Figure 4. Low-field regions of pmr spectrum of calf thymus DNA at 220 Mcps: concentration 30 mg/ml in D_2O, pD 7, 93°, 50 spectra averaged.

distribution of the DNA H-2′ resonance region. Additional investigation of oligonucleotide spectra will be necessary to completely elucidate these interactions.

Finally, the two lowest field resonance absorption regions of calf thymus DNA are shown in Figure 4. The resonance absorption from 5.8 to 6.4 ppm which comprises H-5 protons of cytosine and H-1′ protons shows evidence of structure, but the individual resonances are not sufficiently resolved to permit detailed analysis. From examination of corresponding pmr spectra of monomers and dimers, we have tentatively assigned the partially resolved resonances in the 7.3 to 8.3 ppm region as indicated in Figure 4. The H-8 proton positions of purine bases deuterate at a significant rate in neutral D_2O at 90°[22,23] and, therefore, are probably not exhibiting their full intensity in this spectrum (particularly H-8 of guanine which is more labile than H-8 of adenine). Three or possibly four partially resolved resonances can be seen in the region assigned to the H-6 proton of thymine that presumably reflect different environments for this proton in residual conformational structures of the polymer molecule. However, the intensity in this region is too great to be accounted for from H-6 protons of thymine alone, and we conclude that shifted components of the lower field resonances (for example, from H-6 of cytosine) also contribute intensity in this region. Thus, it appears that if the resonances in this region could be more clearly resolved, it might be possible to obtain additional nearest neighbor base frequency information for thymine and perhaps other bases. Work is in progress to attempt to achieve this required degree of resolution.

Acknowledgments. We are grateful to Dr. M. A. Jesaitis of Rockefeller University for gifts of highly purified samples of C_{16} and T_6 bacteriophage DNA, to Dr. R. C. Ferguson for advice and assistance in the maintenance and operation of the 220-Mcps spectrometer, and to Messrs. F. W. Barney and F. V. Ferrari for technical assistance in obtaining the spectra.

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Hypochromism Accompanying Purine–Pyrimidine Association Interactions

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Abstract: Ultraviolet absorption studies on binary mixtures of model nucleoside derivatives of adenine (A), uracil (U), guanine (G), cytosine (C), and inosine (I) in chloroform solution are reported. Specific band hypochromic or hyperchromic effects are found only in solutions containing the complementary pairs A + U, G + C, and I + C. Comparison of the present results with those of infrared studies shows that hypochromic effects in these systems are due to the formation of hydrogen-bonded complexes between the bases (base pairing). It is therefore demonstrated that parallel stacking of the bases as occurs in undenatured DNA is not the only condition for hypochromism in $\pi^* \leftarrow \pi$ transitions of the bases.

Hypochromism in polynucleotides, which is the decrease in absorbance per chromophore in the polymer compared to that of the monomer, has been attributed by Tinoco[2,3] and Rhodes[4] to dipole–dipole interaction between transition moments in neighboring oscillators.[5] This has been termed an off-resonance interaction since the interacting dipoles originate from different electronic transitions. Large hypochromic effects are predicted for this model[3] when the base

(1) This research was completed while the author was a National Institutes of Health Predoctoral Fellow (1964–1967) and constitutes part of a Ph.D. thesis submitted to the Department of Chemistry, Massachusetts Institute of Technology, Feb 1967. Inquiries may be addressed to the Department of Biophysics, King's College, London.
(2) I. Tinoco, Jr., *J. Chem. Phys.*, **33**, 1332 (1960); **34**, 1087 (1961); *J. Am. Chem. Soc.*, **82**, 4785 (1960).
(3) H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 500, 518 (1962).
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(5) Some authors, including those cited, use the term "dispersion force interaction" to refer to dipole–dipole interactions of various orders. It appears preferable at present to restrict the term "dispersion force" to that which leads to the R^{-6} term in the potential energy.

planes are arranged parallel and one above another ("vertical stacking") with their optical centers relatively close to one another, as, for example, in undenatured DNA.

Alternatively, Bolton and Weiss[6] have attributed hypochromism to a local field effect. The electric field experienced by an absorbing unit is considered to have, in addition to the applied optical field, a component due to the induced electric dipole moments in neighboring oscillators. Since large hypochromic effects are predicted for a single transition in the monomer, originating from self-interaction of a monomer band, this has been termed a resonance interaction. Nesbet[7] has further calculated that this resonance effect by itself can lead to a large hypochromism, comparable to that observed in DNA, for hydrogen-bonded base pairs even in the absence of secondary structure or vertical stacking of the bases.

