Solution Studies of the Nucleic Acid Bases and Related Model Compounds. Solubility in Aqueous Alcohol and Glycol Solutions†

Theodore T. Herskovits* and John P. Harrington

ABSTRACT: The solubilities of some of the nucleic acid bases and related nucleosides (adenine, adenosine, deoxyadenosine, thymine, guanosine, cytosine, and uracil) have been determined in water and various alcohol–water, ethylene glycol– and propylene glycol–water mixtures. Setschenow constants and related free energies of transfer of these bases and nucleosides from water to the various alcohol and glycol solutions were evaluated and are presented. In general the increase in chain length or hydrocarbon content of the alcohols is found to increase the initial solubility of these compounds, giving increasingly more negative Setschenow constants and transfer free energies. In comparison to the alcohols, the glycols appear to give less negative constants. Qualitatively, a close correlation is found between the solubility parameters and the effectiveness of the alcohols and glycols as DNA denaturants. The equations of Peller and Flory employed previously to account for the effects of denaturants and salts on the denaturation transition and destabilization of proteins and polypeptides were extended to DNA denaturation, using the present Setschenow parameters corrected for self-interactions. The latter parameters and the binding constants calculated for the nonpolar portion of the alcohols, assuming a hydrophobic mechanism of alcohol–biopolymer interactions used in previous studies on proteins (Herskovits *et al.* (1970), *J. Biol. Chem. 245*, 2588), are found to predict satisfactorily the various alcohol and glycol denaturation midpoints of T4 bacteriophage DNA at 73°, obtained by Levine *et al.* ((1963), *Biochemistry 2*, 168).

The unfolding of globular proteins in denaturing solvents such as the ureas, alcohols, glycols, and amides has been generally attributed to the increased solubility of the hydrophobic amino acid side chains that in the native state of such proteins would usually be found in their interior, solvent inaccessible regions. Much of our knowledge concerning the factors and forces responsible for the maintenance of the native structure of proteins and the mechanisms of solvent denaturation is based on solubility studies of model compounds for the amino acid side chains, the peptide moiety and the solubilities of the amino acids, themselves in both aqueous and organic solvents (Nozaki and Tanford, 1963, 1971; Wetlaufer *et al.*, 1964; Robinson and Jencks, 1965).

Although the denaturation of the nucleic acids by various organic solvents (including the alcohols, glycols, and some of the water-miscible amides, ureas, and dimethyl sulfoxides) has been reported from various laboratories (Geiduschek and Gray, 1956; Helmkemp and Ts'o, 1961; Duggan, 1961; Herskovits *et al.*, 1961; Herskovits, 1962, 1963; Levine *et al.*, 1963; Eliasson *et al.*, 1963; Aubel-Sadron *et al.*, 1964; Fasman *et al.*, 1964; Brahms and Mommaerts, 1964; Strauss *et al.*, 1968; Massie and Zimm, 1969; McConaughy *et al.*, 1969; Nelson and Johnson, 1970; Green and Mahler, 1971) and the base pairing and stacking interactions of purine and pyrimidine derivatives and bases have been extensively studied in both aqueous solutions (Ts'o *et al.*, 1963; Gill *et al.*, 1967; Van Holde and Rosetti, 1967; Lord and Thompson, 1967; Solie and Schellman, 1968; Nakano and Igarashi, 1970) and pure organic solvents, chloroform (Hamlin *et al.*, 1965; Kyogoku *et al.*, 1967; Miller and Sobell, 1967), carbon tetrachloride (Küchler and Drosch, 1966), and dimethyl sulfoxide (Katz and Penman, 1966; Shoup *et al.*, 1966; Newmark and Cantor, 1968), relatively little work has been carried out and reported on the solubility behavior of the free purine and pyrimidine bases and nucleosides related to the solvent denaturation of the nucleic acids. Levine *et al.* (1963) have reported the solubility of adenine at 37° in various 1 M alcohols, glycols, and several amides, ureas, carbamates, and other neutral water-miscible compounds and Robinson and Grant (1966) have examined the effects of various salts on the solubilities of purines and pyrimidines in aqueous solutions, and their relation to the denaturation of DNA by salts (Hamaguchi and Geiduschek, 1962). The effects of purines, pyrimidines and other heteroaromatic compounds on the solubility of the DNA bases in aqueous solutions have also been reported in relation to the base stacking interactions of these compounds (Ts'o *et al.*, 1963; Nakano and Igarashi, 1970). Recently Scruggs *et al.* (1972) have reported the thermodynamic parameters of transfer of adenine and uracil from organic solvents to water, based on solubility, and direct solvent-transfer measurements.

We have investigated the effects of the various alcohols and some of the glycols on the solubility of the purines and pyrimidines as well as some of the DNA nucleosides in relation to the effects of the alcohols and glycols on the stability of native DNA. This paper presents both the results of our studies and an analysis of some of the DNA denaturation data based on the derived Setschenow parameters and the Peller (1959) and Flory (1957) equations used to account for the destabilization of salts and denaturants on the native structure of proteins and biopolymers (Mandelkern and Stewart, 1964; Schrier *et al.*, 1965; Schrier and Schrier, 1967; Von Hippel and Schleich, 1969).

Experimental Section

Materials. The purine and pyrimidine bases and nucleosides employed were Mann or Schwarz-Mann products. All the

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solvents and reagents used were spectral grade or of the highest purity available commercially, and were used without further purification. The water used was freshly distilled in an all-glass still.

Solutions and Solubility Determination. Saturated solutions of the bases and nucleosides were obtained by 5–11 days of stirring of the solutions in constant temperature baths maintained to ±0.03° at 25.0° (and to about ±0.05° at the other temperatures). Submersible microstirrers (Tri-R-Instruments Inc., Rockville Center, N. Y.), each accommodating eight 5- or 10-ml stoppered erlenmeyer flasks, were used, with the solutions being magnetically stirred. Small 7- or 10-mm Teflon-coated stirring bars were used to facilitate the dissolving of the model compounds. Saturation was usually reached after 2 days of equilibration and stirring of the solutions. For example, with adenine in water the following solubilities were obtained after 1, 2, 3, 9, and 11 days of stirring at 25°: 7.16, 6.80, 7.07, 7.08, and 6.80 × 10^{-3} M. For the 35.0, 45.0, and 55.0° solubility data, shorter periods of 3 and 2 days of stirring were found to be sufficient. The solutions were filtered by use of analytical filter paper or filter sticks of medium porosity. For the experiments at 5–15 and 35–55°, the glassware, filters, and pipets were prewarmed or precooled to temperatures close to those of the water baths. The solubilities reported were based on optical density determinations of the filtered solutions diluted with water by a factor of 50–1000. The following molar extinction coefficients were used for the calculated solubilities: adenine, 13.3 × 10^3 at 260.5 nm; adenosine, 14.9 × 10^3 at 259.5 nm; deoxyadenosine, 14.7 × 10^3 at 260 nm; thymine, 7.89 × 10^3 at 264.5 nm; guanosine, 13.65 × 10^3 at 252.5 nm; cytosine, 6.13 × 10^3 at 267 nm; and uracil, 8.2 × 10^3 at 259.5 nm (Beaven *et al.*, 1963). Absorbance measurements were made on a Cary 14 recording spectrophotometer. Where necessary, the absorbance of the solvent blanks was subtracted from those of the solutions. Our solubility data of adenosine, deoxyadenosine, thymine, and cytosine in water at 25.0° (Table I) agree closely to within ±1–3% of the aqueous data of Robinson and Grant (1966) obtained at the same temperature. Our adenine solubility in water at 25.0° is about 12% lower than their reported value of 8.0 ± 0.2 × 10^{-3} M. Nakano and Igarashi (1970) obtained solubilities of about 7.5–7.8 × 10^{-3} M and have found that depending upon the preparations of adenine 3–7 days of equilibration periods were required before equilibrium solubility was reached. Solubility measurements with satisfactory accuracy and reproducibility could not be made on guanine, partly because of the very low solubility of this base and the apparent turbidity of the filtered solutions. Instead the more soluble ribose derivative of this base, guanosine, was investigated and the derived Setschenow parameters, based on the solubilities, were corrected for self-interaction.

Several of the filtered solutions were allowed to crystallize on standing at room temperature and the crystals were examined under a polarizing microscope. No discernible differences in the crystal forms of the solid phase forming with any of the alcohols with the various bases studied could be detected.

