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Comparative Study of Polyribonucleotides in Aqueous and Glycol Solutions*

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ABSTRACT: In an effort to investigate the nature of the ordered but anomalous structure assumed by deoxyribonucleic acid dissolved in ethylene glycol, we have carried out a systematic study of polyribonucleotides, transfer ribonucleic acid, and stoichiometric equivalents of mononucleotides in aqueous and glycolic solutions. Determination of optical rotatory dispersion, circular dichroism, and absorption spectra, as well as of temperature–absorbance profiles, were the techniques used. The rotatory spectra suggest that deoxyribonucleic acid in glycol must have a structure that is distinctly non-Watson–Crick–Wilkins in character, yet, on the basis of its hypochromicity and the sharpness of its thermal transition, must be a highly ordered one. Glycolic solutions of the polynucleotides and of transfer ribonucleic acid, on the other hand, show rotatory spectra which do not exhibit the anomaly characteristic of deoxyribonucleic acid.

We have recently reported (Green and Mahler, 1968) an apparently anomalous and nonconservative (Bush and Brahms, 1967; Tinoco, 1968) rotatory behavior of DNA in ethylene glycol. In this context, "nonconservative" defines a circular dichroic spectrum of an oligo- or polynucleotide, characterized by a single band *centered* on the wavelength of an absorption maximum, in contrast to "conservative," which contains both a positive and a negative band of equal magnitude with a *crossover* at the wavelength of the absorption maximum: $(rC)_n$[1] is an example of a polymer producing the former, $(rA)_n$ one producing the latter type.

Ethylene glycol, due to its high polarizability and the decreased magnitude of resultant solvophobic forces, exerts profound effects on the transition of a polynucleotide between its ordered (helix) and disordered (coil) forms. Among them are the following (all statements relative to water): to increase the contributions to helix stability due to hydrogen bonding (both between complementary bases and those due to sugar hydroxyls) and to electrostatic interactions, and to diminish all contributions due to solvophobic interactions between stacked bases (Singer, 1962; Sage and Singer, 1962; Sinanoglu and Abdulnur, 1964, 1965; Hanlon and Major, 1968). From studies of the behavior of polynucleotides in such solvents, many authors have implicated hydrophobic interactions as a major contribution to the stability of nucleic acids and polynucleotide structures in water (Ts'o *et al.*, 1961, 1962; Gordon and Jencks, 1963; Levine *et al.*, 1963; Marmur *et al.*, 1963; Brahms *et al.*, 1964). Our own results (Green and Mahler, 1968) raised serious doubts whether DNA dissolved in glycol retained a Watson–Crick–Wilkins (WCW) B helix as suggested by Luzzati *et al.* (1964, 1967), but, we did not venture to suggest an acceptable alternative model.

In an attempt to lay the groundwork for such a model, we have now made a systematic study of the behavior of various synthetic polynucleotides and of mixtures of their component mononucleotides, in both solvents. This present work can also be regarded as an extension of the systematic studies by Ts'o and Helmkamp (Ts'o and Helmkamp, 1961; Helmkamp and Ts'o, 1961, 1962; Ts'o *et al.*, 1962), who previously studied the optical rotatory properties of polynucleotides, as well as their mononucleotide components, in both aqueous and nonaqueous solvents and provided strong evidence for the importance of noncovalent interactions to optical rotatory

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[1] We use here the system of abbreviations for nucleotides and their polymers suggested in the Revised Tentative Rules (1965) of the IUPAC–IUB Combined Commission on Biochemical Nomenclature. In this system, polyriboadenylic acid (3′–5′ linked) becomes $(rA)_n$ or poly rA and its duplex with polyribouridylic acid is $(rA)_n \cdot (rU)_n$. Other abbreviations used are: ϵ_{max}, molar extinction coefficient at wavelength of maximum absorption on a mononucleotide basis; λ_{max}, wavelength of maximum absorption; λ_c wavelength of crossover.

behavior. In the present instance, investigations have been extended to the ultraviolet region into the range of the absorption of the relevant chromophores.

Also, while in many cases very similar inferences can be drawn from rotatory behavior and from thermal hypochromism, both sets of properties have been measured and are included in this report. Since they are sensitive to different structural parameters they are not necessarily complementary (Rosenfeld, 1928; Cox and Littauer, 1960; Davis and Tinoco, 1968). Hypochromism and effects thereon during thermal transitions provide an indication of oscillator strength, *i.e.*, the interaction of the main ultraviolet transition of one base with the excited states of its neighbors. It is therefore most sensitive to changes in the stacking of chromophores but relatively insensitive to the precise geometry within the stack. On the other hand, optical rotatory dispersion and circular dichroism spectra provide a measure of the rotational strength of the room temperature form and depend on the geometries of the component transition dipoles. Thus, they provide a particularly sensitive probe for the positioning in space of the individual chromophores relative to one another (Beychok, 1966, 1968; Bush and Brahms, 1967; Brahms *et al.*, 1967a,b; Tinoco, 1968; Davis and Tinoco, 1968).

While this study has provided additional information concerning the structures of polyribonucleotides in glycol, these structures appear to bear little, if any, resemblance to the one assumed by DNA in this solvent. We, therefore, are still lacking a model for the latter.

Materials and Methods

Chemicals. The ethylene glycol, used in these and previously reported experiments, was Fisher Certified Reagent used without further purification. The potassium fluoride was Baker Analyzed Reagent, the EDTA was from Eastman Organic Chemicals, the Tris (analytical grade) from Sigma Chemical Co., the magnesium chloride (analytical reagent grade) was a product of Mallinckrodt Chemical Works, and the cacodylic acid was obtained from Fisher Scientific.

Biological Materials. The ribopolynucleotides were obtained from Miles Laboratories, Inc., Elkhart, Ind. The control numbers were: $(rA)_n$, poly A-11849; $(rC)_n$, poly C-212726; $(rG)_n$, poly G-5171; and $(rU)_n$, poly U-42749 with sedimentation coefficients, as reported by the manufacturer, of 7.03, 6.68, 12.79, and 4.69 S, respectively. All, therefore, had molecular weights greater than 100,000. Calf thymus DNA was purchased from Worthington Biochemicals, Freehold, N. J. (lot no. 995). Its properties have been previously discussed (Mahler *et al.*, 1964). Yeast tRNA (stripped) was obtained from General Biochemicals, Chagrin Falls, Ohio (lot no. 7238L). The monoribonucleotides were purchased from P-L Biochemicals, Inc., Milwaukee, Wis., and were the 2′(3′)-mixed isomers. The lot numbers for rAMP, rCMP, rGMP, rUMP, were, respectively, 3809, 3911, 4008, and 4108. dAMP and dGMP were products of California Foundation for Biochemical Research, lot no. 150391 and 350131, respectively; dCMP was obtained from Sigma Chemical Corp. (lot no. 128-684) and dTMP was purchased from Schwarz BioResearch (TMP-5903).

Preparation of Samples. DNA, single-stranded polynucleotides, and mononucleotides were dissolved directly in aqueous and glycol media of identical ionic composition containing 0.05 M KF–0.001 M EDTA, pH 5.5 (H_2O). In addition, samples of $(rA)_n$ and $(rC)_n$ were also prepared in 0.05 M KF–0.001 M Tris (pH 8.8) (H_2O) to obtain the "neutral" (or basic) form of these polymers. The poly G:C duplex $(rG)_n \cdot (rC)_n$ was formed in 0.1 M NaCl–0.01 M sodium cacodylate (pH 7.0) (H_2O). The solutions of tRNA and the equivalent mixture of mononucleotides were prepared in a media that contained 1.0×10^{-4} M Mg^{2+} instead of EDTA. All solutions were initially prepared on a weight to volume basis, *i.e.*, 100 μg of polynucleotide/ml of solution, at 4°, and were stored at that temperature. An equilibration time of 5–15 hr at 27° was allowed just prior to the recording of spectra. Mononucleotide solutions were prepared in the same manner. The concentration of all solutions on a mononucleotide (or phosphate) basis in aqueous solution was determined by means of published extinction coefficients (Table I). Since the weight of the nucleic acid or mononucleotide and the volume of solvent used in the glycol and aqueous solutions were identical, concentrations were also taken as identical. Although the final ionic strength and pH of the solutions used here were different than those reported in the references, we found that dialysis from these literature conditions to ours produced a volume change of $\leq 5\%$. This change and the resultant change in polynucleotide concentration was well within the limit of

TABLE I: Molar Extinction Coefficients, ϵ_{max}, of the Polynucleotides (A) and Mononucleotides (B) (Aqueous Solution).

Material	λ_{max}	ϵ_{max}	Reference
		Part A	
DNA	260	6.6	Mahler *et al.* (1964)
tRNA	260	7.6	Sarkar and Yang (1965a)
Acid $(rA)_n$	252	8.6	Ts'o *et al.* (1966)
Neutral $(rA)_n$	257	10.5	Ts'o *et al.* (1966)
Acid $(rC)_n$	275	7.4	Ts'o *et al.* (1966)
Neutral $(rC)_n$	267	6.5	Ts'o *et al.* (1966)
$(rU)_n$	260	9.2	Ts'o *et al.* (1966)
$(rG)_n$	252	9.5	Pochon and Michelson (1965)
$(rG)_n \cdot (rC)_n$	257	7.7	Haselkorn and Fox (1965)
$(rA)_n \cdot (rU)_n$	257	6.3	Warner (1957)
		Part B	
DNA mixture[a]	261	11.0	Spirin *et al.* (1959)
tRNA mixture[b]	260	11.7	Spirin *et al.* (1959)
rAMP, pH 5.5	259	15.4	Pabst Circular OR-10
rAMP, pH 8.8	259	15.4	Pabst Circular OR-10
rCMP, pH 5.5	271	9.0	Pabst Circular OR-10
rCMP, pH 8.8	271	9.0	Pabst Circular OR-10
rUMP	262	10.0	Pabst Circular OR-10
rGMP	252	13.7	Pabst Circular OR-10
rGMP + rCMP[c]	253	10.0	Pabst Circular OR-10 (calculated)
rAMP + rUMP[c]	260	12.5	Warner (1957) or as calculated from Pabst Circular OR-10

[a] Mixture prepared in the following mole ratios: dG-0.205; dC-0.205; dA-0.295; dT-0.295. [b] Mixture prepared in the following mole ratios: rG-0.280; rC-0.280; rA-0.220; rU-0.220. [c] Equimolar mixture.

TABLE II: Thermal Properties of the Nucleic Acids and Polynucleotides (A) and of Riboguanylate-Containing Polymers at Low Ionic Strength (B).[a]

Polynucleotide	T_m (°C)	$\sigma_{2/3}$ (°C)	h	h'
	Part A			
DNA	78.2	4.1	0.36	0.54
DNA (glycol)	35.0	4.5	0.39	0.67
tRNA	49.2	30.2	0.21	0.54
Acid $(rA)_n$	45.4	1.9	0.25	0.76
Neutral $(rA)_n$	44.1	33.9	0.19	0.47
Acid $(rC)_n$	56.1	23.6	0.09	0.20
Neutral $(rC)_n$	49.9	42.0	0.35	0.38
$(rA)_n \cdot (rU)_n$	44.2	7.7	0.58	0.98
$(rA)_n \cdot (rU)_n$ (glycol)	14.8	2.7	0.32	0.38
$(rG)_n \cdot (rC)_n$ (glycol)	74.0	5.7	0.21	0.40
	Part B			
Ionic Strength (M NaCl[b])				
$(rG)_n$				
0.001	75.2	22.7	0.44	
0.0001	75.8	26.1	0.43	
0.00001	73.2	31.3	0.43	
$(rG)_n \cdot (rC)_n$				
0.001	77.0	33.2	0.16	
0.0001	81.5	37.1	0.16	
0.00001	82.5	37.3	0.12	

[a] For part A, all data in aqueous medium (0.05 M KF and 0.001 M EDTA (pH 5.5) or 0.001 M Tris (pH 8.8)) except for those in the second and last two rows. T_m equals the temperature at which $A_t/A_{8°} = 0.5\ A_{max}/A_{8°}$, where A_t, $A_{8°}$, and A_{max} are the absorbances (at the absorption maximum) at any temperature, at 8°, and its maximal value, respectively. $\sigma_{2/3}$ is defined as the temperature span for $(0.67\ A_{max}/A_{8°} - 0.33\ A_{max}/A_{8°})$. $h = (A_{max} - A_{8°})/A_{8°}$ for the polymer and $h' = [A((\text{mononucleotide}) - A_{8°})/A_{8°}]$. [b] Thermal data were recorded at 275 nm for $(rG)_n$ and 280 nm for $(rG)_n \cdot (rC)_n$.

the other experimental errors and was therefore deemed acceptable. For calf thymus DNA and yeast tRNA, the equivalent mononucleotide mixtures were prepared on the basis of 41% G plus C (Michelson, 1963) and 56% G plus C (Ingram and Pierce, 1962), respectively.

