*TAUTOMERIC FORMS IN A POLYNUCLEOTIDE HELIX AND THEIR BEARING ON THE STRUCTURE OF DNA**

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Some years ago the author initiated a study of the infrared spectra of nucleosides and nucleotides with the purpose of determining which tautomeric forms of the nucleotides predominated in aqueous solution.[1, 2, 2a] The biological role of DNA as the chemical repository and transmitter of genetic information confers upon this structural feature of the component bases a particular importance in molecular biology. Hydrogen bonds are known to contribute a large part of the energy which stabilizes and holds together the strands of the nucleic acid helices. From the viewpoint of nucleic acid structure it is thus clearly important to know which atoms have the hydrogens attached to them, since only those hydrogen bonding systems can exist which are compatible with the tautomeric forms present in the nucleic acids. It might be expected, therefore, that convincing evidence on tautomeric forms in aqueous solution could provide a criterion for deciding between certain alternative nucleic acid structures.[1] This possibility has been given present pertinence by a recent study of Hoogsteen,[3] who has shown that 1-methylthymine and 9-methyladenine co-crystallize in the unexpected hydrogen bonding scheme XII (see formulas below). This work and the suggestion[4] that it provides, "a strong indication that an arrangement differing from that of Watson and Crick is involved in nucleic acids...," have given added stimulus to the infrared studies in the hope that they might provide a criterion for distinguishing between two crystallographically possible nucleic acid structures. A note proposing bonding scheme XII specifically for an alternative DNA model has recently appeared.[4a] The application of tautomeric form as a criterion of structure is discussed in a later section.

It may further be noted that the suggestion of Watson and Crick[5, 6] that an infrequent change to a less stable tautomeric form may be the specific chemical change responsible for spontaneous mutation clearly adds interest to the investigation of this chemical property. The extreme sensitivity of the genetic test, however, will obviously present a challenge to chemical or physical methods which may be designed to approach it in sensitivity.

In pursuing the tautomeric studies it was a matter of the first importance to deal with aqueous solutions because of the great difference in physical and chemical properties between the usual organic solvents and the biologically important solvent, water. A further requirement for obtaining information of biological importance is that the method employed should be applicable not only to the mononucleotides but also to the nucleic acids themselves.

Although the highly sensitive tool of ultraviolet spectroscopy is amenable to work in aqueous solution, the similarity of the spectra of many of the nucleotides and the overlap of the component bands to produce single peaks in the nucleic acids make its application to this aspect of nucleic acid structure unreliable.

The determination of tautomeric equilibria of aminopyridines from ratios of dis-

sociation constants of alkylated pyridines has been discussed by Angyal and Angyal.[7] This method is not useful for present purposes, however, even if the assumptions upon which it is based introduce no errors, because the multiplicity of ionizable groups in the nucleic acids precludes the deduction of tautomeric form from the titration curves.

Although the author originally suggested the possibility of employing nuclear magnetic resonance to determine tautomeric structure of the nucleotides,[1] it appears that the method is incapable of supplying this information for nucleotides in neutral solution or for the nucleic acids. The exchange with the solvent prevents the observation of peaks caused by the tautomeric hydrogens,[8] though the nondissociable hydrogens of the nucleotides can be readily observed. A nuclear magnetic resonance study of DNA in water solution has yielded important information about the influence of the DNA upon the solvent,[9] but the hydrogens of the macromolecule, whether dissociable or not, cannot be observed.

Infrared absorption, being directly related to the vibrations of the atoms in the molecules, is responsive to changes in molecular structure in a manner which often closely reflects the chemical properties of the molecules. The infrared spectra of the nucleotides are characteristic, usually complex, and sufficiently different from each other to permit resolution of absorption bands in appropriate mixtures. This last property is important if the question of possible change in tautomeric structure upon helix formation is to be dealt with, and it must be dealt with if the information is to be reliably applied to the nucleic acids themselves.