Results

Solubility Data and Setschenow Constants. The solubility data of the DNA bases and nucleosides as well as uracil obtained in alcohol and ethylene glycol solutions at 25.0° is summarized in Table I. The Setschenow constants, K_s, were estimated from usually linear, initial slopes of the logarithmic

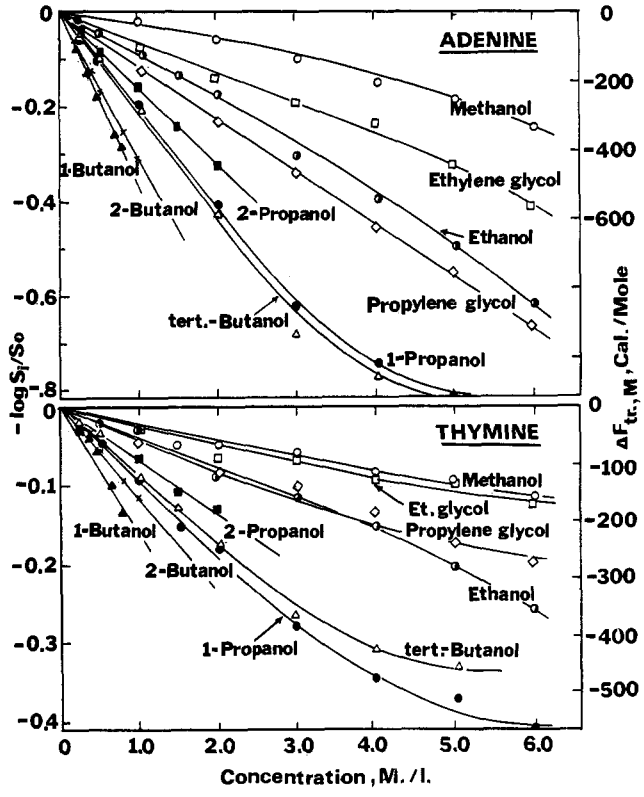

FIGURE 1: Setschenow plots and related $\Delta F_{tr,M}$ plots of various alcohol solutions of adenine and thymine obtained at 25.0°. The data obtained are plotted according to eq 1 and 2 of the text.

solubility ratios, S_i/S_0, *vs.* alcohol or glycol concentration plotted according to the relationship

$$\log S_i/S_0 = -K_s c_s \quad (1)$$

where S_i and S_0 are the solubilities of the solute in the presence and absence of the particular alcohol or glycol employed having a concentration of c_s expressed in moles per liter. If we neglect the small effects of self-interactions of the solute, discussed in the third section of the Results, the related free energy of transfer, $\Delta F_{tr,M}$, on the same concentration scale can be expressed as

$$\Delta F_{tr,M} = -RT \ln S_i/S_0 \quad (2)$$

Figures 1–3 present the 25.0° solubility data plotted according to eq 1 and 2. Only in the case of uracil is there a noticeable curvature in the initial slopes of the methanol and ethylene glycol plots (Figure 3). Consequently no Setschenow constants could be obtained for these two solvent–solute systems. The Setschenow constants based on the data shown are summarized in Table II. It is apparent from the data of this table and Figures 1–3 that in general the alcohols and glycols increase the solubility of the DNA and RNA bases and model compounds. Furthermore, the increase in chain length or hydrocarbon content of the alcohols results in increasingly more favorable, negative free energies of transfer of the bases from water to alcohol and glycol solutions, and also in more negative Setschenow constants.

Self-Interaction Effects and the Effects of the Presence of Ribose and Deoxyribose Moiety. Attachment of the ribose and deoxyribose sugars to the RNA and DNA bases seems to

TABLE I: Solubility of Purine and Pyrimidine Bases and Nucleosides in Aqueous Alcohol and Glycol Solutions at 25.0°.[a]

Concn of Alcohol or Glycol (M)	Solubility (M × 10²)						
	Adenine	Adenosine	Deoxy-adenosine	Thymine	Guanosine	Cytosine	Uracil
Water[a]	0.704 ± 0.008	1.92 ± 0.03	2.69 ± 0.05	2.78 ± 0.06	0.182 ± 0.003	6.58 ± 0.05	2.38 ± 0.02
Methanol							
1.0	0.72	1.92	2.81	2.78	0.186	6.60	2.38
1.5	0.74					6.72	2.35
2.0	0.82	1.96	2.96	3.25	0.193	6.77	2.27
3.0	0.87	1.92	3.21	3.30	0.196	6.81	2.29
4.0	0.98	2.00	3.50	3.36	0.195	6.86	2.25
5.0	1.07	2.04	3.60	3.44	0.202	6.96	2.22
6.0	1.23	2.13	4.01	3.62	0.217	7.04	2.22
Ethanol							
0.25	0.72			2.89			2.26
0.50	0.77	2.02		2.92		6.78	2.22
1.0	0.86	2.15	3.13	2.94	0.192	6.61	2.24
1.5	0.93	2.21		3.13		6.69	2.22
2.0	1.02	2.36	3.83	3.37	0.218	6.92	2.22
3.0	1.40	2.71	4.82	3.62	0.227	7.24	2.23
4.0	1.73	3.21	5.70	3.95	0.258	7.40	2.28
5.0	2.17	3.99	7.24	4.46	0.275	7.71	2.38
6.0	2.88	4.57	8.93	5.09	0.325	7.90	2.53
1-Propanol							
0.25	0.80	2.12	3.58	2.95		6.92	
0.50	0.89	2.33	3.42	3.09	0.183	7.26	2.42
1.0	1.11	2.76	4.49	3.47	0.228	7.50	2.55
1.5		3.31	5.86	4.01	0.265	8.23	2.60
2.0	1.79	4.08	7.10	4.27	0.300	8.55	2.69
3.0	2.90	5.62	10.76	5.37	0.374	8.88	2.85
4.0	3.87	6.83	10.49	6.13	0.415	9.00	3.20
5.0	4.50	7.10	14.77	6.61	0.421	8.65	3.50
6.0		7.05		7.11	0.370	7.96	4.09
2-Propanol							
0.25	0.78	2.02	2.92	2.99	0.186	6.80	2.37
0.50	0.86	2.19	3.25	3.05	0.198	6.91	2.36
1.0	1.01	2.45	3.83	3.23	0.209	7.33	2.37
1.5	1.22	2.75	4.65	3.60	0.238	7.96	2.45
2.0	1.47	3.16	5.60	3.78	0.254	7.58	2.48
3.0		4.29			0.300	7.69	2.66
1-Butanol							
0.25	0.84	2.32	3.46	2.90	0.223	7.21	2.46
0.40	0.94	2.55	3.95	3.04	0.230	7.37	2.49
0.50	1.06	2.76	4.52	3.14	0.237	7.53	2.56
0.70	1.29	3.14	5.64	3.50	0.281	7.81	2.66
0.80	1.35	3.35	5.53	3.81	0.320	8.14	2.70
2-Butanol							
0.25	0.86	2.21	3.30	2.92	0.200	7.24	2.44
0.40	0.92	2.38	3.54	2.99	0.205	7.05	2.52
0.50	1.05	2.53	4.01	3.17	0.212	7.49	2.55
0.80	1.25	2.94	4.75	3.45	0.252	7.81	2.67
1.00	1.43	3.35	5.38	3.65	0.268	8.17	2.72
tert-Butyl Alcohol							
0.25	0.77	2.16	3.13	2.87	0.184	6.48	2.42
0.50	0.88	2.35	3.51	2.95	0.203	6.60	2.44
1.0	1.11	2.79	4.52	3.45	0.228	6.88	2.44
1.5	1.43	3.61	6.10	3.76	0.280	7.22	2.57
2.0	1.83	4.29	7.90	4.28	0.305	7.55	2.64
3.0	3.35	5.87		5.18	0.374	7.24	2.88
4.0	4.09	6.45		5.73		6.59	3.04
5.0	4.41			5.98		5.59	2.73

TABLE I (*Continued*)

Concn of Alcohol or Glycol (M)	Solubility (M × 10^2)						
	Adenine	Adenosine	Deoxy-adenosine	Thymine	Guanosine	Cytosine	Uracil
Ethylene Glycol							
1.0	0.84	2.08	3.07	3.23	0.188	6.89	2.33
2.0	0.98	2.22	3.36	3.28	0.203	7.21	2.29
3.0	1.10	2.38	3.60	3.30	0.238	7.54	2.28
4.0	1.20	2.51	4.17	3.47	0.263	7.86	2.26
5.0	1.48	2.73	4.60	3.48	0.294	8.41	2.29
6.0	1.79	2 90	5.04	3.55	0.344	9.23	2.29
Propylene Glycol							
1.0	0.94			3.06	0.205		2.32
2.0	1.22			3.42	0.231		2.31
3.0	1.50			3.52	0.272		2.34
4.0	1.99			3.82	0.331		2.39
5.0	2.49			4.16	0.384		2.54
6.0	3.19			4.46	0.480		2.57

[a] The solubility values for water represent the average of three to nine determinations; the alcohol and glycol values given represent the average of one to three determinations.

increase significantly their solubilities (Beaven *et al.*, 1963; Robinson and Grant, 1966). For example, the solubility of adenine at 25.0° is found to increase from 7.04 × 10^{-3} to 1.92 × 10^{-2} and 2.69 × 10^{-2} M as a result of this attachment (Table I). The greater self-interaction effects with increasing solubility of the nucleosides may thus in part explain the differences in the observed Setschenow parameters, compared in Table II. It is possible to estimate the effects of self-interactions based on eq 3 and 4

$$K_s c_s = k_s c_s + k_i c_i \quad (3)$$

$$k_s = K_s - k_i(S_i - S_0)/c_s \quad (4)$$

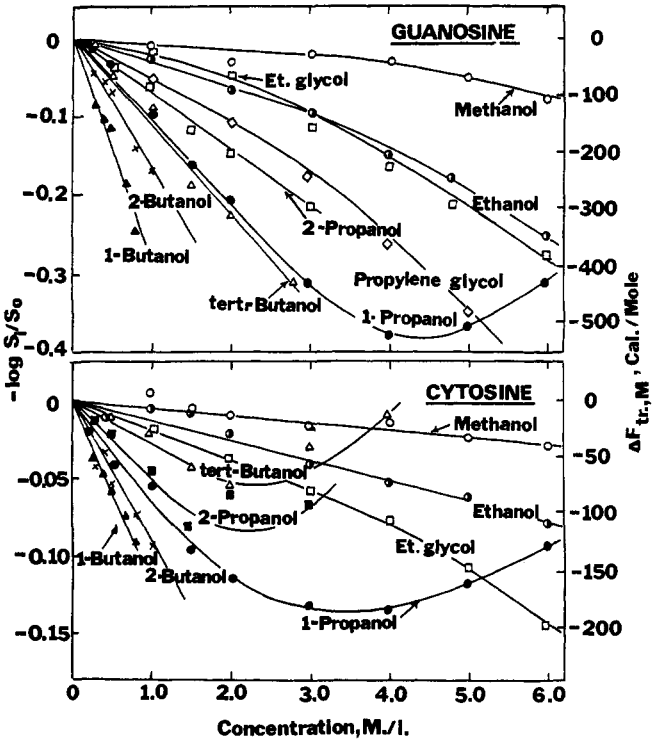

FIGURE 2: Setschenow plots and $\Delta F_{tr,M}$ plots of various alcohol solutions of guanosine and cytosine at 25.0° plotted according to eq 1 and 2 of the text.