Chemical systems have recently been described[8-10] in which model nucleoside components interact with each other through the formation of hydrogen bonds. Infrared studies[11,12] have yielded information on the equilibrium constants for the pairing of adenine (A) with uracil (U) derivatives, and guanine (G) and inosine (I)[13a] with cytosine (C) derivatives. The specificity (*i.e.*, that A interacts only with U and G only with C) of these interactions has also been demonstrated by infrared spectroscopic methods.[12] At the solute concentrations employed, the infrared evidence shows that only dimers are formed and that the mode of interaction is hydrogen bonding. From this evidence, stacking of the bases does not occur at the concentrations used. There is additional supporting evidence for this conclusion from nmr investigations.[14,15]

These systems therefore provide both an experimental basis for determining the contribution to hypochromism or hyperchromism from base pairing alone and a rough test of the predictions of Nesbet.[7] Accordingly, ultraviolet absorption studies have been made of a number of chloroform solutions containing purine and pyrimidine derivatives for which dimerization constants have been determined by infrared spectroscopic measurements.[11-13] The results are reported herewith.

Experimental Section

Materials. The nucleoside derivatives, purchased from Cyclo Chemical Corp., were identical with those used in previous infrared studies[9,11-13] and are referred to by the following symbols: A = 9-ethyladenine, U = 1-cyclohexyluracil, BrU = 1-cyclohexyl-5-bromouracil, G = 2′,3′-benzylidine-5′-tritylguanosine, C = 2′,3′-benzylidine-5′-tritylcytidine, and I = 2′,3′-benzylidine-5′-tritylinosine.

Chloroform (certified ACS grade, Fisher Scientific Co.) was purified by double passage over a 30-cm long alumina-gel column, which satisfactorily removed all infrared-detectable ethanol, water, and other impurities.

The sample of trityl methyl ether was generously supplied by Professor G. Swain, Department of Chemistry, Massachusetts Institute of Technology.

Instrumentation. All spectra were recorded on a Beckman Model DK-2 ratio recording spectrophotometer. Matched pairs of fused quartz cells of approximately 0.010-, 0.10-, and 1.0-cm paths, purchased from the American Instrument Co., permitted the examination of solutions of total solute concentration between the limits 0.01 and 0.00001 *M* in most cases. Precise cell lengths were measured by interference fringes. All spectra were recorded at 24 ± 1°.

In the figures presented below where absorbances are plotted as a function of solution composition ("spectral mixing curves"), the uncertainty in each experimental point is no greater than the size of the point except where error limits are specifically indicated otherwise by the use of markers.

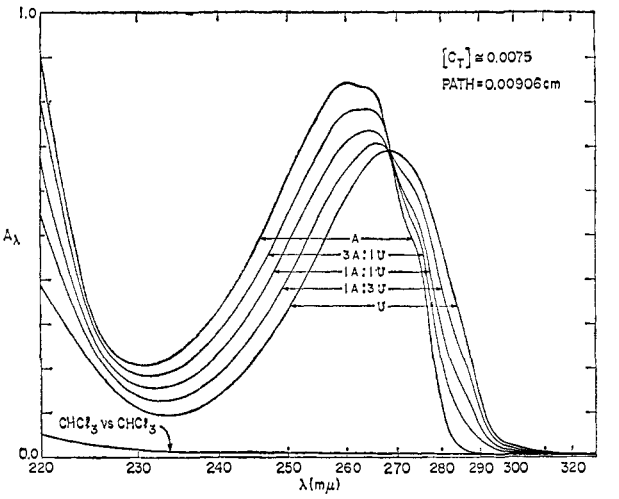

Figure 1. Ultraviolet absorption spectra of chloroform solutions of A and U mixtures as indicated. Total solute concentration is 0.0075 *M* and sample-cell length is 0.00906 cm. In this and in subsequent figures, the lowest curve shows the residual absorption when chloroform is placed in both sample and reference beam cells.

Results

Interaction of A and U. Figure 1 shows the absorption spectra of solutions containing A and U at different relative molar ratios but with a fixed total molar solute concentration ($[C_T]$) of 0.0075. The observed absorbances, at 265 mμ for example, when plotted against the mole fraction of each constituent are found to give a negative deviation from Beer's law at this concentration (Figure 2). The hypochromism is greatest in the wavelength interval 260–280 mμ and is centered near 268 mμ where the absorption maximum of U occurs. The greatest deviation from Beer's law occurs in equimolar solution mixtures of A and U, and therefore the hypochromism appears to be related to the formation of a 1:1 dimer. The equilibrium constant for dimer formation at 25°, as determined by infrared measurements,[11] is $1.0 \times 10^2\ M^{-1}$. In the equimolar mixture, with $[C_T] = 0.0075$, for example, 23% of each base is present in the dimer form, and the relative hypochromism observed is between 2.0 and 2.5%