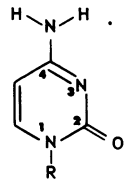
In spite of these potential advantages of infrared spectroscopy, however, the very strong absorption of water rules out the use of this solvent in those regions of the spectrum which are structurally most interesting and useful, i.e., those corresponding to the stretching vibrations of most single and double bonds. Gore *et al.*[10] first pointed out that this difficulty can be circumvented by the use of D_2O, which has a window in the region of double bond absorption.[10a] Sinsheimer *et al.*[11] took advantage of the transparency of D_2O as an infrared solvent in a valuable study of the spectra of a number of pyrimidine nucleotides at different pH values and over a wide range of frequencies. The method of varying the pH, however, while very informative in some respects, is not capable of providing reliable evidence of nucleotide tautomeric structure. When the heterocyclic bases are electrically charged by the loss or addition of a proton, there may be important changes in the electronic distribution in those bonds whose vibrations are responsible for the absorptions. Those views which were expressed[11] on tautomeric structure were generally based on analogy to other methods rather than on the solution spectra themselves. In the case of cytidine the authors preferred the imino form for the neutral molecule and the ammonium form (additional proton on the nitrogen external to the ring) for the cation. In the present paper we shall submit evidence favoring rather the amino and immonium forms (protonation of the ring nitrogen).[11a]

Blout and Lenormant have also employed D_2O to observe various biological materials[12] and highly polymerized DNA.[13] The spectra were too complex, however, to permit unambiguous interpretation on either the macromolecular or the monomer scale, and no tautomeric forms could be assigned.

It is clear that an understanding of the spectral properties of the polymers must

be based on an understanding of the spectral characteristics of the mononucleotides. For this purpose a number of water-soluble N- and O-alkyl model compounds were prepared.[1, 14, 15]

The structures which are important for the present study are shown below (R = methyl for structure IV; R = glucosyl for III; and R = ribosyl for the other structures).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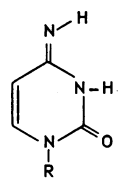
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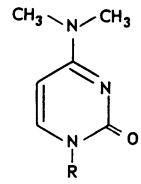
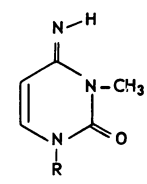
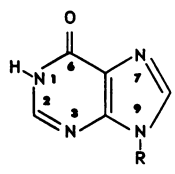
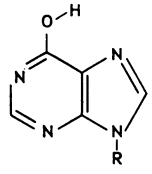
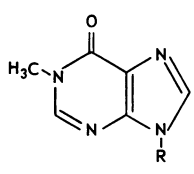

III


IV


V


VI


VII


VIII

The labile hydrogens of the nucleosides could be attached to either of two nitrogens in cytidine (cf. I and II) and to either oxygen or nitrogen in inosine (cf. V and VI).[16] The function of the alkyl groups of the models is to fix these compounds in structures which are isoelectronic with the possible tautomeric forms of the corresponding nucleotides. When the double bond arrangement is then determined by comparison of the spectra, the position of the hydrogen is uniquely determined by the laws of valence.

It was found that the spectrum of cytidine (I or II) in the region of double bond absorption was virtually the same as that of the model compound III and quite different from that of IV (for details of the spectra, see the following sections), thus establishing the structure of cytidine as the amino form I rather than the imino form II.

Similarly, inosine was shown to exist in the keto form, V, because of the close similarity of its spectrum with that of VII and its wide divergence from that of VIII.

After the discovery of the homopolynucleotides[17, 18] and of their interactions[19–24a] it became possible to apply the earlier spectral work on the monomers[1] to the determination of the tautomeric forms which exist in the polynucleotide helices.[2, 2a]

The polynucleotide interactions (A + U) and (I + C) were observed in the infrared,[2, 2a] and with the models available at the time, it was possible to make definite assignments of the tautomeric forms of two of the four bases and probable assignments of the other two. In the case of poly (I + C) it was shown that the inosine units possessed the keto structure both before and after interaction,[2a] in contrast to a previous proposal,[25] based on ultraviolet studies, that the tautomeric form of the inosine units changed upon interaction of poly I with poly C. In the

present paper evidence is presented that the cytidine units exist in the amino form. The possibility of observing the interaction of the polynucleotides by pairs has thus been invaluable in this, as in other studies, in permitting a separation of variables which were too numerous and too complex to be dealt with convincingly in the nucleic acids themselves.

