INTERBASE VIBRATIONAL COUPLING IN
G:C POLYNUCLEOTIDE HELICES

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Abstract.—The carbonyl stretching vibrations of G and C residues undergo frequency changes when a helical complex is formed from nonordered G and C components. We have prepared a series of two-stranded G:C helices having chemical and isotopic (^{18}O) substitutions which lead to variation of the observed vibrational frequencies. Infrared spectra of these helices clearly demonstrate that vibrational coupling occurs between the guanine and cytosine residues in the helix. As a result of this coupling through the hydrogen bonds, the two carbonyl bands undergo frequency shifts of equal magnitude and opposite sign, with mixing of G and C character in the observed interacted vibrations.

Helix formation between complementary polynucleotides leads to marked and characteristic changes in frequency and intensity of infrared bands of the polymers.[1-5] These spectral changes have been observed principally in the region of 1500 to 1800 cm^{-1} which includes the vibrational stretching frequencies of the carbonyl groups and heterocyclic rings. These chemical groupings are directly involved in base pairing and stacking interactions in helices, and thus are most likely to be sensitive to formation of helical secondary structures. The carbonyl bonds of G and C, whose vibrations we observe in the infrared, are indirectly linked by much weaker hydrogen bonds in a precise geometrical arrangement. These G and C carbonyl bonds are vibrationally coupled in the hydrogen-bonded base pairs, whereas other observed vibrations show no evidence of coupling.

Materials and Methods.—The infrared spectra of D$_2$O solutions were measured with a Beckman IR-7 spectrophotometer using matched CaF$_2$ cells of fixed path length.[1-5]

Poly C was purchased from Miles Chemical Co., and poly G from Schwartz Laboratories. Our synthesis and characterization of poly-5-bromocytidylic acid have been reported,[6] and those of poly-5-methylcytidylic acid will be described in a later paper. Syntheses of both of these polymers by somewhat different methods have been described previously.[7, 8] We have also reported previously the synthesis and infrared spectrum[9] of 5'-GMP-6-^{18}O.

We have found that the conditions shown in the legends of Figures 1 to 3 lead to complete formation of 1:1 complexes. From the infrared spectra we can conclude that other molecular species (i.e., complexes having other than 1:1 stoichiometry, self-structures of the components, and uninteracted components) are not present in significant concentrations.[3-5, 10] The spectrum of the fully polymeric helix poly G:poly C (Fig. 4) closely resembles that of GMP:poly C (Figs. 1 and 5), strongly suggesting similar origins of changes in vibrational spectra upon interaction of the components.

Results.—Infrared spectra of the three pairs of chemically distinct *G:C* helices are shown in Figures 1 to 3 and are summarized in Table 1. The second spectrum of each pair is that of a helical G:C molecule differing from the first
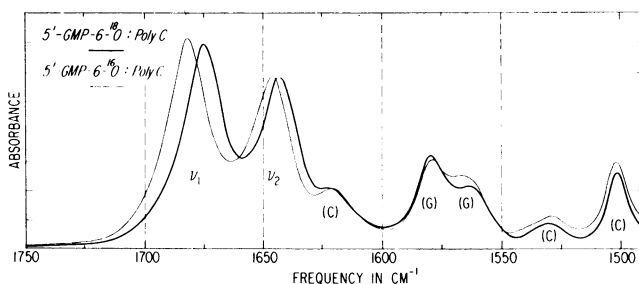
FIG. 1.—Infrared spectra in D_2O solution of two-stranded helices formed between poly C and 5'-GMP. ^{16}O complex: ———; ^{18}O complex———. ^{16}O complex: [GMP] = [poly C] = 0.134 M; [Na$^+$] = 1.9 M; [H$_2$PO$_4^-$ + HPO$_4^=$] = 0.089 M; pD = 8; temp. = $-6°$. ^{18}O complex: [GMP] = [poly C] = 0.128 M; [Na$^+$] = 1.8 M; [H$_2$PO$_4^-$ + HPO$_4^=$] = 0.085 M; pD = 8; temp. = $-7°$. For both spectra scale expansion = 1.4-fold, and path length = 24.8 μ. Ordinate index marks are 0.1 absorbance unit apart in this and all of the following spectra. ν_1 and ν_2 are the coupled carbonyl vibrations of G and C. The bands designated (G) or (C) are almost entirely due to vibrations of the indicated base[3, 5, 6] and show no detectable intermolecular coupling. The small isotope shift in the G band at $\sim$1568 cm^{-1} is observed also in unassociated 5'-GMP.[6] The band designations in this figure apply also to the following spectra, though the (C) frequencies vary somewhat in different molecules.
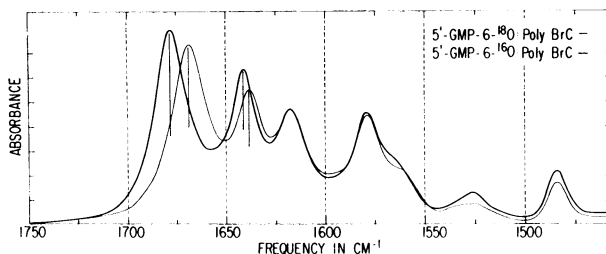

