle X-ray scattering studies of Luzzati, Nicolaieff and Masson (1961).

The specific pairing of the bases in the hydrogen bonded double helix carries with it the implication that for every guanine residue in the DNA molecule there must be an exactly equivalent number of cytosine residues. Similarly adenine and thymine must be present in equivalent amounts. The analytical figures for a large number of samples of DNA show that this is approximately true even though, in nucleic acids from different samples, the ratio of adenine to guanine can vary very considerably. This had been observed by Chargaff (1950) who, prior to the publication of the Watson and Crick structure, had suggested the specific pairing of guanine and cytosine and adenine and thymine in DNA.

Before leaving this discussion, we should consider possible modifications of the Watson and Crick structure. The main possibility is that instead of the two polynucleotide chains in the double helix being continuous, they possess random or specific breaks. Such breaks would not affect the X-ray structure and if occurring at less frequent intervals than once every twenty or thirty nucleotides, could not be detected by titration. Experimental evidence that such breaks do occur has been produced by Dekker and Schachman (1954) from enzymic degradation studies. However, molecular weight studies on the native and denatured DNA do not agree with this view and it is now generally believed that such breaks, if they occur, are artifacts produced during preparation and that in the native material the two intertwined strands are continuous.

Size and Shape of DNA

DNA is a polyelectrolyte inasmuch as it possesses two charged phosphate groups every 3.4 Å along the chain. Synthetic polyelectrolytes such as polymethacrylic acid show marked changes in shape on charging and discharging, being approximately rigid rods when charged and random coils when discharged. However the presence of the hydrogen bonds and other interactions between the chains greatly modifies the expected polyelectrolyte behaviour with the result that DNA polyion behaves in solution as a stiff rod. Even when discharged, in high concentrations of sodium chloride, there is no collapse of the polyion to the random coil form. On denaturing, however, and the rupturing of the bonds between the chains, the stiffness is lost and single stranded DNA, either produced from twin stranded DNA by denaturation, or from the ΦX174 virus of Sinsheimer, shows normal polyelectrolyte behaviour. The stiffness of the DNA double helix is demonstrated by the viscosity data at zero shear in various solutions of sodium chloride obtained by Dr. Porter (1960).

The molecular weight of DNA samples has been regarded until recently to lie in the range 6 - 10×10^6. Such values have generally been obtained from light scattering data or from a combination of sedimentation coefficient and either diffusion or viscosity values. However, recently it has become clear that even these values are too low and that the DNA in the cell possesses molecular weights more in the region of 110×10^6 or higher. Dr. Cairns (1961), by using an extremely mild technique, has been able to isolate DNA in this way and by labelling the DNA with tritium to determine the molecular weight autoradiographically. It has been known for some time that long molecules may be reduced in length by placing them in a shear gradient and the chemist has yet to learn how to handle these very long asymmetric molecules.

One of the most important discoveries which has greatly aided the study of nucleic acids in recent years was made as far back as 1944, when Avery, Macleod and McCarty

(1944) isolated transforming DNA. This biologically active DNA transforms one bacterial cell type into another. This property has been investigated and transforming DNA has now been isolated from many different bacteria.

Denaturation of DNA

If, as we believe, the DNA twin helix separates during cell division and each strand acts as a template for the synthesis of a second strand of DNA to complete the double helix, the study of the denaturation of DNA is of great importance. I shall now discuss the various ways in which DNA can be denatured and the mechanism of the various processes.

DNA may be denatured in aqueous solution by (*a*) heat, (*b*) acid or alkali, and (*c*) solution in very dilute solutions in the absence of sodium chloride. It is worth noting here that ultrasonic degradation does not denature DNA and that γ irradiation also does not denature DNA. Both these forms of radiation produce covalent bond breakage, generally of both chains, so that the DNA is broken down into undenatured smaller fragments. However, in some circumstances denaturation can occur and the behaviour is very dependent on the extent of the irradiation and its intensity.