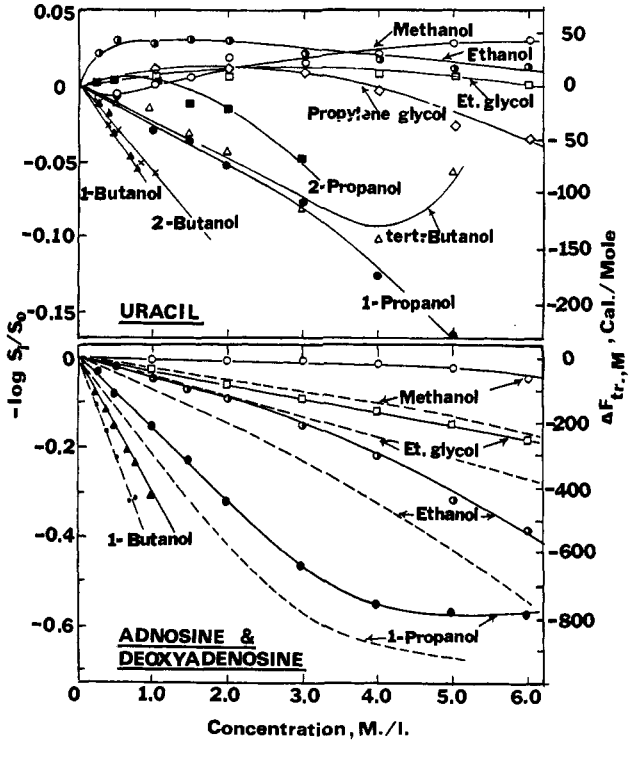

FIGURE 3: Setschenow plots and $\Delta F_{tr,M}$ plots of various alcohol solutions of uracil, adenosine, and deoxyadenosine at 25.0° plotted according to eq 1 and 2. The data obtained on adenosine on the lower part of the figure is represented by solid lines while the deoxyadenosine data is represented by dashed lines.

TABLE II: Solubility Parameters, K_s and k_s, of Purine and Pyrimidine Bases and of Some Ribose and Deoxyribose Nucleosides at 25.0°.[a]

	Adenine		Adenosine		Deoxyadenosine		Thymine		Guanosine		Cytosine		Uracil	
	K_s	k_s	K_s	k_s	K_s	k_s	K_s	k_s	K_s	k_s	K_s	k_s	K_s	k_s
Methanol	−0.015	−0.015	−0.005	−0.005	−0.025	−0.023	−0.020	−0.020	−0.009	−0.009	−0.005	−0.005	*b*	*b*
Ethanol	−0.090	−0.088	−0.048	−0.045	−0.079	−0.073	−0.038	−0.031	−0.030	−0.030	−0.012	−0.012	*b*	*b*
1-Propanol	−0.200	−0.195	−0.160	−0.149	−0.220	−0.197	−0.105	−0.102	−0.105	−0.104	−0.065	−0.064	−0.026	−0.02
2-Propanol	−0.160	−0.156	−0.105	−0.098	−0.160	−0.146	−0.070	−0.068	−0.070	−0.070	−0.040	−0.036	+0.005	+0.005
1-Butanol	−0.355	−0.346	−0.310	−0.289	−0.440	−0.395	−0.150	−0.147	−0.280	−0.279	−0.120	−0.109	−0.072	−0.070
2-Butanol	−0.310	−0.301	−0.240	−0.233	−0.310	−0.276	−0.114	−0.110	−0.165	−0.164	−0.090	−0.081	−0.058	−0.056
tert-Butyl alcohol	−0.200	−0.195	−0.185	−0.174	−0.230	−0.207	−0.093	−0.090	−0.100	−0.099	−0.030	−0.028	−0.024	−0.024
Ethylene glycol	−0.064	−0.062	−0.030	−0.028	−0.046	−0.041	−0.025	−0.023	−0.020	−0.020	−0.020	−0.018	+0.005	+0.005
Propylene glycol	−0.110	−0.107					−0.042	−0.041	−0.052	−0.052			+0.01	+0.01

[a] Setschenow constants, K_s, estimated from initial slopes of plots of $-\log S/S_0$ *vs.* alcohol or glycol concentration (see text and Figures 1–3). Estimates of the corrected k_s parameter were based on eq 4: $k_s = K_s - k_i(S_i - S_0)/c_s$, mostly at c_s of 1.0 M alcohol or glycol concentration. The solute–solute self-interaction constants, k_i, necessary for these calculations were obtained from plots of the log of the activity coefficient *vs.* the concentration of purine (for adenine, adenosine, deoxyadenosine, and guanosine), uridine (for thymine and uracil), and cytidine (for cytosine) in aqueous solutions at 25°. The k_i values used, based on the data of Ts'o *et al.* (1963), were −1.25 for adenine, adenosine, deoxyadenosine, and guanosine, −0.46 for thymine and uracil, and −0.56 for cytosine, respectively. [b] These K_s and k_s could not be estimated because of the strong initial curvature in the $-\log S/S_0$ *vs.* alcohol concentration (Figure 3).

where k_s is the true or corrected solvent–solute interaction constant, k_i is the constant resulting from solute–solute self-interactions, c_s is the alcohol or glycol concentrations, and c_i is the solute concentration (Long and McDevit, 1952). Based on eq 1 and 3 the logarithmic solubility ratios at the solubility limit S_i is equal to

$$\log S_0/S_i = k_s c_s + k_i(S_i - S_0) \quad (5)$$

For dilute aqueous solutions a sufficiently close estimate of the required interaction constant k_i may be obtained using the approximation

$$\log \gamma_i = k_i c_i \quad (6)$$

(Robinson and Grant, 1966) and the known activity coefficients γ_i of the appropriate bases or model compounds (Ts'o *et al.*, 1963). For the estimates of self-interaction effects related to the role of ribose and deoxyribose sugars on the hydrophobicity of the DNA and RNA bases we have used the published activity coefficients of purine, thymidine, cytidine, and uridine in aqueous solutions at 25° (Ts'o *et al.*, 1963). The k_i values used (footnote *a* of Table II) were obtained from the slopes of the log of the activity coefficients *vs.* base concentration.[1]

The data of Table II indicate that the corrected k_s parameters for adenine and deoxyadenosine are not significantly different for the various solvents listed. This suggests that the deoxyribose moiety should have little or no effect on the hydrophobicity of the DNA bases. However, this is not apparently the case with RNA ribose sugar, which seems to lower the K_s and the corrected k_s parameters of adenosine.

Free Energies of Transfer from Water to Alcohol and Ethylene Glycol Solutions. The solubilities and free energies of transfer from water to aqueous methanol, ethanol, 1-propanol, and ethylene glycol solutions of adenine and thymine were also investigated in the intermediate and high alcohol or glycol concentration ranges.[2] The free energies of transfer, ΔF_{tr}, from water to alcohol or glycol solutions of these two bases are shown in Figure 4. Calculation of ΔF_{tr} on the mole fraction scale (Gurney, 1953; Nozaki and Tanford, 1963, 1971) were based on the equation

$$\Delta F_{tr} = -RT \ln N_i/N_0 + RT \ln \gamma_0/\gamma_i \quad (7)$$

[1] As has been suggested by one of the reviewers one can also estimate k_s by use of eq 5 expressed in the form

$$\log (S_0/S_i)/(S_i - S_0) = k_s c_s/(S_i - S_0) + k_i$$

However, in practice such estimates are found to be less reliable than the method employed in this paper based on activity coefficients, because of the relatively large scatter of the log $(S_0/S_i)/(S_i - S_0)$ *vs.* $c_s/(S_i - S_0)$ data in the important 0–1.0 M concentration ranges, cs, of the alcohols and glycols.

[2] Supplementary material describing the solubilities and the Setschenow constants, K_s, of adenine and thymine in aqueous ethanol, 1-propanol, and ethylene glycol, at 15.0–25.0°, and the free energies of transfer, ΔF_{tr}, of these compounds from water to 10–100% methanol, ethanol, 1-propanol, and ethylene glycol at 25.0° will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Department, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N. W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number BIO-72-4800.