The duplex $(rA)_n \cdot (rU)_n$ was formed by mixing equimolar solutions of the homopolynucleotides under conditions when the 1:1 helix is known to be stable, *i.e.*, when the homopolymers are present in an equimolar ratio, at a low polynucleotide concentration, in a low ionic strength medium, at room temperature (Stevens and Felsenfeld, 1964). Our conditions, namely, an ionic strength of 0.05 and a polynucleotide concentration of approximately 1.0×10^{-4} M, are within the range described by the above authors. The high thermal hyperchromicity observed (58%) with a $\sigma_{2/3}$ of 7.7° (Table II) and the room temperature hypochromicity relative to the constituents are adequate indices of duplex formation. In order to monitor duplex formation for $(rG)_n \cdot (rC)_n$ in aqueous solution, a mixing curve at 262 nm (Pochon and Michelson, 1965) was recorded which showed that the structure formed under our experimental conditions was indeed the duplex. For these four duplexes, as well as for $(rG)_n$ which was insoluble in glycol, a gradient dialysis procedure was used, in which the polymers, initially dissolved in aqueous solution, were dialyzed *vs.* increasing proportions of glycol in glycol–water solutions all of identical ionic composition, eventually reaching >99% glycol. Under these conditions solutions remained optically transparent.

Physical Measurements. Thermal denaturation profiles were obtained on a Gilford recording spectrophotometer (Model 2000) equipped with a Colora circulating water bath for temperature control. Its operation has been discussed previously (Mahler *et al.*, 1966), as have the variables which are determined by means of these profiles (Mahler and Dutton, 1964). The conventions used are those of Mahler *et al.* (1966). A check on precipitation during each run was made by monitoring the absorbance at 320 nm. As in previous experiments, none was observed. The experimental procedures used in obtaining optical rotatory dispersion and circular dichroism spectra and the treatment of data so obtained have also been discussed previously (Mahler *et al.*, 1968). All optical rotatory dispersion, circular dichroism, and absorption spectra were obtained by means of a Durrum-Jasco recording spectropolarimeter Model ORD/UV-5 with a circular dichroism attachment. The instrument was calibrated against *d*-10-camphorsulfonic acid for circular dichroism measurements, using a $\epsilon_l - \epsilon_r$ value of 2.2 at 290 nm (Urry *et al.*, 1968). Raw spectra were recorded in duplicate and converted to the presentation shown in the various graphs by performing the appropriate calculations every 2 nm. The units used were: for *rotation*, reduced mean residue rotation, $[m']$, which is equal to $[\alpha]_\lambda \times mrw/100 \times 3/(n^2 + 2)$, where $[\alpha]_\lambda$ is the specific rotation at wavelength, λ, and equals $100\ \alpha_{obsd}/lc$, l = light path in decimeters, c = concentration in g/100 ml, mrw = mean residue weight, and n = refractive index of the solvent (the values used for the Lorentz correction $[3/(n^2 + 2)]$, were 0.774 and 0.733, for the aqueous and glycol solutions, respectively; they were the mean values for the wavelength range employed here and are discussed in Mahler *et al.*, 1968) and has units of deg-cm/dmole; for *dichroism*, molecular ellipticity, $[\theta]$, which is equal to $[\psi]_\lambda \times mrw/100$, where $[\psi]_\lambda$ is the specific ellipticity at wavelength λ and equals $33(A_l - A_r)/lc$; $A_l - A_r$ = the difference in absorbance between the left- and right-handed circularly polarized light, l = light path in decimeters, c = concentration in grams per milliliter, and mrw = mean residue weight, and has units of deg-cm^2/dmole; for absorbance, extinction coefficient per mole of residue ϵ_p, which has units of l./mole-cm. The average spectral band widths for the spectra described are at 350 nm (0.15 mm), at 300 nm (0.50 mm), at 275 nm (1.00 mm), and at 250 nm (1.50 mm). All spectra were determined against blanks that contained all components of the experimental cuvet except for the nucleotide. All measurements were reproducible to the extent of $\leq 5\%$.

A problem of some concern to us has been low values of $[m']$ and $[\theta]$ for some of our sample solutions, in addition to some displacement of the extrema to higher or lower wavelengths, when compared with certain literature values. In almost all cases, the observed differences were no greater than 15% and, in many cases, 5% or less. Those in the latter range were assumed to be insignificant since they were within experimental

error. Other differences were assumed to arise from the procedures used to calculate nucleic acid concentration. We calculated concentrations on the basis of previously published extinction coefficients (Table IA) and not on extinction coefficients specifically determined for each solution, say by phosphate analysis.

The accuracy of our instrument was checked against previously published optical rotatory dispersion spectra of testosterone and cholesterol in dioxane (Djerassi, 1960), cholesterol in chloroform and in methanol (Brand *et al.*, 1954). In these four cases, our values agreed with the published values within 5%, *i.e.*, within experimental error.

We then ran a series of optical rotatory dispersion, circular dichroism, and absorption spectra under our conditions and under published literature conditions, since it was conceivable that the differences in ionic strength and/or pH might produce noticeable changes on conformation and rotation. The following polynucleotides and conditions were used: (1) $(rA)_n$ in 0.1 M acetate–0.1 M NaCl (pH 4.85) (Sarkar and Yang, 1965b) and in 0.05 M KF–0.001 M EDTA (pH 5.5) (our conditions); (2) $(rC)_n$ in 0.1 M acetate (pH 4.1) (Fasman *et al.*, 1964) and in 0.05 M KF–0.001 M EDTA (pH 5.5) (our conditions); (3) $(rA)_n$ in 0.05 M Tris–0.1 M NaCl (pH 7.8) (Sarkar and Yang, 1965b) and in 0.05 M KF–0.001 M Tris (pH 8.8) (our conditions); (4) DNA in 0.15 M KF (pH7.4) (Samejima and Yang, 1965) and in 0.05 M KF–0.001 M EDTA (pH 5.5) (our conditions); (5) tRNA in 0.15 M KF (pH 7.4) (Samejima and Yang, 1965) and in 0.05 M KF–1.0 $\times$ 10^{-4} M Mg^{2+} (pH 7.6) (our conditions). Since $(rA)_n$ and $(rC)_n$ were not directly soluble in the acid solutions described in the literature, these polymers were first dissolved in aqueous solution at neutral pH and then dialyzed against the acid media.

In *all* of the above five cases, all the spectra obtained under our conditions and under literature conditions were identical. Therefore, the ionic strength and pH can be varied over the appropriate range without any drastic effect on any of the optical properties and, hence, on helix geometry. On the other hand, such variations do produce an effect on helix stability, as evidenced by changes in T_m with observed differences of 20–30°.

While we did not notice any pH-induced optical rotatory dispersion or circular dichroism changes for DNA in the pH range from 7.4 to 5.5, Sarkar and Yang (1965a) report that as the pH is decreased from 7 to 4, the rotation decreases even though the overall helical structure is retained. Fasman *et al.* (1964) found $[m']$ for the peak of neutral $(rC)_n$ to vary between 23,800 and 49,000 depending on the previous history of the sample, and Guschlbauer (1967) has provided evidence for profound effects of pH on the *nature* of the transition of acid $(rC)_n$ even over a very narrow range, unaccompanied by any profound changes of the optical rotatory dispersion spectrum. That very significant effects can be produced by protonation (below pH 5.5) of $(rG)_n \cdot (rC)_n$ and of DNA has been discussed by Michelson and Pochon (1969).

Results

Since the main purpose of this report is to provide a comparison of the behavior of polynucleotides and mononucleotides in glycol and aqueous solutions, the three types of optical spectra, *i.e.*, optical rotatory dispersion, circular dichroism, and absorption spectra, are always shown in vertical alignment to permit a direct comparison of the positions of maxima and minima as well as crossovers in the circular dichroism relative to the absorption spectra (*e.g.*, Kauzmann *et al.*, 1940; Moscovitz, 1960; Tinoco, 1968; Eyring *et al.*, 1968). The same symbols are used in all figures: polynucleotides in water and glycol are represented by a solid and a broken line, respectively, while the mononucleotides, in water and glycol, are represented by a dotted and a dotted-dashed line.

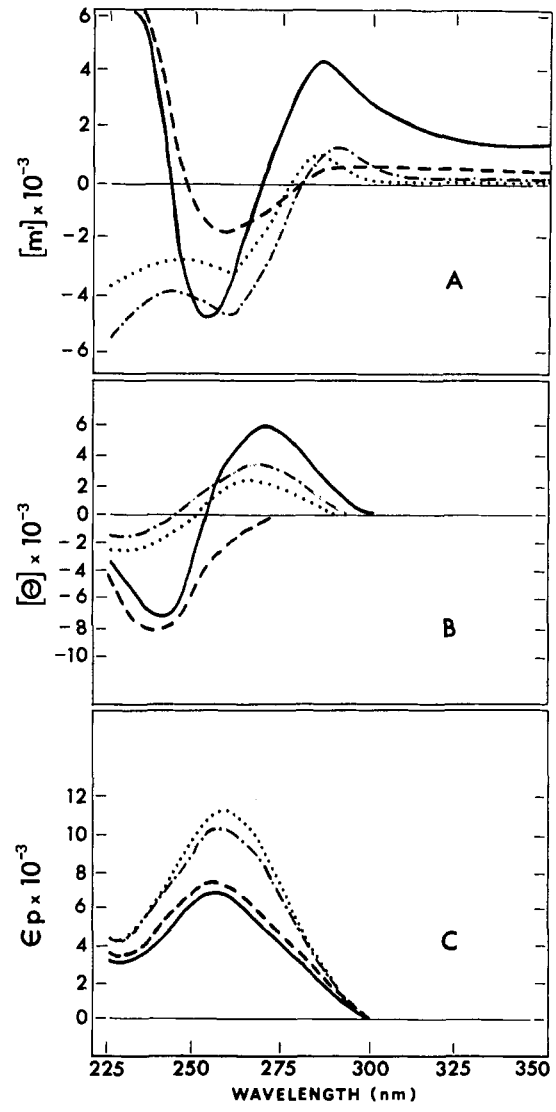

FIGURE 1: Optical spectra of calf thymus DNA and mononucleotide components in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. DNA in water (——), DNA in glycol (– – – –), mononucleotides in water (. . . .), mononucleotides in glycol (-·-·-·-). Concentration for optical rotatory dispersion and circular dichroism was 2.7 $\times$ 10^{-4} M polymer phosphate, while 2.7 $\times$ 10^{-5} M was used for the absorption curves. Spectra were recorded at 27 ± 1° in a 1-cm cell.

DNA. The optical rotatory dispersion, circular dichroism, and absorption spectra of DNA, compared with a component mixture of mononucleotides in glycol and aqueous solution, are presented in Figure 1. Since these curves have been discussed previously (Green and Mahler, 1968), we only

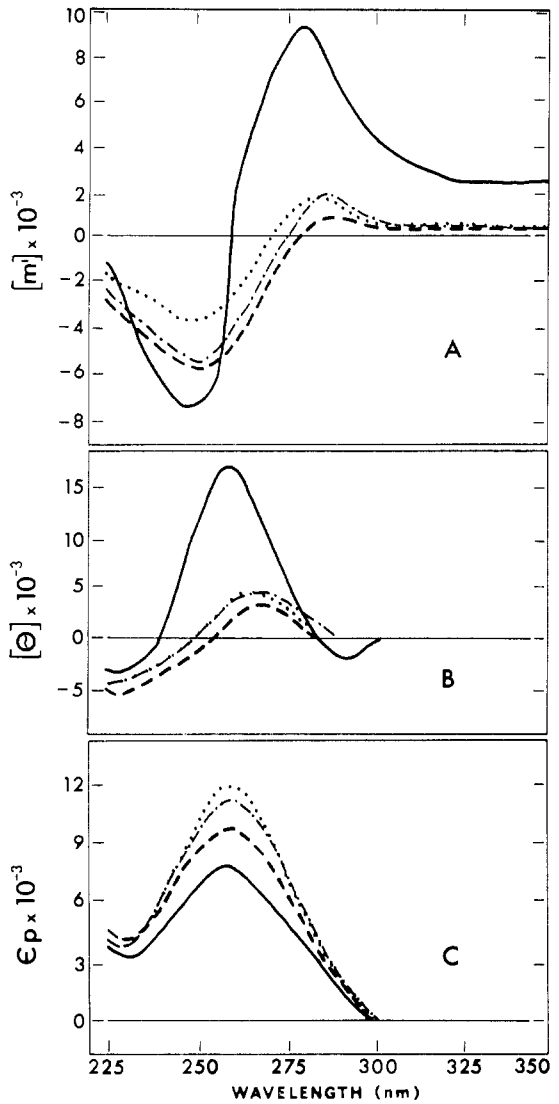

FIGURE 2: Optical spectra of yeast tRNA (stripped) and mononucleotide components in 0.05 M KF–0.0001 M Mg^{2+} (pH 7.6). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. tRNA in water (——), tRNA in glycol (– – –), mononucleotides in water (· · · · ·), mononucleotides in glycol (-·-·-·-). Concentration for optical rotatory dispersion and circular dichroism was 3.0×10^{-4} M polymer phosphate, while 3.0×10^{-5} M polymer phosphate was used for the absorption curves. Spectra were recorded at 27 ± 1° in a 1-cm cell.

mention that DNA apparently undergoes a major structural change when its environment is changed from an aqueous to this particular nonaqueous one: the long-wavelength peak in the optical rotatory dispersion, which is reported to be very sensitive to changes in conformation (Samejima and Yang, 1964, 1965; Sarkar and Yang, 1965a; Gratzer, 1966), is eliminated to the extent that only a background rotation, with an origin in the far-ultraviolet region, remains. The positive dichroic band, a most sensitive indicator of conformation (Brahms and Mommaerts, 1964; Bush and Brahms, 1967; Tinoco, 1968; Mahler *et al.*, 1968), has similarly been eliminated. While there has been a drastic alteration in these conformation-dependent parameters, the vertical base–base interactions have apparently been retained, since the absorption spectra of this polymer, whether dissolved in water or glycol, are virtually identically hypochromic. Different results, however, are observed at lower ionic strengths (reviewed in Green and Mahler, 1968). Also, as reported there, and confirming earlier studies of Luzzati *et al.* (1964, 1967), a sharp and extensive (39%) hyperchromicity is seen when DNA in glycol is heated (*cf.* also Table IIA). These facts provide additional indications of strong base–base interaction, cooperativity and rigidity of conformation (Luzzati *et al.*, 1967). With these spectra for DNA as a basis of comparison, we then proceeded to make a systematic study of all available model polyribonucleotides in an attempt to find the cause(s) of this anomalous behavior of DNA in glycolic solutions. In particular, we raised the following questions: (a) is any particular base or combination of bases in a polynucleotide specifically responsible for this phenomenon by virtue of a selective interaction with glycol and (b) does the explanation lie with a particular conformation characteristic of DNA and lacking in other polynucleotides?