The heat denaturation of DNA may be brought about by heating the DNA in solution above a certain critical temperature. Zamenhof, Alexander and Leidy (1953) were the first to observe that the viscosity of DNA solutions remained constant as temperature was increased until a certain critical temperature is reached, when the viscosity falls rapidly with further increase of temperature. The activity of transforming DNA also falls at the same temperature. Instead of observing the viscosity, it is preferable, and certainly more convenient, to follow the change in optical density. The ultraviolet absorption of DNA at 259 mμ is much less than that calculated from the known absorption coefficients of the nucleotides. This hyperchromic effect is believed to be due to the interaction between the purines and pyrimidines stacked one above the other in the double helix. Removal of the hydrogen-bonded structure markedly reduces the hyperchromic effect and the absorption markedly increases. Thus following the absorption at 259 mμ is a very convenient way of determining the denaturation or "melting" temperature. This temperature is related to the composition of the DNA, those possessing a high guanine and cytosine content have high melting points, and those with a high adenine-thymine content, low melting points. The denaturation process is analogous to the melting of a hydrogen-bonded crystal in which the structure passes from the ordered arrangement in the crystal to the disordered state in the melt. On denaturing DNA we pass from the ordered Watson-Crick structure to the random coils of single or intertwined polynucleotide chains with an analogous entropy change to that occurring in the melting of the crystal. As the temperature is increased, the kinetic energy of the segments of the DNA molecule will also increase until the energy is sufficient to overcome the interaction energy.

Although the denaturation by acid and alkali was observed first, since the titration curves illustrate irreversible denaturation, we know less about the mechanism than we do for other methods. The effect of acid or alkali addition is to affect the N....H-N bonds of the adenine to thymine and guanine to cytosine base pairs, addition of protons or removal of protons will produce positively or negatively charged nitrogen atoms respectively, but will only bring about the rupture of one hydrogen bond, the other bond in the adenine-thymine pair and the other two in the guanine-cytosine pair remaining unaffected. It is difficult to see immediately whether this will be sufficient to lead to dissociation.

However, it must be remembered that dissociation by heat cannot be neglected and both these forms of denaturation will be present at the same time. Thus Cavalieri and Rosenberg (1957) have shown that as the temperature is increased it is necessary to titrate less groups in the acid region (and the same will occur in the alkaline region) to produce denaturation.

The last method of denaturation is one which Dr. Inman and I (1960) investigated a year or so ago. Thomas (1954) showed that if sodium chloride was removed from a solution of DNA the optical density increased and on increasing the sodium chloride concentration again the optical density, although reduced, did not return to the original low value. This process he described as denaturation; he was, in fact, the first to apply this term to nucleic acids. Dr. Inman and I were able to show that denaturation occurred on reducing the DNA concentration of a salt-free solution and if this was followed either by the change in conductivity or by the change in optical density a critical concentration was observed at which denaturation occurred. This critical concentration was dependent on the electrolyte concentration, in agreement with the findings of Thomas. The mechanism of this method of denaturation is most probably that of electrostatic repulsion between the charged groups of the two polynucleotide chains. The effective charge on the phosphate groups will vary with the counterion concentration and dilution of the DNA or removal of electrolyte will bring about the dissociation of the ion pairs which exist at higher concentrations, the charge will thus increase until at infinite dilution the phosphate groups are fully charged. The repulsion energy between the charged groups can be calculated as a function of the charge fraction and from the experimentally determined value of the charge fraction at the critical concentration we can obtain the bonding energy per phosphorus atom; this comes out to be 3.1 k. cals., which is not unreasonable for 1 to 1.5 hydrogen bonds per phosphate group.

Protein denaturing agents such as urea and guanidine hydrochloride do not denature DNA, but do aid denaturation by other means. Thus the melting temperature is lowered in the presence of 8M urea.

The Reversibility of DNA Denaturation

From early studies of the denaturation of calf thymus DNA, it was concluded that the denaturation process was irreversible and the double helix gave an intertwined random coil which could contain some non-specific hydrogen bonds. However, with the realization that the sequence of nucleotides determines the genetic code, it became appreciated that the likelihood of producing reversible denaturation was dependent on having a nucleic acid preparation which did not contain too many different kinds of genetically, and therefore chemically, different DNA. The DNA from mammalian glands such as calf thymus is very complex as has been shown by the fractionation work of Bendich, which has been confirmed and extended by Mr. Colvill and myself. Whether this fractionation is the type which is genetically significant, I very much doubt, but it nevertheless shows that the DNA is complex. Bacterial DNA is less complex genetically and has the advantage of being biologically active, virus DNA would be simpler still since each virus contains only one DNA molecule. The very brilliant researches of Doty (1961) have demonstrated beyond doubt that bacterial DNA can be denatured reversibly. The process used is a simple and obvious one. We have already likened the denaturation by heat to a melting of a crystal, so the reverse process of renaturation should be possible by a process akin to crystallization. Heat denaturation followed by a slow cooling should therefore reproduce native DNA. This is what Doty observed. The slow cooling process allows the individual

