# Nonaqueous Solutions of DNA; Denaturation by Urea and Its Methyl Derivatives

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# Nonaqueous Solutions of DNA; Denaturation by Urea and Its Methyl Derivatives*

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The denaturation of deoxyribonucleic acid (DNA) by urea and its methyl derivatives has been investigated by optical density and optical rotation methods. Denaturation in urea, dimethylurea, and tetramethylurea solution of salmon DNA (in the presence of $10^{-3}$ M salt) is accompanied by a 46–48% change in hypochromicity and a decrease in optical rotation at 436 mμ of 230 to 340°. In the presence of more than $10^{-3}$ M salt, DNA cannot be denatured in saturated urea solutions at 25°. The effectiveness of the ureas as DNA denaturants is enhanced with increasing alkyl-substitution of the $NH_2$ groups of urea. Thus 1,3-dimethylurea and tetramethylurea are found to be more effective denaturants than urea. These observations support the conclusions, based on previous studies (Herskovits *et al.*, 1961; Herskovits, 1962), that hydrophobic forces play an important role in determining the native aqueous structure of DNA. Density banding experiments with $N^{14}N^{15}$ hybrid *E. coli* DNA, denatured in 9 M dimethylurea and 5.4–5.8 M tetramethylurea solutions, indicate that the dissociation of DNA into its constituent subunits can be accomplished in these solvents. However, *E. coli* DNA is not denatured fully in 10 M urea at 25°, and strand dissociation is not achieved in this solvent. On the basis of these and other experiments it is concluded that both the ionizing power of the solvent and its hydrogen-bonding capacity are important in the process of full dismemberment of the DNA molecule.

The effects of urea on the secondary structure of the nucleic acids have been investigated by a number of workers in the past decade (Conway and Butler, 1952; Alexander and Stacey, 1955; Rice and Doty, 1957; Sturtevant *et al.*, 1958). Alexander and Stacey (1955) studied the light scattering of herring sperm DNA in the presence of 4 M urea and after rapid removal of urea by dialysis and reported that the molecular weight of DNA decreased to about one-half of its original value. On reexamining the effects of urea on DNA, Rice and Doty (1957) found no changes in the molecular weight of DNA after exposure to 8 M urea. Furthermore, no significant changes were observed in the intrinsic viscosity of DNA in 8 M urea solutions in the presence of salt (Rice, 1955) and in the hypochromicity of 5 M urea solutions (Blout and Asadourian, 1954), although a substantial lowering in the denaturation temperature was observed (Rice, 1955; Doty, 1955). Since in the absence of salt DNA is denatured at room temperatures, it was suggested (Rice and Doty, 1957) that the results of Conway and Butler may have been caused by the absence of salt in parts of the experiments involving the addition and removal of urea. In fact, Meselson and Stahl (1958) and, recently, Doty and his co-workers (Doty *et al.*, 1960; Schildkraut *et al.*, 1961) have reported that DNA can be thermally dissociated into two molecular subunits.

The denaturing ability of urea has been widely attributed to its ability to disrupt interpeptide or interchain hydrogen bonds. Recent studies on the effects of urea on suitable model compounds (Levy and Magoulas, 1961) and detergent micelles (Bruning and Holtzer, 1961) have suggested that this mode of action of urea is grossly oversimplified and that, at least in part, the denaturing action of urea should be attributed to the destabilization of hydrophobic interactions. The denaturing effects of a number of structurally related organic solvents on the structure of proteins (Tanford *et al.*, 1960; Tanford and De, 1961) and DNA (Geiduschek and Holtzer, 1958; Herskovits *et al.*, 1961; Helmkamp and Ts'o, 1961; Herskovits, 1962) have led to the thesis that hydrophobic interactions play an important role in determining the aqueous configuration of proteins and nucleic acids.

These observations have offered a plausible explanation of the fact that the helical organization of DNA remains largely unaffected in concentrated urea solutions in the presence of moderate quantities of salt. In a previous paper it was noted that the denaturing ability of the solvent is enhanced with increasing hydrocarbon content and increasing alkyl substitution; thus ethanol and propanol were found to be more effective than methanol, and the methylated solvents *N,N'*-dimethylformamide and dimethylsulfoxide were found to be appreciably more effective DNA denaturants than formamide (Herskovits, 1962). This has suggested the present study, dealing with the comparative effects of urea, dimethylurea, and tetramethylurea on the stability of DNA secondary structure. In addition, the full separation of the complementary strands of DNA by these denaturing agents was investigated by CsCl density gradient centrifugation (Meselson and Stahl, 1958), with the use of half-labeled $N^{14}N^{15}$ hybrid DNA isolated from *Escherichia coli*.