(6) H. C. Bolton and J. J. Weiss, *Nature*, **195**, 666 (1962); J. J. Weiss, *ibid.*, **197**, 1296 (1963).
(7) R. K. Nesbet, *Biopolymers Symp.*, **1**, 129 (1964); *Mol. Phys.*, **7**, 211 (1964).
(8) S. Basu and L. Loh, *Biochim. Biophys. Acta*, **76**, 131 (1963), have noted the nonadditivity of absorption and fluorescence spectra of aqueous nucleoside mixtures. The mode of interaction, however, between nucleosides in water and the stability constants involved could not be determined.
(9) R. M. Hamlin, Jr., R. C. Lord, and A. Rich, *Science*, **148**, 1734 (1965).
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(13) (a) Y. Kyogoku, unpublished work; (b) Y. Kyogoku, R. C. Lord, and A. Rich, *Proc. Natl. Acad. Sci. U. S.* **57**, 250 (1967).
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(15) R. R. Shoup, H. T. Miles, and E. D. Becker, *Biochem. Biophys. Res. Commun.*, **23**, 194 (1966).

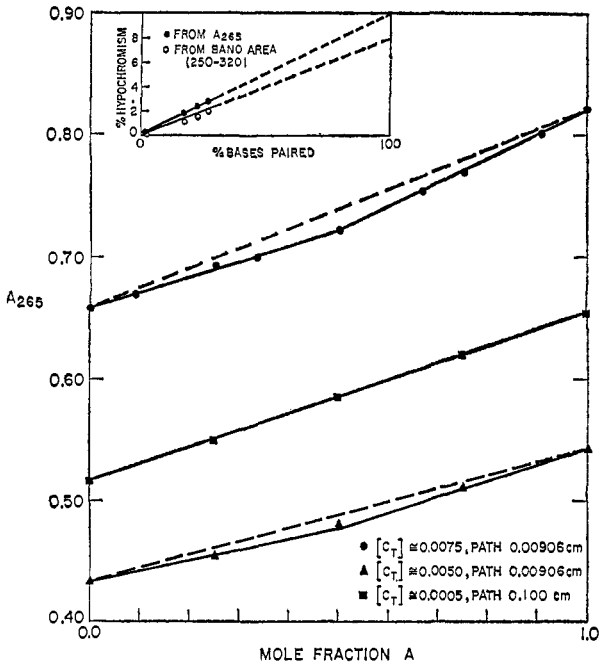

Figure 2. Comparison of observed and Beer's law absorbances (dashed line) at 265 mμ for solutions containing different relative amounts of A and U at the indicated total molar solute concentration levels ([C_T]). Inset: per cent relative hypochromism observed as a function of the percentage of bases paired assuming $K_{AU} = 1.0 \times 10^2\ M^{-1}$. The hypochromism was calculated both from absorbances at 265 mμ (A_{265}) and from integrated band intensities from 250 to 320 mμ in equimolar mixtures of A and U. Similar notation is used in subsequent figures.

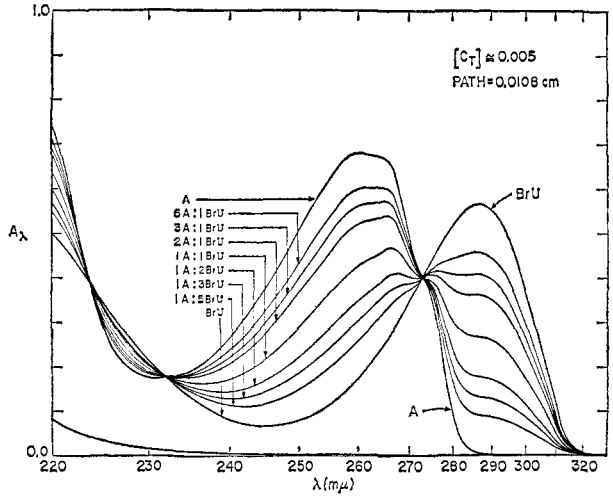

Figure 3. Ultraviolet absorption spectra of chloroform solutions of A and BrU mixtures as indicated. Total solute concentration is 0.005 *M* and sample-cell length is 0.0108 cm.

of the expected band absorbance.[16,17] At $[C_T] \leqslant 0.0005$ less than 2% of the bases remain paired and no hypochromism can be detected within the experimental error of these measurements. The results for several values of [C_T] are summarized in the Figure 2 inset, where it can be seen that for complete pairing of the bases an extrapolated value of 8–10% relative hypochromism would be expected.