FIG. 2.—Infrared spectra in D_2O solution of two-stranded helices formed between poly BrC and 5'-GMP. ^{16}O complex———: [GMP] = [poly C] = 0.089 M; [Na$^+$] = 1.9 M; [H$_2$PO$_4^-$ + HPO$_4^=$] = 0.09 M; pD = 8; temp. = 0°. ^{18}O complex———: [GMP] = [poly C] = 0.084 M; [Na$^+$] = 1.8 M; [H$_2$PO$_4^-$ + HPO$_4^=$] = 0.84 M; pD = 8; temp. = $-2°$. For both spectra scale expansion = 2.0-fold, and path length = 24.8 μ.

only in having a G component specifically labelled with ^{18}O in the 6-carbonyl group. In each case there is an isotopic frequency shift in the highest frequency carbonyl band, ν_1, and, more strikingly, a frequency decrease also in the band next lower in frequency, ν_2. Since isotopic shifts occur in the bands of *both* G and C whereas isotopic substitution occurs only in G, this mixing or distribution of isotopic shift must result from *interstrand* vibrational coupling between the hydrogen-bonded base pairs. A detailed statement of this relationship is given in the discussion.[11]

The frequency shifts upon helix formation of the two carbonyl bands in non-isotopically substituted helices (increase of one and decrease of the other frequency from uninteracted values) are themselves suggestive of vibrational couplings and can be formally rationalized in these terms.[12] Other explanations could be advanced to account for the observations, however, and the coupling
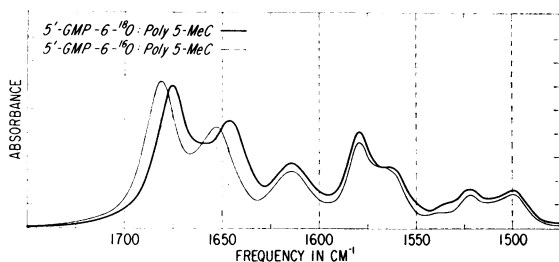
FIG. 3.—Infrared spectra in D_2O solution of two-stranded helices formed between poly *MeC* and 5'-GMP. ^{16}O complex ——: [GMP] = [poly C] = 0.043 M; [Na$^+$] = 1.8 M; [H$_2$PO$_4^-$ + HPO$_4^-$] = 0.86 M; pD = 8; temp. = $-6°$; scale expansion = 1.5 fold; path length = 57.8 μ. ^{18}O complex ——: [GMP] = [poly C] = 0.045 M; [Na$^+$] = 1.9 M; [H$_2$PO$_4^-$ + HPO$_4^+$] = 0.090 M; pD = 8; temp. = $-6°$; scale expansion = 3.6 fold; path length = 24.9 μ.
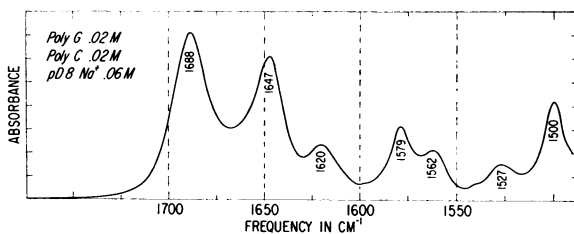

FIG. 4.—Infrared spectrum in D_2O solution of two-stranded helix formed between poly G and poly C. The G:C complex did not form spontaneously upon mixing but required an annealing procedure because of strong poly G self structure. Ordinate scale expansion = 5.0-fold. Path length = 57.6 μ. The spectrum closely resembles that of Fig. 1 (^{16}O complex) except that the frequency of ν_1 is somewhat higher. Frequencies of bands are indicated in the figure.