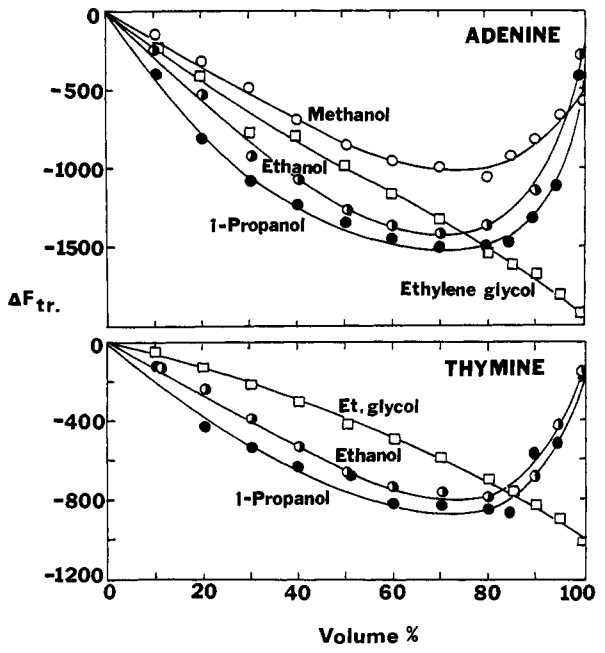

FIGURE 4: The free energies of transfer of adenine and thymine from water to methanol, ethanol, 1-propanol, and ethylene glycol as a function of alcohol and glycol concentration at 25.0°; ΔF_{tr} on the mole fraction scale was corrected for self-interaction according to eq 7 and 8.

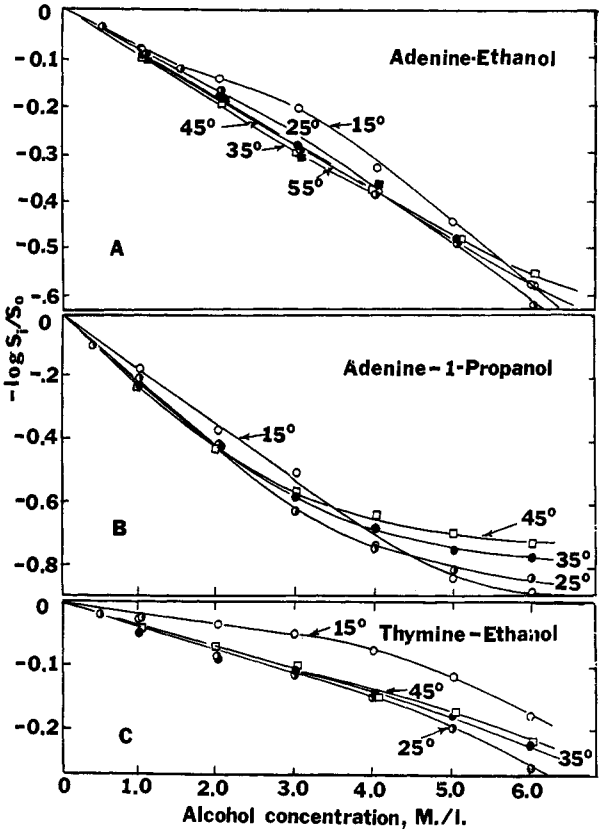

FIGURE 5: The effects of temperature on the Setschenow plots of adenine and thymine obtained with aqueous ethanol and 1-propanol solutions at 15–55°.

where N_i and N_0 and γ_i and γ_0 represent the mole fraction and activity coefficient of the solute in the alcohol, alcohol–water, or ethylene glycol–water mixture and in pure water, respectively. As in the case of the Setschenow constant corrections, described in the previous section, estimates of the self-interaction term $RT \ln \gamma_0/\gamma_i$ were based on the purine and pyrimidine activity coefficient data of Ts'o *et al.* (1963) in aqueous solutions at 25°. In the absence of activity coefficient data in the alcohol and glycol solutions it was assumed that the self-interactions of the bases in these solutions are the same as in water (Nozaki and Tanford, 1963, 1971). As with the K_s parameters mentioned, a good estimate of this term, based on the measured saturation values of the solute concentrations, S_i and S_0, and constant k_i can be obtained using eq 8. Based on eq 6 and 5

$$2.303\ RT \log \gamma_0/\gamma_i = -2.303\ RTk_i(S_i - S_0) \qquad (8)$$

The corrections applied due to the second, activity coefficient term have been found to be relatively small, at most of the order of 2–5% of first ΔF_{tr} term. For example, in the neighborhood of the solubility maxima of adenine, at 60% methanol, ethanol, 1-propanol, and 100% ethylene glycol the ΔF_{tr} correction terms obtained ranged from +33 to 106 cal per mol. The corresponding corrections for thymine for the solutions in 60% ethanol, 1-propanol, and ethylene glycol were respectively +20, 20, and 14 cal per mol. Plots of ΔF_{tr} for both adenine and thymine shown in Figure 4 indicate that the maximum values of ΔF_{tr} and also the maximum solubilities are reached at about 60–80% alcohol and 100% ethylene glycol.

Effects of Temperature and Thermodynamic Parameters of Solution. The effects of temperature on the Setschenow plots of adenine and thymine in some of the alcohol–water systems investigated[2] are shown in Figure 5. The Setschenow constants K_s based on such plots show an initial decrease in K_s followed by a relatively constant-temperature dependence above 25–35° for both ethanol and 1-propanol (Figure 6). The ethylene glycol data, however, show a distinct increase in the K_s parameters for both adenine and thymine to less negative K_s values at higher temperatures.

The free energies, enthalpies, and entropies of solutions, $\Delta F°_{sol}$, $\Delta H°_{sol}$, and $\Delta S°_{sol}$, of adenine and thymine were evaluated on the basis of the following thermodynamic relationships

$$\Delta F°_{sol} = -RT \ln N_i \qquad (9)$$

$$\log N_i = -\Delta H°_{sol}/2.303RT + \Delta S°_{sol}/2.303R \qquad (10)$$

$$\Delta S°_{sol} = (\Delta H°_{sol} - \Delta F°_{sol})/T \qquad (11)$$

Figure 7 shows some of the van't Hoff $\log N_i$ *vs.* $1/T$ plots and $\Delta F°_{sol}$ *vs.* temperature plots for both adenine and thymine. The family of lines with slopes representing the enthalpies and entropies of solutions indicates noticeable curvature above 5° and below 25°. The curvature indicates that for many of the systems examined (*i.e.*, the adenine curves in 3 and 6 M ethanol, 6 M ethylene glycol, and the thymine curves in water, ethanol, and 1-propanol) the heats of solutions are not strictly constant and that heat capacity effects contribute significantly to the observed thermodynamic parameters. Because of the greater curvature in the neighborhood of 25°, the thermodynamic parameters of solution summarized in Table III have been evaluated at 35°, using the tangent of the curves at 35° for the evaluation of $\Delta H°_{sol}$ and the eq 9–11 calculating $\Delta F°_{sol}$ and $\Delta S°_{sol}$ of solution. The enthalpies of

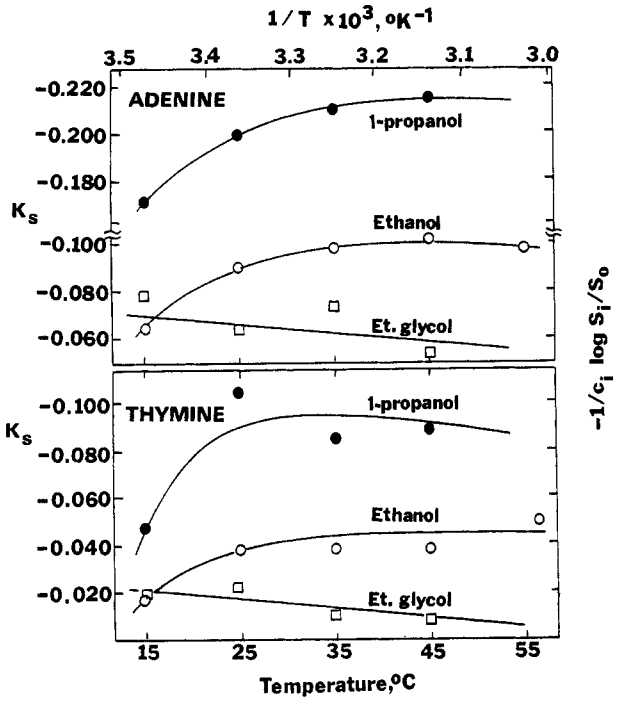

FIGURE 6: The effects of temperature on the Setschenow constants K_s of adenine and thymine obtained with aqueous ethanol, 1-propanol, and ethylene glycol solutions.