While larger hypochromic effects are to be expected when more of the bases are paired, there are other difficulties which arise at significantly higher solute concentrations. Nmr measurements[14] give evidence for some trimer formation (A + 2U), and appreciable self-association of each base is also expected.[11] These complicating factors are essentially eliminated by keeping [C_T] below 0.01 and the observed hypochromism can be ascribed solely to heterologous base pairing.

Bromine substitution at the C-5 position of U enhances the tendency for pairing with A, the dimerization constant for the A–BrU complex being $2.4 \times 10^2\ M^{-1}$ at 25°.[13b] Accordingly at comparable values of [C_T] a somewhat larger hypochromic effect is expected for A + BrU than for A + U mixtures. Spectra for the A + BrU system are shown in Figure 3 where [C_T] is 0.005. The hypochromism is 6–7% at this concentration (30% bases paired), 1.5% at [C_T] = 0.0005 (5.4% bases paired), and not detectable at [C_T] = 0.00005 (less than 2% bases paired). Thus about 20% relative hypochromism is expected for this interaction when 100% of the bases are paired. The fact that this maximum value is larger by a factor of 2 than that for the A + U system is attributable to the greater separation of the absorption bands of A and BrU (compare Figures 1 and 3). This permits a more precise evaluation of the hypochromicity apparently localized in the 287-mμ band of BrU. It is noteworthy that the bands of A at 260 and 266 mμ are unperturbed by base pairing in this case, the hypochromism being confined to the band of the pyrimidine base.

The A + BrU system represents the only case in the present study for which a meaningful quantitative value can be assigned to the band intensity change due to base pairing, since the separation of the A and BrU bands permits the area of the latter to be reliably calculated. In the other systems examined, notably I + C and G + C below, overlapping of bands introduces arbitrariness into the evaluation of band areas, and therefore hypo- or hyperchromism calculated from areas over the wavelength intervals cited is only semiquantitative. The intervals were chosen to include the entire region over which deviation from Beer's law was measurable.

Interaction of I and C. The system I + C is of general interest because of the formation of stable helical complexes between polyinosinic and polycytidylic acids. This system is also more readily examined than the G + C system since in the latter case the self-association of G does not allow a simple interpretation of the spectral results to be given, as will be seen below.

Figure 4 shows spectra of solutions containing I and C at different relative molar ratios with [C_T] = 0.0025.[18] There is a positive deviation from Beer's law (hyperchromism) in the region 260–300 mμ and a negative deviation (hypochromism) in the region 240–260 mμ where the base derivatives have high absorption. Both

(16) The per cent relative hypochromism at wavelength λ for an equimolar mixture of interactants X and Y is defined as $100\{1 - [2 \cdot A_\lambda^{x+y}/(A_\lambda^x + A_\lambda^y)]\}$ where A_λ^x, A_λ^y, and A_λ^{x+y} are absorbances at λ for solutions of X, Y, and the mixture of X and Y, respectively, at the same total solute concentration. To obtain the over-all band hypochromism, the respective integrated band intensities are substituted for absorbances. Unfortunately overlapping of bands makes quantitative evaluation of band areas rather difficult.

(17) The equilibrium constant for dimer formation between interactants X and Y is defined as $K_{xy} = [C_{xy}]/[C_x][C_y]\ M^{-1}$ where the C's are appropriate molar concentrations. At total concentration $[C_T] = 2[C_x]_0 = 2[C_y]_0$ the percentage of paired bases is accordingly $100[C_{xy}]/[C_x]_0$, the subscript zero indicating initial concentration.

(18) The solubility limit of I in chloroform is 0.003 *M*.

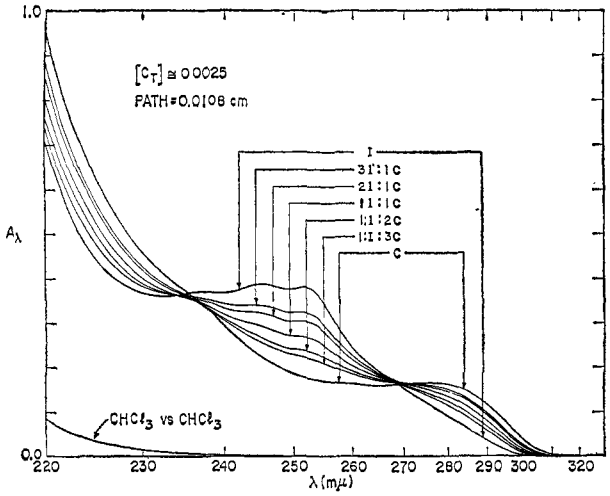

Figure 4. Ultraviolet absorption spectra of chloroform solutions of I and C mixtures as indicated. Total solute concentration is 0.0025 M and sample-cell path is 0.0108 cm.