## EXPERIMENTAL

*DNA Samples.*—The two salmon DNA samples employed throughout this study have been fully described in a previous paper of this series (Herskovits *et al.*, 1961). Half-labeled hybrid $N^{14}N^{15}$ DNA (Meselson and Stahl, 1958) was prepared from *E. coli*. Bacteria were first grown in a $N^{15}$ medium containing $NH_4Cl$ as the only source of nitrogen, and then for one generation in a medium containing a 10-fold excess of unlabeled $NH_4Cl$. Growth was terminated in the logarithmic phase by the addition of sodium azide (final concentration, 0.01 M) and chilling on ice. The DNA was isolated by a method described by Marmur (1961). The crude hybrid DNA was purified by CsCl density gradient centrifugation, followed by fractionation and removal of CsCl by dialysis in the cold. The dialysate employed was 0.3 to 2 $\times$ $10^{-3}$ M NaCl-Tris buffer, *p*H 7.5, containing 2 $\times$ $10^{-4}$ M versene. The

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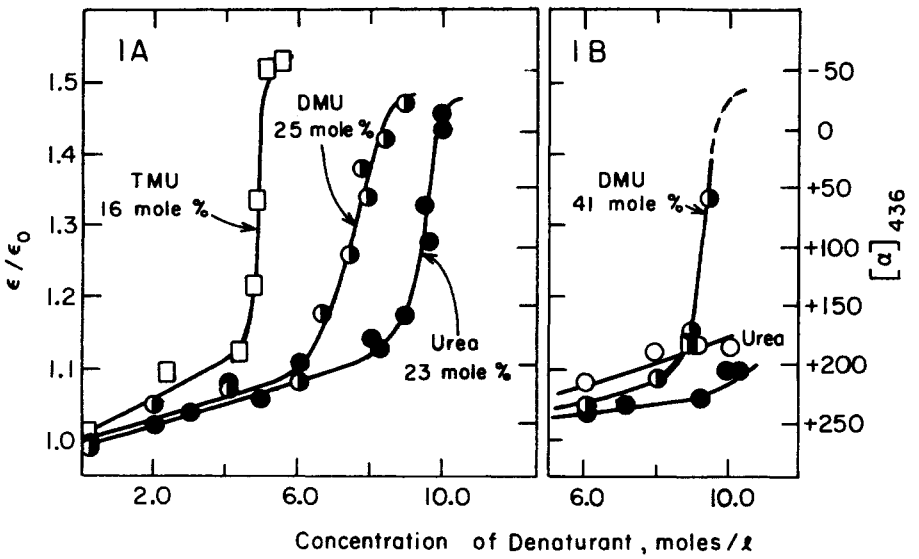


FIG. 1.—Denaturation of DNA in urea, 1,3-dimethylurea, and tetramethylurea solutions at 25°. $\epsilon/\epsilon_0$, extinction coefficients related to native DNA, $\lambda$ = 259 m$\mu$ (data represented by circles); $[\alpha]_{436}$, optical rotation at 436 m$\mu$ (squares). *A*, Denaturation in the presence of $10^{-3}$ M salt. Denaturant: ●, urea; ◐, 1,3-dimethylurea; □, tetramethylurea. *B*, Denaturation in the presence of 1–5 × $10^{-2}$ M salt. ○, urea, ionic strength ($\mu$) = 1 × $10^{-2}$; ●, urea, $\mu$ = 5 × $10^{-2}$; ◐, ◨, 1,3-dimethylurea, $\mu$ = 1 × $10^{-2}$. DNA sample H-I; concentration = 0.1–0.4 mg/ml; buffer 1 × $10^{-3}$ – 5 × $10^{-2}$ M Tris, 5 × $10^{-5}$–1 × $10^{-4}$ M NaCl, *p*H 7.5.

purified labeled material was stored in solution form in the presence of 2 × $10^{-3}$ M NaCl-Tris, 2 × $10^{-4}$ M versene. $N^{14}$ *E. coli* DNA and T-2 bacteriophage DNA samples were a gift of Dr. E. P. Geiduschek. A detailed description of these samples has been given by Hamaguchi and Geiduschek (1962).

*Materials.*—1,3-Dimethylurea (Matheson, Coleman and Bell) was purified by two successive crystallizations from hot ethanol. Before use, dimethylurea stock solutions were decolorized with activated charcoal. Tetramethylurea was obtained from John Deere Chemical Company (Pryor, Okla.) and used without further purification. All the other reagents were analytical grade.