tRNA. Figure 2 shows the observed optical data for tRNA in the presence of a stoichiometric quantity of Mg^{2+}. This molecule can be considered a DNA-like polymer both in the sense that all four bases are bound in a defined sequence and in possessing a high degree of order and base pairing (Madison, 1968; Cox, 1968). Its optical behavior is, however, quite different. Its optical rotatory dispersion spectrum in aqueous solution has been reported by Lamborg *et al.* (1965), Fasman *et al.* (1965), and Kay and Oikawa, (1966; see also Cox, 1968) even though these investigators give numerical values differing from one another. The transition from an aqueous to a nonaqueous environment produces the following optical rotatory dispersion changes: (a) a shift of the positive extremum to a longer wavelength, (b) a sharp decrease in peak to trough magnitude, (c) a shift in crossover point to longer wavelength, and (d) a shift of the trough to longer wavelength. These changes are those expected on denaturation (Fasman *et al.*, 1964; Kay and Oikawa, 1966; Mahler *et al.*, 1968; Samejima *et al.*, 1968), and some form of order–disorder conversion must have occurred: the decrease in the magnitude of the Cotton effect in glycol is due to a loss of rotational strength and the most likely transition causing such a loss is from one containing helical regions to a random coil. A truly featureless random coil, lacking any structural organization, should exhibit a transition moment that does not differ significantly from that of a solution of mixed mononucleotides in either solvent, and indeed that is what is observed in Figure 2A and Table III. Even in the presence of Mg^{2+}, a cation that is known to stabilize the secondary structure of tRNA in solution (Boedtker, 1960; Cox, 1968) and without which these molecules are lacking in functional activity (Nirenberg and Leder, 1964), glycol is apparently able to exert this effect as a structure breaker. Fasman *et al.* (1965) and Kay and Oikawa (1966) had previously observed, by means of optical rotatory dispersion studies, this collapse of secondary structure for tRNA in ethylene glycol. They attributed it to a weakening of solvophobic forces and argued that, conversely, such base–base interactions play the principal role in maintaining the dissymmetric native structure (see Table III). The circular dichroism spectrum (Figure 2B) lends support to this suggestion: there is a shift of the positive dichroic band to longer wavelength together with a drastic

TABLE III: Optical Rotatory Dispersion Data.

Material	First Extremum (nm)	$[m']_1$	First Crossover (nm)	Second Extremum (nm)	$[m']_2$	Second Crossover (nm)	$\pm([m']_1 - [m']_2)$
DNA (water)	287	+4,430	269	255	−4,980	244	9,410
DNA (glycol)				259	−1,800	249	
dAMP–dCMP–dGMP–dTMP (water)	287	+850	276	262	−3,260		4,110
dAMP–dCMP–dGMP–dTMP (glycol)	291	+1,225	280	263	−4,760		5,985
tRNA (water)	280	+9,440	262	248	−7,800		17,240
tRNA (glycol)	289	+930	279	252	−5,920		6,850
rAMP–rCMP–rGMP–rUMP (water)	284	+1,740	271	250	−3,750		5,490
rAMP–rCMP–rGMP–rUMP (glycol)	289	−1,930	275	252	−5,650		7,580
$(rA)_n$–acid (water)	288	+32,000	269	250	−75,000		107,000
$(rA)_n$–acid (glycol)	262	−3,700	243	232	+2,500		6,200
rAMP–acid (water)	270	−1,800	237	231	+900	225	2,700
rAMP–acid (glycol)	260	−3,550	240	237	+680	232	4,230
$(rA)_n$–neutral (water)	280	+16,680	268	255	−48,400	243	65,080
$(rA)_n$–neutral (glycol)	258	−920	238	227	+895		1,815
rAMP–neutral (water)	267	−3,490	241	236	+800	233	4,290
rAMP–neutral (glycol)	259	−3,680	248	242	+1,375	233	5,055
$(rC)_n$–acid (water)	303	+23,200	288	274	−52,400		75,600
$(rC)_n$–acid (glycol)	292	+3,810	280	245	−10,300		14,110
rCMP–acid (water)	288	+4,040	273	241	−7,680		11,720
rCMP–acid (glycol)	288	+4,750	275	244	−8,800		13,550
$(rC)_n$–neutral (water)	291	+27,400	276	266	−35,000		62,400
$(rC)_n$–neutral (glycol)	292	+4,440	275	240	−9,450		13,890
rCMP–neutral (water)	286	+5,200	269	238	−9,800		15,000
rCMP–neutral (glycol)	289	+8,000	269	240	−10,000		18,000
$(rU)_n$ (water)	282	+8,050	273	258	−18,250	232	26,300
$(rU)_n$ (glycol)	284	+4,390	275	259	−12,050	237	16,440
rUMP (water)	278	+2,680	265	247	−7,350		10,030
rUMP (glycol)	285	+4,020	272	256	−6,700	227	10,720
$(rG)_n$ (water)	270[a]	−10,300	263	247	−26,000		36,300
$(rG)_n$ (glycol)	272	+8,250	264	249	−21,400	227	29,650
rGMP (water)	267	−1,270	242				
rGMP (glycol)	267	−635	246				
$(rG)_n \cdot (rC)_n$ (water)	291	+9,300	271	245[a]	−12,525		21,825
$(rG)_n \cdot (rC)_n$ (glycol)	280	+8,200	271	243	−7,550		15,750
rGMP–rCMP (water)	288	+2,400	275	248	−5,050		7,450
rGMP–rCMP (glycol)	290	+2,100	276	245	−5,050		7,150
$(rG)_n \cdot (rG)_n$–$(rC)_n \cdot (rC)_n$ (water)	298	+12,700	282	271	−12,200		24,900
$(rG)_n \cdot (rG)_n$–$(rC)_n \cdot (rC)_n$ (glycol)	291	+3,880	269	247	−12,300		16,180
rGMP–rCMP (water)	289	+2,960	275	245	−7,710		10,670
rGMP–rCMP (glycol)	287	+3,860	278	245	−9,500		13,360
$(rA)_n \cdot (rU)_n$ (water)	285	+16,400	266	251	−26,610		43,010
$(rA)_n \cdot (rU)_n$ (glycol)	282	+2,450	273	254	−1,645	237	4,095
rAMP–rUMP (water)	290	+1,860	277	257	−2,990	231	4,850
rAMP–rUMP (glycol)	290	+930	281	260	−6,500	240	7,430

[a] For $(rG)_n$ and $(rG)_n \cdot (rC)_n$, the incompletely resolved shoulder on the Cotton effect has not been taken into account.

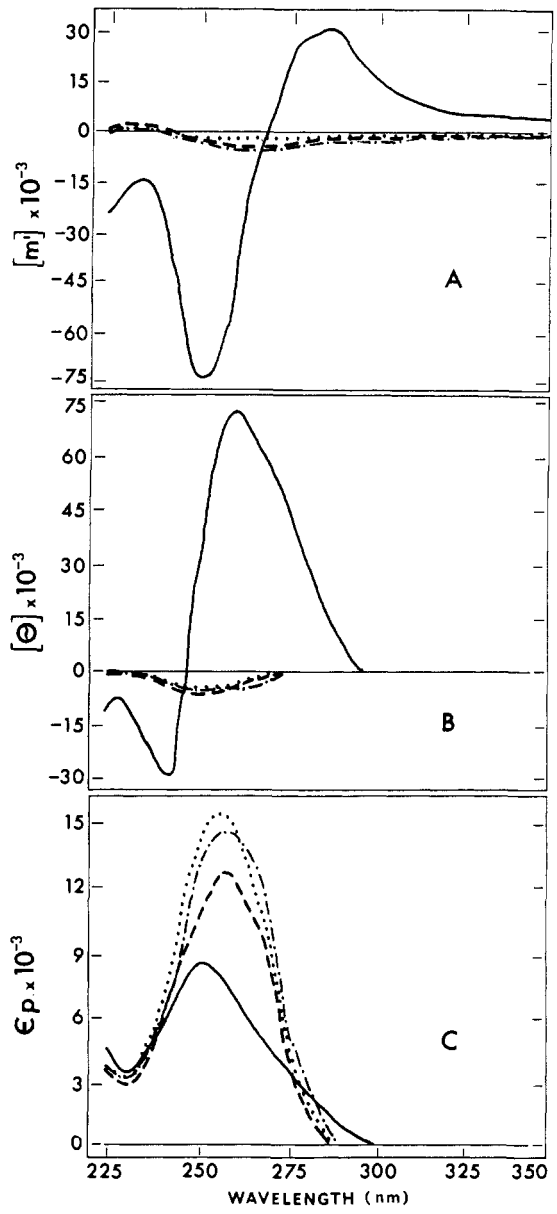

FIGURE 3: Optical spectra for acid $(rA)_n$ and rAMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(rA)_n$ in water (——),$(rA)_n$ in glycol (– – – –), rAMP in water (· · · · ·), rAMP in glycol (-·-·-·-). Concentration for optical rotatory dispersion and circular dichroism was 1.5×10^{-4} M polymer phosphate, while 3.0×10^{-5} M was used for the absorption curves. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

decrease in rotatory power and a shift to longer wavelength for the crossover point. The mixture of mononucleotides in water or glycol shows similar values for the positive band and the crossover point, although the positive band maximum occurs at longer wavelength for the glycol solution.

In addition, aqueous solutions of tRNA exhibit a negative circular dichroism band of low intensity centered at 295 nm. This band was first reported by Beychok (1966) and later by Sarkar *et al.* (1967), who showed it to be conformation dependent, since its magnitude was sharply reduced on increasing the temperature. In solutions of tRNA in glycol or other organic solvents (Wolfe *et al.*, 1968), this band is absent. It is absent also in solutions of stoichiometric mixtures of mononucleotides in either glycol or water.

As expected, the absorbance of tRNA is increased when it is dissolved in glycol instead of water. Some hypochromism relative to solutions of the mononucleotide mixtures in either solvent is, however, retained. From this we conclude that although no extended helical regions can still be present in the molecule, some base–base interactions must be retained. The most pertinent inference to be drawn from Figure 2 is that in spite of the many structural similarities between DNA and tRNA (Luborsky and Cantoni, 1962; Felsenfeld and Sandeen, 1962; Spirin, 1963; Henley *et al.*, 1966; Vournakis and Scheraga, 1966; Cantor *et al.*, 1966; Lake and Beeman, 1968; Cox, 1968) the two macromolecules must differ with regard to at least one structural parameter responsible for this differential effect elicited by ethylene glycol. Such differences in structure and, consequently, differential responses of the two types of nucleic acids to solvent-induced perturbations are not unexpected, since previous reports (Samejima and Yang, 1965; Brahms and Mommaerts, 1964) had already indicated that even-ordered RNA and DNA differ from one another in both their optical rotatory dispersion and circular dichroism spectra. The possible implications of these findings have been discussed by a number of investigators (*e.g.*, Ts'o *et al.*, 1966; Cantor *et al.*, 1966; Tinoco, 1968; Adler *et al.*, 1968; Maurizot *et al.*, 1968; Yang and Samejima, 1968; Arnott *et al.*, 1968).

Polyriboadenylic Acid, $(rA)_n$. The optical data for this polymer are presented in Figures 3 and 4. At an acid pH, the conditions of the experiment summarized in Figure 3, the molecule is known to assume a relatively rigid structure, probably constituted, as in the fiber, by a parallel double helix stabilized not only by hydrogen bonds and hydrophobic interactions but also by means of electrostatic links between the protonated N^1 of adenine bases in one-strand and phosphate residues on the opposite strand. When heated, this structure collapses over a very narrow temperature range to a singly stranded conformation with minimal residual helical features (Fresco and Doty, 1957; Fresco and Klemperer, 1959; Steiner and Beers, 1959; Rich *et al.*, 1961; Luzzati *et al.*, 1964, 1967; Holcomb and Tinoco, 1965; Hanlon and Major, 1968). At higher pH values, *i.e.*, under the conditions of the experiment presented in Figure 4, deprotonated $(rA)_n$ is the stable structure. Hydrodynamically, it exhibits a high degree of flexibility (Fresco and Klemperer, 1959; Luzzati *et al.*, 1964, 1967; Stevens and Felsenfeld, 1964) consistent with a single-stranded, base-stacked helix. This conformation is similar to that assumed by di- and higher oligomers of rAMP under comparable conditions and melts out broadly over an extended range of temperatures (*e.g.*, Brahms, 1964; Van Holde *et al.*, 1965; Holcomb and Tinoco, 1965; Cantor *et al.*, 1966; Warshaw and Tinoco, 1966; Michelson *et al.*, 1966; Poland *et al.*, 1966; Brahms *et al.*, 1967b).