interpretation would remain inconclusive. The isotopic experiments demonstrate the phenomenon, though the nature of the coupling is obviously the same in all the examples. It is important to observe that the frequencies of the five bands below $\sim$1640 cm^{-1} show no detectable isotopic shift in any of the systems studied (Figs. 1–3). A slight qualification of this statement is appropriate for the G vibration at $\sim$1568 cm^{-1} (^{16}O-GMP), which does show a slight isotopic shift. This change, however, is present in uninteracted 5'-GMP and is not appreciably influenced by helix formation. We can show that the sum of the isotopic frequency shifts in ν_1 and ν_2 is equal to the isotopic shift in uninteracted G alone: $(^{16}\nu_1 - {}^{18}\nu_1) + ({}^{16}\nu_2 - {}^{18}\nu_2) = ({}^{16}\nu G - {}^{18}\nu G)$.

We observe a slight deviation of this sum from constancy in the three helices and attribute it to minor variation in the isotopic content of the GMP-^{18}O used in preparing them.[13] We have used this summation value in subsequent calculations.

Discussion.—^{18}O substitution produces vibrational frequency shifts in the carbonyl groups because of the mass change, the force constants being unaffected by the substitution. Changes in chemical structure, as in the substitution of a

TABLE 1.   *Observed carbonyl absorption maxima and frequency shifts in G:C helices.**

Helix†	Observed Carbonyl Frequencies in G:C Helices‡, §				Difference between Two Carbonyl Frequencies‡, §			
	^{16}O		^{18}O		^{16}O		^{18}O	
					Unasso-ciated	Helix	Unasso-ciated	Helix
	ν_1	ν_2	ν_1	ν_2	$\nu_G - \nu_C$	$\nu_1 - \nu_2$	$\nu_G - \nu_C$	$\nu_1 - \nu_2$
GMP:poly C	1682.4	1646.3	1675.0	1643.2	8.5	36.1	−4.0	31.8
GMP:poly BrC	1677.5	1640.6	1668.5	1638.5	21.5	36.9	9.0	30.0
GMP:poly MeC	1681.2	1653.0	1675.5	1646.5	4.5	28.2	−8.0	29.0
poly G:poly C	1688.0	1647.0	...	...				

	Isotope Shifts§,**			Carbonyl Absorption Maxima of Nonassociated G and C Components			
	$^{16}\nu_1 - {}^{18}\nu_1$	$^{16}\nu_2 - {}^{18}\nu_2$	$(^{16}\nu_1 - {}^{18}\nu_1) + (^{16}\nu_2 - {}^{18}\nu_2)$				
GMP:poly C	7.4	3.1	10.5	5'-GMP	1664.5	poly C	1656.0
GMP:poly BrC	9.0	2.1	11.1	5'-GMP-^{18}O	1652.0	poly BrC	1643.0
GMP:poly MeC	5.7	6.5	12.2			poly MeC	1660.0

* All entries in the table are observed values or differences of observed values. Frequencies are expressed in cm^{-1}, and are estimated to be accurate to 0.5 cm^{-1}. The values for helical structures were measured at temperatures which led to complete interaction, as indicated in legends of Figs. 1 to 3. The values for the nonassociated components were measured at 33° and in a few cases vary slightly with temperature. The largest such variation occurs with poly C, which has $\nu_{max} = 1656.0$ cm^{-1} at 33° and 1657.5 cm^{-1} at +4°.

† These abbreviated notations signify two-stranded helices formed between 5'-GMP (either the ordinary or 6-^{18}O labeled molecule) and polycytidylic acids, as follows: GMP:poly C, GMP, and polycytidylic acid; GMP:poly BrC, GMP, and poly-5-bromocytidylic acid; GMP:poly MeC, GMP, and poly-5-methylcytidylic acid. Poly G:poly C is the two-stranded helix formed between polyguanylic acid and polycytidylic acid.

‡ The notation ^{18}O indicates specific ^{18}O substitution ($\sim$90 atom %) in the 6 position of the 5'-GMP (cf. ref. 3) used to prepare the G:C helices. The notation ^{16}O indicates that ordinary 5'-GMP was used. Poly C and poly G were not labelled with ^{18}O in any of the experiments.