*Preparation of DNA Solutions.*—DNA stock solutions were prepared and stored in the presence of a minimum of $10^{-3}$ M salt at 1–5°. Nonaqueous solutions of DNA were prepared by volumetric or gravimetric dilution. The dilution procedures were designed to minimize the possibility of thermal denaturation (Geiduschek and Herskovits, 1961). Urea, dimethylurea, and tetramethylurea solutions of $N^{14}N^{15}$ hybrid DNA were prepared by 10 to 40 fold dilution. After 2 hours at 26 ± 1° the denaturing agent was removed by rapid dialysis against 2 × $10^{-3}$ M Tris-NaCl, 1 × $10^{-4}$ M versene, *p*H 7.5, at 1–5°. For the density gradient experiments 7.7 molal CsCl solutions were prepared by the addition of solid CsCl to buffered DNA solutions. Where adjustment of density was necessary, small quantities of solid salt or buffer were added to give refractive index readings $n_D^{25}$ = 1.4020.

*Methods.*—Optical density measurements were made in a Beckman DU spectrophotometer at 25°. Measurements were made against solvent blanks in matched 1.0-cm silica cells, usually with 0.70- or 0.90-cm spacers to reduce the path length.

Optical rotation measurements were made in a Rudolph Model 80S polarimeter, with a mercury vapor lamp used as a source and a narrow-band interference filter or a Bausch and Lomb monochromator used for the isolation of the 436 m$\mu$ band.

CsCl density-gradient experiments were performed in a Spinco Model E analytical ultracentrifuge at 44,770 rpm. Ultraviolet absorption photographs were taken after 20–22 hours of centrifugation at 25°. Density tracings were made with a Joyce-Loebl double-beam recording microdensitometer.

## EXPERIMENTAL RESULTS

*A. Denaturation in Urea and Its Methyl Derivatives.*—Studies on the denaturation of DNA by a number of organic solvents (Herskovits *et al.*, 1961; Helmkamp and Ts'o, 1961; Herskovits, 1962) have shown that the disorganization of the native structure is in all cases accompanied by a 35 to 50% increase in absorbance and a decrease in optical rotation ranging from 200 to 350° at 436 m$\mu$. The change in hypochromicity and optical rotation accompanying the disorganization of the native structure of DNA by urea and its methyl derivatives is equally pronounced (Table I). Moreover, as can be seen in Figure 1, the collapse of the native helical structure of DNA in the presence of salt occurs over a quite narrow range of solvent composition. No such abrupt transition is observed with DNA that has been

TABLE I

SUMMARY OF THE OPTICAL PROPERTIES OF NATIVE AND DENATURED SALMON DNA AT 25°

| Denaturant | Ionic Strength | $[\alpha]_{436}$ | $E(P)^a$ |
|---|---|---|---|
| None | 1 × $10^{-3}$ [c] | +265 | 6470 |
| Heat[b] | 1 × $10^{-3}$ [c] | +59 | 8700 |
| 10 M urea | 1 × $10^{-3}$ [c] | +15 | 9300 |
| 10 M urea | 1 × $10^{-4}$ [d] | +32 | 9100 |
| 9.0 M 1,3-dimethylurea | 1 × $10^{-3}$ [c] | −78 | 9500 |
| 5.4 M tetramethylurea (65 vol. %) | 1 × $10^{-3}$ [c] | −70 | 9600 |

[a] Molar extinction coefficient, cm²/mole P, at 259 m$\mu$. [b] Denatured in $10^{-3}$ M salt by heating at 98 ± 2° for 15 minutes. [c] 1 × $10^{-3}$ M Tris-NaCl, *p*H 7.5. [d] 1 × $10^{-4}$ M NaCl.

first denatured by heat in water (shown as the upper line in Fig. 2). In this latter case, the gradual change in optical density and optical rotation reflects the disorganization of shorter, imperfectly base-paired regions of the molecule (Doty *et al.*, 1960).

For a given temperature and ionic strength, $S_{1/2}$, the midpoint of the denaturation transition is a measure of the denaturing power of the solvent (Herskovits, 1962). The $S_{1/2}$ values and the changes in $[\alpha]_{436}$, shown in Figure 1 and Table I, indicate that urea is an appreciably less effective denaturant than its fully methylated derivative tetramethylurea. The denaturing power of dimethylurea is intermediate. It should be noted that DNA is not denatured in concentrated urea solutions in the presence of more than $10^{-3}$ M salt (Fig. 1*b*).