A more detailed discussion of the deduction of tautomeric form from the spectra and the application of this information as a criterion of nucleic acid structure is given in the following sections.

In addition to permitting the determination of tautomeric form the spectra have shown certain characteristic changes in the frequency and intensity of the absorption bands[2, 2a] as the helix is formed. The most prominent changes have been decreases in intensity and increases in frequency—both changes in the opposite directions from those to be expected simply on the basis of increased strength of hydrogen bonding. The author has proposed[26] that as a first approximation these changes are the resultant of shifts produced by two major effects: (1) stronger hydrogen bonding and (2) a decrease in dielectric constant of the immediate environment of the vibrating groups (caused by the forcing of water away from the surfaces of the planar bases as they are packed tightly in the helix). Spectroscopic studies[27] of small molecules have shown that a decrease of dielectric constant produces a decrease in intensity and increase in frequency of carbonyl bands, though more specific interactions may also make important contributions to the spectral changes. It may be expected that further studies of the origins of the infrared spectral shifts will permit an improved insight into the nature of the chemical interactions in the core of the nucleic acid helix.

It is clear that the spectral changes[13] observed on the disruption of the DNA helix must have the same origins as those discussed above and that, in principle at least, it should be possible to construct a nucleic acid spectrum from its component parts.

Results and Discussion.—(a) *Tautomeric form of cytidine and poly C:* It is seen that cytidine (I), the amino model (III), and poly C (Fig. 1, *A*, *B*, *K* resp.) all have essentially the same spectrum in the double bond region, with a strong band at 1651 ± 2 cm^{-1} and a weaker band at somewhat lower frequency (1618, 1625, and 1617 cm^{-1}, resp.). The imino model (IV)[27a] (Fig. 1, *C*), however, has a markedly different spectrum with a strong band at 1657 cm^{-1} and moderate to strong bands at 1671 cm^{-1} and 1579 cm^{-1}. These spectra are believed to establish beyond any reasonable doubt that cytidine and poly C have the amino rather than the imino form in solution.

It is considered probable, though not essential to the above argument, that the strong bands at 1651 ± 2 cm^{-1} (Fig. 1, *A*, *B*, *K*) are caused to a large extent by the vibration of $C_2 = O$ bond and that the weaker bands at 1618 cm^{-1}, 1625 cm^{-1}, and 1617 cm^{-1} have a large contribution from C = N vibrations in cytidine, III, and poly C, respectively. It is not necessary to consider NH_2 deformations, which fall in the same general region, because the solvent is D_2O rather than water and because replacement of NH_2 with $N(CH_3)_2$ does not cause the disappearance of a band.

It is suggested that the 1657 cm^{-1} band of the imino model (IV) may be attributed largely to a C = O vibration and that the 1671 cm^{-1} absorption may