§ ν_G signifies the vibration frequency of uninteracted GMP and ν_C that of the polycytidylic acid listed in the first column. ν_1 refers to the highest frequency band in the helical complexes (about 1680 cm^{-1}) and ν_2 that of the next highest frequency (about 1645 cm^{-1}). There is mixing of G and C character as a result of vibrational coupling (see text), the calculated amount of mixing being given by a^2 in Table 2.

** $^{18}\nu_1$ and $^{18}\nu_2$ are the higher and lower carbonyl frequencies, respectively, of helices in which the GMP component is labelled with ^{18}O. ν_1 and ν_2 refer to frequencies of the corresponding bands of helices in which the GMP component is unlabelled. Where it is desired for clarity, the superscript 16 may also be used to indicate that the frequency refers to an unlabeled molecule, as in $^{16}\nu_1$. The differences $^{16}\nu_1 - {}^{18}\nu_1$ and $^{16}\nu_2 - {}^{18}\nu_2$ correspond to the separations of the vertical bars in Figs. 2 and 3. As we have indicated in the text, the summation of the first two columns of this entry, given in the third column, is equal to the isotopic shift of the uninteracted GMP-^{18}O used in the preparation of that particular complex. The value of 1652 cm^{-1} given in this Table for GMP-^{18}O is that of the material when it was originally prepared.

methyl group or bromine for hydrogen, on the other hand, cause frequency shifts both as a result of mass differences and of changes in force constants.

In the G:C pair we may consider the observed interaction to be composed of two parts, one of which is itself complex: The first part consists of hypothetical association without coupling, in which the frequencies ν_G and ν_C assume new values, ν_G^0 and ν_C^0. The elements which compose this hypothetical first part of the interaction include hydrogen bonding, specific and nonspecific solvent effects, dipole interactions, and other factors.[14, 15] These factors may influence vibrational frequencies and force constants of any of the vibrations of the molecule including, but not restricted to, the two higher frequency vibrations which undergo coupling. Presumably, these environmental factors are responsible for the changes of the lower frequency bands upon interaction (see Fig. 5).

The second part of the overall interaction is vibrational coupling. This purely
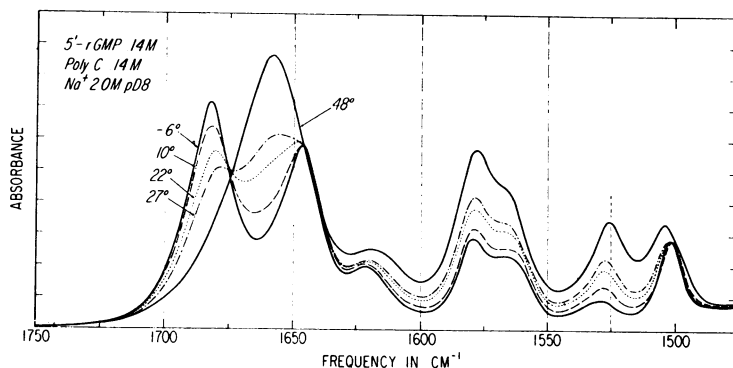
FIG. 5.—Temperature dependence of infrared spectrum of two-stranded helix formed between poly C and 5'-GMP. On cooling, the spectrum becomes essentially constant by $\sim0°$. $T_m = 26°$ under these conditions; ordinate scale expansion = 1.4-fold; path length = 24.8 μ.

mechanical interaction occurs with mixing of the two vibrations and without change of force constants.

The coupling may be formulated in terms of standard first-order perturbation theory (see, for example, Herzberg,[16] Eq. II, p. 291) as

$$E = \bar{E} \pm \frac{1}{2} \sqrt{4W^2 + \delta^2}, \qquad (1)$$

where the two values of E are the perturbed energies (in our case the observed frequencies of the interacted pair ν_1 and ν_2, $\bar{E} = \frac{1}{2}(E_1^0 + E_2^0)$, the mean of the two uncoupled energy levels (or equivalently the mean of the coupled frequencies observed in the helix). W is a matrix element (given as Wni by Herzberg[16]) which measures the strength of the coupling interaction, and $\delta = E_1^0 - E_2^0$ is the difference between the uncoupled levels. Use of this equation would also require that other vibrations be sufficiently distant not to be involved in the intermolecular coupling, a condition which we assume to be satisfied in these cases in view of the lack of detectable isotope effect in the other bands in the helices (Figs. 1–3). From a knowledge of δ and W it is possible to calculate the coefficients a and b in the eigenfunctions of the coupled states.[17] The fractional mixing (percentage of G or C character) is then expressed by a^2 and b^2.