*B. Effect of Electrolyte on the Denaturation Transition.*—Previous studies on the solvent denaturation of DNA have shown that electrolyte has a pronounced effect on the location of the denaturation transition in highly polar solvents (Herskovits, 1962). This has been attributed to the predominance of electrostatic effects in these solvents at low salt concentrations. Much less electrolyte is required to suppress these effects in less polar solvents such as the alcohols, where a large degree of charge neutralization has been observed (Herskovits *et al.*, 1961). Since the dielectric constants of formamide, urea, and dimethylurea solutions in the transition region are comparably high (about 95–110; Wyman, 1933; Cohn and Edsall, 1943) one should expect that electrostatic destabilization would be roughly comparable. In turn, this should be reflected in the salt concentration dependence of $S_{1/2}$ for these three denaturants. As shown in Figure 3 the strong ionic strength dependence of $S_{1/2}$ for dimethylurea is comparable to that of DNA in formamide. While the data of Figure 1 suggest a similarly pronounced electrolyte effect for urea, the low denaturing power and limited solubility of this reagent have not permitted a similar investigation above $10^{-3}$ M salt. It is significant that in the much less polar denaturant, tetramethylurea (dielectric constant = 23; Gaumann, 1958) $S_{1/2}$ shows appreciably smaller dependence on electrolyte concentration. In fact, the salt concentration dependence of $S_{1/2}$ seems to disappear at $5 \times 10^{-3}$ M salt (Fig. 3). The $S_{1/2}$ above this salt concentration is 19 mole % (61 vol. %). On the other hand, appreciably larger quantities of dimethylurea are required to denature DNA at these salt concentrations. In the presence of $10^{-2}$ M salt, $S_{1/2}$ is 41 mole % (75 vol. %) for dimethylurea.

*C. Strand Separation and Reversibility Experiments.*—The structural changes accompanying the denaturation of salmon DNA by the ureas are largely irreversible (Table II). This has suggested the possibility that the DNA molecule is fully dissociated in these solvents. The dissociation of DNA has in fact been achieved in 9 M dimethylurea and 65–70 vol. % tetramethylurea.[1] The buoyant density distributions of half-labeled $N^{14}N^{15}$ DNA, after exposure to these denaturants at 26°, show the characteristic band-patterns (Fig. 4, curves *C* to *E*) associated with strand separation (Meselson and Stahl, 1958; Doty *et al.*, 1960; Schildkraut *et al.*, 1961).

[1] Tetramethylurea is less polar than *N,N'*-dimethylformamide or dimethylsulfoxide. Probably as a result, loss of some DNA due to aggregation in the tetramethylurea-rich region (above 65 vol. %) in the presence of $10^{-3}$ M salt has been observed. This has made this solvent the least suitable for a study concerning the role of water in strand dissociation above the transition region (T. T. Herskovits, to be published).

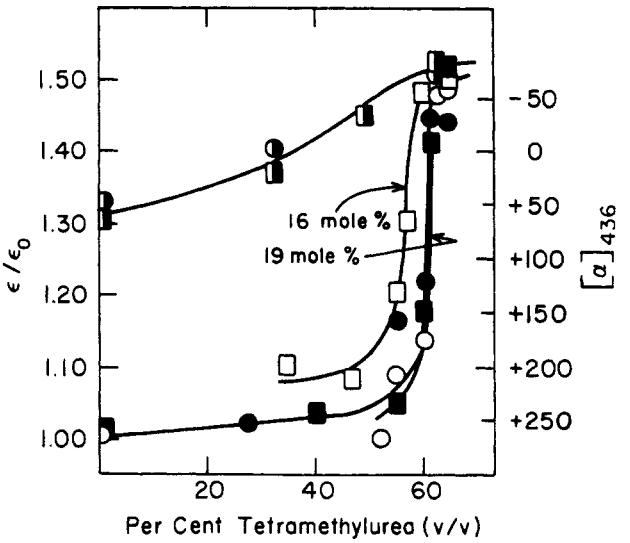


FIG. 2.—Effect of electrolyte on the denaturation of DNA in tetramethylurea-water mixture at 25°. $\epsilon/\epsilon_0$, extinction coefficients related to native DNA in aqueous salt solutions, $\lambda$ = 259 m$\mu$ (circles); $[\alpha]_{436}$, optical rotation measured at 436 m$\mu$ (squares). □, $1 \times 10^{-3}$ M salt; ○, $5 \times 10^{-3}$ M salt; ■, ●, $1 \times 10^{-2}$ M salt; ◨, ◑, DNA previously heat-denatured in aqueous salt solutions in the presence of $10^{-3}$ salt. DNA sample H-I concentration = 0.1–0.4 mg/ml; buffer: $1 \times 10^{-3}$ M Tris, 0.5–1 $\times 10^{-4}$ M NaCl, *p*H 7.5.

