MACROMOLECULAR STRUCTURE AND PROPERTIES OF DEOXYRIBONUCLEIC ACID

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DURING the last decade the role of deoxyribonucleic acid (DNA) as the carrier of genetic information has been studied in great detail. The essential simplicity of the basic metabolic process whereby DNA acts as a template for the synthesis of messenger RNA (ribonucleic acid) which in turn acts as a template for protein synthesis has been shown to be involved in a complex web of control mechanisms in which it now seems probable that proteins can control the operation of sections of the DNA template so that specific enzymes are made only when required by the cell. The key to the elucidation of the properties of DNA has been the structure proposed by Watson and Crick.[1] As is shown in Fig. 1, native DNA consists of two polyphosphate chains, the phosphate groups linking the 3′,5′ positions of neighbouring deoxyribose moieties. The bases are linked to the 1′ position of the sugar and the two chains are held together by hydrogen bonds formed between pairs of bases. The fundamental requirement is that adenine (A) on one chain pairs with thymine (T) on the other and guanine (G) with cytidine (C). It should be noted that the two chains run in opposite directions (antiparallel).* In the hydrated molecule the pairs of bases lie stacked on top of each other in planes at right angles to the long axis of the molecule; the two chains are coiled, one full turn being made every ten pairs of bases. Replication of the molecule involves the separation of the two chains and the synthesis of a new complementary strand on each, the composition of the new strands being dictated by the requirement for the pairing of A with T and G with C. In contrast to this the formation of single-stranded messenger RNA on the DNA template does not appear to require the separation of the two chains of the DNA molecule.[2] It is possible that the messenger RNA is complementary to only one of the strands of DNA, *i.e.*, that one strand contains the genetic message, the other strand being a nonsense code.

The preparation[3] of DNA involves disrupting the cell or organism, the isolation of DNA or DNA–protein complex free from other cell constituents, particularly RNA and polysaccharides, and the removal of the protein bound to the DNA. The main methods so far applied depend upon

*The abbreviations A, T, G, and C have been used for adenine, thymine, guanine, and cytosine and for the corresponding deoxyribose derivatives where no confusion occurs. dATP, dCTP, dGTP and dTTP are the deoxyribose triphosphates of the four bases. poly-dAT is a twin-stranded, synthetic polymer with regularly alternating deoxyadenosine and thymidine linked 3′-5′ by phosphate groups in each chain. poly-dGdC is a twin-stranded, synthetic polymer of deoxyguanosine and deoxycytidine, one strand containing only guanine moieties and the other cytosine only.

[1] J. D. Watson and F. H. C. Crick, *Nature*, 1953, **171**, 737.

[2] J. Hurwitz and J. T. August, *Progr. Nucleic Acid Research*, 1963, **1**, 59.

[3] K. S. Kirby, *Progr. Nucleic Acid Research*, 1965, **3**, 1.

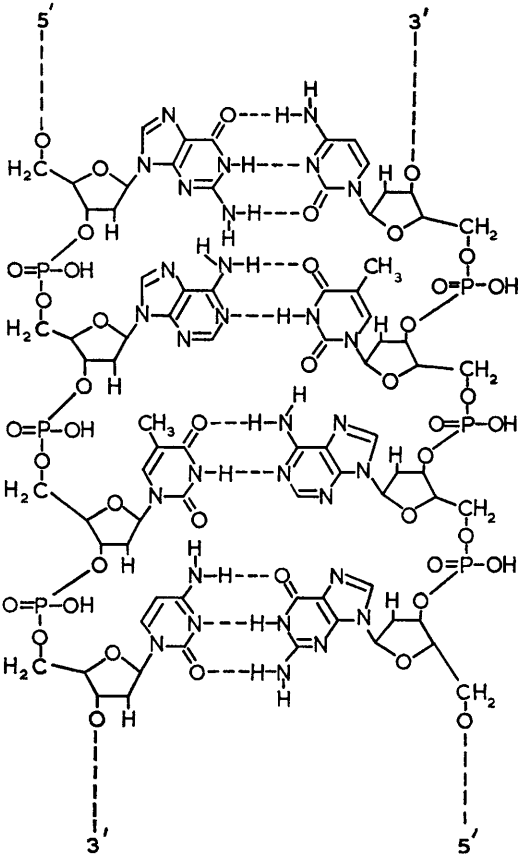

FIG. 1. *A schematic diagram of part of a DNA helix showing the pairing of adenine with thymine and guanine with cytosine. Reading from the top of the diagram the pairs of bases shown are guanine–cytosine, thymine–adenine, adenine–thymine, and cytosine–guanine.*

the use of either a detergent or of phenol to inhibit enzyme action and to denature and remove protein. It has always been accepted that the preparation should have a high molecular weight and a low protein content. Most preparations of mammalian DNA with less than 0·1% protein were found to have molecular weights in the range 6—8 $\times$ 10^6. The presence of some residual protein, in the most extensively purified preparations, posed the question whether small proteins or amino-acids were part of the structure of DNA, linking units of the molecule end to end within the cell and whether breaking occurred at some of these points during isolation.[4,5] However, no evidence of a deoxynucleotide covalently linked to an

[4] A. Bendich and H. S. Rosenkranz, *Progr. Nucleic Acid Res.*, 1963, **1**, 219.
[5] C. Sadron, *J. Chim. phys.*, 1961, **58**, 877; G. Bernardi and C. Sadron, "Acidi nucleici e loro funzione biologia," Istituto Lombardo, 1964, 62.

amino-acid has yet been found. Within the last few years it has been shown that the whole of the DNA of a bacteriophage can be isolated as a single unit.[6] A homogeneous preparation of DNA from T2 bacteriophage can be obtained with a molecular weight of 120×10^6. Following this, Cairns[7] published radioautographs showing the isolated DNA of the bacterium *Escherischia coli* as a single unit of length 400 μ ($M = 1000 \times 10^6$). Extreme care has to be taken in the isolation of DNA of these high molecular weights to avoid degradation of the molecules by shear. It appears that the polydisperse preparations of DNA normally obtained from mammalian sources are degradation products of much larger molecules. The possibility that the DNA of a single chromosome may be a single unit cannot be ignored. Homogeneous preparations of DNA of high molecular weight have not so far been made in sufficient quantity to allow analysis for the presence of protein residues.