polynucleotide chains to seek out their partners in the solutions and form the specific hydrogen bonds with the complementary sequence. DNA which has been denatured by heat and slowly cooled is found to regain its original melting temperature and also much of its original biological activity.

Renaturation of DNA in this way does not necessarily involve strand separation during the denaturation process. That strand separation does occur was brilliantly proved by Doty (1961) by using two preparations of DNA obtained from the same bacteria, one grown on media containing N^{14} (*sic*, ^{14}N etc.) and H^1 and the other N^{15} and D. Such DNA's can be separated analytically by the technique of density gradient ultracentrifugation. A mixture of N^{14} and N^{15} labelled DNA was heated so as to produce denaturation; if strand separation *did not* occur only two species would result, but if strand separation *did* occur five species would result, *viz*. single strand N^{14}, double strand N^{14}, double strand hybrid N^{14},N^{15}, single strand N^{15}, and double strand N^{15}. Five strands were observed in density gradient ultracentrifugation and if the renatured solution was treated with an enzyme which hydrolyses only single strand DNA, the single stranded moieties are removed and also any single strand "tails" at the ends of double strands. Three sharp fractions of the double stranded molecule are then obtained.

The Rate of Denaturation

Now that the mechanism of the denaturation process is firmly established, the rate of this process and the renaturation process warrants examination. Kinetic studies on the denaturation and renaturation of DNA have not been made, but Sturtevant and Ross (1960) have shown that the reaction between synthetic polyadenylic acid and polyuridylic acid is very rapid. In this reaction there is no slow process such as there is in DNA renaturation when the DNA strands have to seek the complementary sequence, since any adenylic acid residues on one chain can bond to a uridylic acid residue of the other. However, it does show that the formation of the double helix can be a rapid process. From theoretical considerations, Kuhn (1961) and Longuet-Higgins and Zimm (1961) have concluded that a Watson-Crick helix can unwind by partial rotational or torsional Brownian movement round the axis in 50 to 80 seconds. In the renaturation process, there is not only the problem of forming the helix which is rapid, as shown by the formation of the polyadenylic acid-polyuridylic acid complex, but also the problem of forming the helix of minimum free energy which will have the maximum interaction between the strands.

The Macro Structure of RNA

Compared to DNA, our knowledge of the structure of RNA is still rather rudimentary; evidence exists for the formation of both stranded and double stranded RNA. In the single stranded RNA there is also evidence for twin stranded sections, but whether these are biologically important or artifacts formed due to the ease of helix formation, is not clear.

Future Work

In his will, Archibald Liversidge directs the lecturer to draw "attention to the research work which should be undertaken". In the nucleic acid field, immense possibilities exist and I will confine my comments to those aspects which we are developing. One of the main difficulties confronting a physical chemist in nucleic acid chemistry is the difficulty

that arises in handling a large, charged macromolecule in aqueous solution. Some years ago it was observed that the cetyl trimethyl ammonium salts of DNA were soluble in non-aqueous solvents and recently it has been shown that these salts can be reversibly formed from nucleic acids and polynucleotides. This opens up the possibility of making studies in non-aqueous media. We expect this technique to help greatly in the elucidation of the size and shape studies and also in the study of denaturation mechanism. We are furthering our studies of denaturation by extending the dilution method to other nucleic acids and have already observed that the critical concentration is dependent on composition. Much can be done with rapid reaction techniques on denaturation and renaturation mechanisms and we are particularly interested in the possibilities of studying reactions by suddenly altering the environment, either as regards concentration or temperature.

Then there is the problem of fractionation. Procedures so far have employed basic columns which have thus used only phosphate groups as points of attachment. Fractionation has thus largely been concerned with molecular size. What is required is a surface which will specifically adsorb only certain DNA molecules. Perhaps the answer is DNA supported on cellulose or combined and held in an oriented structure by some other means. It is important to realize that the problem of fractionation must be completely mastered before sequence determinations will have any significance.