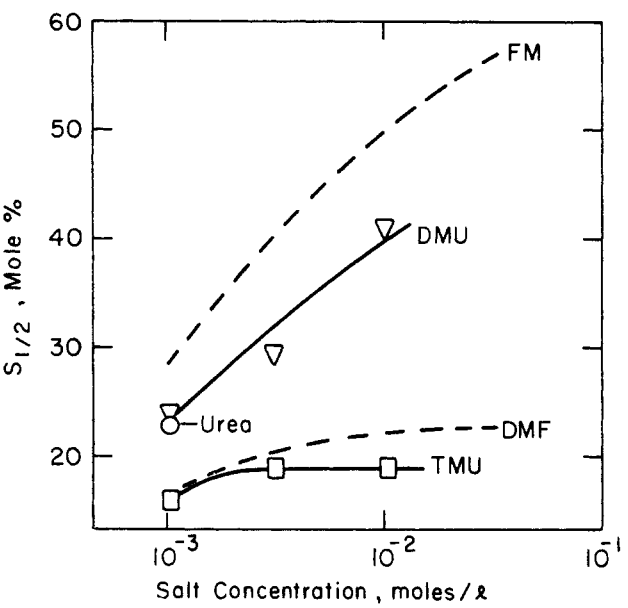


FIG. 3.—Comparison between the effects of electrolyte on the denaturation midpoints, $S_{1/2}$, of urea and formamide and their methyl-substituted derivatives. Denaturing solvent: ○, urea; ▽, 1,3-dimethylurea, □, tetramethylurea; - - - -, formamide (FM) and *N,N'*-dimethylformamide (DMF). The $S_{1/2}$ values refer to the mole % of the nonaqueous component of buffered solutions. $S_{1/2}$ values due to the ureas are taken from Figs. 1 and 2; the FM and DMF data are taken from a previous paper (Herskovits, 1962).

DNA with high guanine-cytosine content is slightly more stable and more resistant to solvent denaturation (Geiduschek and Herskovits, 1961). As a result *E. coli* DNA (guanine-cytosine content 51%, as compared with 43% for salmon DNA) is only partly denatured in 10 M urea. The change in hypochromicity accompanying denaturation at 25° is only 20%, whereas in the case of salmon DNA the corresponding change is 46% (Table I). Both the reversibility experiments

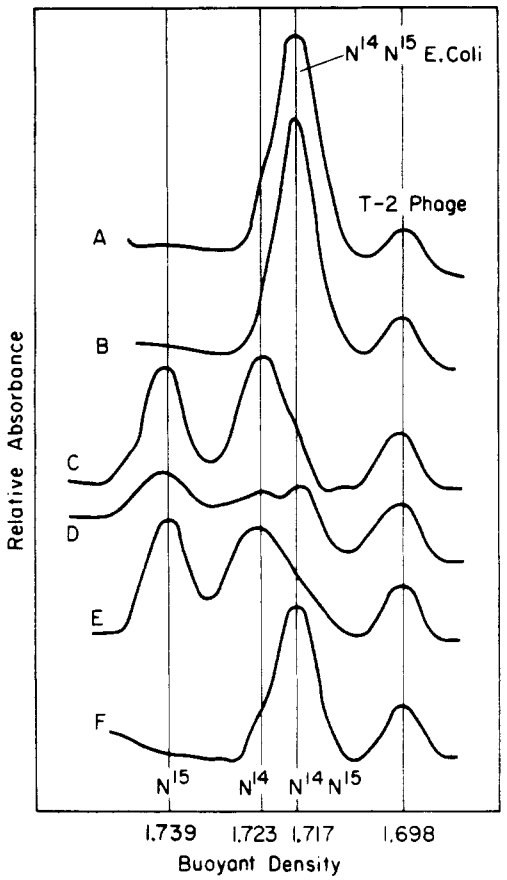


FIG. 4.—Density gradient centrifugation tracings of half-labeled $N^{14}N^{15}$ *E. coli* DNA before and after denaturation by urea, dimethylurea, and tetramethylurea and *N,N'*-dimethylformamide. *Curve A*, Native $N^{14}N^{15}$ DNA. *Curve B*, $N^{14}N^{15}$ DNA exposed to 10 M urea, $1 \times 10^{-3}$ M Tris-NaCl, $1 \times 10^{-5}$ M versene. *Curve C*, $N^{14}N^{15}$ DNA exposed to 9 M 1,3-dimethylurea, $1 \times 10^{-3}$ M Tris-NaCl, $2 \times 10^{-5}$ M versene. *Curve D*, $N^{14}N^{15}$ DNA exposed to 70 vol. % (5.8 M) tetramethylurea, $2 \times 10^{-1}$ M Tris-NaCl, $2 \times 10^{-5}$ M versene. *Curve E*, $N^{14}N^{15}$ DNA exposed to 76 vol. % *N,N'*-dimethylformamide, $1 \times 10^{-3}$ M Tris-NaCl, $1 \times 10^{-5}$ M versene. *Curve F*, $N^{14}N^{15}$ DNA exposed to 95 vol. % (7.9 M) tetramethylurea, $1 \times 10^{-3}$ M Tris-NaCl, $1 \times 10^{-5}$ M versene. Urea and 1,3-dimethylurea solutions were prepared at room temperature. Tetramethylurea and *N,N'*-dimethylformamide solutions were prepared by rapid dilution at 0°. After 2 hours at 26 ± 1° the denaturant was removed by dialysis at 0–5°. Since dialysis is a relatively slow process, in order to minimize the possibility of strand dissociation in tetramethylurea and *N,N'*-dimethylformamide solutions in the transition region, after 2 hours of equilibration at 26° solutions were cooled on ice, rapidly diluted with cold $1 \times 10^{-3}$ M Tris-NaCl buffer to the level of 40–50 vol. % tetramethylurea or *N,N'*-dimethylformamide, and then dialyzed. DNA solutions (6–16 μg/ml) were centrifuged in 7.7 molal CsCl, $7 \times 10^{-3}$ M Tris, $7 \times 10^{-5}$ M versene, *p*H 7.5, at 44,770 rpm, 25°, until equilibrium was attained. The density marker used was T-2 bacteriophage DNA.