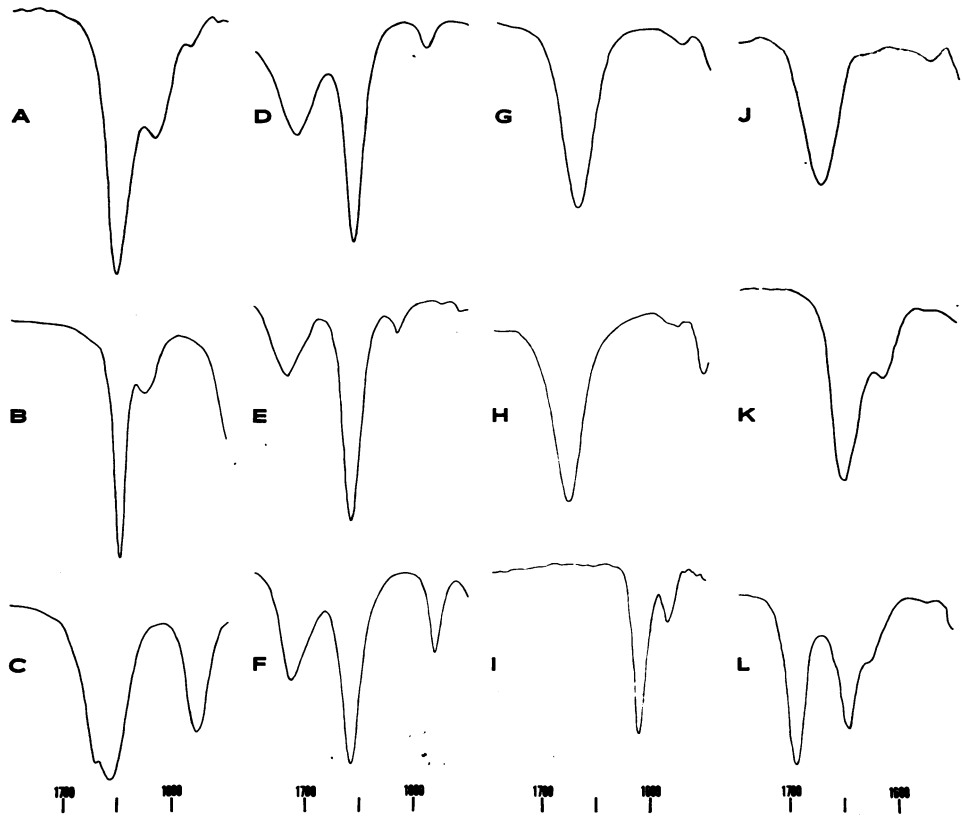


FIG. 1.—Infrared absorption spectra from 1550 cm^{-1} to 1750 cm^{-1} in D_2O solution. Abscissa, frequency of absorption in cm^{-1}; ordinate, per cent transmission on arbitrary scale. Conditions indicated in footnotes to Table 1. A, Cytidine; B, III (1-(β-D-glucopyranosyl-4-dimethylamino-2-pyrimidone). C, IV (1,3-dimethyl-cytosine). D, Acid solution of cytidine. E, Acid solution of III. F, Acid solution of IV. G, Inosine. H, VII (1-methylinosine). I, VIII (6-Methyoxy-9-β-D-ribofuranosylpurine). J, Poly I. K, Poly C. L, Poly (I + C).

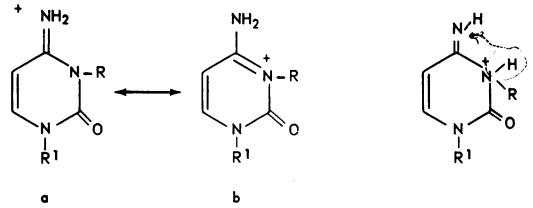
have a large contribution from a C = N vibration.

It was found that the result of changing the solvent from D_2O to chloroform is to increase the separation of the two strongest absorptions of IV (peaks at 1689 cm^{-1} and 1663 cm^{-1} in chloroform). With III in chloroform (R = tetraacetyl-β-D-glucopyranosyl) there is also an increase in the frequency of the two strongest bands (to 1657 cm^{-1} and 1638 cm^{-1}) but a decrease rather than increase in band separation.

The possibility that the difference between the spectra of III and IV is due to protonation of IV is ruled out by the quite different acid spectra in the following section and by the fact that the spectrum of IV was determined in neutral, buffered solution (pH 7.4.)

(b) *Tautomeric form of cytidine in acid solution:* The spectra of cytidine and of compounds III and IV (Fig. 1, *D*, *E*, *F*) are all nearly identical in acid solution, indicating that they have an isoelectronic[28] structure. It is the model compound, IV, which shows that this common cation must have the structure IX because this structure would be more stable than the alternative, X. IX (R = R′ = CH_3)

can be stabilized by resonance between the equivalent forms a and b, whereas X cannot be so stabilized. The cytidine cation must therefore have structure IX (R = H; R′ = ribofuranosyl) and hence have the additional proton on the ring nitrogen rather than on the external amino group.[11a]



IX X

(c) *Tautomeric form of inosine and poly I:* Inosine (V: Fig. 1, *G*), poly I (Fig. 1, *J*) and 1-methylinosine (VII: Fig. 1, *H*) all have spectra with a single strong band in the region of double bond absorption (1673 cm^{-1}, 1677 cm^{-1}, and 1678 cm^{-1} resp.), suggesting a common keto structure. The radically different spectrum of the enol model (VIII: Fig. 1, *I:* bands at 1611 cm^{-1} and 1586 cm^{-1}) establishes the correctness of this interpretation and rules out the enol structure VI.[29]