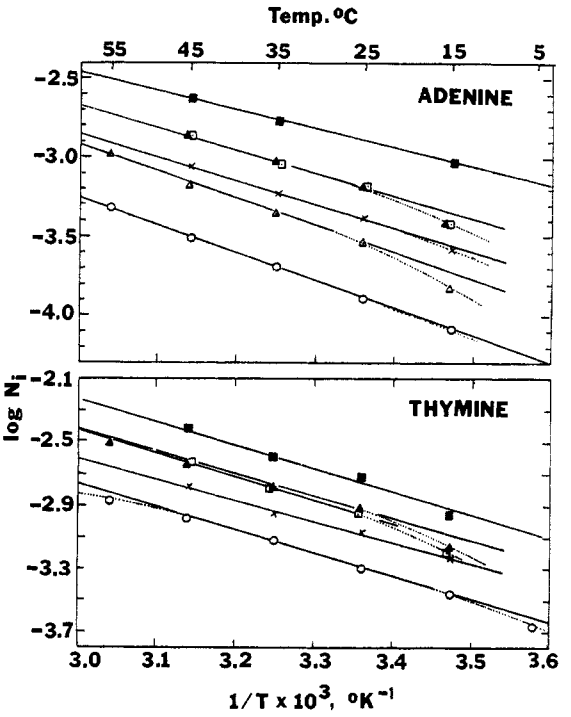

FIGURE 7: van't Hoff plots of adenine and thymine solubility data in water and several alcohol solutions, plotted according to eq 10. N_i is the mol fraction of adenine or thymine at saturation. (○) Water; (△) 3.0 M ethanol; (▲) 6.0 M ethanol; (□) 3.0 M 1-propanol. (■) 6.0 M 1-propanol; (×) 6.0 M ethylene glycol.

solution of adenine appear to be less positive in aqueous alcohol and ethylene glycol solutions than in pure water, while those of thymine seem to be essentially the same for the aqueous and mixed-solvent systems. Differences between the thermodynamic parameters of solution for the mixed-solvent systems and the aqueous solution values are equal to the free energies, enthalpies, and entropies of transfer from water to the mixed-solvent systems listed. For the 6 M ethanol, 1-propanol, and ethylene glycol solutions the ΔF_{tr} values given in column 1 of this table were found to be the same by both the differences in the ΔF_{sol} and the directly calculated ΔF_{tr} values based on eq 7. For both sets of calculations the secondary effects of self-interaction were neglected. Direct estimates of the ΔH_{tr} and ΔS_{tr} are difficult to obtain because of the pronounced curvature in the van't Hoff log S_i/S_0 *vs.* $1/T$ plots in the neighborhood of 25° (data not shown). The curvature in the data obtained is similar to the related K_s *vs.* T or $1/T$ plots shown in Figure 6. For these reasons the ΔH_{tr} and ΔS_{tr} listed should only be considered as approximate values, indicating the general trend of these parameters as a function of increasing alcohol and ethylene glycol concentration. More exact estimates will probably require calorimetric measurements, similar to those reported by Cassel and Wen (1972), on the transfer functions of tetraalkylammonium bromides from water to urea solutions.

Scruggs *et al.* (1972) reported recently the entropies of solution and transfer from methanol into water for both adenine and uracil. Their $\Delta S°_{sol}$ of +5 to +11 eu are similar to our values for adenine and thymine.

The hydrogen-bonding tendencies of the mixed purine and pyrimidine derivatives in organic solvents (Hamlin *et al.*, 1965; Küchler and Drosch, 1966; Katz and Penman, 1966; Shoup *et al.*, 1966; Kyogoku *et al.*, 1967; Miller and Sobell, 1967; Newmark and Cantor, 1968), as opposed to base-stacking interaction observed in aqueous solutions (Ts'o *et al.*, 1963; Gill *et al.*, 1967; Van Holde and Rosetti, 1967; Lord and Thompson, 1967; Solie and Schellman, 1968; Nakano and Igarashi, 1970), are somewhat puzzling. In view of the findings that the enthalpies and entropies of transfer of adenine and thymine from water to alcohol and glycol solutions tend to be negative (Table III), further studies will have to be made in order to clarify the significance of these parameters as a function of solvent composition, going from the mainly aqueous to the nonaqueous phase.

Discussion

The increasing solubility of the various nucleic acid bases and nucleosides with increasing hydrocarbon content of the various alcohols of this study (Table I) are in general accord with the widely accepted view that hydrophobic interactions contribute significantly to DNA base–denaturant interactions in alcohol solutions and thereby cause the destabilization of the DNA helix in such solutions (Herskovits *et al.*, 1961; Herskovits, 1962; Ts'o *et al.*, 1962; DeVoe and Tinoco, 1962; Levine *et al.*, 1963; Michelson, 1963; Sinanoglu and Abdulnur, 1965). The relative destabilization of native DNA by a given alcohol may be viewed in terms of the following thermodynamic arguments. The free energy of transfer of a DNA base or nucleotide from the DNA interior (x) into water (H_2O) or alcohol–water solutions (R–OH) may be expressed as

$$\Delta F_{tr}(x \rightarrow H_2O) = -RT \ln S_{H_2O}/S_x - RT \ln \gamma_{H_2O}/\gamma_x \quad (12)$$

$$\Delta F_{tr}(x \rightarrow R\text{–}OH) = -RT \ln S_{R\text{–}OH}/S_x - RT \ln \gamma_{R\text{–}OH}/\gamma_x \quad (13)$$

where S_{H_2O}, S_{R-OH} and S_x are respectively the concentrations at saturation (solubility) of the bases in question, in water, alcohol, and the DNA interior. The difference between eq 12 and 13 eliminates the experimentally undefined solvent condition S_x, or the corresponding ln S_x term, giving us the expression

$$\Delta F_{tr}(H_2O \rightarrow R\text{-}OH) = -RT \ln S_{R-OH}/S_{H_2O} + RT \ln \gamma_{H_2O}/\gamma_{R-OH} \quad (14)$$

Equation 14 is identical with eq 2 of the Results section except for the second, self-interaction term and the difference in subscripts. It represents the free energy of transfer of a given DNA base from water to the alcohol solution, R–OH. For a DNA isolated from a given species of varying base composition, the ΔF_{tr} from water to 1.0 M alcohol solutions per average base can be expressed in terms of the individual Setschenow constants corrected for self-interaction, k_s

$$\Delta F_{tr}(H_2O \rightarrow R\text{-}OH) = 2.303RT \sum_i f_i k_s \quad (15)$$

where f_i represents the mole fraction of each of the four bases in the DNA. With denaturation data of T4 bacteriophage DNA listed in Table IV, the ΔF_{tr} values given were calculated with f_i values of 0.32 for adenine and thymine and 0.18 for guanine and cytosine, respectively (Josse *et al.*, 1961).

As was found for several proteins examined in our laboratory (Herskovits and Jaillet, 1969; Herskovits *et al.*, 1970a) there should be a close correlation between the magnitude of ΔF_{tr} and the relative stability of the biopolymer examined in various alcohol solutions. Table IV presents such a comparison for the alcohol denaturation of T4 bacteriophage DNA at 73° obtained by Levine *et al.*, (1963). We have also included several other indices that may be associated with the hydrophobicity and relative effectiveness of the alcohols as denaturing agents. Among these we have listed the molar refraction of the alcohols (Hanlon, 1966), the distribution coefficients of some of the alcohols in the olive oil–water system (Collander, 1954), the free energies of transfer of the alkyl side chains of the various alcohols listed, from water to ethanol (Herskovits *et al.*, 1970a), the denaturation midpoints of a representative protein, α-chymotrypsinogen, and the associated free energies of transfer of indole from water to these alcohol solutions (Herskovits *et al.*, 1970a). It is significant that both the relative order of effectiveness of the alcohols and glycols studied as DNA and protein denaturants seem to be the same. Moreover, a striking feature of this correlation is the increasing effectiveness of the alcohols with increasing chain length and hydrocarbon content as DNA and protein denaturants and solvents for the DNA bases and indole (the latter expressed in terms of the free energy of transfer, ΔF_{tr}, from water to alcohol).

A more quantitative assessment of the DNA denaturation data in conjunction with the solubility parameters of the DNA bases can be made by means of the equations of Peller (1959) and Flory (1957), used to examine the effects of salts (Mandelkern and Stewart, 1964; Von Hippel and Schleich, 1969), alcohols (Schrier *et al.*, 1965; Herskovits *et al.*, 1970a), ureas, and amides (Herskovits *et al.*, 1970b,c) on the stability of proteins and polypeptides. The lowering of the melting or denaturation temperature of a biopolymer, ΔT_m (Peller, 1959), and the denaturation midpoint, S_m (Herskovits *et al.*, 1970a), due to preferential binding of the denaturing agent to the unfolded or the denatured form of the biopolymer[3] can be expressed as

$$\Delta T_m = T_m^\circ - T_m = (RT_mT_m^\circ/\Delta h)\bar{\nu} \ln (1 + K_B a_s) \quad (16)$$

$$S_m = c_s\gamma_s = (\Delta T_m \Delta h/RT_mT_m^\circ\bar{\nu})1/K_B \quad (17)$$

$$S_m = (-\Delta T_m \Delta h/2.303RT_mT_m^\circ\bar{\nu})1/k_s \quad (18)$$

where T_m and T_m° are the midpoints of the denaturation transition in the presence and absence of denaturant, Δh is the enthalpy change of unfolding per monomer unit of the biopolymer under investigation, $\bar{\nu}$ is the effective number of binding sites per monomer unit (treated here as an adjustable parameter), K_B is the association or binding constant of the denaturant with the average monomer unit,[3] k_s is the Setschenow constant per average DNA base (corrected for self-interaction), and a_s is the activity of the denaturant taken as the concentration in moles per liter assuming that the activity coefficient, γ_s, is unity. For our calculations a Δh value of 4000 cal/mol per average DNA base was used (Bunville *et al.*, 1965), with the average k_s value per T4 bacteriophage DNA base averaged according to eq 15 using the corrected Setschenow constants, k_s, of Table II. We have also calculated S_m values for this DNA based on the binding constants K_B using eq 17. These K_B parameters have been estimated from the free energies of transfer of the nonpolar portions of the various alcohols (Herskovits *et al.*, 1970a) associated with hydrophobic interactions of the amino acid side chains in proteins[3] (Kauzmann, 1959; Nemethy and Scheraga, 1962; Tanford, 1962). The k_s and K_B parameters used are listed in Table V, together with the calculated and experimental denaturation midpoints, S_m, obtained at 73° (Levine *et al.*, 1963). With the possible exception of denaturation midpoints of *sec*-butyl alcohol, the calculated and experimental S_m values are in satisfactory accord, considering some of the assumptions and approximations used for the evaluation of the Setschenow and binding parameters, k_s and K_B.[3] It was assumed that both of these constants are unaffected by changes in temperature, or that the slight varia-