(Table II) and the density-banding experiments (Fig. 4, curve *B*) indicate that no strand separation is achieved with *E. coli* DNA after recovery from 10 M urea.

## DISCUSSION

From the data presented in this paper it is apparent that urea is a relatively ineffective DNA denaturing agent. In fact, DNA cannot be denatured by urea at 25° in the presence of more than $10^{-3}$ M salt (Fig. 1*b*). In the somewhat more soluble and more effective denaturing solvent, 1,3-dimethylurea (in the presence of $10^{-2}$ M salt at 25°), about 75% of the water has to be replaced before DNA is denatured. Appreciably lower concentrations of tetramethylurea are required to denature DNA (19 mole %, 61 vol. %). Moreover, electrostatic destabilization is more easily suppressed in this latter solvent system (Fig. 3). Analysis of the conductance data of DNA in solvents of dielectric constant comparable to tetramethylurea, such as methanol (Herskovits *et al.*, 1961) and ethanol (Coates and Jordan, 1960), have shown that a large fraction of the counterions are associated with the charged phosphate groups in these solvents (the dielectric constants of methanol, ethanol, and tetramethylurea at 25° are, respectively, 32, 29, and 23). The attendant charge neutralization along the two opposite chains of DNA results in a substantial decrease in electrostatic destabilization of the molecule. These effects play an important role in the case of the more polar solvents (Herskovits, 1962).

A significant conclusion from the present findings is the observation that the denaturing power of the ureas is increased with increasing alkyl-substitution of the $NH_2$ groups.[2] In this relation the denaturing effects of the ureas and formamides are closely analogous (Herskovits, 1962). These and other observations based on the denaturation of DNA in a number of structurally related organic solvents have led to the conclusion that hydrophobic forces play an important role in determining native helical structure of DNA in aqueous media (Herskovits *et al.*, 1961; Herskovits, 1962). Pertinent to these conclusions was the correlation between the denaturing power of these solvents and their effectiveness as micelle breakers (Corrin and Harkins, 1946; Marchi, 1962). Recently Bruning and Holtzer (1961) have observed that urea also destabilizes the structure of detergent micelles, albeit less effectively than acetone.

It has been suggested that a very important factor in the maintenance of hydrophobic structures in solution is the hydrogen-bonded structure of the supporting solvent (Kauzmann, 1959). Solvents capable of strong solvent-solvent interactions seem to be the

[2] Alkyl substitution of the ureas and amides may increase their hydrogen bond acceptor strength (Tamres *et al.*, 1954). In turn, this may increase the denaturing power of the solvent, through its effect on the structure of water and, perhaps, through the enhancement of its ability to break solute-solute hydrogen bonds. It has been our contention that the major factor contributing to the denaturation of DNA is the effect of the solvent on the hydrogen-bonded structure of water (Herskovits *et al.*, 1961; Hamaguchi and Geiduschek, 1962). Solvents such as dioxane, *N,N'*-dimethylformamide, and dimethylsulfoxide, which can act only as hydrogen-bond acceptors or donors, and solvents such as 2-chloroethanol, which can satisfy their hydrogen-bonding requirements internally, are known to promote helix formation in proteins and synthetic polypeptides (Weber and Tanford, 1958; Tanford *et al.*, 1960; Urnes and Doty, 1961; Fasman, 1962). This means that these solvents are unable to compete successfully for the C=O . . . HN hydrogen bonds of the α-helix. On the other hand, in concentrated urea and formamide solutions, β-lactoglobulin is in the random-coiled configuration (Tanford and De, 1961). Similarly, poly-γ-benzyl-L-glutamate is in a random configuration in strongly hydrogen-bonding solvents such as dichloroacetic acid and hydrazine (Yang and Doty, 1957). Thus it appears that the effects of blocking the hydrogen-bond donor groups in urea and formamide more than compensate for the possible effects of alkyl-substitution on the hydrogen-bonding strength of the C=O moiety in these compounds.