Careful isolation techniques in which shearing is avoided have also shown that in some cases the DNA molecule exists as a closed circle. The existence of circular DNA has been demonstrated in the bacteriophage λ,[8] in the bacterium *E. coli*[7] and in the polyoma[9] and papilloma viruses.[10] The DNA of the bacteriophage ϕX174 is single-stranded and both this form and the double-stranded molecule formed after infection of a bacterium as a preliminary to replication, are circular.[11]

The use of density-gradient centrifugation for characterising DNA preparations has proved to be extremely useful.[12] In these experiments the DNA is dissolved in a high concentration of one of the soluble cæsium salts, usually the chloride or sulphate. Centrifugation at speeds of about 30,000—40,000 r.p.m. generates in time an equilibrium density gradient of the salt, the DNA sedimenting to form a band within the gradient. It has been found that the buoyant density of DNA preparations is a linear function of the base composition, increasing with G + C content. By including a marker DNA of known density and composition the technique can be used to determine the average base composition of a preparation. Theoretically, a homogeneous preparation should exhibit a Gaussian distribution at equilibrium, the variance being inversely related to the molecular weight. Attempts have been made to use this method for determining the molecular weight of the homogeneous preparations from bacteriophage, but difficulties are encountered owing to light scattering by the large molecules. The variance was found to depend on the wavelength of the light used in the absorption optical system and measurements

[6] A. D. Hershey and E. Burgi, *J. Mol. Biol.*, 1960, **2**, 143.
[7] J. Cairns, *J. Mol. Biol.*, 1962, **4**, 407; 1963, **6**, 208.
[8] C. A. Thomas, "Acidi nucleici e loro funzione biologia," Istituto Lombardo, 1964, 17.
[9] R. Dulbecco, "Acidi nucleici e loro funzione biologia," Istituto Lombardo, 1964, p. 278; R. Dulbecco and M. Vogt, *Proc. Nat. Acad. Sci. U.S.A.*, 1963, **50**, 236.
[10] J. D. Watson and J. W. Littlefield, *J. Mol. Biol.*, 1950, **2**, 161.
[11] W. Fiers and R. L. Sinsheimer, *J. Mol. Biol.*, 1962, **5**, 424; A. K. Kleinschmidt, A. Burton, and R. L. Sinsheimer, *Science*, 1963, **142**, 961.
[12] J. Vinograd and J. E. Hearst, *Fortschr. Chem. org. Naturstoffe*, 1962, **20**, 372.

could not be made over a sufficient range of wavelengths to allow extrapolation to the correct value.[13] With degraded preparations of lower molecular weight, the variance of the distribution is increased by the density heterogeneity of the molecules resulting from differences in base composition. The method has however been used successfully to demonstrate that circular DNA from papilloma or polyoma virus has the same molecular weight as the linear form of these molecules. With degraded samples of bacteriophage and bacterial DNA (M about 20×10^6) the bands are extremely sharp and small differences in overall composition between two species are sufficient to give completely separated bands. Preparations of mammalian DNA of molecular weight $10—20 \times 10^6$ give much broader bands. Detailed analyses have shown that the equilibrium distributions of some DNA preparations contain satellite bands. DNA from herring sperm and mouse tissue for example exhibits a satellite band lighter than the average (low G + C content) while guinea pig and calf thymus DNA have satellite bands denser than the average. The light satellite band accounts for 30% of the DNA from the crab *Cancer borealis* and 11% in *C. irroratus*. In the last two cases the minor band contains 93% adenine and thymine and has been found to correspond closely with the synthetic copolymer poly-dAT (see footnote, p. 369) in which adenine and thymine residues alternate in each chain. The significance of these satellite bands is not yet understood. From studies of the buoyant density in a variety of cæsium salts Hearst and Vinograd[14] were able to show that the net hydration of the cæsium salt of DNA is 30% by weight in the physiological range of water activities from 0·9—1·0.

The observation that the DNA of a bacteriophage can be isolated as a unit has helped to solve many problems relating to the characterisation of DNA in solution. Attempts have been made to correlate the molecular weight of DNA with easily measurable parameters such as sedimentation coefficient and viscosity. The basis for the comparisons has usually been the equation derived by Mandelkern and Flory

$$\frac{s^\circ[\eta]^{\frac{1}{3}}}{M^{\frac{1}{3}}} = \frac{\beta\,(1 - \bar{v}\rho)}{N\eta_0}$$

where s° is the sedimentation coefficient, $\bar{v}$ is the partial specific volume of DNA, ρ and η_0 the density and viscosity of the solvent, and N Avogadro's number. β is a factor dependent only on the geometry of the molecule, *e.g.*, for random coils $\beta = 2{\cdot}5 \times 10^6$ and for stiff rods $3{\cdot}1—3{\cdot}4 \times 10^6$. Most determinations of molecular weight have been made by use of light-scattering. The primary difficulty here has been to decide how high a molecular weight can be measured. Recently Froelich *et al.*[15] have constructed a light-scattering apparatus with which measurements can be

[13] D. J. Cummings, *Biochim. Biophys. Acta*, 1963, **72**, 475.
[14] J. E. Hearst and J. Vinograd, *Proc. Nat. Acad. Sci. U.S.A.*, 1961, **47**, 825, 1005.
[15] D. Froelich, C. Strazielle, G. Bernardi, and H. Benoit, *Biophys. J.*, 1963, **3**, 115.