We assume that the coupling constant W is not significantly affected by isotopic substitution[18] and thus is the same in the ^{18}O and ^{16}O helices for a given G:C pair. Since the factors which alter the G frequency on going to the associated but uncoupled state will be the same for G $- ^{18}O$ as for G $- ^{16}O$, $^{18}\nu_G^0 = ^{16}\nu_G^0 - (^{16}\nu_G - ^{18}\nu_G)$. Further, the associated but uncoupled C frequency ν_C^0 must be the same in both ^{16}O and ^{18}O helices, so that $(^{18}\nu_G^0 - \nu_C^0) = (^{16}\nu_G^0 - \nu_C^0) - (^{16}\nu_G - ^{18}\nu_G)$.

We may now solve equation (1) for δ and W using these isotopic relationships, and from the values of δ obtain ν_G^0 and ν_C^0 for the different helices. Alternatively and equivalently, we may re-express equation (1) in terms of the unknown hypothetical associated frequencies as follows:

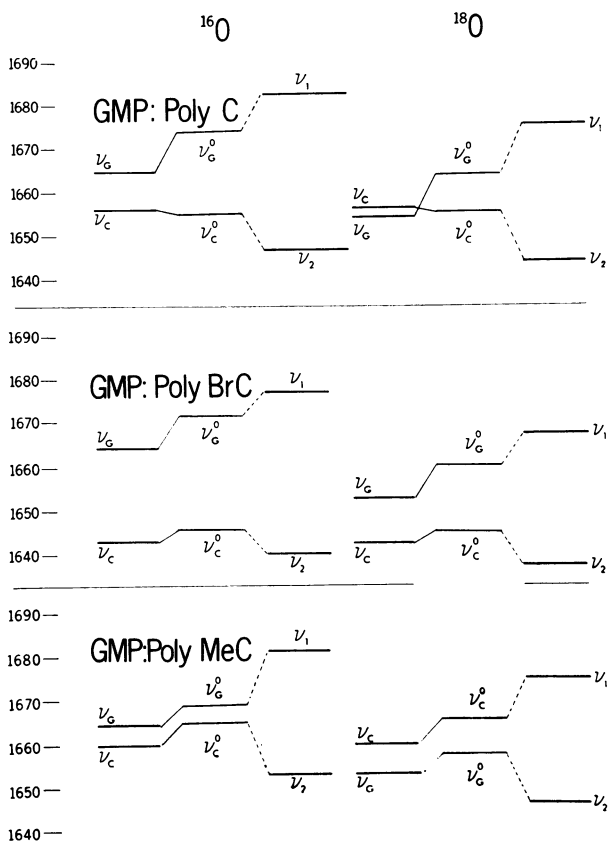$$^k\nu_i = \frac{1}{2}(^k\nu_G^0 + \nu_C^0) \pm \frac{1}{2} \sqrt{4W^2 + (^k\nu_G^0 - \nu_C^0)^2}, \qquad (2)$$

FIG. 6.—Vibrational energy level diagrams for G:C helices. The ^{16}O and ^{18}O headings refer to complexes formed with 5'-GMP-6-^{16}O and 5'-GMP-6-^{18}O, respectively. Ordinate is frequency in cm^{-1}. Symbols and abbreviations are given in footnotes to Tables 1 and 2.

where $i = 1$ or 2 designates the higher or lower frequency band and $k = 16$ or 18 designates the isotopic substitution. These four equations may then be solved for the four unknowns W, $^{16}\nu_G{}^0$, $^{18}\nu_G{}^0$, and $\nu_C{}^0$. Values are given in Table 2.