We first had to establish that the acid and neutral forms of $(rA)_n$ had indeed been formed under our particular conditions in aqueous solution. Acid $(rA)_n$ exhibits an absorption maximum at 252 nm while that of the neutral form has been reported at 257 nm (Sarkar and Yang, 1965b; Holcomb and Tinoco, 1965); the results summarized in Table V are in good agreement with these values. The minimum in the circular dichroism spectra of the acid and neutral forms have

been reported at 243 and 248 nm, respectively (Brahms *et al.*, 1966); again the values observed by us (Table IV) are in satisfactory agreement. Finally, as shown in Table II, the highly hypochromic acid form melted at 45.4° with extreme cooperativeness (*i.e.*, $\sigma_{2/3} = 1.9°$) while the neutral form exhibited a considerably decreased hypochromicity and melted over a wide range ($\sigma_{2/3} = 33.9°$), a behavior in conformity with that expected on the basis of previous reports (Holcomb and Tinoco, 1965).

When the acid form is transferred from water to glycol, it exhibits changes in its optical properties indicative of a profound structural transformation (Figure 3). The powerful, positive Cotton effect in the former is replaced by a weak, broad negative one as shown in Figure 3A and Table III. In this regard, the polymer and the monomer in glycol appear quite similar both with respect to their qualitative and quantitative features. Certain differences, however, become apparent on examining the circular dichroism and optical spectra. The former (Figure 3B and Table IV) again is indicative of a profound structural change in that a polymer spectrum with considerable conservative exciton splitting is replaced by one similar to that of a monomer exhibiting only nonconservative interactions (Bush and Brahms, 1967; Tinoco, 1968). However, while the qualitative agreement between the circular dichroism spectra of monomer and polymer is good, we see that the center of the resultant negative dichroic band is displaced. That this displacement is probably ascribable to a residual conformation feature in the polymer and not just due to an analogous displacement in the absorption band becomes apparent from the data of Figure 3C and Table V. Only the polymer in glycol exhibits a shift of the dichroic band relative to the absorption band; the two bands coincide for the monomer in either solvent, and for the polymer in water the wavelength of maximal absorbance coincides with the crossover, as expected for exciton splitting (Tinoco, 1964; Bush and Tinoco, 1967; Bush and Brahms, 1967; Eyring *et al.*, 1968). Furthermore, the polymer remains partially hypochromic relative to the monomer (Table V) to an extent comparable with that characteristic of the molecule after heat denaturation in aqueous solution. The data are consistent with a conformation lacking the particular residual helical features still present in aqueous solutions of denatured $(rA)_n$ at high temperatures (Brahms and Mommaerts, 1964; Holcomb and Tinoco, 1965; Van Holde *et al.*, 1965; Warshaw and Tinoco, 1966; Bush and Tinoco, 1967); but, based on the partial hypochromism in the absorption spectrum, some base–base interactions must still be present. While the observed change in the circular dichroism might be due to a structural alteration in nucleoside conformation resulting in an altered coupling between base and sugar chromophores, this residual hypochromicity and the coincidence of the peak wavelength in absorption and circular dichroism spectra of the monomer and the divergence of the latter in the polymer suggest that the altered spectra are probably due to coupling of two different electronic (presumably the long- and short-wavelength π–π^*) transitions in adjacent bases (Brahms *et al.*, 1967a,b). Such coupling requires reasonably close contact (*i.e.*, a spacing of the order of 3.4 Å) between relatively isolated neighbors or small clusters [ϵ_p for $(rA)_n$ in glycol at 8° = 12.8×10^3; for ApA in water at 4° = 13.8×10^3 (Van Holde *et al.*, 1965)]. The absence of a conservative term in the circular dichroism

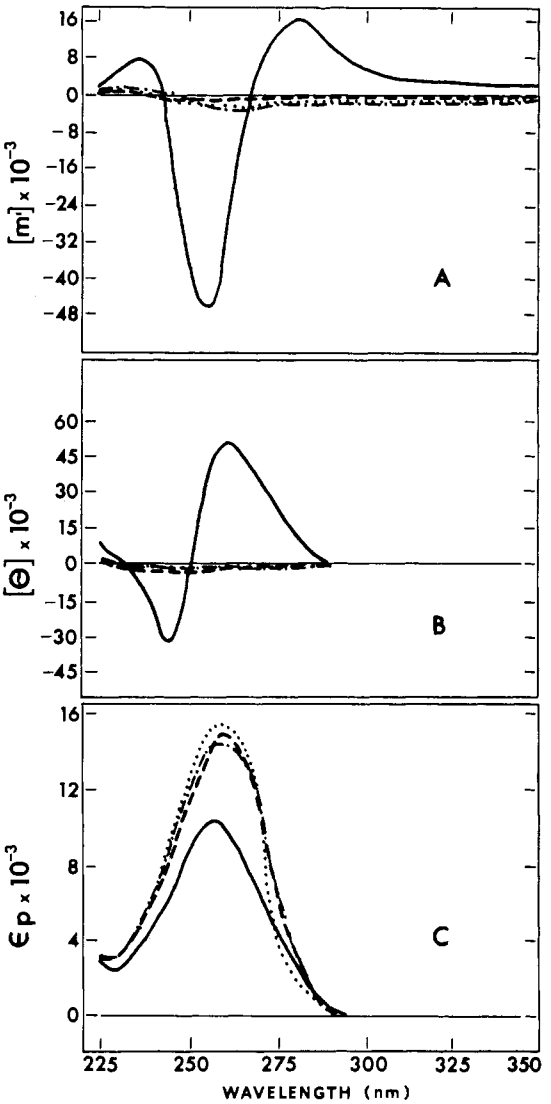

FIGURE 4: Optical spectra for neutral $(rA)_n$ and rAMP in 0.05 M KF–0.001 M Tris (pH 8.8). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(rA)_n$ in water (——), $(rA)_n$ in glycol (-----), rAMP in water (· · · · ·), rAMP in glycol (-·-·-·-). Concentration for optical rotatory dipersion and circular dichroism was 1.1×10^{-4} M polymer phosphate while 2.2×10^{-5} M was used for the absorption spectra. Spectra were recorded at 27 ± 1° in a 1-cm cell.

spectra requires the rotation between any two such stacked bases to be either 0 or 180° (Van Holde *et al.*, 1965; Bush and Tinoco, 1967; Tinoco, 1968; Eyring *et al.*, 1968). Perhaps the high viscosity of glycol stabilizes this particular conformation. Its formation appears to be at least partially dependent on electrostatic forces since in slightly alkaline solutions (Figure 4) there is no longer any indication of such residual hypochromism (Figure 4C, Table V). Except for a smaller red shift in the dichroic band, relative to the absorption band, the optical properties of this neutral form of $(rA)_n$ in glycol closely resemble those of the monomer. Thus, its conformation is likely to be that of a single-stranded, virtually random, coil. Incidentally, the close similarity of the circular dichroism minima of the neutral and acid forms of $(rA)_n$ in glycol

TABLE IV: Circular Dichroism Data.

Material	Band Max (nm)	$[\theta]_{max}$	First Crossover (nm)	Band Min (nm)	$[\theta]_{min}$	Second Crossover (nm)
DNA (water)	270	+5,775	255	242	−7,400	
DNA (glycol)				240	−8,325	
dAMP–dCMP–dGMP–dTMP (water)	264	+2,200	251			
dAMP–dCMP–dGMP–dTMP (glycol)	269	+3,110	245			
tRNA (water)	261[a]	+17,300	240	227	−3,500	
tRNA (glycol)	269	+3,520	255	227	−6,000	
rAMP–rCMP–rGMP–rUMP (water)	263	+4,300	249			
rAMP–rCMP–rGMP–rUMP (glycol)	270	+4,300	249			
$(rA)_n$–acid (water)	260	+72,750	248	242	−31,000	
$(rA)_n$–acid (glycol)				251	−6,200	225
rAMP–acid (water)				255	−3,900	229
rAMP–acid (glycol)				258	−5,600	225
$(rA)_n$–neutral (water)	262	+51,000	252	245	−34,000	230
$(rA)_n$–neutral (glycol)				249	−3,500	227
rAMP–neutral (water)				260	−2,040	240
rAMP–neutral (glycol)				260	−2,420	240
$(rC)_n$–acid (water)	282	−53,500	270	263	−17,900	
$(rC)_n$–acid (glycol)	276	+9,150	250			
rCMP–acid (water)	270	+8,450	240			
rCMP–acid (glycol)	274	+10,300	240			
$(rC)_n$–neutral (water)	275	+58,900	245	232	−10,800	
$(rC)_n$–neutral (glycol)	272	+14,000	242			
rCMP–neutral (water)	265	+12,700	232			
rCMP–neutral (glycol)	265	+14,500	234			
$(rU)_n$ (water)	269	+11,130	255	244	−5,460	
$(rU)_n$ (glycol)	272	+5,670	260	245	−5,460	
rUMP (water)	267	+6,180	250	232	−4,940	
rUMP (glycol)	273	+7,650	254	234	−5,350	
$(rG)_n$ (water)	258[a]	+27,600	245	237	−8,700	225
$(rG)_n$ (glycol)	260[a]	+21,100	247	239	−7,960	225
rGMP (water)				242	−4,040	
rGMP (glycol)				242	−5,890	
$(rG)_n \cdot (rC)_n$ (water)	273	+20,200	242			
$(rG)_n \cdot (rC)_n$ (glycol)	274	+13,300	247			
rGMP–rCMP (water)	264	+7,000	250			
rGMP–rCMP (glycol)	268	+7,000	251			
$(rG)_n \cdot (rG)_n \cdot (rC)_n \cdot (rC)_n$ (water)	283	+18,400	248	237	−4,410	
$(rG)_n \cdot (rG)_n$–$(rC)_n \cdot (rC)_n$ (glycol)	264	+9,650	247	230	−8,250	
rGMP–rCMP (water)	266	+8,700	247			
rGMP–rCMP (glycol)	270	+12,000	247			
$(rA)_n \cdot (rU)_n$ (water)	261	+32,800	249	244	−8,400	230
$(rA)_n \cdot (rU)_n$ (glycol)	272	+1,160	261	246	−4,180	230
rAMP–rUMP (water)	266	+2,820	247	232	−1,880	
rAMP–rUMP (glycol)	277	+1,570	272	256	−2,510	

[a] Only principal bands (*e.g.*, for tRNA and $r(G)_n$) are shown.

TABLE V: Absorption Data.

Material	λ_{max}	ϵ_{max}	Material	λ_{max}	ϵ_{max}
DNA (water)	258	6,600	$(rA)_n$–neutral (water)	257	10,540
DNA (glycol)	259	6,960	$(rA)_n$–neutral (glycol)	259	15,080
dAMP–dCMP–dGMP–dTMP (water)	259	11,260	rAMP–neutral (water)	258	15,400
dAMP–dCMP–dGMP–dTMP (glycol)	260	10,360	rAMP–neutral (glycol)	258	14,300
tRNA (water)	259	7,600	$(rC)_n$–acid (water)	273	7,400
tRNA (glycol)	260	9,680	$(rC)_n$–acid (glycol)	272	8,120
rAMP–rCMP–rGMP–rUMP (water)	259	11,720	rCMP–acid (water)	270	9,000
rAMP–rCMP–rGMP–rUMP (glycol)	260	11,210	rCMP–acid (glycol)	272	8,300
$(rA)_n$–acid (water)	251	8,600	$(rC)_n$–neutral (water)	268	6,500
$(rA)_n$–acid (glycol)	258	12,800	$(rC)_n$–neutral (glycol)	273	8,240
rAMP–acid (water)	257	15,400	rCMP–neutral (water)	270	9,000
rAMP–acid (glycol)	258	14,620	rCMP–neutral (glycol)	273	8,285
$(rU)_n$ (water)	261	9,200	$(rG)_n \cdot (rG)_n$–$(rC)_n \cdot (rC)_n$ (water)	259	6,200
$(rU)_n$ (glycol)	261	9,750	$(rG)_n \cdot (rG)_n$–$(rC)_n \cdot (rC)_n$ (glycol)	256	6,350
rUMP (water)	263	10,000	rGMP–rCMP (water)	253	8,050
rUMP (glycol)	261	9,800	rGMP–rCMP (glycol)	254	7,250
$(rG)_n$ (water)	253	9,500	$(rA)_n \cdot (rU)_n$ (water)	257	6,300
$(rG)_n$ (glycol)	255	9,350	$(rA)_n \cdot (rU)_n$ (glycol)	264	9,150
rGMP (water)	250	13,700	rAMP–rUMP (water)	259	12,500
rGMP (glycol)	255	15,100	rAMP–rUMP (glycol)	262	12,720
$(rG)_n \cdot (rC)_n$ (water)	263	7,700			
$(rG)_n \cdot (rC)_n$ (glycol)	261	6,800			
rGMP–rCMP (water)	254	9,900			
rGMP–rCMP (glycol)	255	9,600			

makes it highly likely that whatever its precise conformation in this solvent, the acid form at this pH is single stranded also. Finally, it should be mentioned that at a more acid pH, *i.e.*, at a ratio of 10 protons/base, the polynucleotide appears to retain its double-stranded structure even in glycol as already reported by Hanlon and Major (1968).