made down to 16° from the main beam; the limit for most instruments previously used was 30°. For DNA of molecular weight up to 6×10^6, measurements to 30° or 16° gave identical results. At higher molecular weights the values calculated diverged rapidly, *e.g.*, one preparation gave $M = 8{\cdot}5 \times 10^6$ if data to 30° were used and $M = 17 \times 10^6$ from data to 16°. Light-scattering measurements can therefore only be used with certainty to establish the correlations between M and s and M and $[\eta]$ up to about 6×10^6. The high-molecular weight end of the curves can be established from measurements on the homogeneous preparations of DNA from bacteriophage. Careful measurements of the sedimentation coefficient and viscosity of preparations of DNA from T2, T4, and T7 bacteriophage have been made in several laboratories and good agreement has been achieved demonstrating that present techniques can be used with these very large molecules.[16,17,18] Sedimentation studies have to be made at low rotor speeds to avoid intertwining of the molecules and the formation of rapidly sedimenting aggregates.[19] The zonal sedimentation technique of Vinograd *et al.*[20] avoids this difficulty and eliminates the problems resulting from self-sharpening of the boundary due to the Johnson–Ogston effect. The molecular weights of the DNA preparations have been determined from measurements of the lengths of the molecules in electron micrographs, from determinations of the specific radioactivity of the DNA and the activity of the whole bacteriophage and from determinations of the weight of DNA per bacteriophage (see ref. 18). The molecular weights obtained show considerable scatter: T2 and T4 DNA, $M = 97—162 \times 10^6$; T7 DNA, $19—27 \times 10^6$. Burgi and Hershey found that when the DNA of bacteriophage T2 is broken by carefully controlled shearing, the breaks occur most frequently near the middle of the molecule giving a reasonably homogeneous solution of half molecules which can in turn be sheared to quarter molecules.[21] These preparations provide further standards of known molecular weight. Eigner and Doty[18] have reviewed all the published data and have found that in the range $M = 0{\cdot}3—130 \times 10^6$ the intrinsic viscosity and sedimentation coefficient at infinite dilution are monotonically related. The relationship between M and s and M and $[\eta]$ changes with molecular weight; at high molecular weights the molecules behave as random coils with a transition towards the characteristics of stiff rods as the molecular weight decreases, although even at $M = 1—4 \times 10^6$ the limit of rod-like behaviour is not reached. It seems probable that all samples of native DNA have a characteristic chain stiffness. The bending of the molecules to form random coils could be associated with a limited degree of flexibility of the twin-strand structure or to the presence of junction

[16] D. M. Crothers and B. Zimm, *J. Mol. Biol.*, 1965, **12**, 525.
[17] J. B. T. Aten and J. A. Cohen, *J. Mol. Biol.*, 1965, **12**, 537.
[18] J. Eigner and P. Doty, *J. Mol. Biol.*, 1965, **12**, 549.
[19] J. Rosenbloom and V. N. Schumaker, *Biochemistry*, 1963, **2**, 1206.
[20] J. Vinograd, R. Bruner, R. Kent, and J. Weigle, *Proc. Nat. Acad. Sci. U.S.A.*, 1963, **49**, 902.
[21] E. Burgi and A. D. Hershey, *J. Mol. Biol.*, 1961, **3**, 458.

points along the molecule at which free rotation is possible. No distinction between these possibilities can be made from the above data since both models lead to the same functional relationship between s and $[\eta]$ and M. A small proportion of breaks in the single strands of the helix does not appear to increase the flexibility of the molecule.

So far preparations of twin-stranded circular DNA from only two sources have been studied in detail. It has been found that, in each case, because of the more compact structure imposed upon it the circular DNA has a higher sedimentation coefficient than the corresponding linear form: papilloma DNA, circular 28*S*, linear 21*S*:[10] polyoma DNA, circular 20*S*, linear 16*S*.[9] The identity of the circular and linear forms of these molecules has been confirmed by measurements of the molecular weight from the spread of the equilibrium distribution curve in cæsium chloride density gradients.

The stiffness of the twin-stranded DNA molecule may be contrasted with the flexibility of the single chains. Changes in the ionic strength of the solution, for example, have little effect on the sedimentation rate of native DNA (Fig. 2) indicating that the shape of the molecule remains essentially

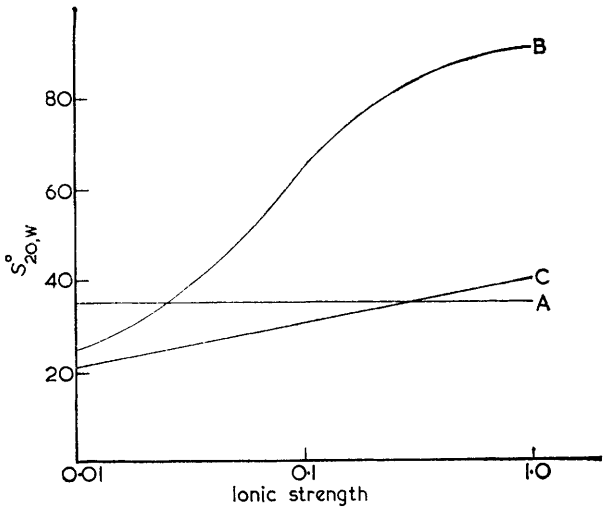

FIG. 2. *The effect of ionic strength on the sedimentation coefficient at infinite dilution. A, native DNA; B, denatured, single-stranded DNA at pH* 8*; C, denatured DNA at pH* 12–13. (Reproduced by permission from F. W. Studier, *J. Mol. Biol.*, 1965, **11**, 373.)

unchanged. With the single chains there is a marked increase in sedimentation rate as the masking of the charge effects of the phosphate groups increases.[22] At pH 8 interaction and stacking of the bases occurs, leading to a much tighter coiling of the molecule than is observed at pH 12—13

[22] F. W. Studier, *J. Mol. Biol.*, 1965, **11**, 373.

where charged groups on the nucleotides prevent the stacking of the bases. No change in the absorption at 260 mμ occurs at pH 12—13 when the ionic strength is increased but at pH 8 the progressive ordering of the bases in parallel arrays is accompanied by a decreasing absorption. Using first-order perturbation theory, Tinoco[23] has related this hypochromicity to the geometry of the transition moments of the nucleotide moieties. Treating the phenomenon as a local field effect, Devoe[24] has demonstrated that the π–π^* transition at 260 mμ should exhibit hypochromism in the ordered structure of layers of bases and that the n–π^* transition near 280 mμ should be hyperchromic.