A knowledge of the precise location of the protons in protonated DNA and polynucleotides in solution is required before the mechanism of acid and alkali denaturation can be elucidated. We hope nuclear magnetic resonance will help us here. We also have to learn how to handle large, asymmetric molecules such as have been isolated by Dr. Cairns, since subjecting these to even low shear gradients causes degradation.

Finally, there remains the problem of the small chemical difference between DNA and RNA which appears to cause a fundamental difference in the biological function. There is thus much to do; the main problem therefore is what should be done first. This emphasizes the most important aspect of research, the proper design of meaningful experiments.

References

Avery, O.T., Macleod, C.M., and McCarty, M.J., 1944. *J. Exp. Med.,* **79**, 137.
Broomhead, J.M., 1951. *Acta Cyyst.,* **4**, 92.
Cairns, J., 1961. *J. Mol. Biol.,* **3**, 756.
Cavalieri, L. F., and Rosenberg, B.H., 1957. *J. Amer. Chem. Soc.,* **79**, 5354.
Chargaff, E., 1950. *Experientia,* **6**, 20.
Clark, V.M., Todd, A.R., and Zussmann, J., 1951. *J. Chem. Soc.,* 2952.
Cochran, W., 1951. *Acta Cryst.,* **4**, 81.
Creeth, J. M., Gulland, J. M., and Jordan, D.O., 1947. *J. Chem. Soc.,* 1141.
Davoll, J., Lythgoe, B., and Todd, A.R., 1944. *J. Chem. Soc.,* 833.
Dekker, C.A., and Schachman, H.K., 1954. *Proc. Natl. Acad. Sci. Wash.,* **40**, 894.
Franklin, R.E., and Gosling, R.G., 1953. *Acta Cryst.,* **6,** 673, 678.
Furberg, S., 1950. *Acta Cryst.,* **3**, 325.
Furberg, S., 1952. *Acta Chem. Scand.,* **6**, 637.
Gulland, J. M., Jordan, D.O., and Taylor, H.F.W., 1947. *J. Chem. Soc.*, 1131.
Huber, M., 1957. *Acta Cryst.,* **10**, 128.

Inman, R.B., and Jordan, D.O., 1960. *Biochim. et Biophys. Acta,* **42**, 421, 427 ; **43**, 9, 206.
Jardetzky, C.D., and Jardetzky, O., 1960. *J. Amer. Chem. Soc.,* **82**, 2222.
Kuhn W., 1961. *J. Mol. Biol.,* **3**, 473.
Longuet-Higgins, H.C., and Zimm, B.H., 1961. *J. Mol. Biol.,* **3**, 475.
Luzzati, V., Nicolaieff, A., and Masson, F., 1961. *J. Mol. Biol.,* **3,** 185.
Lythgoe, B., Smith, H., and Todd, A.R., 1947. *J. Chem. Soc.,* 355.
Mathieson, A.R., and Matty, S., 1957. *J. Polymer Sci.,* **23**, 747.
Pauling, L., and Corey, R.B., 1956. *Arch. Biochem. Biophys.,* **65**, 164.
Porter, M.R., and Jordan, D.O., 1960. *In* Jordan, D.O., " Chemistry of Nucleic Acids ". Butterworths, London, p. 200.
Schildkraut C.L., Marmur, J., and Doty, P., 1961. *J. Mol. Biol.,* **3**, 595.
Sturtevant, J.M., and Ross, P.D., 1960. *J. Polymer Sci.,* **47**, 111.
Thomas, R., 1954. *Biochim. et Biophys. Acta,* **14**, 233.
Watson, J.D., and Crick, F.H.C., 1953. *Nature* (London), **171**, 737; Cold Spring Harbour Symp. Quant. Biol., **18**, 123.
Watson, J.D., and Crick, F.H.C., 1954. *Proc.* Roy. Soc., **A**, **223**, 80.
Wilkins, M.H.F., Langridge, R., Wilson, H.R., Hooper, C.W., and Hamilton, L.D., 1960. *J. Mol. Biol.,* **2**, 19.
Zamenhof, S., Alexander, H.E., and Leidy, G., 1953. *J. Exp. Med.,* **98**, 379.