[3] In our treatment the questions of specific binding (through use of the binding constant K_B) *vs.* nonspecific solvent effects implied by the use of the corrected Setschenow constants k_s is left open, since it was shown that with the adjustable parameter $\bar{\nu} = 1.0$, $K_B = -2.303K_s$ (see footnote 2 in Herskovits *et al.*, 1970a). The K_B parameters were evaluated by assuming group additivity of the free energy of interaction of the aliphatic portion of the alcohols to the average hydrophobic site on the biopolymer. The polar –OH contributions to the interactions were neglected assuming that only the hydrocarbon portion of the alcohol contributes significantly to the interaction energy (Schrier *et al.*, 1965). Presumably in the presence of water the hydroxyl groups in both the free and bound forms of the alcohols can hydrogen bond to the solvent. The hydrophobic interaction term of the free energy which seems to govern K_B is a net effect of the overall free-energy change of the complex process termed as the "hydrophobic interaction" or "hydrophobic bond" (Kauzmann, 1959; Nemethy and Scheraga, 1962). K_B is thus calculated by means of the equation $\Delta F^\circ_B = -2.303RT \log K_B$, where $\Delta F^\circ_B = \Delta F_{tr} - T\Delta S^\circ$; the ΔF_{tr} values used to evaluate K_B are listed in column 2 of Table IV. A ΔS° value of −11 eu was used for all these calculations suggested by Schrier *et al.* (1965) on the basis of the average ΔS° of dimerization of urea, *N*-methylacetamide, and formic acid in aqueous solutions. The ΔF_{tr} values used were based on estimates of the free energies of transfer of amino acid side chains from water to 95–100% ethanol taken from the literature (Cohn and Edsall, 1943; Tanford, 1962; Brandts, 1964). The ΔF_{tr} values involved represent −610 per $-CH_3$, −570 cal/mol per $-CH_2-$ group, and a loss of 300 cal/mol per branching group in case of the secondary and tertiary alcohols (Herskovits *et al.*, 1970a).

TABLE III: Approximate Thermodynamic Functions of Solution and Transfer of Adenine and Thymine from Water to Alcohol and Ethylene Glycol Solutions at 35°.

Solvent	Adenine			Thymine		
	ΔF°_{sol} (kcal/mole)	ΔH°_{sol} (kcal/mole)	ΔS°_{sol} (eu/mole)	ΔF°_{sol} (kcal/mole)	ΔH°_{sol} (kcal/mole)	ΔS°_{sol} (eu/mole)
A. Functions of Solution at 35°						
Water	5.20	8.1	9.4	4.40	6.7	7.5
3.0 M Ethanol	4.73	8.1	10.9	4.17	6.5	7.6
6.0 M Ethanol	4.25	7.0	8.9	3.94	6.1	7.0
3.0 M 1-Propanol	4.27	7.0	8.9	3.92	6.8	9.4
6.0 M 1-Propanol	3.89	5.8	6.2	3.69	6.8	10.1
3.0 M Ethylene glycol	4.81	7.1	7.4	4.29	6.1	5.9
6.0 M Ethylene glycol	4.54	6.8	7.3	4.17	6.1	6.3
	Adenine			Thymine		
	ΔF_{tr} (kcal/mole)	ΔH_{tr} (kcal/mole)	ΔS_{tr} (eu/mole)	ΔF_{tr} (kcal/mole)	ΔH_{tr} (kcal/mole)	ΔS_{tr} (eu/mole)
B. Approximate Transfer Parameters from Water to Alcohol and Ethylene Glycol at 35°						
6.0 M Ethanol	−0.95	−1.1	−0.5	−0.47	−0.6	−0.5
6.0 M 1-Propanol	−1.3	−2.3	−3.2	−0.72	+0.1	+2.6
6.0 M Ethylene glycol	−0.66	−1.3	−2.1	−0.23	−0.6	−1.2

TABLE IV: Correlation between Various Properties of the Alcohols and Glycols Related to Their Effectiveness as DNA and Protein Denaturants.[a]

Alcohol or Glycol	Molar Refraction (cm^3)[b]	ΔF_{tr} of Alkyl Side Chain of Alcohol[c]	Distribution Coef of Olive Oil–H_2O System	Denaturation Midpoints of T4 Phage DNA at 73°	ΔF_{tr} of Av T4 Phage DNA Base at 25°[d] (cal/mol)	Denaturation Midpoint of α-Chymotrypsinogen at 25°	ΔF_{tr} of Indole at 25°[d] (cal/mol)
Methanol	8.2	−610	780	3.5 M	−19	7.2 M	−103
Ethanol	12.9	−1180	3200	1.2	−63	3.8	−151
1-Propanol	17.8	−1750	13000	0.54	−170	1.6	−232
2-Propanol	17.6	−1450		0.90	−124	2.6	−186
1-Butanol	22.2	−2320		0.33	−311	0.7	−302
2-Butanol	22.1	−2020	25000	0.62	−240	1.1	−240
tert-Butyl alcohol	22.2	−1720		0.60	−155	2.0	−195
Ethylene glycol	14.5	−610	49	2.2	−46	11.0	−108
Propylene glycol	18.1	−1180	170		−80	8.5	−160

[a] Some of the molar refraction, distribution coefficient and phage DNA denaturation data were taken, respectively, from Hanlon (1966), Collander (1954), and Levine *et al.* (1963). The α-chymotrypsinogen data and the ΔF_{tr} data of the alcohol side chains and indole were taken from Herskovits *et al.* (1970a). [b] Molar refraction, R, defined as $M/d_T(n_D^2 - 1)/(n_D^2 + 2)$, where M is the molecular weight, d_T is the density of the pure alcohol or glycol at temperature T (at 20 or 25°), and n_D is the refractive index at the sodium D line measured at the same temperature. The n_D and d_T values were taken from the Handbook of Chemistry and Physics. [c] Estimated values based on the free energies of transfer of amino acid side chains from water into 95 or 100% ethanol (Herskovits *et al.*, 1970a). [d] Free energies of transfer of the average T4 bacteriophage DNA base and indole from water to 1.0 M alcohol and glycol solutions.

tion with temperature, such as those seen with the K_S parameters of adenine and thymine in some of the alcohols above 25° (Figure 6), will be cancelled out in the averaging of the computed k_s. It was also assumed that the enthalpy change of unfolding is largely unaffected in the various alcohols at the concentrations of the denaturation midpoints. The enthalpies of transfer of adenine and thymine, given in Table III, suggest that at concentrations below about 3 moles the transfer of the average DNA base from the DNA helix into solution and the ensuing interaction with the alcohol present should not alter significantly the effective Δh of unfolding.

The $\bar{\nu}$ value of 0.35 used with the corrected Setschenow constant to predict the denaturation midpoints of bacteriophage DNA represents less than a full base, suggesting some

preferential alcohol to base interaction with the exposed portions of the bases in the two grooves of native DNA and that some of the residual base–base interactions in denatured DNA exclude some solvent relative to the solvation of the free bases. This is consistent with the base–base interactions present in oligonucleotides and single-stranded polyribonucleotides suggested by their optical rotatory properties and hypochromism (Michelson, 1963; Warshaw and Tinoco, 1965; Brahms *et al.*, 1966, 1967; Felsenfeld and Miles, 1967). The $\bar{\nu}$ value of 1.2, most consistent with the calculated S_m value based on the free energies of transfer from water to ethanol of the nonpolar alkyl portions of the alcohols, indicates that the average DNA base has comparable effects on the solvent with the average amino acid group in proteins.[3] A $\bar{\nu}$ value of 1.0 was used with the denaturation studies of γ-chymotrypsinogen, myoglobin and cytochrome *c*, using the same binding constants (Herskovits *et al.*, 1970a).

The use of the Setschenow constants for calculating the S_m parameters of the biopolymer assumed no mechanism for solvent–polymer interaction. The binding constant, based on the free energies of transfer of nonpolar portion of the alcohols, on the other hand, assumes a hydrophobic mechanism of interaction. The close correlation between the experimental and calculated S_m parameters is noteworthy, especially if we realize that the DNA bases are relatively polar and their interactions with hydrocarbons may differ from that of hydrocarbon to hydrocarbon interactions. Our findings again demonstrate the relative importance of hydrophobic interactions between the alcohols and the DNA bases in the process of denaturation.