The effects of changes of pH on the properties of native and single-stranded DNA are illustrated in Fig. 3. Titration of the bases of the single strands at high pH leads to an elongation of the molecule, the increase in frictional resistance resulting in a decreased sedimentation rate.[22] At low pH the beginning of a similar effect is observed but aggregation then ensues and the sedimentation rate increases. In native DNA titration of the bases occurs at more extreme pHs than for the single strands.[25] As is shown in Fig. 3, the shape of the native DNA molecules remains constant

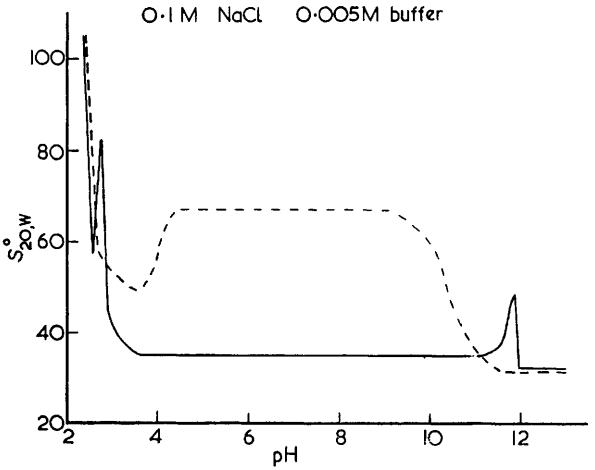

FIG. 3. *The effect of pH on the sedimentation coefficient at infinite dilution.* ——— *native DNA;* - - - - - - *denatured DNA.* (Reproduced by permission from F. W. Studier, *J. Mol. Biol.*, 1965, **11**, 373.)

over a wider range of pH than is observed with the single strands. The maxima observed at high and low pH with native DNA are associated with the collapse of the twin helix and indicate the existence of a metastable structure in which the hydrogen bonding between the chains has been broken but the two chains are not completely separated.

[23] I. Tinoco, *J. Amer. Chem. Soc.*, 1960, **82**, 4785; 1961, **83**, 5047.
[24] H. Devoe, *Biopolymers Symposia*, No. 1, 1964, 251.
[25] A. R. Peacocke, *Progr. Biophysics Biophys. Chem.*, 1960, **10**, 55.

Denaturation of DNA,[26] the separation of the two chains, can be achieved by raising or lowering the pH as described above or by heating or by lowering the ionic strength of the solution. The coiling of the separated strands can lead to the formation of intermolecular aggregates and thus make it difficult to relate the size of the denatured product to that of the original material. Working at comparatively low ionic strength and at low concentration Eigner and Doty[18] found that the molecular weight of DNA from bacteria is halved upon denaturation. The integrity of the single chains of the DNA molecule is not however necessary for biological activity. Davison, Freifelder, and Holloway[27] found that denaturation of DNA from bacteriophages T2, T4, and T5 gave a completely polydisperse product as judged by the shape of the sedimenting boundary. Denaturation of DNA from nine other sources tested yielded products containing 15—100% of polydisperse material. Since the amount of polydisperse material found was characteristic of the bacteriophage rather than of the method of denaturation it must be concluded that breaks can occur naturally in the single chains.

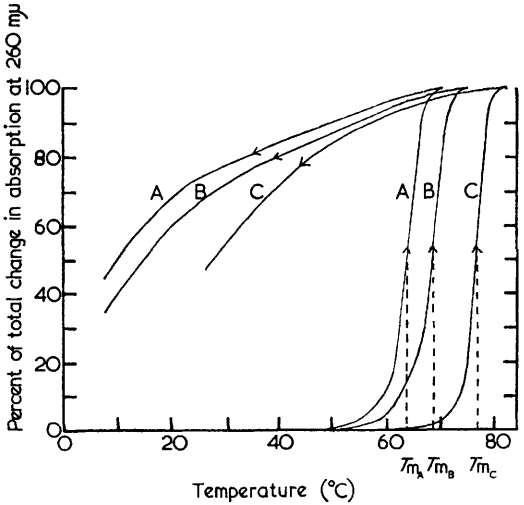

FIG. 4. *The changes in absorption at* 260 mμ *observed when native DNA is heated and then cooled. The total increment in absorption observed on heating is about* 40% *of the absorption of the solution of native DNA: in the Figure the curves have been normalised and the changes in absorption are shown as fractions of the total increment observed on heating. The dashed lines drawn from the mid-points of the curves obtained on heating give the* T_m *values which are characteristic of the base composition of the DNA. The DNA preparations are from:*

A, D. pneumoniae, 38% G + C
B, E. coli, 52% G + C
C, Ps. aeruginosa, 66% G + C

(Reproduced by permission from J. Eigner and P. Doty, *J. Mol. Biol.*, 1965, **12**, 549)

[26] J. Marmur, R. Rownd, and C. L. Schildkraut, *Progr. Nucleic Acid Research*, 1963, **1**, 232.
[27] P. F. Davison, D. Freifelder, and B. W. Holloway, *J. Mol. Biol.*, 1964, **8**, 1.

The process of denaturation can be readily followed by observing the changes in absorption at 260 mμ (Fig. 4). At a given ionic strength the mid-point of the hyperchromic change, T_m, is a characteristic of each DNA and increases with the G + C content of the DNA. Reducing the ionic strength of the solution progressively lowers T_m until at very low ionic strengths denaturation occurs spontaneously owing to the repulsive forces between the unshielded phosphate groups. When solutions of DNA which have been heated above the denaturation temperature cool, the absorption at 260 mμ falls, largely as a result of the random coiling and stacking of the bases of the single chains. A partial reversal of denaturation and the re-forming of some double-stranded molecules does however occur if the temperature has not been raised high enough to separate all the twin-stranded regions. Denaturation in the presence of formaldehyde which prevents the re-formation of the twin helix shows that all the double-stranded regions are not melted out until the full hyperchromic effect has been achieved.[28] If the two strands of the DNA helix are cross-linked (*e.g.*, by reaction with mitomycin, actinomycin, or bifunctional alkylating agents or as occurs naturally in circular DNA) the separation of the two chains is prevented and reversible denaturation is observed. With mammalian DNA one cross-link per 10^6 molecular-weight units may be required to ensure complete re-formation of the double helix.