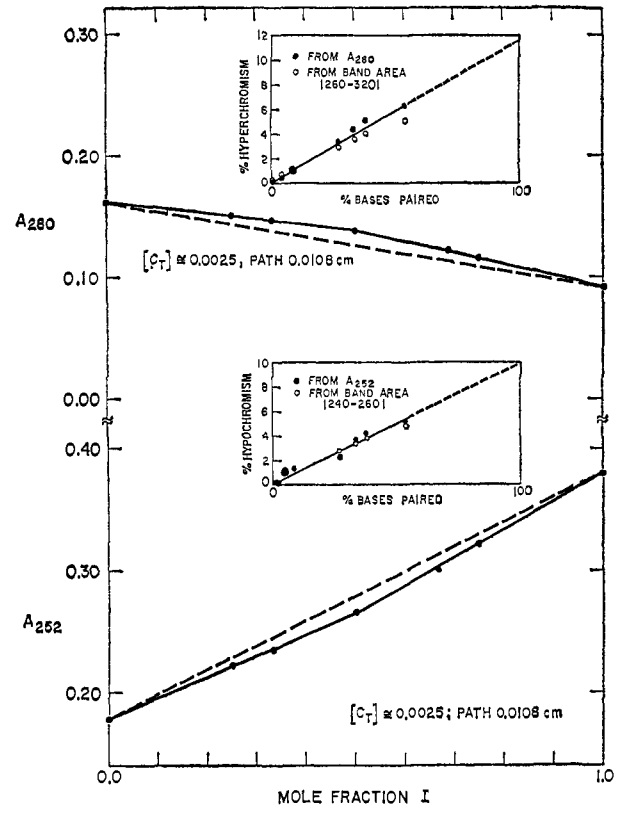

Figure 5. Comparison of observed and Beer's law absorbances at 252 and 280 mμ for solutions containing different relative amounts of I and C with $[C_T]$ = 0.0025. Lower (upper) inset: per cent relative hypo- (hyper-) chromism observed at 252 (280) mμ as a function of the percentage of bases paired assuming $K_{IC} = 2 \times 10^3\ M^{-1}$.

of these effects are greatest in equimolar mixtures, and both diminish in magnitude with decreasing solute concentration. They are therefore attributable to base pairing. The equilibrium constant at 25° for pairing of I with C is $2 \times 10^3\ M^{-1}$ as determined from infrared measurements,[13a] so that when $[C_T]$ = 0.0025 about 54% of the bases are paired and when $[C_T]$ = 0.000025 only about 2% remain paired. Accordingly at the former concentration about 5 and 6% hyper- and hypochromicities, respectively, are observed in the two spectral regions, and at the latter concentration no substantial deviations from Beer's law can be detected. Therefore neither effect is expected to exceed 10–12% when all the bases are paired. These results are summarized in Figure 5.

It should be mentioned that the self-association of I need not be considered in these results, as K_{II} can be estimated as between 200 and 400 M^{-1} from the infrared data.[13a] Therefore the dimer II contributes only a small fraction (less than 0.1) to the total number of paired bases in a mixture of I and C. The absorption spectrum of I also does not appear to be significantly affected by self-association.[19]

The nucleoside derivatives I, C, and G all contain phenyl substituents on the sugar moiety necessary to carry them into chloroform solution. These substituents contribute only slightly to absorption in the 250–277-mμ region and not at all at higher wavelengths. Extinction coefficients of the bases are found to be at least an order of magnitude larger than those of the phenyl substituents combined for $\lambda \geq 250$ mμ.

Interaction of G and C. Figure 6 shows the spectra of solutions containing different molar ratios of G and C when $[C_T]$ = 0.0075. With both G and C present the observed absorbances show a negative Beer's law deviation (hypochromism) at wavelengths higher than 277 mμ and a positive deviation (hyperchromism) at shorter wavelengths in regions of absorbance due to the heterocyclic ring bases.