The most general definition of hydrophobic interactions or bonding given by Kauzmann (1959) (Kozak *et al.*, 1968) "is the tendency of nonpolar groups to associate or adhere to one another" in aqueous solutions. With hydrocarbons and nonpolar amino acid side chains usually found in proteins, the enthalpies of hydrophobic bond formation are close to zero or slightly positive (Kauzmann, 1959; Nemethy and Scheraga, 1962; Bigelow, 1967). Hydrophobic bonding associated with the interactions of nonpolar amino acid side chains in proteins are thus largely entropically driven processes. However, the tendency of the more polar DNA bases to associate and form stacked base–base complexes in aqueous solutions is accompanied by a significant negative change in enthalpy. Brahms and coworkers (1966, 1967), as well as others (cited by Felsenfeld and Miles, 1967), have found that the enthalpies of stacking interactions of various 3′,5′-dinucleoside phosphates, adenylate oligomers, and poly(U) and poly(A) vary from about −6 to −8 kcal per mol of base pair.[4] At the present there is no general agreement concerning the origin of the favorable, negative enthalpy change accompanying the formation of stacked DNA bases in aqueous solutions (Felsenfeld and Miles, 1967). Undoubtedly, van der Waals and other short-range interactions (DeVoe and Tinoco, 1962; Nash and Bradley, 1966) must contribute significantly to the enthalpy of base stacking. The role of the supporting solvent, water, necessary for the maintenance of the integrity of the native, helical form of DNA has been generally recognized (Herskovits *et al.*, 1961; Ts'o *et al.*, 1962; DeVoe *et al.*, 1962; Levine *et al.*, 1963; Falk *et al.*, 1963; Michelson, 1963; Sinanoglu and Abdulnur, 1965).[5] The destabilization of the native structure of DNA by various organic denaturants suggests that either the internal hydrogen bonds of the Watson–Crick helix are destabilized or that base-to-base stacking interactions are weakened. The less positive enthalpies of solution of adenine and thymine in 3–6 M alcohol and glycol solutions relative to water, and the less negative enthalpies of transfer (Table III), suggest that a part of the destabilization may be due to the increasing strength of alcohol–base hydrogen bonds formed at moderate to high denaturant concentration. However, this hydrogen-bonding effect of the alcohols could not explain the increasing effectiveness of the denaturant, with increasing hydrocarbon content and alkyl substitution of the functional amino groups in the case of the ureas and amides (Herskovits, 1962, 1963; Levine *et al.*, 1963). This is reflected by the larger binding constants, K_B, of the nonpolar part of the denaturant listed in Table V (see also, Herskovits *et al.*, 1970a–c). Thus one could reasonably argue that the destabiliza-

TABLE V: Comparison between the Calculated and Experimental Denaturation Midpoints, S_m, of Bacteriophage T4 DNA at 73°.[a]

Alcohol or Glycol	$k_s \times 10^2$ [b]	$K_B \times 10^2$ [c]	Calcd S_m: From k_s Data Eq 18 (mol/l.)	Calcd S_m: From K_B Eq 17 (mol/l.)	Exptl S_m[d] (mol/l.)
Methanol	−1.37	1.01	3.9	3.9	3.5
Ethanol	−4.6	2.88	1.26	1.35	1.2
1-Propanol	−12.4	7.59	0.47	0.51	0.54
2-Propanol	−9.1	4.57	0.64	0.85	0.90
1-Butanol	−22.7	20.0	0.26	0.20	0.33
2-Butanol	−17.8	10.2	0.33	0.38	0.62
tert-Butyl alcohol	−11.4	7.25	0.51	0.54	0.60
Ethylene glycol	−3.4	1.01	1.7	3.9	2.2

[a] Parameters used with eq 17 and 18 were T_m = 349.1°K, $T_m°$ = 346.3, Δh = 4000 cal/mole, $\bar{\nu}$ = 0.35 with eq 18 and 1.2 with eq 17. [b] Calculated using the relationship, k_s = $0.32k_s$(adenine) + $0.32k_s$(thymine) + $0.18k_s$(guanosine) + $0.18k_s$(cytosine) using the k_s data of Table II. [c] Calculated by use of the relationship, $\Delta F°_B = -RT \ln K_B$, where $\Delta F°_B = \Delta F_{tr} - T\Delta S°_B$ with the ΔF_{tr} values used listed in column 2 of Table IV and $\Delta S_{tr} = -11$ eu (Herskovits *et al.*, 1970a). [d] Data taken from Levine *et al.* (1963).

[4] According to Brahms *et al.* (1967) the internal hydrogen bonding potential of the 2′-hydroxyl group of the ribose moiety of the 3′,5′-dinucleotide phosphates probably contributes little to the observed enthalpy change accompanying the stacking of these compounds. Somewhat lower enthalpies of self-association ranging from −2 to −4.2 kcal per mole have been reported by Gill *et al.* (1967) for purine and purine derivatives (purine ribonucleoside, deoxyadenosine, and caffeine). From among the latter compounds only purine riboside has the internally hydrogen bonding 2′-ribose hydroxyl group.

[5] In our earlier publications (Herskovits *et al.*, 1961; Herskovits, 1962, 1963) it was pointed out that helical DNA is stabilized, because this structure permits the maximum number of water–water and base–base interactions to form in solution. Falk *et al.* (1963) have found that the dehydration of DNA results in unstacking and disordering of the bases. The latter finding suggests that water plays an important role in defining and maintaining the native, helical structure of DNA. These authors have also presented evidence showing that the hydrogen bonds between the bases in native DNA have about the same bond strength as the remaining base–water and base–base hydrogen bonds in the unordered DNA.

tion of the DNA helix by the alcohols and other organic denaturants must be due to greater competition of the denaturant with the interstrand hydrophobic bonds or sites of stacking interactions, as suggested by Ts'o *et al.* (1962), or that the observed effects are due to the net increase in dispersion interactions (van der Waals' and other short-range interactions) between the average base and the denaturing solvent.

References

Aubel-Sadron, G., Beck, G., and Ebel, J. P. (1964), *Biochim. Biophys. Acta 80*, 448.

Beaven, G. H., Holiday, E. R., and Johnson, E. A. (1963), *Nucleic Acids 1*, 493.

Bigelow, C. C. (1967), *J. Theor. Biol. 16*, 187.

Brahms, J., Maurizot, J. C., and Michelson, A. M. (1967), *J. Mol. Biol. 25*, 481.

Brahms, J., Michelson, A. M., and Van Holde, K. E. (1966), *J. Mol. Biol. 15*, 467.

Brahms, J., and Mommaerts, W. F. H. M. (1964), *J. Mol. Biol. 10*, 73.

Brandts, J. F. (1964), *J. Amer. Chem. Soc. 86*, 4302.

Bunville, L. G., Geiduschek, E. P., Rawitscher, M. A., and Sturtevant, J. M. (1965), *Biopolymers 3*, 213.

Cassel, R. B., and Wen, W.-Y. (1972), *J. Phys. Chem. 76*, 1369.

Cohn, E. J., and Edsall ,J. T. (1943), Proteins, Amino Acids and Peptides, New York, N. Y., Reinhold Publishing Corp., Chapter 9.

Collander, R. (1954), *Physiol. Plant 7*, 420.

DeVoe, H., and Tinoco, I., Jr. (1962), *J. Mol. Biol. 15*, 500.

Duggan, E. L. (1961), *Biochem. Biophys. Res. Commun. 6*, 93.

Eliasson, R., Hammarsten, E., Lindahl, T., Bjork, I., and Laurent, T. C. (1963), *Biochim. Biophys. Acta 68*, 234.

Falk, M., Hartman, K. A., and Lord, R. C. (1963), *J. Amer. Chem. Soc. 85*, 391.

Fasman, G. D., Lindblow, C., and Grossman, L. (1964), *Biochemistry 3*, 1015.

Felsenfeld, G., and Miles, H. T. (1967), *Annu. Rev. Biochem. 36*, 407.

Flory, P. J. (1957), *J. Cell. Comp. Physiol. 49*, 175.

Geiduschek, E. P., and Gray, I. (1956), *J. Amer. Chem. Soc. 78*, 879.

Gill, S. J., Downing, M., and Sheats, G. F. (1967), *Biochemistry 6*, 272.

Green, G., and Mahler, H. R. (1971), *Biochemistry 10*, 2200.

Gurney, R. W. (1953), Ionic Processes in Solution, New York, N. Y., McGraw-Hill Book Co., Inc., p 90.

Hanlon, S. (1966), *Biochem. Biophys. Res. Commun. 23*, 861.

Hamaguchi, K., and Geiduschek, E. P. (1962), *J. Amer. Chem. Soc. 84*, 1329.

Hamlin, R. M., Jr., Lord, R. C., and Rich, A. (1965), *Science 148*, 1734.

Helmkemp, G. K., and Ts'o, P. O. P. (1961), *J. Amer. Chem. Soc. 83*, 138.

Herskovits, T. T. (1962), *Arch. Biochem. Biophys. 97*, 477.

Herskovits, T. T. (1963), *Biochemistry 2*, 335.

Herskovits, T. T., Gadegbeku, B., and Jaillet, H. (1970a), *J. Biol. Chem. 245*, 2588.

Herskovits, T. T., and Jaillet, H. (1969), *Science 163*, 282.