The observation that the temperature of denaturation increases with G + C content was at first attributed to the presence of three hydrogen bonds linking guanine and cytidine as opposed to the two linking adenine and thymine. Studies of denaturation in the presence of a variety of solutes and solvents showed that effects on the melting temperature could not be correlated with the hydrogen-bonding capacities of the various agents. Substances which are not in the usual sense hydrogen-bond breaking agents, can alter the differential stability of DNA containing a high proportion of G + C relative to DNA rich in A + T as is shown in Table 1.[29] These results indicate that the hydrogen bonding of the base pairs contributes little to the stability of the helix. Theoretical treatments of the thermo-

TABLE 1

Solvent	dT_m/d (G + C)
6·5M-CF_3CO_2Na, versene, pH 7	0·60
7·2M-$NaClO_4$, versene, pH 7	0·56
0·15M-NaCl, citrate, pH 7	0·41
51% (v/v) MeOH, 10^{-3}M-NaCl, 10^{-3}M-tris, pH 7	0·35
81% (v/v) MeOH, 10^{-3}M-NaCl, 10^{-3}M-tris, pH 7	about 0

Slope of the curve relating denaturation temperature, T_m, of DNA to the content of guanine and cytidine, (G + C), in different solvent systems. $dT_m/d(G + C)$ is the change in T_m for a change in (G + C) content of 1 mole %.
(Reproduced by permission of K. Hamaguchi and P. Geiduschek, *J. Amer. Chem. Soc.*, 1962, **84**, 1329.)

[28] D. Freifelder and P. F. Davison, *Biophys. J.*, 1962, **2**, 249.
[29] K. Hamaguchi and P. E. Geiduschek, *J. Amer. Chem. Soc.*, 1962, **84**, 1329.

dynamics of denaturation and the difficulties encountered have been summarised by Crothers and Zimm.[30] These authors have related the free-energy change which occurs on stacking hydrogen-bonded base pairs in the helix to the slope of the thermal denaturation curves. For the synthetic polymer poly-dAT the calculated free-energy change amounts to about −7 kcal./mole of base pairs compared with the estimate of −2 to −3 kcal./mole for the initial hydrogen bonding of the bases. The problem of the strength and nature of the bonding between the superimposed bases in the helix has been approached from quantum mechanical considerations. Calculations made by Ladik and Hoffman[31] show that overlap of the π orbitals of the bases occurs, but that the interaction is non-bonding in character. Assuming a van der Waals's type of interaction, Devoe and Tinoco[32] found that dipole–dipole, dipole–induced dipole, and London force interactions between the bases are large and that, as is observed, the stability of the helix should increase with G + C content. Although, therefore, the hydrogen bonding between the pairs of bases is essential in dictating the specificity of the structure these bonds contribute little to the stability of the helix.

If solutions of denatured bacteriophage DNA are held at a temperature 25° below T_m for several hours, realignment of some of the molecules and the formation of twin-stranded DNA occurs.[26] Theoretical considerations suggest that the process begins by a slow second-order reaction involving the formation of nucleation centres, followed by the bonding of short sections of the molecules with appropriate base sequences, and this is followed by an annealing process in which further sections of the chains become properly matched. This reversal of denaturation occurs only to a limited extent with bacteriophage DNA, to a much smaller degree with bacterial DNA, and has not yet been demonstrated with mammalian DNA. The greater the amount of DNA required by a cell or organism to express its genetic complexity the lower is the probability that complementary strands will meet during the annealing. When DNA from *E. coli* grown in a medium containing nitrogen-14 is mixed with DNA from the bacterium grown in medium containing ^{15}N, denatured and annealed, half of the "renatured" DNA molecules formed are labelled in one strand with ^{14}N and in the other strand with ^{15}N (Fig. 5). Formation of native DNA from complementary strands from different cells is thus possible. No hybrid formation has been detected between DNA molecules from organisms which are not genetically related even though the base composition of the DNA may be the same for both. The existence within the bacterial cell of RNA complementary in composition to the DNA has been demonstrated by the formation of RNA–DNA hybrids under the conditions for the renaturation of DNA described above.

If bacteria or cells from dividing tissues are disrupted and the particu-

[30] D. M. Crothers and B. Zimm, *J. Mol. Biol.*, 1964, **9**, 1.
[31] J. Ladik and T. A. Hoffman, *Biopolymers Symposia*, No. 1, 1964, 117.
[32] H. Devoe and I. Tinoco, *J. Mol. Biol.*, 1962, **4**, 500.

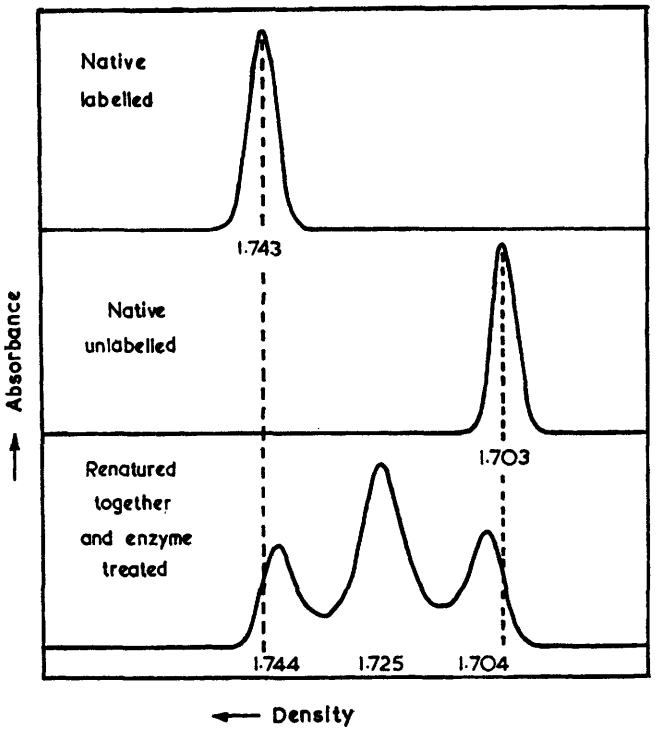

FIG. 5. *Banding of native and renatured DNA from* E. coli *in cæsium chloride. The DNA of* coli *grown in medium containing* ^{15}N *(upper figure) has a higher density than DNA from* coli *grown in normal medium (middle figure). Samples of these two preparations of DNA were denatured, mixed, and allowed to renature together. Before banding, denatured DNA left was degraded preferentially using* E. coli *phosphodiesterase. The lower figure shows that, as would be expected for random renaturation, equal amounts of the two original native DNAs are formed and twice as much of the hybrid of intermediate density.*