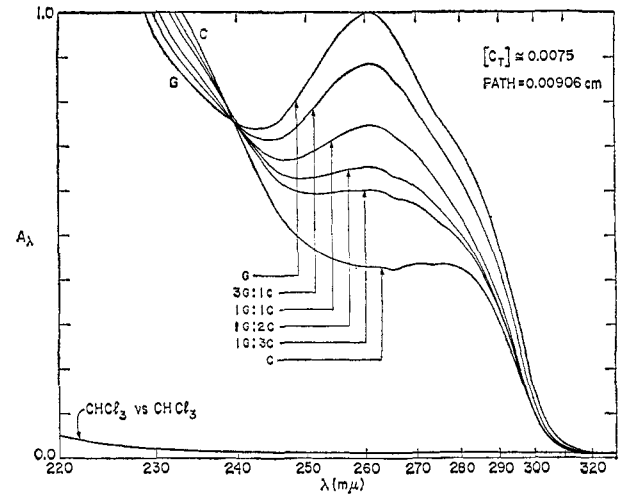

Figure 6. Ultraviolet absorption spectra of chloroform solutions of G and C mixtures as indicated. Total solute concentration is 0.0075 M and sample-cell length is 0.00906 cm.

Interpretation of the hypochromism in the high wavelength region (277–320 mμ), which appears to be localized in the band of C,[20] is straightforward: (1) the effect is greatest in equimolar mixtures and is concen-

(19) Spectra of I follow Beer's law closely at wavlengths below 285 mμ over the concentration range 0.000025–0.0025 M. In the region 285–300 mμ, changes in extinction coefficients accompany dilution. This probably accounts for the nonlinear trend of hyperchromicities determined from band areas (open circles of upper inset of Figure 5), but the self-association of I is clearly not an important factor in the interpretation of the results in the 240–260-mμ region.

(20) As noted for the I + C interaction, the perturbation in this wavelength region appears more probably in the band of C. The oscillator strength of the C band at *ca.* 280 mμ is several times larger than that of the G band at *ca.* 285 mμ, which appears as a weak shoulder in the spectrum of G (see ref 3).

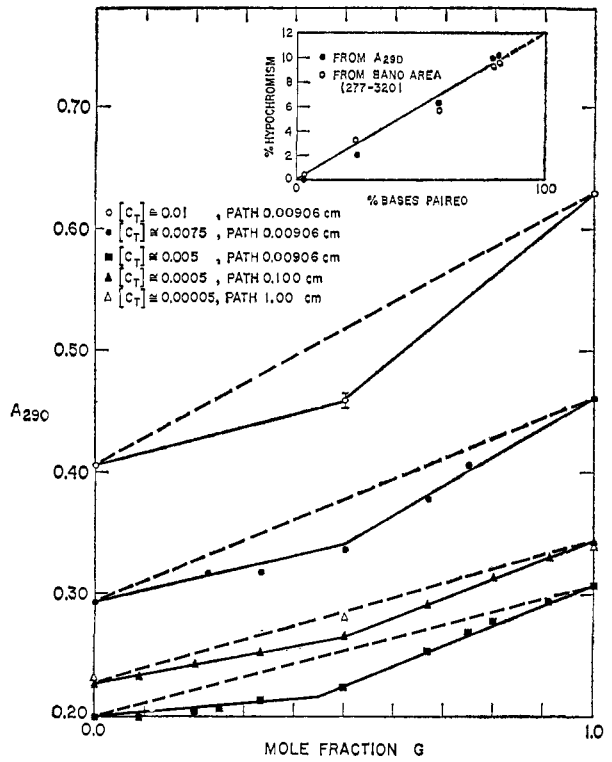

Figure 7. Comparison of observed and Beer's law absorbances at 290 mμ for solutions containing different relative amounts of G and C with [C_T] as indicated. Inset: per cent relative hypochromism observed as a function of the percentage of bases paired assuming $K_{GC} = 2 \times 10^4\ M^{-1}$ and $K_{GG} = 5 \times 10^3\ M^{-1}$.

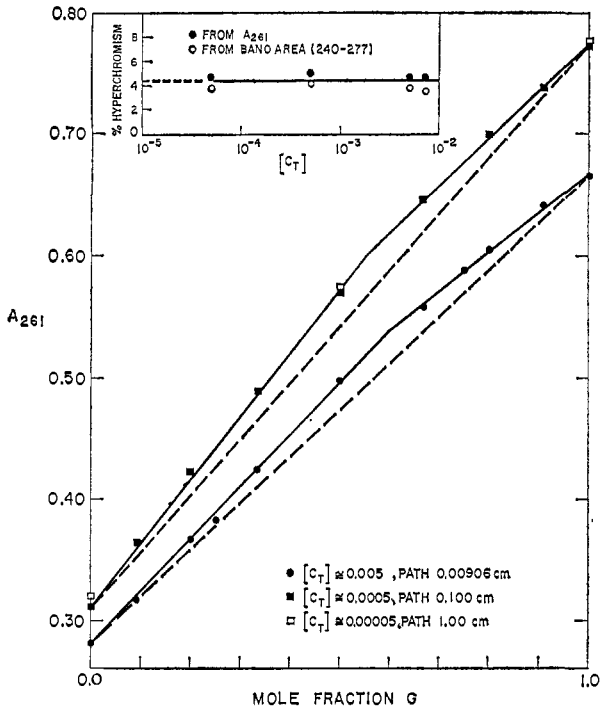

Figure 8. Comparison of observed and Beer's law absorbance at 261 mμ for solutions containing different relative amounts of G and C with [C_T] as indicated. Inset: per cent relative hyperchromism observed as a function of [C_T].