(d) *Tautomeric forms in poly (I + C):* The spectrum (Fig. 1, *L*) of this interaction product shows a strong band at 1697 cm^{-1}, another at 1648 cm^{-1} and a shoulder of moderate intensity at 1630 cm^{-1}. The band at 1697 cm^{-1} is attributed to the inosine units (the reason for the shift from 1677 cm^{-1} is discussed in the introduction and in ref. 26) and shows that they are in the keto form since the N-alkyl model absorbs at 1678 cm^{-1} whereas the enol model absorbs at 1611 cm^{-1}, a region quite free of strong absorption in the poly (I + C) spectrum (Fig. 1, *L*). The remainder of the curve (Fig. 1, *L*) shows a close similarity in general shape and appearance to the curve of the amino model, III (Fig. 1, *B*) and of poly C (Fig. 1, *K*) and marked differences from the curve of the imino model, IV (Fig. 1, *C*). The following specific points may be observed: both major cytidine absorptions are present (at 1648 cm^{-1} and ~1630 cm^{-1} (sh.)) with approximately the same relative intensities, though with slightly shifted frequencies and with lower resolution.

Two of the absorptions characteristic of the imino form (1671 cm^{-1} and 1579 cm^{-1}; Fig. 1, *C*) are completely lacking in the poly (I + C) spectrum (Fig. 1, *L*). These spectra, therefore, lead to the conclusion that in this interaction product the cytosine units are in the amino rather than the imino form. It should be noted that this conclusion does not depend upon X-ray data, nor does it presuppose any particular helical model.

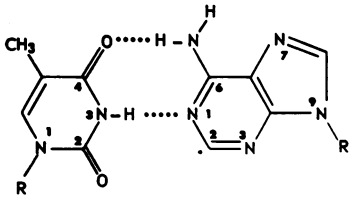
Nature of the helical interaction product, poly (I + C): The first report of the interaction of poly I with poly C indicated that a 1:1 complex was formed and that the fibers obtained from the interaction product had an X-ray diffraction pattern resembling that of RNA,[22] whereas the diffraction pattern of poly (A + U) had resembled that of DNA.[20]

Observations of the infrared spectra were employed to study the tautomeric form[28] and a preliminary attempt to obtain further information about the structure

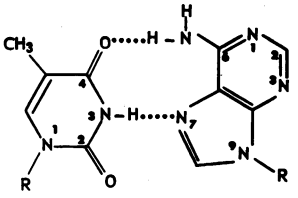
in solution was made by measuring the optical rotation of poly (I + C) and comparing it to that of poly (A + U). The author found that poly (I + C) had the same specific rotation as poly (A + U) $[\alpha]_D^{25°} = 290°$; $[\alpha]_{365}^{25°} = 1200°$; Doty *et al.*[24] report $[\alpha]_D^{25°} = 300°$ for poly (A + U)) and a similarly sharp decrease in optical rotation upon heating (melting-out curve).[30] These experiments suggested the possibility that poly (I + C) might, like poly (A + U), have a DNA-like structure in solution, though the possibility of drawing a definite conclusion from such evidence will have to await a better knowledge of the dependence of rotation upon structure.

In further experiments with poly (I + C) Davies has obtained highly crystalline fibers with an X-ray diffraction pattern characteristic of a DNA-like structure.[31] It thus appears that the poly (I + C) helix has a structure like that of DNA but that this structure could be composed either of a hydrogen bonding scheme of the Watson-Crick type (XIII) or of the alternative arrangement, XIV (see following section).