Polyribocytidylic Acid, $(rC)_n$. The relevant data for this polymer are presented in Figures 5 and 6. It, like $(rA)_n$, has been reported to exist in two conformations. In aqueous solutions at pH around 5, the so-called acid form consists of a double-helical structure, stabilized by hydrophobic forces and hydrogen bonding, including a bond by means of a shared proton between the N^3 of two cytosines on the two parallel strands (Rich *et al.*, 1961; Ts'o *et al.*, 1962; Akinrimisi *et al.*, 1963; Langridge and Rich, 1963; Fasman *et al.*, 1964; Hartman and Rich, 1965; Guschlbauer, 1967; Adler *et al.*, 1967). Removal of the proton in more alkaline solutions results in the neutral form of $(rC)_n$: a single-stranded helix, the stability and conformation of which is determined by the base-stacking proclivities of cytidine nucleotides (Brahms, 1964; Fasman *et al.*, 1964; Bush and Tinoco, 1967; Bush and Brahms, 1967; Adler *et al.*, 1967, 1968; Cantor and Tinoco, 1967; Brahms *et al.*, 1967a,b). The melting behavior and ionic strength dependence of the two forms is in accord with the two postulated conformations. Again, we first established that the two different forms were indeed present under our experimental conditions. Discriminatory probes used were: (a) the reported positions of the Cotton effect: positive and negative extrema at ≥ 300 and 275 nm for the acid *vs.* 290 and 265 nm for the neutral form; (b) the observation that while the acid form is characterized by a significant conservative contribution to its circular dichroism spectrum (Brahms *et al.*, 1967a,b), the neutral form provides the prime example of one that appears almost completely nonconservative (Brahms, 1967; Bush and Brahms, 1967; Adler *et al.*, 1967); and (c) the higher T_m and greater cooperativity (smaller $\sigma_{2/3}$) in the thermal transition of the acid compared with the neutral form (Fasman *et al.*, 1964; Adler *et al.*, 1967; Guschlbauer, 1967). Reference to the two figures, and especially to Tables II–IV, shows that all these criteria have been met.

The interpretation of the changes occurring in glycol (Figures 5 and 6) are straightforward. Both the neutral and acid form assume a conformation, presumably close to that of a random coil, characterized by optical properties virtually superimposable on those of the monomer. This behavior is somewhat different from that just discussed for $(rA)_n$. The

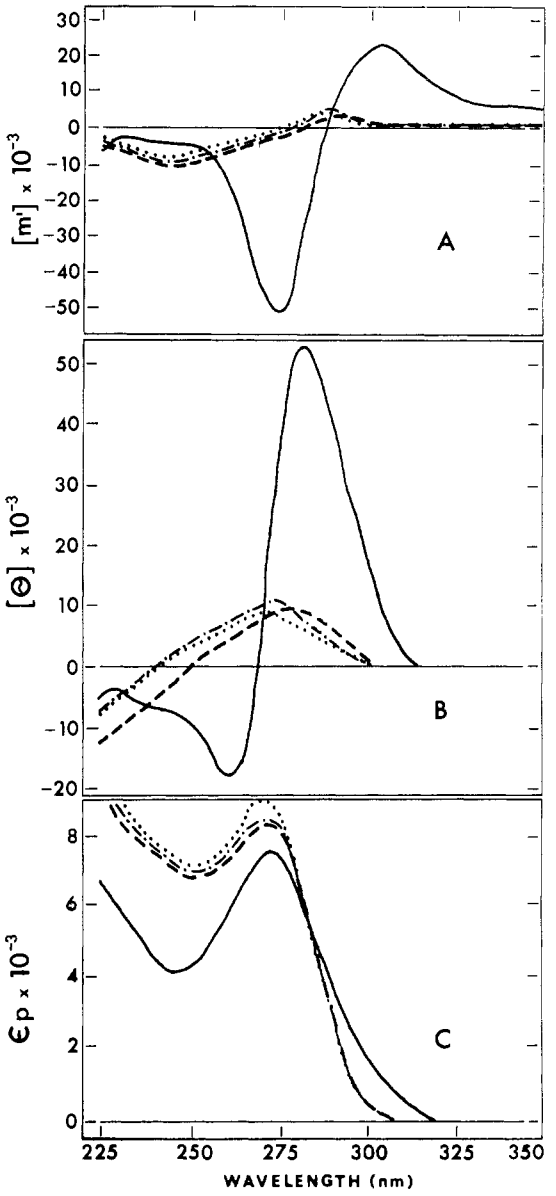

FIGURE 5: Optical spectra for acid $(rC)_n$ and rCMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption, $(rC)_n$ in water (——), $(rC)_n$ in glycol (-----), rCMP in water (· · · · ·), rCMP in glycol (-·-·-·-). Concentration for optical rotatory dispersion and circular dichroism was 1.2×10^{-4} M, while 5.0×10^{-5} M was used for the absorption spectra. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

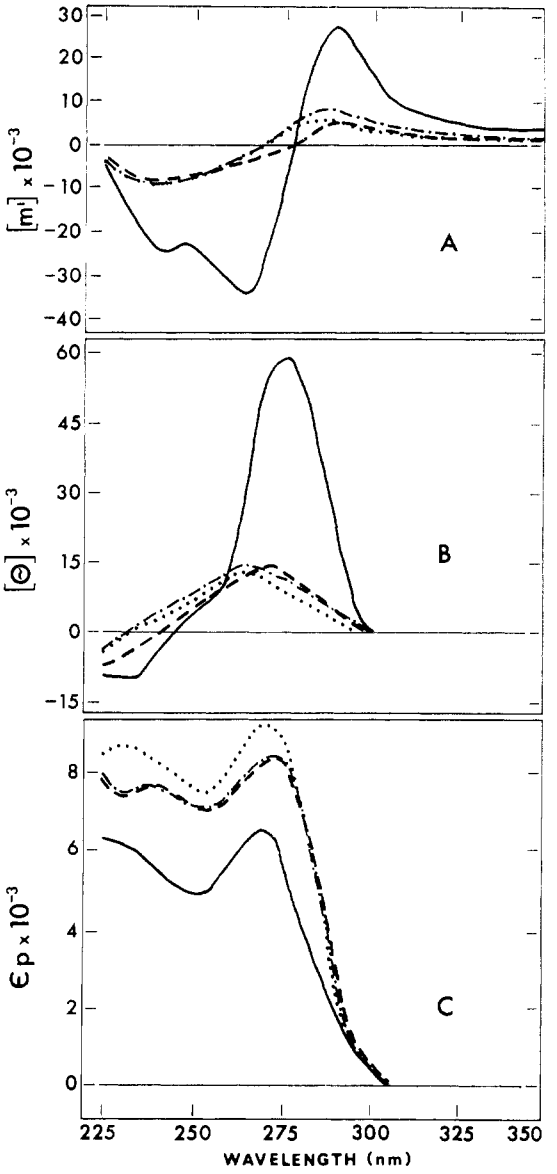

FIGURE 6: Optical spectra for neutral $(rC)_n$ and rCMP in 0.05 M KF–0.001 M Tris (pH 8.8). (A) Optical rotatory dispersion, (B) circular dichroism, (C) absorption. $(rC)_n$ in water (——), $(rC)_n$ in glycol (-----), rCMP in water (· · · · ·), rCMP in glycol (-·-·-·-). Concentration for optical rotatory dispersion and circular dichroism curves was 1.4×10^{-4} M polymer phosphate, while 5.5×10^{-5} M polymer phosphate was used for the absorption spectra. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

solvent-induced denaturation is accompanied by a blue shift in the absorption spectrum of acid $(rC)_n$ (Figure 5C), similar to that accompanying its denaturation by heating of aqueous solutions (Akinrimisi *et al.*, 1963; Guschlbauer, 1967) and to be interpreted in similar terms: loss of a proton during the helix-coil transition. Thus, the conformation of $(rC)_n$ in glycol appears to be virtually independent of pH and, in this regard also, acid $(rC)_n$ appears to behave somewhat differently from acid $(rA)_n$. In essence, the conclusions just stated concerning the conformation of $(rC)_n$ in glycol confirm the ones previously published by Fasman *et al.* (1964) and Adler *et al.* (1967). In addition, a limited participation of a short-wavelength electronic transition is suggested by the greater rotational strength—relative to the neutral form—of the incipient negative dichroic band at wavelength <240 nm, also seen in the short-wavelength region of the optical rotatory dichroism spectrum.

The mononucleotide in glycol (Figures 5C and 6C, Table V) appears to be somewhat hypochromic relative to water at both pH values. This is surprising in view of the efficacy of glycol, again demonstrated here, in disrupting all interactions between neighboring bases capable of inducing helix formation. Perhaps, some interaction between solute dipoles due to hydrogen bonding is still possible and favored by a solvent

of high viscosity and low dielectric constant. One prediction would be the absence of such interactions in dCMP (Adler *et al.*, 1968; Maurizot *et al.*, 1968).

Again, as in the case of $(rA)_n$, the spectra of $(rC)_n$ show no resemblance to those of DNA in glycol.

Polyribouridylic Acid, $(rU)_n$. Representative spectra for $(rU)_n$ are seen in Figure 7. As expected, they indicate relatively little conformational change between polynucleotide solutions in water or glycol. At the temperatures employed, this polymer had previously been shown to exhibit little secondary structure beyond that already present in oligomers (Rich, 1957; Lipsett, 1960; Richards *et al.*, 1963; Michelson and Monny, 1966; Brahms *et al.*, 1967a,b), and, therefore, no drastic reduction in rotational strength would be expected on transfer to glycol solutions. This prediction is borne out by the measurements. Optical rotatory dispersion spectra in water and glycol show a very similar wavelength dependence with a reduction in both peak and trough height. The optical rotatory dispersion spectra of rUMP, in water and glycol, also show a positive Cotton effect with peak to trough magnitudes less than that for $(rU)_n$ in either solvent. An analogous picture emerges from a study of circular dichroism spectra: rUMP in either solvent and $(rU)_n$ in glycol exhibits conservative exciton splitting, *i.e.*, two dichroic bands of almost equal magnitude, a positive one at longer, and a negative one at shorter wavelength with a crossover at wavelengths close to the absorption maximum (Table V). The maxima and minima, for both monomer and polymer in glycol, are red shifted relative to water. No such red shift is evident for the absorption band: all the maxima are within ~1 nm of 262 nm.

Polyriboguanylic Acid, $(rG)_n$. Representative spectra for this polymer are displayed in Figure 8. The optical rotatory dispersion in aqueous solution agrees quite well with the one previously published by Ulbricht *et al.* (1966). Specifically, it exhibits two positive, incompletely resolved extrema, followed by a negative extremum. All these features are strongly structure dependent since the dissymmetry is lost in going from polymer to monomer in either solvent. On the other hand, the principal features of the structure appear to be retained in glycol since the spectrum remains unchanged qualitatively, but with a reduction in peak to trough height.

Additional information can be derived from the circular dichroism spectrum which has not been previously reported. Three bands are again observed: a strongly positive one centered at 258 nm flanked by a broad, weakly positive one at ~295 nm and a negative one at 237 nm. Qualitatively, this spectrum bears a great deal of resemblance to one reported by Brahms and Sadron (1967) for GMP at low temperatures in 0.1 M KF–0.05 M sodium acetate (pH 5.0) where peaks at ~300 (weak) and 245 nm were seen; however, in this case there was a minimum at 278 nm and a trough at ~225 nm.

The two positive dichroic bands of $(rG)_n$ are strongly dependent on polymer conformation. They are absent in the spectra of rGMP under the conditions studied by us in either solvent. The negative band, on the other hand, appears to be due in part to features also found in the monomer. For the related compound 2′,3′-*O*-isopropylidene-3,5′-guanosine cyclonucleoside, Miles *et al.* (1967) report a minimum at 254 nm ($[\theta] \simeq -11{,}000$) assigned to B_{1u} π–π^* transition, and a weaker positive one at ~263 nm ($[\theta] \simeq 4000$) assigned predominantly to the B_{2u} π–π^* transition.