tration dependent, as shown by the data of Figure 7, so that the hypochromism is thus attributable to the formation of a 1:1 GC dimer; (2) absorption spectra of G and C solutions alone follow Beer's law closely at

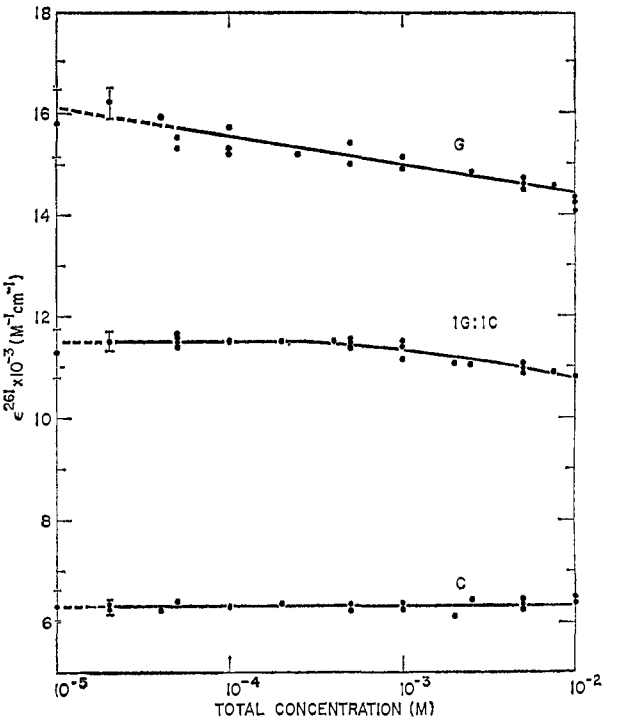

Figure 9. Molar extinction coefficients at 261 mμ of chloroform solutions of G, C, and G + C (1:1 mixture) as a function of [C_T]. Several determinations were made on samples of independent preparation.

these higher wavelengths, indicating that the hypochromism in G + C mixtures is not complicated by self-association; and (3) assumption of reasonable values for the dimerization constants K_{GC} and K_{GG} show that the hypochromism is linearly related to the fraction of GC dimers in mixed solutions (Figure 7 inset).

Such a simple explanation for the apparent hyperchromism in the 240–277-mμ region in solutions containing G and C cannot be given, however, for the following reasons: (1) the effect seems to be independent of concentration and is not convincingly greatest in equimolar mixtures, as shown in Figure 8. (2) Absorption spectra of G alone do not follow Beer's law at these lower wavelengths but instead the extinction coefficient of G decreases significantly (~10–20%) with increasing concentration as is shown, for example, at 261 mμ in Figure 9. This large hypochromism in solutions of G alone must be attributed to self-association.[21] (3) Assumption of a large mean self-association constant for G (by comparison with self-association constants for other base derivatives) accounts for this hypochromism in the 261-mμ band of G but renders impossible a simple explanation of the hyperchromism in G + C mixtures at this wavelength since no reference spectrum of unassociated G is available.

The present results are thoroughly consistent with those of infrared investigators,[10,11] who find the GC dimerization constant to lie between 10^4 and $10^5\ M^{-1}$ at 25°, but also observe extensive self-association of G[12] even in dilute solutions. The present results re-

(21) Accompanying this hypochromism there is also a shift of the band maximum from 260 mμ at 10^{-5} M to 261 mμ at 10^{-2} M in solutions of G. Since the band is very broad this amounts to only a few thousandths absorbance units and a very small fraction of the observed hypochromism. Such red shifts are generally considered to accompany hydrogen-bonding interactions.

quire that K_{GC} lie in the interval $1-2 \times 10^4\ M^{-1}$ but are not sensitive to the value of K_{GG} (if indeed only GG dimers are of significance) so long as $K_{GC} > K_{GG} > 10^3\ M^{-1}$. The value $5 \times 10^3\ M^{-1}$ for K_{GG} used in Figure 7 (inset) was chosen arbitrarily but within the range suggested by the ultraviolet and infrared results.