Herskovits, T. T., Jaillet, H., and DeSena, A. T. (1970b), *J. Biol. Chem. 245*, 6511.

Herskovits, T. T., Jaillet, H., and Gadegbeku, B. (1970c), *J. Biol. Chem. 245*, 4544.

Herskovits, T. T., Singer, S. J., and Geiduschek, E. P. (1961), *Arch. Biochem. Biophys. 94*, 99.

Josse, J., Kaiser, A. D., and Kornberg, A. (1961), *J. Biol. Chem. 236*, 864.

Katz, L., and Penman, S. (1966), *J. Mol. Biol. 15*, 220.

Kauzmann, W. (1959), *Advan. Protein Chem. 14*, 1.

Kozak, J. J., Knight, W. S., and Kauzmann, W. (1968), *J. Chem. Phys. 48*, 675.

Küchler, E., and Drosch, J. (1966), *Z. Naturforsch. B21*, 209.

Kurihara, K., Hachimori, Y., and Shibata, K. (1963), *Biochim. Biophys. Acta 68*, 434.

Kyogoku, Y., Lord, R. C., and Rich, A. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 250.

Levine, L., Gordon, J. A., and Jencks, W. P. (1963), *Biochemistry 2*, 168.

Long, F. A., and McDevit, W. F. (1952), *Chem. Rev. 51*, 119.

Lord, R. C., and Thompson, G. J., Jr. (1967), *Biochim. Biophys. Acta 142*, 1.

Mandelkern, L., and Stewart, W. E. (1964), *Biochemistry 3*, 1135.

Massie, H. R., and Zimm, B. H. (1969), *Biopolymers 7*, 475.

McConaughy, B. L., Laird, C. D., and McCarthy, B. J. (1969), *Biochemistry 8*, 3289.

Michelson, A. M. (1963), Chemistry of Nucleotides and Nucleosides, New York, N. Y., Academic Press.

Miller, J. H., and Sobell, H. M. (1967), *J. Mol. Biol. 24*, 345.

Nakano, N. I., and Igarashi, S. J. (1970), *Biochemistry 9*, 577.

Nash, H. A., and Bradley, D. F. (1966), *J. Phys. Chem. 45*, 1380.

Nelson, R. G., and Johnson, W. C., Jr. (1970), *Biochem. Biophys. Res. Commun. 41*, 211.

Nemethy, G., and Scheraga, H. A. (1962), *J. Phys. Chem. 66*, 1773.

Newmark, R. A., and Cantor, C. R. (1968), *J. Amer. Chem. Soc. 90*, 5010.

Nozaki, Y., and Tanford, C. (1963), *J. Biol. Chem. 238*, 4074.

Nozaki, Y., and Tanford, C. (1971), *J. Biol. Chem. 246*, 2211.

Peller, L. (1959), *J. Phys. Chem. 63*, 1199.

Robinson, D., and Grant, M. E. (1966), *J. Biol. Chem. 241*, 4030.

Robinson, D., and Jencks, W. P. (1965), *J. Amer. Chem. Soc. 87*, 2462.

Schrier, E. E., Ingwall, R. T., and Scheraga, H. A. (1965), *J. Phys. Chem. 69*, 298.

Schrier, E. E., and Schrier, E. B. (1967), *J. Phys. Chem. 71*, 1851.

Scruggs, R. L., Achter, E. K., and Ross, P. D. (1972), *Biophys. Soc.*, Abstr. 174a.

Shoup, R. R., Miles, H. T., and Becker, E. D. (1966), *Biochem. Biophys. Res. Commun. 23*, 194.

Sinanoglu, O., and Abdulnur, S. (1965), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 24*, S-12.

Solie, T. N., and Schellman, J. A. (1968), *J. Mol. Biol. 33*, 61.

Strauss, J. H., Kelly, R. B., and Sinsheimer, R. L. (1968), *Biopolymers 6*, 793.

Tanford, C. (1962), *J. Amer. Chem. Soc. 84*, 4240.

Tanford, C. (1968), *Advan. Protein Chem. 23*, 121.

Ts'o, P. O. P., Helmkamp, G. K., and Sanders, C. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 686.

Ts'o, P. O. P., Melvin, I. S., and Olson, A. C. (1963), *J. Amer. Chem. Soc. 85*, 1289.

Van Holde, K. E., and Rossetti, G. P. (1967), *Biochemistry 6*, 2189.

Von Hippel, P., and Schleich, T. (1969), *in* Structure and Stability of Biological Macromolecules, Timasheff, S. N., and Fasman, G. D., Ed., New York, N. Y., Marcel Dekker, p 417.

Warshaw, M. M., and Tinoco, I., Jr. (1965), *J. Mol. Biol. 13*, 54.

Wetlaufer, D. B., Malik, S. K., Stoller, L., and Coffin, R. L. (1964), *J. Amer. Chem. Soc. 86*, 508.

Studies on the Structure of Metaphase and Interphase Chromatin of Chinese Hamster Cells by Circular Dichroism and Thermal Denaturation†

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ABSTRACT: As a step toward understanding the molecular basis of chromosome changes during mitosis, deoxyribonucleoprotein was isolated from Chinese hamster cell cultures arrested at metaphase with vinblastine (metaphase chromatin) and compared to chromatin of interphase cells. Metaphase chromatin possesses a histone complement similar to interphase chromatin; however, some difference in nonhistone protein is evident by polyacrylamide gel electrophoresis. Thermal denaturation profiles of metaphase and interphase chromatin in either 2.5×10^{-4} M EDTA (pH 8.0) or in medium containing 5 M urea, 0.01 M NaCl, and 0.001 M Tris (pH 7.0) are very similar. Metaphase and interphase chromatin in the latter medium demonstrate recognizable thermal transitions of DNA at 72 and 65° due to association of histones which stabilize the DNA double helix, and a third transition at about 54° due to DNA that is not covered by histone. Circular dichroism (CD) spectra of metaphase and interphase chromatin are similar. Both show positive CD bands at 277 nm which are 45% in magnitude of that of isolated DNA or of chromatin in 0.5% sodium dodecyl sulfate in which chromosomal proteins are dissociated from DNA. The CD spectral alteration of chromatin DNA is partially reversed to that of isolated DNA in the presence of urea (5 M urea, 0.01 M NaCl, and 0.001 M Tris, pH 7.0), even though there is no histone dissociation as revealed by gel electrophoresis. Thus, DNA conformational changes appear to result from the organization of nucleohistone into a complex structure such as supercoil model which is unfolded in urea solution. The increased histone phosphorylation in metaphase chromatin that is observed in the native complex does not affect CD changes. The present study suggests that an essentially similar elementary structure of nucleohistone persists throughout the interphase and metaphase of the cell cycle, and thereby ensures that the same chromatin structure is passed down to the identical daughter cells.

Chromosomes of eukaryotic cells are complexes of DNA with basic histone, acidic nonhistone protein, and some RNA. During the process of mitosis, the dispersed chromatin of interphase cells is gradually condensed to a metaphase chromosome which possesses a characteristic morphology.[1] The present study is an attempt to understand the molecular basis of this cellular process.

Procedures for isolation of metaphase chromosomes in quantities large enough for biochemical and biophysical studies have been developed (Salzman *et al.*, 1966; Mendelsohn *et al.*, 1968; Cantor and Hearst, 1966; Maio and Schildkraut, 1967; Huberman and Attardi, 1966), and some structural studies by physicochemical methods were reported (Cantor and Hearst, 1970). There are problems in studies to determine the properties of metaphase chromosomes due to their large particle size and possible cellular contamination, *e.g.*, of the isolated chromosomes by ribosomes. In this paper, the elementary deoxyribonucleoprotein complex was extracted from cells arrested at metaphase by vinblastine and from interphase cells and they were studied in order to reveal whether there is an altered structure of elementary nucleohistone during the process of mitosis. However, the superstructure characteristic of chromosome morphological appearance is not preserved in the present isolation procedure. The methods employed to probe the structure are circular dichroism and thermal denaturation. Circular dichroism studies of chromatin of interphase cells (Shih and Fasman, 1970; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Wilhelm *et al.*, 1970; Henson and Walker, 1970; Wagner and Spelsberg, 1971; Matsuyama *et al.*, 1971), and DNA–histone complexes (Shih and Fasman, 1971, 1972; Fasman *et al.*, 1970; Olins and Olins, 1971; Gottesfeld *et al.*, 1972; Wagner and Vandegrift, 1972) demonstrated altered spectra of DNA due to association with histones which suggest conformational changes of DNA. Chromatin and DNA–histone complexes also exhibit characteristic melting profiles indicating stabilization of the DNA double-helical structure by histone binding (Shih and Bonner, 1970; Li and Bonner, 1971; Smart and Bonner, 1971; Ansevin *et al.*, 1971).

Materials and Methods

Materials. A clonal line of Chinese hamster cells (V79-589FR) was grown in Tricine-buffered Eagle's 2 medium supplemented with 10% fetal calf serum as previously described (Lake *et al.*, 1972). Metaphase cells were harvested after 4-hr exposure to 0.1 μg/ml of vinblastine sulfate (Eli Lilly, Indianapolis, Ind.) by selective detachment. The re-

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[1] The term, metaphase chromatin, refers to deoxyribonucleoprotein isolated from metaphase cells.