(Reproduced by permission from C. L. Schildkraut, J. Marmur and P. Doty, *J. Mol. Biol.*, 1961, **3**, 595.)

late matter removed by centrifugation the clear supernatant fraction contains all the enzymes required for the synthesis of DNA. Using this fraction with an added energy source, one can demonstrate the successive phosphorylation of the four deoxynucleosides to the mono-, di, and triphosphates. The reactions normally stop when the triphosphates have been produced. If however DNA is added, polymerisation of the triphosphates occurs with the formation of a polymer possessing all the properties associated with native, twin-stranded DNA.[33] As a result of the presence of nucleases in most of the enzyme preparations degradation

[33] A. Kornberg, "Enzymatic Synthesis of DNA," John Wiley and Sons Inc., New York, 1961.

proceeds simultaneously with synthesis and the product has a lower molecular weight and is more polydisperse than the original primer. The polymerase enzyme has an absolute requirement for the deoxynucleoside triphosphates and the priming polymer has itself to be a deoxyribose derivative. Specificity for the synthesis of DNA does not reside in the enzyme since preparations from any one source can use DNA from any other source or a synthetic deoxyribose polymer as primer. The composition and base sequences of the primer dictate the nature of the product; for example, when the synthetic copolymer of deoxyadenine and thymidine is used as primer in the presence of all four triphosphates, less than one guanine is incorporated for every 28,000 adenine and thymine residues. The fundamental correctness of the mechanism of replication proposed by Watson and Crick has been confirmed by analysis of the frequency of nearest neighbours in the polymer formed when a normal DNA is used as primer. In these experiments one of the four 5′-triphosphates in the reaction was labelled with ^{32}P. During synthesis the phosphate group is attached to the 3′-OH of the sugar at the end of the growing chain. By degrading the product of the polymerisation with an enzyme that cleaves the bond joining the phosphate group to the 5′-OH of the sugar the ^{32}P label is transferred from its original base to its neighbour (Fig. 6). Repeti-

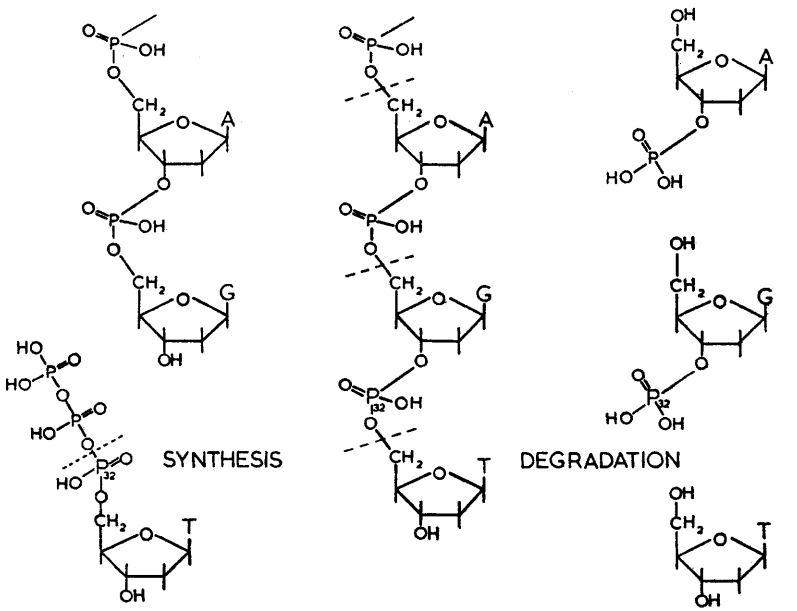

FIG. 6. *Synthesis of DNA occurs by addition of a nucleoside 5′-phosphate to the 3′-OH at the end of the growing DNA chain with the elimination of pyrophosphate from the nucleoside 5-triphosphate. Degradation by micrococcal or spleen DNAase cleaves the bond linking the 5′-OH to the phosphate group thus transferring the labelled phosphorus atom from its original nucleoside to its neighbour.*

tion of the experiment using a different labelled base each time provides data for calculating the relative frequencies of the sixteen possible pairs of bases. As an example of the application of the method, data for the product obtained using DNA from *Mycobacterium phlei* as primer is given in Table 2. The sums of the four columns show that the amounts

TABLE 2

Reaction No.	Labelled triphosphate	Isolated 3′-Deoxyribonucleotide			
		T	A	C	G
1	$dAT^{32}P$	TA	AA	CA	GA
		0·012	0·024	0·063	0·065
2	$dTT^{32}P$	TT	AT	CT	GT
		0·026	0·031	0·045	0·060
3	$dGT^{32}P$	TG	AG	CG	GG
		0·063	0·045	0·139	0·090
4	$dCT^{32}P$	TC	AC	CC	GC
		0·061	0·064	0·090	0·122
	Totals	0·162	0·164	0·337	0·337

Nearest-neighbour frequencies in the product formed using DNA from *Mycobacterium phlei* as primer in a polymerisation reaction. The totals of the four columns give the molar proportions of the bases in the product. The base composition of the primer DNA obtained from chemical analysis is: thymine 0·165, adenine 0·162, cytosine 0·335, guanine 0·338.