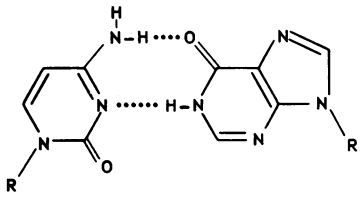
Application to the structures of poly (I + C) and of DNA: The structural model of DNA proposed by Watson and Crick[32] required the presence of the purines and pyrimidines in two specific complementary bonding arrangements, one between adenine and thymine (XI) and the other between guanine and cytosine (the addition of the third hydrogen bond as shown in XV follows the proposal of Pauling and Corey.)[33] The first specific proposal of a fundamentally different bonding arrangement in a case clearly applicable to the nucleic acids was that the second



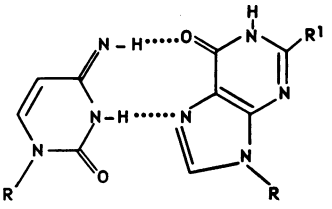
XI



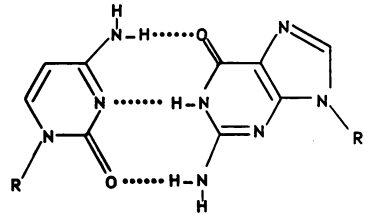
XII



XIII



XIV



~~XIV~~ XV

strand of poly U in the three-stranded helix poly (A + 2U) was bonded to the N_7 position of adenine[34, 35] as shown in XII (the other poly U chain was believed to be bonded in the Watson-Crick manner, XI, at N_1 of adenine). It has since been found[3] that 1-methyl-thymine and 9-methyladenine co-crystallize in the arrangement XII rather than XI, and this has led to the proposal[4] cited in the introduction that an arrangement differing from that of Watson and Crick may be involved

TABLE 1

INFRARED SPECTRA IN D_2O SOLUTION[a]

Compound	ν_{max} (cm^{-1})		
A Cytidine	1650	1618	...
B III[b] (amino model)	1649	1625	1548
C IV[c] (imino model)	1671	1657	1579
D Acid cytidine[d]	1712	1657	1591
E Acid III[d]	1718	1658	1617
F Acid IV[d]	1716	1658	1581
G Inosine	1673	...	...
H VII[e] (keto model)	1678	1555	...
I VIII[f] (enol model)	1611	1588	...
J Poly I[g]	1677	...	...
K Poly C[g]	1653	1617	...
L Poly (I + C)[g]	1697	1648[h]	~1630 (sh.)

[a] These qualitative spectra were measured on a Beckman IR7 grating-prism spectrophotometer in D_2O solution, solvent compensated (0.025 mm path length). The instrument was purged continuously with dried air, and for some of the spectra, high scale expansion was employed.

[b] See references 1 and 14.

[c] Prepared by the method of Hilbert[27a] and purified by crystallization and sublimation (mp 150°). This spectrum was measured in 0.1 *M* sodium cacodylate buffer, pH 7.4, and in unbuffered D_2O, the same result being obtained in each case.

[d] The cytidine solution was made approximately 0.1 *N* in acid by adding D_2SO_4. The crystalline hydroperchlorate[1] of III was used. The crystalline hydroiodide (called the methiodide by Hilbert because of its method of preparation) of IV and a sulfuric acid solution of IV (pH 2) were both measured and gave the same spectrum.

[e] Bredereck and Martini[43] reported that the product of the reaction of diazomethane with triacetylinosine was 1-methylinosine but made little serious attempt to purify or characterize the product. It has now been found that the reaction product, after ether precipitation as described, contains the 6-methoxy and 1-methyl derivatives as well as other substances. Pure 1-methylinosine has been obtained by column chromatography of the reaction mixture and found to be a crystalline substance of mp 209–210°, λ_{max} = 251 in H_2O. Details of the purification and characterization will be published at a later date.

[f] The author is indebted to Dr. H. Schaeffer for a sample of the O-methyl compound.

[g] The polynucleotides used were the very generous gift of Dr. Marie Lipsett and Dr. Leon Heppel. The solutions were 0.05 *M* in sodium chloride and 0.01 *M* in sodium cacodylate buffer, pH 7.0. The poly I was versene-dialyzed and the poly C water dialyzed.