An apparent concentration independence of the hyperchromism could result from two or more competitive equilibria that cause intensity changes in the 261-mμ band of G. For example, if it is assumed that GC pairing causes a small hypochromism compared to that caused by G self-association, a net hyperchromism will be observed. This results from the breaking up of a large percentage of GG pairs (and possibly higher G aggregates) by the addition of C, which overcompensates for the hypochromism caused by GC pairing. Little concentration dependence will appear over the range examined as long as the fraction of liberated G aggregates is comparable to the fraction of GC pairs formed. Since neither the stoichiometry of the G aggregates nor accurate equilibrium constants governing this self-association are obtainable from the ultraviolet or infrared results, a more detailed quantitative treatment of perturbations in the 261-mμ band of G cannot be given. The above discussion serves only to show that the concentration-independent hyperchromism can be understood when the self-association of G is taken into account.

Noninteracting Systems. In addition to the above, solutions containing pairs of noncomplementary bases, *i.e.*, A + C, A + G, U + G, and U + C, were also examined but showed no unusual intensity effects attributable to heterologous base-pairing interactions. Further, no strong pairing interactions are evident with BrU or with I except in the presence of their respective complementary bases A or C. These results are in complete agreement with those of infrared investigations[12,13] which reinforce the base-pairing specificity found in double-stranded nucleic acids and polynucleotides. A summary of the results reported herewith is given in Table I.

Table I. Band Intensity Changes from Base Pairing[a]

Base	Band,[b] mμ	A	U	G	C	I
A	275 (S)		0			
	266 (S)	N	0	N	N	N
	260 (M)		0			
U	286 (S)	0				
	268 (M)	−10	N	N	N	N
G	285 (S)			0	0	
	261 (M)	N	N	−15[c]	−5[c]	N
C	280 (M)	N	N	−12	N	+10
I	280 (S)				0	−5[e]
	252 (M)	N[d]	N	N	−10	0

[a] The table gives the per cent intensity change of a band (column 2) of a given base (column 1) upon interaction with another base (row 1). The perturbation is negative (hypochromic), positive (hyperchromic), or zero as indicated. Pairs of noninteracting bases, *i.e.*, no bands of either base are perturbed, are denoted by N. [b] S, shoulder; M, maximum. [c] Estimated (see text). [d] A very weak interaction between I and A is observed in the infrared.[13a]

Conclusions

The results of the previous section show that associative interaction between purine and pyrimidine base derivatives in chloroform solution gives rise to hypo- or hyperchromism in the stronger absorption bands of the heterocyclic ring bases which are assigned to $\pi^* \leftarrow \pi$ transitions.[3] In view of the chemical systems chosen, and on the basis of previous infrared[9-13] and nmr[14,15] results, it is reasonably certain that the intermolecular aggregates are hydrogen-bonded base pairs. The hypochromic effects, moreover, are quantitatively related to the fraction of dimers formed in the binary mixtures of interacting molecules. Thus it has been shown that vertical stacking of purine or pyrimidine bases as occurs in double helical DNA and synthetic polynucleotides is not the only condition for ultraviolet absorption band hypochromism of base $\pi^* \leftarrow \pi$ transitions, as has been commonly assumed.[22] From the present data, a maximum hypochromic effect of about 10–12% is expected for complete base pairing at wavelengths where both bases have comparable absorption but where only a band of one base is perturbed. This corresponds to a maximum of roughly 20–25% hypochromism in the given band (compare A + U and A + BrU systems, above).

Since a rigorous comparison of the present results with those of studies of aqueous solutions is not justifiable,[23] it is impossible to state whether the hypochromism reported here could account entirely for the larger effect (~40%) arising in the 260-mμ band of DNA and related polymers.[22] Further the effect of base pairing on ultraviolet absorption is apparently highly specific both with regard to the band(s) suffering intensity change in a given base pair and to the sign of this intensity change. Although the treatment of Nesbet[7] has been criticized on theoretical grounds,[24-26] his prediction that a large hypochromism can result from the formation of coplanar base pairs appears substantiated. It should be emphasized, however, that there is only scant agreement between the experimental results (Table I) and the specific band intensity changes due to resonance interaction computed by Nesbet.[7]

One of us has applied[27] the hypochromism equation derived by DeVoe and Tinoco[3] to the hydrogen-bonded dimers in order to estimate the band intensity changes due to dipole–dipole interaction in isolated base pairs. There is, however, essentially no qualitative agreement between the calculated and experimental results. This lack of agreement may be due in part to the assumptions which were made in applying the hypochromism equation[3] to the systems examined here.[27] The present study provides data that may be useful for further theoretical treatment of the hypochromic effect.

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(23) While solvent effects can be neglected in the present case, this, is not necessarily true of the denaturation of aqueous DNA where hypochromism contributions from solvent groups outside the helix may be significant.[3]

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