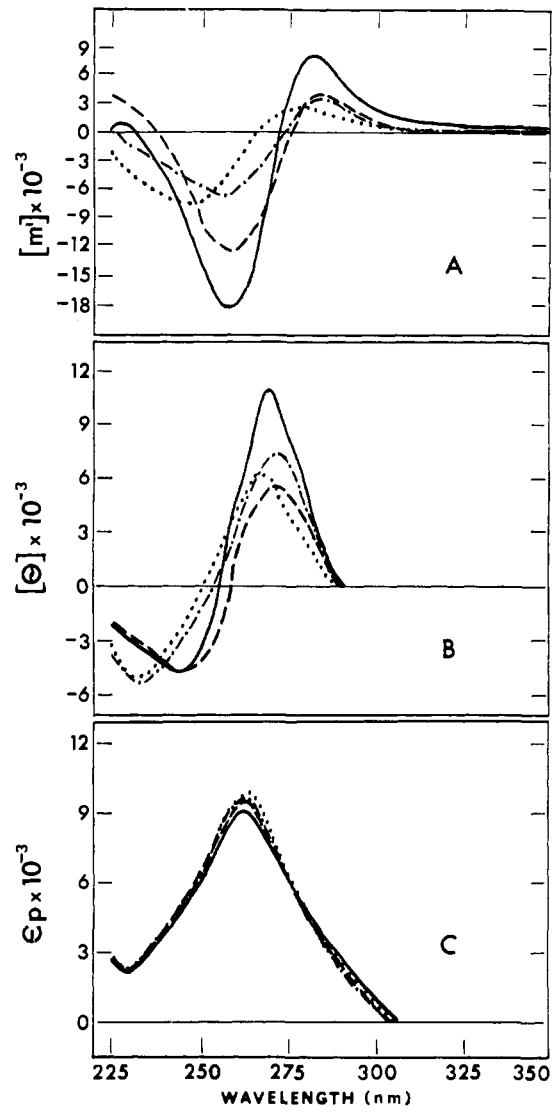

FIGURE 7: Optical spectra for $(rU)_n$ and rUMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(rU)_n$ in water (———), $(rU)_n$ in glycol (– – – –), rUMP in water (· · · · · · ·), rUMP in glycol (— · — · —). Concentration for optical rotatory dispersion and circular dichroism curves was 1.3×10^{-4} M polymer phosphate, while 6.6×10^{-5} M polymer phosphate was used for the absorption curves. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

Since the B_{1u} transition in GMP is responsible for the band with the absorption maximum at 250 nm in aqueous solution (red shifted to 255 nm in glycol and to 253–255 nm for $(rG)_n$ in either solvent), it is evident that the corresponding rotationally active transition in $(rG)_n$ is a partially conservative one (*cf.* Tinoco, 1968; Figure 1). It is thus qualitatively similar to the ones previously described for poly rC at acid pH, and for structured monomeric (Brahms and Sadron, 1967), and oligomeric riboguanylic acids (Brahms *et al.*, 1967). The assignment of the positive circular dichroism band at longer wavelengths is more difficult, particularly in view of the uncertainty concerning the λ_{max} of the long-wavelength B_{2u} transition, located in the monomer at ~280 nm (*cf.* Clark and Tinoco, 1965; Miles *et al.*, 1967). If we assume that this

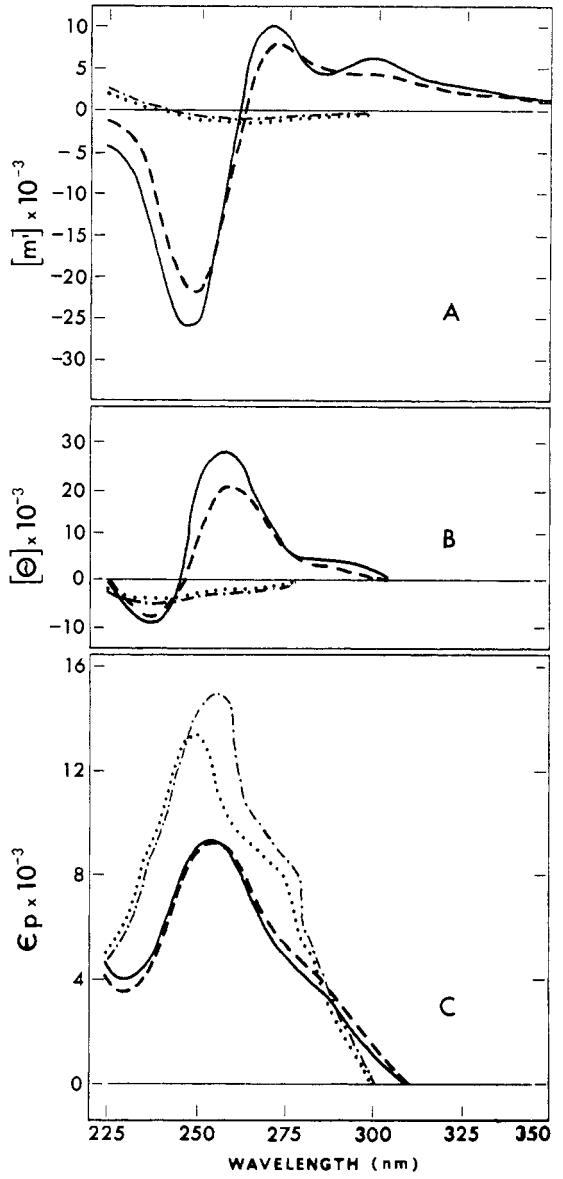

FIGURE 8: Optical spectra for $(rG)_n$ and rGMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) Circular dichroism, and (C) absorption. $(rG)_n$ in water (———), $(rG)_n$ in glycol (———), rGMP in water (· · · · · · ·), rGMP in glycol. (– · – · –) Concentration for optical rotatory dispersion and circular dichroism spectra was 7.7 x 10^{-5} M polymer phosphate, while 3.8 x 10^{-5} M was used for the absorption curves. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

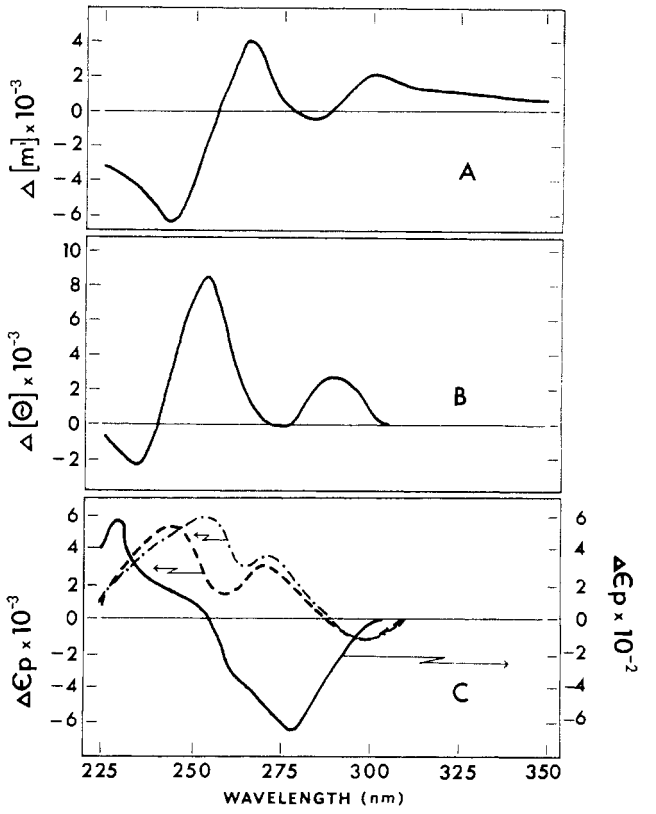

FIGURE 9: Difference spectra of $(rG)_n$ and rGMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion difference spectrum of $(rG)_n$ plotted as $(rG)_n$ water minus $(rG)_n$ glycol. (B) Circular dichroism difference spectrum of $(rG)_n$ plotted as $(rG)_n$ water minus $(rG)_n$ glycol. (C) Absorption difference spectra: $(rG)_n$ water minus $(rG)_n$ glycol (———), rGMP water minus $(rG)_n$ water (– – – –), rGMP glycol minus $(rG)_n$ glycol (· · · · · ·).

peak in the polymer is not red shifted by more than 18 nm, then the positive circular dichroism band observed might constitute the positive component of a completely conservative transition with a crossover in the 275–285-nm region (the negative component would be masked by the great rotational strength of the main band). Alternatively, this band may be generated by the weak n–π^* transition, with an electric vector polarized at right angle to the base plane, known to be a component of purine spectra in the wavelength range of 295–305 nm (Rich and Kasha, 1960; Clark and Tinoco, 1965; Miles *et al.*, 1967). That this transition can contribute to polymer spectra and is strongly structure dependent is indicated by the hyperchromic nature of the absorption spectrum relative to the mononucleotide (Figure 8C) in this wavelength range as contrasted to its hypochromism at all wavelengths $\leq$290 nm.

There are some lines of evidence that suggest to us that the n–π^* transition is responsible for the long-wavelength circular dichroism peak: (a) the hyperchromism at long wavelength with a maxima in the difference spectrum at 299 nm (Figure 9C); (b) the qualitative similarity between the circular dichroism spectra of $(rG)_n$ and ordered monomeric rG—the latter also exhibits a positive peak in this region but, in this instance, it is known that the B_{2u} transition generates a *negative* band (Miles *et al.*, 1967); (c) at acid pH, the cyclonucleoside studied by Miles *et al.* shows a circular dichroism spectrum that is qualitatively a mirror image of that given by $(rG)_n$ and here an n–π^* contribution is quite evident; (d) the quantitative differences between the two circular dichroism peaks observed on transfer to glycol to be discussed below; (e) the presence of an analogous circular dichroism band, but of opposite magnitude, in naturally occurring nucleic acids, also tentatively assigned to an n–π^* transition by Sarkar *et al.* (1967); [however, the broadness of the band suggests that it may well constitute an envelope composed of two different positive bands]; and (f) certain features of the absorption spectra discussed below.

On transfer to glycol, the qualitative features of the circular dichroism spectrum are retained. However, quantitatively, the main peak appears reduced to a lesser extent than does the satellite (Figure 9A,B). The change in solvent, similarly, leads to a decrement in the short-wavelength and to an increment in the long-wavelength region of the absorption spectrum (Figure 9C). These observations suggest that two (or more) transitions with *different* geometries are responsible for these changes.

Additional information can be extracted from the quantitative optical rotatory dispersion and circular dichroism difference spectra for $(rG)_n$ between an aqueous and a glycolic medium (Figure 9A,B). All three electronic transitions are affected by the change in solvent, with *all* of them indicating a decrease in dissymetry in going from the aqueous to glycolic medium. Recent work by Bush and Scheraga (1969) using monomers, oligomers, and polymers of adenylic acid also suggest that the long-wavelength band is indeed due to a nonconservative $n-\pi^*$ transition and is not the positive component of the splitting of a $\pi-\pi^*$ transition. Their calculations show that exciton splitting of the strong $\pi-\pi^*$ transition should leave most of the rotational strength on the short-wavelength side of the 260-nm band and thus the 290–295-nm band, on the long-wavelength side, cannot be assigned to such transition. They further suggest that solvation would shift the $n-\pi^*$ band to shorter wavelengths so that it would be masked by the strong $\pi-\pi^*$ transition, and, for this reason, a long-wavelength transition is not seen for monomers and dimers. Our data for $(rG)_n$ tend to support their conclusions. $(rG)_n$ in water and glycol, as can be seen from Figure 8, shows qualitatively the same spectra in both media and, in particular, the long-wavelength transition is retained in glycol. This shows that even a strong structure-breaking solvent, such as ethylene glycol, is incapable of destroying the strong intermolecular forces responsible for maintaining the structure of the $(rG)_n$ complex, since its destruction and the subsequent solvation of the guanine residues should lead to the disappearance of the long-wavelength transition. The circular dichroism difference spectrum does show that the magnitude of the magnetic transition in glycol is reduced to approximately two-thirds of its value in aqueous solution, which indicates that some slight weakening or deformation of the $(rG)_n$ macrostructure may have been introduced either by a change in geometry or a greater exposure of the bases to solvation.

Of further interest is our observation that the circular dichroism band on the short-wavelength side of the main $\pi-\pi^*$ transition is relatively insensitive to solvent (Table IV). This would tend to indicate that this particular electronic transition is a weak $\pi-\pi^*$ transition since, as already discussed, these are less sensitive to solvent changes than are $n-\pi^*$ transitions (Bush and Scheraga, 1969). In addition, molecular orbital calculations predict that a $\pi-\pi^*$ transition should occur in this region (Bush and Scheraga, 1969).

Figure 9C shows the absorption difference spectra of $(rG)_n$ in water minus $(rG)_n$ in glycol, rGMP in water minus $(rG)_n$ in water, and rGMP in glycol minus $(rG)_n$ in glycol. A significant degree of base stacking present in the polymer (in water or glycol), is lacking in the monomer (in water or glycol) under the conditions studied here, provided the long-wavelength transition is interpreted as a measure of base–base stacking interactions. These spectra also provide additional evidence for an alteration of geometry of solvation when $(rG)_n$ is placed in glycol.

The structure of polyriboguanylate in aqueous solution is known to be a highly ordered and stable one, but its precise geometry, including the number of strands comprising the helix, is uncertain (*cf.* the discussion by Pochon and Michelson, 1965). These authors also point out that the extreme thermal stability of the polymer does not necessarily indicate the presence of more than two strands (Fresco and Massoulie, 1963). The properties of the circular dichroism spectrum are such as to suggest a structure consisting of a right-handed helix with the base plane transition moments tilted relative to the helix axis (Bush and Brahms, 1967; Brahms *et al.*, 1967a,b; Tinoco, 1968; Yang and Samejima, 1968). We have also measured the thermal denaturation profiles of aqueous $(rG)_n$ at low ionic strengths (Table IIB). Although not as broad as that reported in the complete absence of salt (Pochon and Michelson, 1965), the transitions appear to be lacking in cooperativeness, and are relatively insensitive to ionic strength. The optical spectra in this range are, however, very similar to those observed under standard conditions. Hence, we conclude that the hydrogen bonding, postulated by Pochon and Michelson as being responsible for the structure of $(rG)_n$ at relatively high salt concentrations, makes virtually no contributions to the optical properties of the polymer. These can be accounted for by features already present in its pure aqueous solution—where the tendency to form a duplex helix must be slight—and are probably largely intrastrand in nature.

The structure of the polymer in glycol must bear a good deal of resemblance to its aqueous counterpart. Apparently, the inherent conformational stability is such as to permit retention of most of the interactions between transition moments characterizing the aqueous form, in spite of the great reduction in all solvophobic interactions induced by the change in solvent. Neither the purely solvent-dependent ones (Sinanoglu and Abdulnur, 1965) nor the contact London forces (DeVoe and Tinoco, 1962) would be expected to be qualitatively different for a single-stranded $(rG)_n$ compared with a $(rA)_n$ or $(rC)_n$ helix. The main contributor capable of stabilizing the former must, therefore, be either additional H bonds between the sugar 2′-OH and a component specifically found in $(rG)_n$ and not present in other ribohomopolymers, or else interstrand H bonds. A study of the behavior of $(dG)_n$ should permit a choice between these two alternatives. The detailed structure of $(rG)_n$ in glycol is of course also unknown. However, it must be one that permits a simultaneous *decrease* (compared with the aqueous form) in *both* the rotational and the oscillator strength due to the B_{1u} transition, not a common occurrence in the polynucleotide field. This requirement suggests a subtle alteration in helix dimensions, perhaps combining an increase in base–base contact, *e.g.*, by altering the degree of tilt, with a change in the rotational angle between successive bases in the helix (Tinoco, 1968). This suggestion is compatible with the apparent differential effects on spectra due to $\pi-\pi^*$ and $n-\pi^*$ transitions.