(Reproduced by permission from A. Kornberg, "Enzymatic Synthesis of DNA," p. 22, John Wiley and Sons Inc., New York, 1961.)

of adenine and thymine incorporated are equal as are the amounts of guanine and cytidine and that the chemical composition of the product is close to that of the primer. Comparison of the frequencies of the sixteen different pairs establishes that in the synthetic polymer base-pairing occurs as required by the Watson and Crick model and proves that the two chains of the helix are antiparallel. The frequency of AA and TT pairs are equal; CC and GG pairs are also equal as would be expected for either parallel or antiparallel chains. Matching of the other base pairs depends upon the polarity of the strands (Fig. 7). For antiparallel chains a pair CA will be

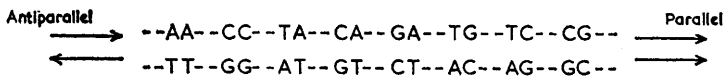

FIG. 7. *Representation of the two chains of a native DNA molecule to show the relation of different pairs of bases on the two strands. For parallel strands the pairs on each chain are read from left to right. For antiparallel chains pairs on the top strand are read from left to right and on the lower strand from right to left.*

matched on the other chain by TG, CT with AG, GA with TC, and GT with AC. Inspection of the data shows that there is good agreement for the frequencies of the two pairs in each of these four sets. A pair AT on one chain will be matched by an AT pair on the second chain; the frequency of this matching cannot therefore be determined. The same argument applies

to the pairs TA, CG, and GC. If the two chains of the helix were parallel equal frequencies would be expected for the pairs TA = AT, CA = GT, GA = CT, TG = AC, TC = AG, and CG = GC. Of these six sets only two show nearly equal frequencies for the pairs.

Observations on polymerase preparations from a variety of sources have shown that, while native DNA can act as primer, denatured DNA is more effective. A preparation of the enzyme from calf thymus is the only one so far made which is free of nuclease activity.[34] With this preparation, in the presence of denatured DNA as primer, the reaction stops when an amount of DNA equivalent to the added primer has been produced (Fig. 8). Richardson, Inman, and Kornberg[35] have studied the action of

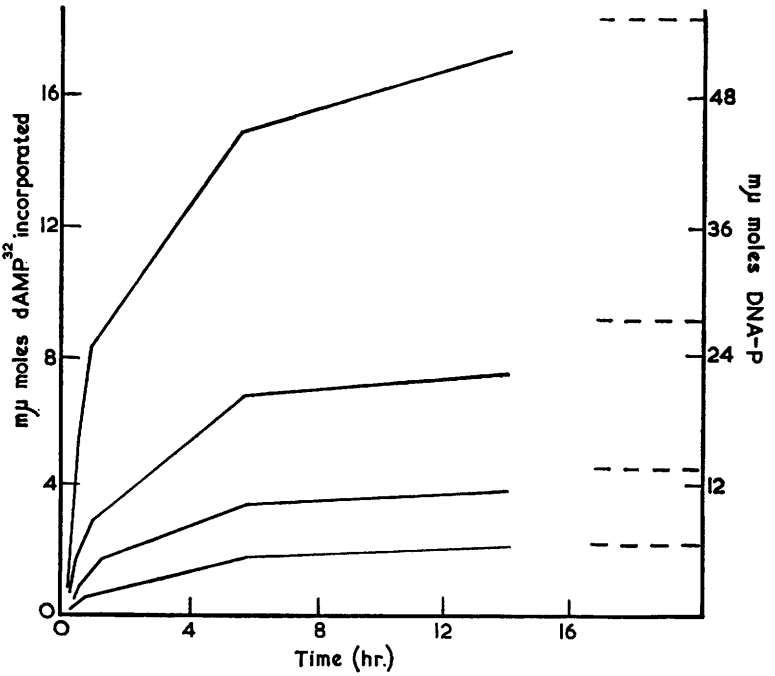

FIG. 8. *The amounts of DNA from the bacteriophage ϕX 174 used as primer in the presence of DNA polymerase from calf thymus and [^{32}P] dATP are shown as dashed lines at the right of the diagram. The curves show that polymerisation approaches a limit when an equal amount of DNA has been synthesised. The incorporation of 10^{-6} mole dAMP^{32} corresponds approximately to the synthesis of 3×10^{-6} mole of DNA phosphorus.* (Reproduced by permission from F. J. Bollum, *Progr. Nucleic Acid Research*, 1963, **1**, 17.)

purified polymerase from *E. coli* (still containing some nuclease activity), using as primer DNA from bacteriophage T7 which had been degraded with exonuclease III, an enzyme which removes nucleotides sequentially

[34] F. J. Bollum, *Progr. Nucleic Acid Res.*, 1963, **1**, 1.
[35] C. C. Richardson, R. B. Inman, and A. Kornberg, *J. Mol. Biol.*, 1964, **9**, 46.

from the 3'-OH ends of the two chains, thus leaving a central piece of twin helix with single chains at the ends. They observed initially a synthesis at five times the rate for the undegraded primer until an amount of DNA had been formed equivalent to that removed by the enzymic degradation. Following this, the reaction rate fell to that observed when undegraded primer was used. Electron micrographs showed the molecules present at the end of the first stage of the reaction to be linear rods with no evidence of single chains at the ends. The product of the second stage of the reaction consisted mainly of molecules showing branching.[36] The difference between the two types of polymerisation reaction is further emphasised by the observation that the first stage of the reaction, the repair of the degraded DNA, occurs when the system is incubated at 20°. Polymerisation based on the native DNA as primer proceeds rapidly at 37° but cannot be detected at 20°. It appears that, in the absence of specific nucleases, the polymerase cannot induce the separation of the strands of native DNA. Activation of native DNA by nuclease action enables it to act as primer but the product is not a succession of molecules identical with the initial primer molecules.