It is useful to define an association shift and a coupling shift corresponding to the two parts of the overall interaction (see Fig. 6 and Table 2). The association shifts of G and C are the frequency changes $(\nu_G{}^0 - \nu_G)$ and $(\nu_C{}^0 - \nu_C)$, respectively, on going from the uninteracted state to the hypothetical associated but uncoupled state. These shifts are caused by various environmental factors, as mentioned above, and are equal in both ^{16}O and ^{18}O members of a given G:C pair. The coupling shift gives the increase or decrease on going from the hypothetical associated frequencies $\nu_G{}^0$ and $\nu_C{}^0$ to the observed coupled frequencies ν_1 and ν_2. The relatively small magnitudes of some of the association shifts probably arise from the composite nature of this quantity. Thus, for example, the frequency decrease upon hydrogen bonding is of opposite sign and appears in

TABLE 2. *Calculated spectroscopic quantities.**

		Helix			Coupling Shift*	Association Shift	
	$\nu_G{}^0$	$\nu_C{}^0$	δ	W	a^2	$(\nu_1 - \nu_G{}^0, r;)$	$(\nu_G{}^0 - \nu_G)$ $(\nu_C{}^0 - \nu_C)$
^{16}O								
GMP:poly C	1673.9	1654.8	19.2	15.3	0.77	8.5	9.4	−1.2
GMP:poly BrC	1672.2	1645.9	26.3	12.9	0.86	5.3	7.7	2.9
GMP:poly MeC	1669.2	1665.0	4.2	13.9	0.58	12.0	4.7	5.0
^{18}O								
GMP:poly C	1663.4	1654.8	8.7	15.3	0.64	11.6	9.4	−1.2
GMP:poly BrC	1661.1	1645.9	15.2	12.9	0.75	7.4	7.7	2.9
GMP:poly MeC	1657.0	1665.0	−8.0	13.9	0.36	18.5	4.7	5.0

* Quantities calculated from equation (1) or (2). Definitions: $\nu_G{}^0$ and $\nu_C{}^0$, hypothetical associated but uncoupled frequencies of G and C, respectively. δ, separation of associated uncoupled frequencies $= \nu_G{}^0 - \nu_C{}^0$. W, coupling constant in equations (1) and (2). a^2, square of mixing coefficient, a. a^2 represents here fractional G character of ν_1, and b^2 fractional C character of $\nu_1 = 1 - a^2$. Coupling shift, frequency shift up or down from $\nu_G{}^0$ or $\nu_C{}^0$ to ν_1 or ν_2. It is equal to $\frac{1}{2}[(\nu_1 - \nu_2) - |\delta|]$ and is the separation between associated and coupled frequencies (see Fig. 6). Association shift for G and C $(\nu_G{}^0 - \nu_G)$ and $(\nu_C{}^0 - \nu_C)$, respectively.

relevant examples to be roughly comparable in magnitude to the frequency increase on going from more polar to less polar solvents.[10, 14, 15]

The quantity W, which measures the strength of the coupling interaction, is of chemical interest in that it probably depends primarily on the strength of hydrogen bonding of base pairs in a helical environment. While it is important to recognize that the values of W do not show wide variation (implying that differences in hydrogen bonding are not large), we suggest that they may be used to order a series of chemically related molecules with respect to the strength of their hydrogen bonds. The separation of interbase hydrogen bonding from other energetic factors such as polymer solvation or random coil stability should be valuable in studies of helix stability.[6]

[1] Miles, H. T., *Biochim. Biophys. Acta*, **30**, 324 (1958).

[2] Miles, H. T., and J. Frazier, *Biochem. Biophys. Res. Commun.*, **14**, 21, 129 (1964).

[3] Howard, F. B., J. Frazier, M. N. Lipsett, and H. T. Miles, *Biochem. Biophys. Res. Commun.*, **17**, 93 (1964).

[4] Howard, F. B., J. Frazier, M. F. Singer, and H. T. Miles, *J. Mol. Biol.*, **16**, 415 (1966).

[5] Miles, H. T., in *Methods in Enzymology*, eds. S. P. Colowick and N. O. Kaplan, (New York: Academic Press, 1968), vol. 12B, p. 256.

[6] Howard, F. B., J. Frazier, and H. T. Miles, *J. Biol. Chem.*, **244**, 1291 (1969).

[7] Grunberg-Manago, M., and A. M. Michelson, *Biochim. Biophys. Acta*, **80**, 431 (1964).

[8] Szer, W., and D. Shugar, *J. Mol. Biol.*, **17**, 174 (1966).

[9] Howard, F. B., and H. T. Miles, *J. Biol. Chem.*, **240**, 801 (1965).

[10] Howard, F. B., J. Frazier, and H. T. Miles, manuscript in preparation.