Polyriboguanylic·Polyribocytidylic Duplex $(rG)_n\cdot(rC)_n$. The optical data for this complex are presented in Figure 10. Its formation was first studied by Haselkorn and Fox (1965), who also presented evidence for its extreme thermal stability. These investigators, as well as Pochon and Michelson (1965), described its optical spectra (see also Michelson and Pochon,

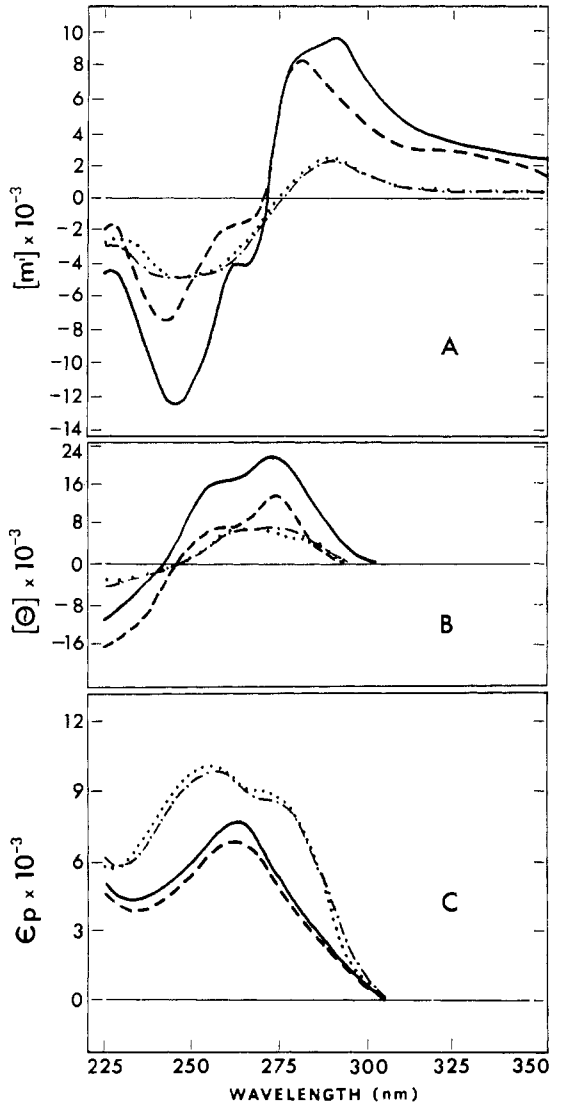

FIGURE 10: Optical spectra for $(rG)_n \cdot (rC)_n$ and a 1:1 mixture of rGMP and rCMP in 0.1 M NaCl–0.01 M sodium cacodylate (pH 7.0). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(rG)_n \cdot (rC)_n$ in water (———), $(rG)_n \cdot (rC)_n$ in glycol (– – – –), rGMP plus rCMP in water (· · · · · · ·), rGMP plus rCMP in glycol (-·-·-·-). Concentration for optical rotatory dispersion and circular dichroism spectra was 1.9×10^{-4} M polymer phosphate, while 9.7×10^{-5} M polymer phosphate was used for the absorption curves. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

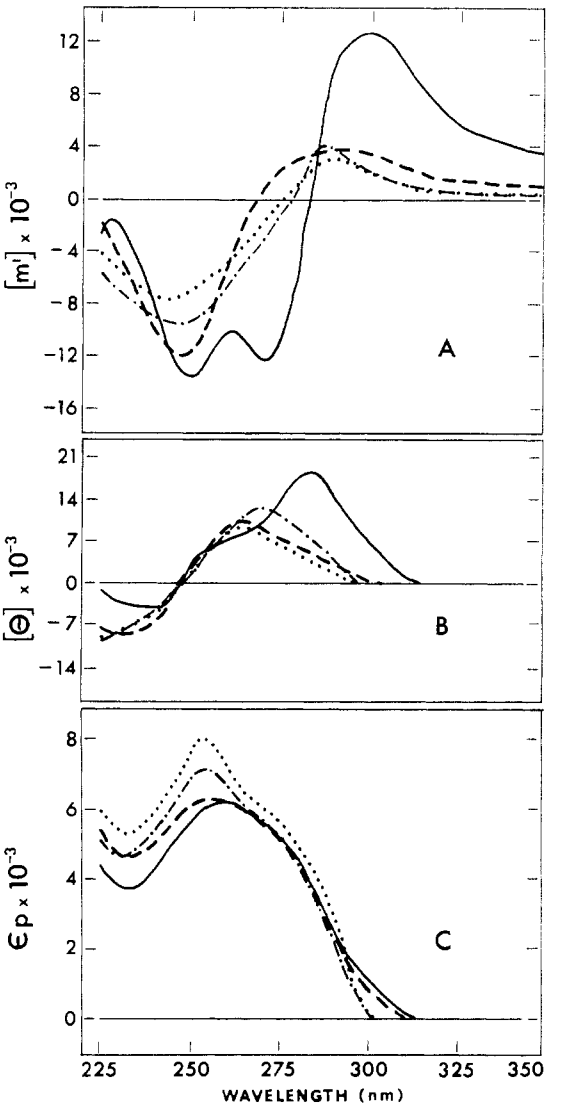

FIGURE 11: Optical spectra for $(rG)_n \cdot (rG)_n/(rC)_n \cdot (rC)_n$ and a 1:1 mixture of rGMP and rCMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(rG)_n \cdot (rG)_n/(rC)_n \cdot (rC)_n$ in water (———), $(rG)_n \cdot (rG)_n/(rC)_n \cdot (rC)_n$ in glycol (– – – –), rGMP plus rCMP in water (· · · · · · ·), rGMP plus rCMP in glycol (-·-·-·-). Concentration for optical rotatory dispersion and circular dichroism spectra was 2.5×10^{-4} M polymer phosphate, while 6.3×10^{-5} M polymer phosphate was used for the absorption curves. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

1969) as well as the difference spectra relative to the constituents and provided evidence for the 1:1 stoichiometry indicated, at *neutral* pH. At acidic pH, *i.e.*, 5.5, there is seen (Figure 11) the formation of a complex or complexes, which are distinctly different from the $(rG)_n \cdot (rC)_n$ duplex observed at neutral pH. This conclusion is based on a comparison of the rotatory and absorption spectra (see Figures 10 and 11) as well as on the thermal data, to be presented later, which show that a conformational transition is observed in glycol under neutral conditions, while none is seen under acidic conditions at the same ionic strength.

Figure 10 and Table III show the rotatory spectra for the $(rG)_n \cdot (rC)_n$ duplex. These values in aqueous media compare favorably with those reported by Ulbricht *et al.* (1966) in a different buffer. The circular dichroism spectrum presents one conformation-dependent, exceedingly broad band, evidently composed of more than one component. One can estimate that the band maxima must be roughly centered at 275 and 255 nm, *i.e.*, the regions at which the strong dichroic transitions of the component *homo*polymer helices are known to occur. As was seen for $(rG)_n$, when the duplex is dissolved in glycol, the essential features of the aqueous form of the polymer are retained, but are reduced in value. If the aqueous $(rG)_n \cdot (rC)_n$ duplex is slowly dialyzed against a medium of the same composition and ionic strength but at a lower pH

(*i.e.*, 5.5), we observe the optical rotatory dispersion spectrum seen in Figure 11. This differs from the one observed at neutral pH; specifically, the maximum is red shifted as is the crossover. Whereas only one trough is seen in the neutral form, at acidic pH two distinct troughs approximately equal in magnitude, are observed. One also notices differences in the absorption spectra: that at acidic pH appears skewed on the long-wavelength side, while the neutral form is more Gaussian in appearance. The effect of glycol on the acid form appears to be a reduction of dissymmetry to that characteristic of the mononucleotides, while still leaving a high degree of hypochromicity and hence, presumably of base stacking (see Figure 11C).

These results raise some doubts concerning certain conclusions by previous authors (Pochon and Michelson, 1965; Ulbricht *et al.*, 1966; Michelson and Pochon, 1969) that the $(rG)_n \cdot (rC)_n$ duplex, because of its extreme stability, can exist as such, over a wide range of pHs (*i.e.*, 5–13). While it is probably true that *some* form of highly ordered complexes of polyriboguanylate and of polyribocytidylate exist throughout this range, the conclusion that the complex in question is the *same* $(rG)_n \cdot (rC)_n$ duplex throughout is open to question.

Two additional pieces of evidence confirm this conclusion. First, we ran a Job (1928) (or mixing) plot of $(rG)_n$ and $(rC)_n$ at acidic pH, keeping the total molar concentration of polymer constant. Optical densities were recorded at three wavelengths (*i.e.*, 280, 262, and 245 nm), and plotted against the mole per cent G (or mole per cent C). *All* three plots show a complete absence of interaction between the components, since they consisted of straight lines connecting the two values for the pure components. This indicates that, at acidic pH, the homopolymers themselves are the thermodynamically stable species with no tendency to interact and to form heterocomplexes. It can therefore be assumed that, if the heterocomplex formed at neutral pH were exposed to acidic pH values, it would disproportionate to form the corresponding homopolymer complexes.

Second, as seen in Figure 12, when the reconstructed optical rotatory dispersion, circular dichroism, and absorption spectra for the sums of acid $[(rC)_n$ plus $(rG)_n]$, and of neutral $[(rC)_n$ plus $(rG)_n]$, respectively, are compared with those determined experimentally, we see that the former, but not the latter, produce a quite satisfactory agreement with respect to all qualitative features (*i.e.*, the extrema and crossovers are *all* within 2 nm of each other).

We propose that in the pH range of 5–6 the $(rG)_n \cdot (rC)_n$ duplex can disproportionate to form two homopolymer complexes: one being the partially protonated "acid" form of $(rC)_n$ and the other a multistranded form of $(rG)_n$ capable of accommodating additional protons (Pochon and Michelson, 1965). Using the techniques available to us, we cannot determine whether the latter is double or triple stranded.

$$2[(rG)_n \cdot (rC)_n] \overset{H^+}{\rightleftharpoons} (rG)_n \cdot (rG)_n + [(rC)_n \cdot (rC)_n]^+$$

$$3[(rG)_n \cdot (rG)_n] \overset{?}{\rightleftharpoons} 2[(rG)_n \cdot (rG)_n \cdot (rG)_n]$$

Confirmatory evidence, for the above scheme, is found in the optical rotatory dispersion spectra of $(rC)_n$-"acid" and $(rG)_n$ (see Figures 5 and 8). The two long-wavelength extrema in Figure 11 occur within 5 nm of that seen for $(rC)_n$ while the trough at 248 nm is only 1 nm removed from the trough for $(rG)_n$.

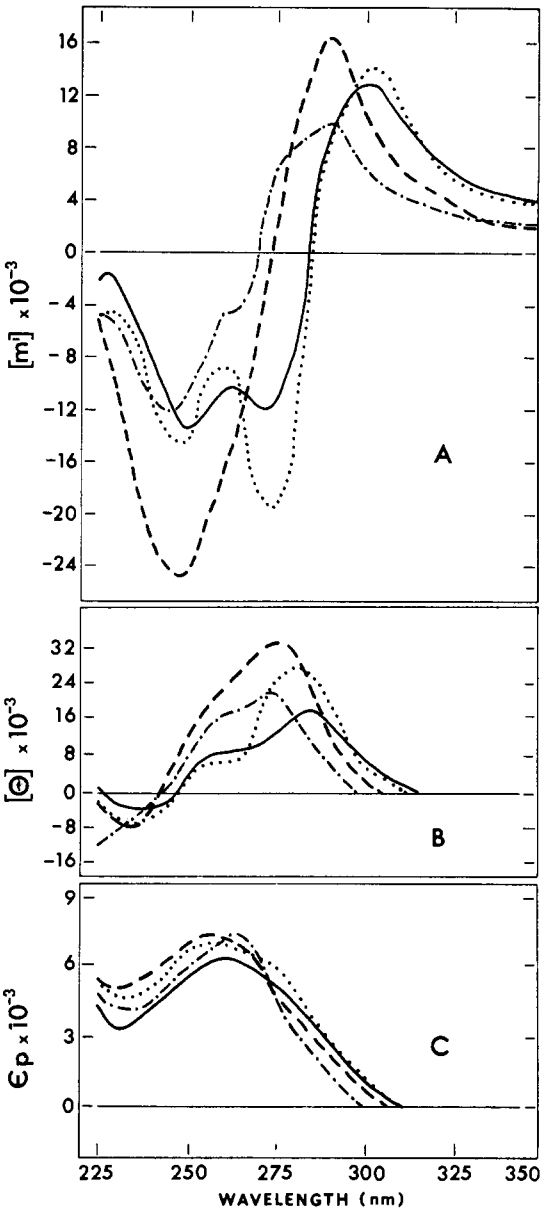

FIGURE 12: Calculated spectra for the $[(rG)_n, (rC)_n]$ complexes where $[m']$, $[\theta]$, or $\epsilon)^{\lambda} = {}^1/_2([m']$, $[\theta]$, or $\epsilon)^{\lambda}_{(rC)_n} + [m']$, $[\theta]$, or $\epsilon)^{\lambda}_{(rG)_n}$. (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. Acid $(rC)_n$ plus $(rG)_n$ calculated (· · · · · · · ·). Neutral $(rC)_n$ plus $(rG)_n$ calculated (– – – – – –), $(rG)_n \cdot (rC)_n$ duplex under neutral conditions (· – · – · – ·) and $[(rG)_n, (rC)_n]$ complexes under acidic conditions (———).