[h] The poly (I + C) spectra obtained in these experiments are essentially the same as those reported previously,[2a] but are of considerably higher quality because of the purging of the spectrophotometer in these experiments with dry air. At the high scale expansion used (90–100% T expanded full scale) atmospheric water vapor was not completely compensated in the earlier spectra. With these dilute solutions (about 0.009 *M* in bases) slow pen response and scanning speed are employed to minimize noise.

in nucleic acids. A recent note[4a] has proposed this bonding scheme, (XII), specifically for DNA, the cytosine units in the G-C pairs being in the imino tautomeric form, although the reason for the selection of the imino form was not stated. The authors proposed the alternative model[4a] for DNA but stated that it, "...introduces some stereochemical difficulties which make it less likely than the Watson-Crick model." Some pertinent aspects of the alternative bonding schemes and of the DNA model based upon them are discussed below.

It should be understood, as background for the subsequent argument, that a requirement of any two-stranded model for DNA is that there be equivalence of the glycosidic bonds of the base pairs (i.e., that it be possible to superimpose simultaneously both of the glycosidic bonds of successive base pairs; a more detailed statement of this condition has been given by Pauling and Corey).[33]

It is possible that the arrangement shown in XII could form the basis of a DNA structure which satisfied the above-mentioned principle of equivalence. But if this is the adenine-thymine pairing in DNA, then in order to preserve the equivalence of the glycosidic bonds of the base pairs, the guanine-cytosine bonding pattern

would have to be that indicated in XIV ($R' = NH_2$).[36] A necessary feature of this structure is that the cytosine units must exist in the imino form. In the Watson and Crick bonding scheme the cytosine units are in the amino form.

The spectral evidence presented above demonstrates that in poly (I + C) the cytosines exist in the amino form, and, therefore, that the bonding scheme cannot be that shown in XIV (R = H). Further, since cytosine has been shown to have the amino form in cytidine, in poly C, and in the DNA-like helix, poly (I + C), it is most reasonable to expect that it will maintain in DNA the tautomeric form which has proved to be the more stable one throughout this range of molecular structures.

Having stated this conclusion, however, let us ask whether it is likely to be changed by a more explicit consideration of two obvious structural differences between DNA and the polynucleotide helices. The latter have hydroxyl groups on the C_2 atoms of the sugar residues where the former has hydrogen atoms. It was found in the case of poly (A + U) that this difference resulted in a slight increase in the diameter of the molecule,[25] but the arrangements of the bases deduced from the X-ray data[25, 31] indicate that this increase is not the result of an increased separation of the coplanar bases (if the separation of the bases were increased very much, of course, effective hydrogen bonds could not be made). It is concluded that the presence of an additional hydroxyl group on the sugar is quite unlikely to alter the tautomeric forms of the bases.

The second point may then be considered: is the difference between inosine and guanosine sufficient to result in a change in the tautomeric form of the cytidine units on going from poly (I + C) to DNA? It can be seen that in XIV the amino group (R') by which the two bases differ is now remote from the cytosine and can no longer interact with it as in XV. We conclude that since the structure XIV ($R' = H$) is not sufficiently stable to exist in preference to XIII, it is most unlikely that the presence of an additional amino group ($R' = NH_2$) at a point remote from the area of hydrogen bonding would render XIV ($R' = NH_2$) more stable than XV (for structure XIV the argument would be the same if this functional group should exist in the imino tautomeric form).

The following arguments from the stabilities of different base pairings are presented in support of the structural conclusion reached above on the basis of the criterion of tautomeric form. Pauling and Corey[33] have pointed out that a third hydrogen bond can be formed between guanine and cytosine (XV) and suggested that this conclusion strengthens the arguments of Watson and Crick concerning the role of complementariness of structure of the two DNA polynucleotide chains in the duplication of the gene. The additional hydrogen bond in XV might be expected to confer higher stability upon the GC pair than the two-bond AT pair (IX) possesses, and, in fact, the work of Marmur and Doty[37] has shown a linear increase in melting temperature of DNA samples as the GC content is increased. The most reasonable, although not the only possible conclusion, is that the source of this greater stability is the additional hydrogen bond possible in the GC pair. While it could be supposed that GC forms only two hydrogen bonds, and that this pair possesses greater stability in a helix because these hydrogen bonds are intrinsically stronger than those in the AT pair, serious doubt is cast on this possibility by the fact that poly (I + C) has a lower melting temperature than poly (A + U)[10a].

Structure XIV (R′ = NH_2) would thus require the loss of the unique possibility of forming three hydrogen bonds, which the GC pair possesses, and which is a presumed major source of its higher stability.