TABLE II
REVERSIBLE AND IRREVERSIBLE DENATURATION OF DNA
(Ionic strength $1 \times 10^{-3}$)

| Denaturing Agent[a] | % Irreversibly Denatured[b] | |
|---|---|---|
| | Salmon DNA-I | *E. coli* DNA |
| 10 M urea | 67 | 25 |
| 9.0 M 1,3-dimethylurea | 85 | 92 |
| 5.3 M tetramethylurea[c] (64 vol. %) | 100 | 95 |
| 5.6 M tetramethylurea[c] (68 vol. %) | — | 100 |

[a] Exposed to 26 ± 1° for 2 hours; DNA concentration = 0.15–0.2 mg/ml. [b] After 2 hours 5-fold dilutions were made with $1 \times 10^{-3}$ M Tris-NaCl buffer, and the absorbance (at 259 mμ) was determined. The degree of irreversibility was estimated by comparing the relative absorbancies to native DNA (*N*) and heat-denatured DNA (*D*) (100°, 10 min.) in the presence of the same amount of denaturant (1.06 to 2 M). The "% irreversibility" was calculated by use of the relation $[(\epsilon/\epsilon_0)_{S\text{-}N}/(\epsilon/\epsilon_0)_{D\text{-}N}] \times 100\%$, $(\epsilon/\epsilon_0)_{S\text{-}N}$ and $(\epsilon/\epsilon_0)_{D\text{-}N}$ being the differences between the $\epsilon/\epsilon_0$ values of DNA exposed to the denaturing solvent (*S*) and native DNA and DNA denatured by heating. [c] Prepared by rapid dilution at 0–5° after 2 hours at 26 ± 1°, cooled on ice, and diluted rapidly with cold $10^{-3}$ M Tris-NaCl buffer.

less effective DNA denaturants.[3] The importance of solvent-solute interactions appears to be secondary, since blocking the $NH_2$ groups in urea and formamide leads not to a decrease in the denaturing ability of the solvent but rather to an enhancement.

Tanford and his co-workers (Tanford *et al.*, 1960; Tanford and De, 1961) have made the important observation that, while intramolecular hydrogen bonds contribute little to the conformational stability of native β-lactoglobulin, once the native conformation of the protein is destroyed strongly hydrogen-bonding substances such as urea and formamide prevent the helix formation which is observed in other organic solvents, presumably by successfully competing for the CO...HN hydrogen bonds in the polypeptide chain. Such interactions, in the case of nucleic acids, may be important, since they could facilitate the separation of the complementary strands, once the secondary structure is destroyed and the nucleotides are made accessible to the approach of solvent.

Full separation of the complementary strands of DNA has been realized in a number of polar and hydrogen-bonding solvents. Among the solvents discovered in recent years are formamide (Marmur and Ts'o, 1961), *N*-methylformamide, and ethylene glycol (95 vol. % solutions in the presence of $10^{-3}$ M salt[4]). The irreversible denaturation of *E. coli* DNA by 9.0 M 1,3-dimethylurea is also accompanied by strand disentanglement. However, we find no evidence for strand dissociation of *E. coli* DNA in 10 M urea (see Fig. 4, curves *B* and *C*). Since *E. coli* DNA is only partly denatured in 10 M urea solutions, this is to be expected. On the other hand, the changes in absorbance attending urea denaturation of salmon DNA (46% increase in absorption as compared with 20% for *E. coli* DNA) suggests that the splitting of less stable DNA's from other sources, with lower guanine-cytosine contents, may in fact be accomplished at room temperature in concentrated urea solutions.

[3] These solvents include the unsubstituted and partly substituted amides and urea, the alcohols, and the glycols (Herskovits, 1962).

[4] T. T. Herskovits, to be published.

Only partial strand dissociation can be achieved with tetramethylurea solutions (Fig. 4, curve *D*), despite the fact that the secondary structure of the molecule is largely destroyed by this denaturant. Moreover, the complementary strands are not dissociated in water-poor solutions (Fig. 4, curve *F*). Limited aggregation, associated with the low charge density of the two DNA chains due to ion-pair formation (Herskovits *et al.*, 1961), has been observed in concentrated solutions,[1] which may, in part, explain these observations. However, full strand dissociation can be achieved with the other more polar methylsubstituted solvents, employed in our previous studies (Herskovits, 1962), that produce no obvious signs of aggregation. Thus it is found that full dissociation is achieved in 60–76 vol. % *N,N'*-dimethylformamide (Fig. 4, curve *E*) and 65 vol. % dimethylsulfoxide (both in the presence of $10^{-3}$ M salt at 26°), while again, as in the case of tetramethylurea, no strand dissociation can be achieved in 90–95 vol. % solutions.[4]

Unlike water, which can participate as both donor and acceptor of solute hydrogen bonds, these three fully methylated solvents can act only as hydrogen bond acceptors. Thus it appears that both the polarity or ionizing power of the solvent and its hydrogen bonding capacity are important in the process of full dismemberment of the DNA molecule in solution.