[11] A chemically interesting example of intermolecular vibrational coupling is that of carboxylic acid dimers (cf. Davies, M. M., and G. B. B. M. Sutherland, *J. Chem. Phys.*, **6**, 755 (1938), and Millikan, R. C., and K. S. Pitzer, *J. Am. Chem. Soc.*, **80**, 3515 (1958)), in which the identical $>C = O$ vibrations lose their separate identities as a result of coupling and become symmetrical and antisymmetrical vibrations. The former is Raman active and the latter infrared active as a consequence of the selection rules. It would be interesting to see whether the G:C bands ν_1 and ν_2 have markedly different relative intensities in the Raman and in the infrared,

since the $G:C$ pair could be considered to have approximate local C_{2h} symmetry if only the bonding atoms are considered. A further relevant spectroscopic interpretation in terms of intermolecular vibrational coupling as well as intramolecular coupling is that of Miyazawa and Blout (*J. Am. Chem. Soc.*, **83**, 712 (1961)), for polypeptides.

[12] Spectroscopic data on three related polynucleotide helices (poly I:poly C, poly I:poly BrC, and poly I:poly MeC)[5, 6, 10] may indicate that vibrational coupling occurs in these complexes also. In each of these cases one carbonyl band increases and the other decreases in observed frequency upon complex formation, a relationship which is suggestive but not in itself decisive. We have not yet carried out the isotopic experiments which should permit an unambiguous determination of this point.

[13] Because of broadening of carbonyl bands in D_2O, incomplete isotopic substitution leads to an apparent single band at a frequency intermediate between ^{16}O and ^{18}O frequencies.[9] In a nonbonding organic solvent, the bands are narrower, and resolved ^{16}O and ^{18}O bands can be observed.[9]

[14] Studies of solvent effects on vibrational spectra of small molecules have been made by Bayliss, N. S., A. R. W. Cole, and L. H. Little, *Australian J. Chem.*, **8**, 26 (1955); Archibald, L. B., and A. D. E. Pullin, *Spectrochim. Acta*, **12**, 34 (1958); Bellamy, L. H., and R. L. Williams, *Trans. Faraday Soc.*, **55**, 14 (1959); Bellamy, L. H., and H. E. Hallam, *Trans. Faraday Soc.*, **55**, 220 (1959); Caldow, G. L., and H. W. Thompson, *Proc. Roy. Soc.*, (*London*), **A254**, 1 (1960); Caldow, G. L., D. Cunliffe-Jones, and H. W. Thompson, *Proc. Roy. Soc.*, (*London*), **A254**, 17 (1960); Buckingham, A. D., *Proc. Roy. Soc. (London)*, **A248**, 169 (1958) and *Trans. Faraday Soc.*, **56**, 753 (1960), and other references in these papers.

[15] The relevance of small molecule studies to polynucleotide spectral shifts has been pointed out (Miles, H. T., *Nature*, **181**, 1814 (1959)). We have sought more directly applicable information on solvent effects by observing spectra of a number of purines and pyrimidines in organic solvents in the double bond region. Some of the carbonyl groups show both unbonded peaks and resolved bonded peaks, shifted to lower frequency. There are also shifts of carbonyl bands to higher frequency on going from more to less polar solvents.[10]

[16] Herzberg, G., in *Infrared and Raman Spectra* (Princeton, N.J., D. Van Nostrand Co., Inc., 1954), p. 216.

[17] The mixing of the eigenfunctions of the perturbed states (ψ_1 and ψ_2) in terms of those of the unperturbed states (ψ_1^0 and ψ_2^0) is given by $\psi_1 = a\psi_1^0 - b\psi_2^0$, and $\psi_2 = b\psi_1^0 + a\psi_2^0$. The mixing coefficients a and b can be obtained from the equations (see, for example, Herzberg, Eq. II, p. 294, loc. cit.), $a^2 = (\sqrt{4W^2 + \delta^2} + \delta)/2\sqrt{4W^2 + \delta^2}$, and $b^2 = 1 - a^2$. a^2 and b^2 measure the fractional mixing, or in our case percentages of G and C character, respectively, in the higher frequency mode, ν_1.

[18] This assumption is not strictly correct, since ψ has a functional dependence on the frequency ν. Since the fractional change in frequency on ^{18}O substitution ($\sim$12 parts in 1660) is so small, however, the effect on W should be negligible in our calculations.