The equivalence of base ratios[38] is a fundamental chemical fact which has been of great importance to our understanding of the nueleic acids and one which should be accommodated by any model for DNA. In the present case, however, it provides no criterion for distinguishing between the two models since either pair of bonding schemes would satisfy this condition.

The crystallographic data on DNA gave evidence only of rough compatibility with the Watson and Crick model[32, 39, 40] at the time of its publication, but more detailed studies have greatly improved the agreement between the model and the X-ray data.[41, 42]

The DNA model of Watson and Crick is thus supported by a variety of positive evidence, and no seriously contradictory evidence has as yet been advanced. While the alternative model based on structures XII and XIV is also compatible with some of the experimental evidence, in our view the present experiments and deductions from them make this alternative model quite improbable.

* Abbreviations and definitions: Poly I, polyinosinic acid sodium salt;[17] poly C, polycytidylic acid sodium salt; poly (I + C), the interaction product of poly I and poly C; poly A, polyadenylic acid sodium salt; poly U, polyuridylic acid sodium salt; poly (A + U), the 1:1 interaction product of poly A and poly U; poly (A + 2U), the 1:2 interaction product[34] of poly A and poly U; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; AT, adenine-thymine; GC, guanine-cytosine. The tautomeric forms of the nucleosides are those structures which differ in the position of attachment of a labile or exchangeable hydrogen and consequently in the positions of the double bonds. Thus, for example, the amino form of cytidine has the structure I and the imino form, the structure II; the keto form of inosine has the structure V and the enol form, the structure VI. The numbering system used for the purines and pyrimidines is indicated in I and V.

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[28] That is to say, the structures are the same in those structural features which govern the distribution of the double bonds; there will be a similar electron distribution whether R = H or R = methyl, the important difference being that when R = H it is dissociable and when R = methyl it is not.

[29] The interesting study of D. J. Brown, and S. F. Mason, *J. Chem. Soc.*, 682 (1957), has reported the infrared spectra of a number of methylated purines in chloroform solution and in the solid state. Though the authors generally favor the ketonic form of the oxo purines, as far as the tautomeric form of inosine is concerned the evidence cannot be considered conclusive. The required evidence may be provided by the compounds V, VII, and VIII. Of these only V, (R = CH_3) was reported. The O-methyl and N-methyl compounds corresponding to VII and VIII, but with R = hydrogen, were reported. Since the tautomeric structure of the imidazole ring is not defined when R = H, however, the result can be applied to inosine only by assuming that the spectrum of the pyrimidine ring is independent of the imidazole ring with which it is conjugated. Further, as indicated earlier, it cannot be assumed that the tautomeric forms in organic solvents are necessarily the ones existing in the nucleic acids. The authors presented a number of valuable correlations and proposed that the strong absorption of 9-methylhypoxanthine (1679 cm^{-1} in

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*NUCLEOSIDEDIPHOSPHATASE ACTIVITY IN THE GOLGI APPARATUS AND ITS USEFULNESS FOR CYTOLOGICAL STUDIES**

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Cytochemical information regarding Golgi material is limited. Early staining methods suggested the presence of alkaline phosphatase activity in the Golgi substance of intestinal mucosa and other cells, and of acid phosphatase activity in the Golgi "zone" of epithelial cells and macrophages.[1] However, it is clear now that the integrity of both Golgi apparatus and acid phosphatase-rich granules associated with it[2] could not survive the acetone fixation and paraffin embedding then employed. Biochemical studies of Golgi fractions isolated from homogenates of epididymis have indicated the presence of acid phosphatase activity in the Golgi apparatus.[3] Electron microscopy established the presence in the Golgi fractions of numerous membranous arrays characteristic of the organelle, but a critical study of the purity of the fractions is needed, in view of the observations to be presented here, before attributing the acid phosphatase activity to the Golgi apparatus.

We are presenting evidence indicating that in epididymis, as in all but two of the many cell types studied, acid phosphatase activity is concentrated not in the Golgi apparatus but in small granules located in the Golgi region of the cell. From the effects of the detergent, Triton X-100, to be described here and from considerations