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# Properties of Helical Polycytidylic Acid*

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The $T_m$ of polycytidylic acid in acidic solutions of various *p*H levels, ionic strengths, and urea concentrations has been investigated by measurements of absorbancy and specific rotation *versus* temperature. Other physicochemical properties of polycytidylic acid in solution were also studied, such as optical rotatory dispersion, viscosity, and sedimentation coefficients. The data support the notion that the polycytidylic acid in acidic solution assumes a helical structure with a hydrogen bonding scheme similar to that of the cytosine-5-acetic acid in crystal. This scheme consists of a pair of hydrogen bonds formed between the amino groups and the keto groups of the two cytosine residues in the helix, with an added proton in between shared by the two ring nitrogen atoms.

Studies of the helical structures formed by synthetic polynucleotides have provided considerable insight into the problem of the specificity and interaction of natural nucleic acids (Doty *et al.*, 1959; Steiner and Beers, 1961). All available homopolymers, *i.e.*, polyadenylic acid, polyinosinic acid, polyuridylic acid, and polycytidylic acid,[1] appear to form regular and helical structures by themselves under certain conditions. Among these, the purine-polynucleotides, especially poly A, are better known (Fresco and Klemperer, 1959; Rich *et al.*, 1961; Ts'o *et al.*, 1962a), while less is known about the pyrimidine-polynucleotides. The structures and the hydrogen-bonding schemes of the homohelices formed by poly U and poly C are of great interest in view of the fact that they are both biochemically active in the synthesis of polypeptides by the ribosomal system of *E. coli* (Nirenberg and Matthaei, 1961; Speyer *et al.*, 1962).

The optical properties of poly C in both neutral and acidic solution have been reported (Ts'o *et al.*, 1962a). The relationship between the optical properties of poly C and its secondary structure was found to be complicated compared to that of poly A and other nucleic acids (Ts'o *et al.*, 1962a; Helmkamp and Ts'o, 1962). This paper contains additional information pertinent to the understanding of the helical structure of poly C in acidic solution. The information is especially relevant in view of the hydrogen-bonding scheme of cytosine-5-acetic acid in the crystal form as recently described by Marsh *et al.* (1962) and the x-ray fiber diagram of helical poly C as described by Langridge and Rich (1963).

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[1] Abbreviations: poly A, polyadenylic acid; poly I, polyinosinic acid; poly U, polyuridylic acid; poly C, polycytidylic acid.

## Materials

The poly C was purchased from Miles Chemical Co., Clifton, N. J. The polymer was further deproteinized by the detergent–amyl alcohol–chloroform method as previously described (Ts'o *et al.*, 1962a). The polymer was dialyzed against 0.2 M ammonium acetate in the cold for 40 hours and then precipitated with three volumes of cold alcohol. The precipitate was washed twice with alcohol and twice with ether and then dried *in vacuo*. All other compounds were of reagent grade.

## Instrumentation and Methods of Analysis

Optical rotation measurements were made with a Rudolph Model 200S polarimeter equipped with an oscillating polarizer and xenon and mercury arc lamps (Ts'o *et al.*, 1962a). The 20-cm polarimeter tubes utilized glass construction with water jacket and quartz window. The temperature of the polarimeter tube was read directly with a thermometer and held at any desired temperature to $\pm 0.1°$ by the flow of water through both the compartments and the tube. Measurements of specific rotation $[\alpha]$ were determined at concentrations of 0.4–0.6 mg/ml.

Optical density measurements were made with a Beckman DK-2 recording spectrophotometer fitted with a modified temperature-control device. Quartz cells were fitted with a 20-mm immersion standard taper thermometer for direct reading of solution temperatures to $\pm 0.1°$.

*p*H measurements were made with a Radiometer *p*H meter 22, Copenhagen, Denmark, and to an accuracy of $\pm 0.01$ *p*H units.

Resistance of the solutions was measured to an accuracy of $\pm 1\%$ at $22.2 \pm 0.05°$ with a portable A.C. Electrolytic conductivity bridge manufactured by Leeds and Northrop Co., New York.

Viscosities were determined with a four-bulb dilution-type viscometer (Ubbelohde) designed and constructed by the Cannon Instrument Co., State College, Pa.