One apparent inconsistency in the above scheme is that if $(rC)_n$ were indeed present as a homopolymer duplex, one would expect it to be susceptible to thermal denaturation in aqueous solution. None is observed. Perhaps $(rG)_n$ is, somehow, protecting the $(rC)_n$ in a way that is not immediately obvious.

Polyriboadenylic·Polyribouridylic Duplex, $(rA)_n \cdot (rU)_n$. The spectra of this duplex are shown in Figure 13. Under appropriate conditions, equimolar mixtures of $(rA)_n$ and $(rU)_n$ are

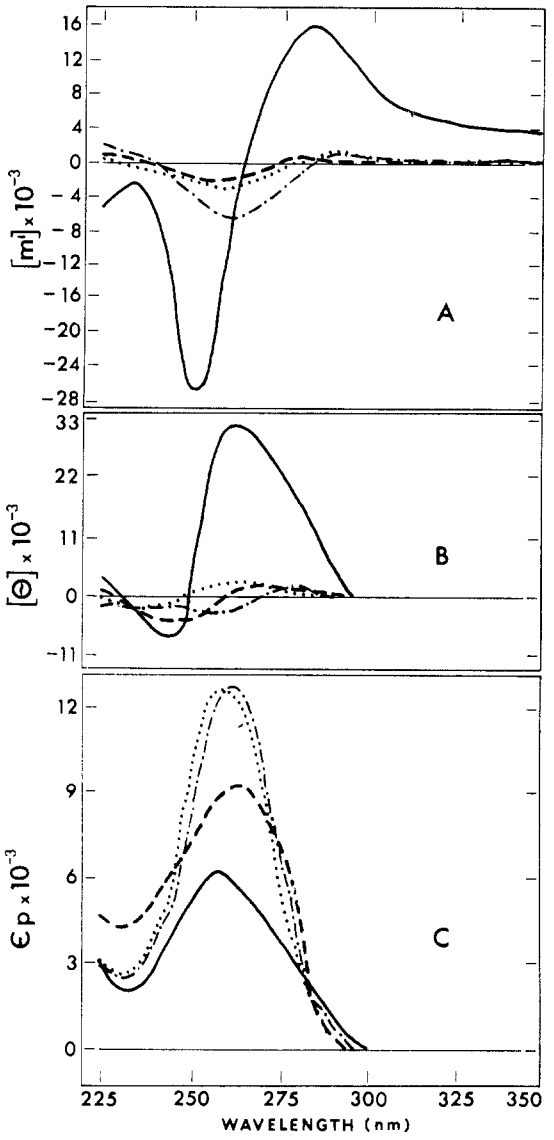

FIGURE 13: Optical spectra for $(rA)_n \cdot (rU)_n$ and a 1:1 mixture of rAMP plus rUMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(rA)_n \cdot (rU)_n$ in water (———), $(rA)_n \cdot (rU)_n$ in glycol (– – – – –), rAMP + rUMP in water (· · · · · ·), rAMP + rUMP in glycol (– · – · – · –). Concentration for optical rotatory dispersion and circular dichroism spectra was 1.5×10^{-4} M polymer phosphate, while 3.7×10^{-5} M was used for the absorption curves. Rotatory spectra were recorded at 27 ± 1° in a 2-cm cell.

known to form a stable one to one complex rather than the three-stranded structure $2(rU)_n \cdot (rA)_n$ (Felsenfeld and Rich, 1957). The dimensions of the resultant antiparallel double helix have been shown to be similar to, but not identical with, that of DNA (Rich, 1957; Miles and Frazier, 1964; Davies, 1967). Optical rotatory dispersion and circular dichroism spectra of aqueous solutions have been previously reported (Brahms, 1965; Sarkar and Yang, 1965b; Ts'o *et al.*, 1966; Hashizume and Imahori, 1967). Our values (Table IV) for the positive peak are in good agreement, but we find a somewhat smaller rotational strength. The optical rotatory dispersion spectra, for aqueous and glycol solutions, are characterized by a sharp decrease in peak to trough magnitude, and a red shift in crossover point and trough. It is instructive in this instance also to obtain a comparison not only between the duplex and mixture of mononucleotides but also between it and the calculated value for the sum of the homopolymers. For instance, $(rA)_n \cdot (rU)_n$ in glycol exhibits a peak to trough magnitude of 4095; the observed value for a mixture of rAMP plus rUMP in this solvent in 7430 (4850 in H_2O), while the calculated value for $(rA)_n$ plus $(rU)_n$ in glycol equals 9130. Similar conclusions can be drawn from the circular dichroism data (Table IV).

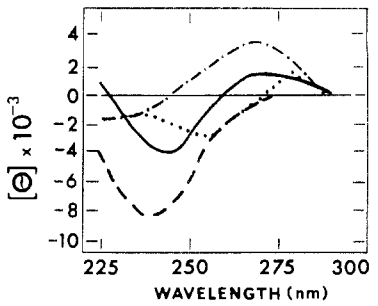

FIGURE 14: Comparison of the circular dichroism spectra of DNA (– – – – –) and its mononucleotide component mixture (– · – · – · –) with $(rA)_n \cdot (rU)_n$ (———) and its mononucleotide component mixture (· · · · · · · · ·). All spectra were recorded in glycol 0.05 M KF–0.001 M EDTA (pH 5.5).

In glycol solution, the strong positive band center is red shifted by 12 nm with a profound decrease in magnitude while the negative band has been decreased to a lesser extent. Comparison with the spectrum of a stoichiometric mixture of mononucleotides discloses that, except for a further red shift in the latter, the magnitude and the qualitative features of the spectra, including their apparently conservative nature, are similar (Figure 14). The ribopolymer duplex, therefore, exhibits a behavior quite different from that of DNA.

The absorption curves for the duplex are summarized in Table V. In glycol, the position of the peak is red shifted with an increase in extinction coefficient. This value represents considerable residual hypochromicity relative either to the sum of the mononucleotides or that of the two homopolymers. The properties of the duplex so far described are consistent with a structure that retains considerable elements of order over a relatively long range (see also next section on thermal denaturation) but one in which the angles between the transition moments of adjacent bases approach 0 or 180°, perhaps with an increase in contact distance between bases and a resultant release of the steric strain caused by the presence of 2′-OH groups in the aqueous form of a polyribonucleotide helix. An alternative, suggested by one of the referees, would be the torsional oscillation model of Glaubiger *et al.* (1968). In its properties, the structure may resemble that ascribed to DNA in aqueous and particularly in glycol solutions at elevated temperatures (Luzzati *et al.*, 1964, 1967; Green and Mahler, 1968).

Thermal Measurements. A summary of the data obtained from temperature–absorbance profiles is presented in Table II. All T_m's were determined at the wavelength of maximum absorption. The only polynucleotides which showed hyperchromic thermal transitions in glycol in the temperature range

accessible to us, *i.e.*, between 8 and 99°, were DNA, $(rA)_n \cdot (rU)_n$ and $(rG)_n \cdot (rC)_n$. This behavior is consistent with the properties deduced from the changes in optical parameters in this solvent. The lack of stability of tRNA in glycol is somewhat surprising, but Strauss *et al.* (1968) have recently reported analogous observations in Me_2SO, a solvent with somewhat similar properties. No evidence for thermally induced conformational transitions at any wavelength could be obtained for aqueous solutions of $(rU)_n$, $(rG)_n$, and $(rG)_n \cdot (rC)_n$ under our conditions. The G-containing polymers form structures so strongly stabilized by inter- and intranucleotide interaction that temperatures above the range tested are required for their disruption. Pochon and Michelson (1965), for instance, were unable to observe a thermal transition for $(rG)_n$ in pure aqueous solution. Such transitions were, however, observed by them at extremely low ionic strength and in the presence of methanol. We have repeated and extended these studies to pure aqueous salt solution. The results are shown in Table IIB. In contrast, $(rU)_n$ is already devoid of sufficient secondary structure, even at 8°, to yield a measurable change on heating (Michelson and Monny, 1966). There is, therefore, no reason to expect a melting out and its absorbance remains constant over the entire temperature range.

Comparisons and Conclusions

Polynucleotides. The most detailed recent study comparing the behavior of a polyribonucleotide in water and glycol is the one by Hanlon and Major (1968) on $(rA)_n$. They demonstrated that the fully protonated "acid" form of this polymer appeared to retain a duplex helical structure in glycol while the deprotonated "neutral" form collapsed to a structure probably identical with that of random coil in the same solvent. These findings were interpreted in terms of an increase, in this solvent, of the electrostatic contribution to the free energy of stabilization of the ordered form of the polymer compensating for the complete elimination of the contribution by stacking forces. Our results for the relevant optical parameters for the helical forms in water, as well as for the helical form (at low pH) and the disordered form (at pH $\geq$ 5.5) in glycol, are in good agreement with these observations. The fact that in water at pH 5.5,—where it can be estimated that $\leq$10% of the total residues are actually protonated—the optical properties are quite comparable with those obtained under conditions where this protonation is complete, plus the observation that at this pH in glycol there is almost complete collapse to a random coil, leads to the conclusion that in aqueous solution the electrostatic contributions to the total free-energy term for the helix–coil transition may be relatively unimportant, while it becomes the preponderant one only in solvents of high polarizability and low dielectric constant. There is an apparent discrepancy between the broad transitions observed by us (Table II) and those reported by Fasman *et al.* (1964) for the thermal denaturation of the acid form of poly rC. The difference may be referable to the degree of protonation in the two experiments. Ours were performed under our standard conditions of pH 5.5 and an ionic strength of 0.05 while those of Fasman *et al.*, were conducted at a pH of 4.2 at an ionic strength of 0.1. Under the latter conditions, as shown by Guschlbauer (1967), denaturation of the protonated polymer involves a transition without loss of protons while at higher pH values a second process involving deprotonation comes into play. Not only are the transition widths for the two processes different, but at any one ionic strength they each occur with their own characteristic T_m. Thus, at intermediate pH values, where both processes may well be operative, the thermal transition would consist of two steps and the apparent T_m would be the mean of the two characteristic individual T_m's, each with its own dispersion. Under our conditions, >0.4 of the cytosine residue is protonated—with 0.5 the value required for maximal stability of the double-helical form of $(rC)_n$ (Hartman and Rich, 1965)—yet denaturation is as complete for this protonated, hydrogen-bonded, duplex form of $(rC)_n$ on exposure to glycol as it is for the unprotonated single-stranded helix (see also Fasman *et al.*, 1964; Adler *et al.*, 1967).

Either the low ionic strength used has reduced the electrostatic contribution to the free-energy term to a value that is incapable of providing an adequate compensation for the loss of the stacking contribution in going from aqueous to glycol solutions (Hanlon and Major, 1968) or else the pK, and hence the degree of protonation, of $(rC)_n$ is different in the two solvents.

One additional phenomenon deserves some comment; for many of the polymers studied, the rotational strength, as measured by $[m']$ or $[\theta]$, in glycol was reduced to the monomer value, while the oscillator strength, as measured by the extinction coefficient, appeared to retain a value intermediate between the one characteristic of the polymer in water, and its monomer in either solvent, *i.e.*, the polymers remained partially hypochromic [*cf.* $(rA)_n$, Figure 3; $(rC)_n$, Figure 6; and $(rA)_n \cdot (rU)_n$, Figure 13]. Although a reciprocal relation between rotational and oscillator strengths of helical (or other inherently dissymmetrical) polymeric or oligomeric aggregates is generally observed one can certainly conceive of polymeric structures in which adjacent transition dipoles are oriented essentially randomly relative to one another yet permit some coupling. To mention but one example—not considered appropriate for the present instance—a polymer with adjacent and equal segments of right-handed and left-handed helices would exhibit zero net rotational strength combined with the maximal possible hypochromism. Similarly, stacks with base planes parallel but with the electronic transitions of nearest neighbors in the stack (which can be separated by long loops of unoriented bases) oriented at 0 or 180° to one another will lead to a complete absence of polymer induced dichroism but may still exhibit hypochroism, etc. Finally, the model of Glaubiger *et al.* (1968) also can account for an absence of reciprocity.

Mononucleotides. The rotational data for aqueous solutions presented here are in good agreement and are to be considered confirmatory to studies published previously (Fasman *et al.*, 1964, 1965; Yang *et al.*, 1966; Adler *et al.*, 1967). No previous work has been reported on the circular dichroism spectra of DNA and tRNA component mixtures, nor for any of the rotational spectra in glycol. Of particular interest is the close agreement between our optical rotatory dispersion data on ribonucleotides and those of Yang *et al.*, on deoxyribonucleotides all in aqueous solution. The position and magnitude of the extrema are quite comparable as are the qualitative generalizations that purine nucleotides exhibit a negative and pyrimidine nucleotides a positive Cotton effect. Clear evidence for association is provided by the behavior of an equimolar mixture of cytidine and guanine nucleotides in both studies.

All these findings tend to rule out significant contributions by the sugar to the structure of the monomer in solution, in marked contrast to the situation existing in oligomers and polymers (Ts'o *et al.*, 1966; Adler *et al.*, 1967; Yang and Samejima, 1968).

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