In the *in vitro* system, synthesis proceeds in only one direction along each chain, successive nucleotides being added to the 3'-OH of the growing chains. In contrast to this, replication of DNA within the cell proceeds along the twin helix in one direction only, both chains being synthesised together.[7] The replication of DNA in *E. coli* has been studied by Cairns.[7] Cells were grown in the presence of [^{3}H]thymidine, then carefully lysed, and the DNA deposited on millipore filters. Radioautographs showed the DNA molecules as continuous circles. Replication begins at a specific point on the ring and proceeds continuously round the circle. Cells which had been labelled for more than one generation time showed the parent DNA molecule as a continuous ring together with a new, partially duplicated section. The surprising observation was that both ends of the replicating section were still attached to the parent ring. Since the two chains of the molecule have to be untwisted to allow the daughter molecules to separate, there must clearly be some point in the DNA ring where free rotation is possible. This swivel point cannot be detected in electron micrographs of circular DNA. The implications inherent in these observations have been reviewed by Sibatani and Hiai.[37] They have considered the mechanisms involved in the separation of the daughter molecules, discussed the system of enzymes which appears to be required, and suggested a model for the replication of circular DNA.

Crothers[38] has developed a theory for the relaxation kinetics of the helix–coil transition assuming that the rate-limiting factor in the separation of the two chains is the viscous resistance to the rotation of the molecule. The frictional resistance to rotation calculated is about a thousand times

[36] R. B. Inman, C. L. Schildkraut, and A. Kornberg, *J. Mol. Biol.*, 1965, **11**, 285.
[37] A. Sibatani and S. Hiai, *J. Theoret. Biol.*, 1964, **7**, 393.
[38] D. M. Crothers, *J. Mol. Biol.*, 1964, **9**, 712.

greater than would be expected for the helix rotating in water. This large resistance may result from the high viscosity of the ordered structure of the water bound to the helix. Applying the calculated value of the frictional resistance suggests that DNA synthesis can proceed at the rate of $M = 7 \times 10^6$ per minute. From the extent of labelling of the circular *E. coli* DNA following incubation with [^{3}H]thymidine for various times Cairns calculates that DNA is replicated at a rate of $M = 40—60 \times 10^6$ per minute.[7]

The polymerase isolated from *E. coli* has been found to possess the further property of acting as a polymerisation initiator.[33,34] In the presence of dATP and TTP (for abbreviations, see footnote, p. 369) and in the absence of any added primer there is a lag period of up to 2 hours, then a stage of rapid synthesis of high-molecular weight material, followed finally by a period in which the polymer formed is degraded by the nucleases present in the solution. The polymer formed in this reaction was found to contain equal amounts of adenine and thymine. Nearest-neighbour analyses were performed and showed that the frequency of the pairs AT and TA were both 0·5 while the pairs AA and TT could not be detected. The product is thus a copolymer of A and T in regular alternating sequence. Similar experiments using mixtures of dGTP and dCTP were found to give, again after an initial lag period, a polymer containing G and C, but not necessarily in equal proportions, the molar G content ranging from 50 to 81%. Nearest-neighbour analysis showed G always associated with G and C with C. The polymer thus consists of a polydeoxyguanylate chain hydrogen bonded to a polydeoxycytidylate chain. Both of these synthetic polymers were found to be excellent primers, polymerisation in the presence of the appropriate triphosphates beginning immediately with no observable lag period. Investigations have shown that during the lag period there is a slow synthesis of a few macromolecules poly-dAT or poly-dGdC (see footnote, p. 369) and this is followed by an autocatalytic replication of these molecules, the rate of the reaction increasing exponentially. The mechanisms by which the first macromolecules are formed is not yet understood. Measurement of the molecular weight of the polymers after about 5% total reaction and at later stages has shown that the size of the macromolecules does not change during the course of the autocatalytic reaction. The smaller the amount of enzyme used in the reaction the higher is the molecular weight of the product.

When poly-dAT is used as primer, no synthesis of polymer occurs if dATP or dTTP are present alone. With poly-dGdC as primers on the other hand polymerisation occurs if either dGTP or dCTP are present. This mode of replication where only one strand of the double helix is used as primer is similar to the synthesis of messenger RNA on a native DNA template.

Since DNA carries the genetic information required for general metabolism its immutability seems to be a prerequisite of a normally functioning

cell or organism. Mutations or carcinogenesis which can be effected by various forms of radiation or by treatment with a variety of chemical compounds are believed to be produced primarily by the action of these agents on DNA. Carcinogenic hydrocarbons for example can be intercalated between the bases;[39] *X*-irradiation results in attack by the radicals formed by the radiolysis of water mainly on the cytosine moieties,[40] ultraviolet irradiation acts primarily by inducing dimer formation between neighbouring thymidine moieties on the same chain,[41] and the various derivatives developed from mustard gas and sulphur mustard aklylate the 7-position of guanine.[42] All these effects could lead to the insertion in the sequence of a wrong base at the next replication step and hence to a miscoding for some essential protein. A surprising discovery has been that many cells and organisms contain a system for the deletion of damaged parts of DNA molecules and the subsequent repair of the chain. If the DNA of a bacterium is labelled with [^{3}H]thymidine and then irradiated with ultraviolet light, partial degradation of the DNA and elimination of the label can be demonstrated. Concomitant with the degradation there is a synthesis of DNA. [^{14}C]Bromouracil (which can replace thymidine) present in the medium is incorporated into the DNA during the degradation phase. Studies of the DNA formed have shown that the bromouracil is incorporated in short sections distributed along the length of the molecule as would be required by a repair mechanism acting on damaged units randomly distributed throughout the DNA.[43] More recent work has suggested that such repair mechanisms may exist in mammalian cells, possibly even in brain, heart, and muscle cells which do not divide in the adult animal.[44] How DNA codes for its own repair is an interesting problem.

[39] L. S. Lerman, *J. Mol. Biol.*, 1964, **10**, 367.
[40] G. Scholes, *Progr. Biophysics Biophys. Chem.*, 1963, **13**, 59.
[41] A. Wacker, *Progr. Nucleic Acid Research*, 1963, **1**, 369.
[42] P. D. Lawley, "Acidi nucleici e loro funzione biologia," Istituto Lombardo, 1964, 25; P. D. Lawley and P. Brookes, *Nature*, 1961, **192**, 1081.
[43] D. Pettijohn and P. Hanawalt, *J. Mol. Biol.*, 1964, **9**, 395.
[44] S. R. Pelc, *J. Cell. Biol.*, 1964, **22**, 21.