portions of diethyl ether, leaving 22 g (some dimethyl sulfoxide still present) of a colorless solid. A 17-g portion of the solid was suspended in 300 ml of dry dioxane. To the stirred suspension were added 30.4 g (0.280 mol) of trimethylsilyl chloride and, dropwise, a solution of 28.4 g (0.280 mol) of triethylamine in 40 ml of dioxane. The resulting suspension was stirred at ambient temperature for 20 hr and filtered under nitrogen, and the filtrate was concentrated *in vacuo*, leaving a viscous yellow oil. An 18.6-g portion of bis(trimethylsilyl)uracil, bp 68–70° (0.2 mm) [lit. bp 116° (12 mm)], was removed from the oil by distillation, leaving a red-brown pot residue. The residue was dissolved in concentrated aqueous ammonia. Partial removal of solvent *in vacuo*, followed by overnight standing at ambient temperature, led to deposit of 1.53 g (29%) of a pale yellow, amorphous solid, mp 277–287°. The solid was dissolved in formic acid, and the formic acid solution was applied to a column containing 200 g of silica gel packed in chloroform. The desired product was eluted with methanol–chloroform (1:9, v:v) as 1.34 g (25%) of an amorphous, colorless solid, mp 280–290° dec. A 140-mg portion of a middle fraction was recrystallized twice from water to yield 100 mg of an amorphous solid, mp 287–293° dec. A final recrystallization from methanol–water gave analytically pure material as colorless needles, mp 289–293° dec; nmr (TFA) $\tau$ 2.24 and 3.80 (2d, 4, $J$ = 8 Hz, $UrC_{5,6}$–H), 5.89 (t, 4, $J$ = 7 Hz, Ur–$CH_2$), and 7.65 (m, 2, $J$ = 7 Hz, C–$CH_2$–C); mass spectrum (70 eV) *m/e* (relative intensity) 264 (4, $M^+$), 221 (2, $M^+$ − HNCO), 152 (100, Ur–$C_3H_5^+$), 139 (35, Ur–$C_2H_4^+$), 126 (32, Ur–$CH_3^+$), 112 (28, Ur–$H^+$), 109 (23, Ur–$C_3H_5^+$ − HNCO), 69 (24, Ur–$H^+$ − HNCO), and 55 [31, Ur–$CH_3^+$ − HNCO and CO)].

*Anal.* Calcd for $C_{11}H_{12}N_4O_4$: C, 49.99; H, 4.56; N, 21.20. Found: C, 49.86; H, 4.54; N, 21.12.

**Acknowledgment.** The authors wish to thank Dr. A. A. Lamola for his generous assistance with the emission and photochemical studies, Dr. J. Jonas for help with computer programming, and Drs. G. Weber and R. W. Woody for many stimulating discussions. We appreciate also the technical assistance of Mrs. Marjorie Claybrook and Mrs. B. Feuer.

# Interaction of Metal Ions with Polynucleotides and Related Compounds. XII. The Relative Effect of Various Metal Ions on DNA Helicity[1]

**Gunther L. Eichhorn and Yong Ae Shin**

*Contribution from the Gerontology Research Center, National Institute of Child Health and Human Development, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Maryland, and Baltimore City Hospitals, Baltimore, Maryland 21224.* *Received February 29, 1968*

**Abstract:** The ability of Cu(II) and Zn(II) to bring about the unwinding and rewinding of the DNA double helix under appropriate conditions has been previously demonstrated. The present study reveals that other divalent metal ions have similar effects upon DNA and can be placed in a sequence that indicates the magnitude of their influence on DNA structure, as follows: Mg(II), Co(II), Ni(II), Mn(II), Zn(II), Cd(II), Cu(II). This sequence is deduced from the following phenomena. (1) The melting temperature ($T_m$) of DNA increases with increasing metal concentration for the metals at the left of the series, whereas $T_m$ passes through a maximum for the metals to the right, the maximum occurring at lowest concentration at the far right. (2) Mg(II) at the far left cannot induce any detectable rewinding of the double helix; Co, Ni, and Mn produce partial rewinding, whereas the metals on the extreme right bring about complete renaturation of DNA. (3) These metals produce an increasing shift in the absorption maximum of DNA, from left to right. The placement of the metals in the same sequence by these three criteria is readily explained by their ability to bind both phosphate and heterocyclic "base" sites with the ratio of affinity for "base" to phosphate increasing from left to right.

The ability of metal ions to react with a variety of electron-donor sites on polynucleotides has received considerable attention. There are two main sites of interaction, the phosphate moieties of the ribose phosphate backbone, and the electron-donor groups on the bases.[2,3] Two types of interaction carry with them some rather interesting consequences. Reaction with phosphate means stabilization of ordered structures,[4–8] but cleavage of phosphodiester bonds with polyribonucleotides at high temperature.[9–17] Reaction with bases means destabilization of ordered structures.[2]

The differences in the behavior of various metal ions with polynucleotides have made it apparent that some metal ions prefer the phosphate sites and other metal

(1) Presented in part at the 154th National Meeting of the American Chemical Society, Chicago, Ill., 1967, Abstract No. C-44.
(2) G. L. Eichhorn, *Nature*, **194**, 474 (1962).
(3) G. L. Eichhorn, *Advan. Chem.*, **25**, 378 (1966).
(4) J. Shack, R. T. Jenkins, and J. M. Thompsett, *J. Biol. Chem.*, **203**, 373 (1953).
(5) R. Thomas, *Trans. Faraday Soc.*, **50**, 324 (1954).
(6) R. F. Steiner and R. F. Beers, *Biochim. Biophys. Acta*, **32**, 166 (1956).
(7) K. Fuwa, W. E. C. Wacker, R. Druyon, A. F. Bartholomay, and B. L. Vallee, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1298 (1960).
(8) W. F. Dove and N. Davidson, *J. Mol. Biol.*, **5**, 467 (1962).
(9) K. Dimroth, L. Jaenicke, and D. Heinzel, *Ann. Chem.*, **566**, 206 (1950).
(10) K. Dimroth, L. Jaenicke, and I. Vollbrechtshausen, *Z. Physiol. Chem.*, **289**, 71 (1952).
(11) E. Bamann, H. Trapman, and F. Fischler, *Biochem. Z.*, **328**, 89 (1954).
(12) K. Dimroth and H. Witzel, *Ann. Chem.*, **620**, 109 (1959).
(13) K. Dimroth, H. Witzel, W. Hülsen, and H. Mirbach, *ibid.*, **620**, 94 (1959).
(14) J. W. Huff, R. S. Sastry, M. P. Gordon, and W. E. C. Wacker, *Biochemistry*, **3**, 501 (1964).
(15) G. L. Eichhorn and J. J. Butzow, *Biopolymers*, **3**, 79 (1965).
(16) J. J. Butzow and G. L. Eichhorn, *ibid.*, **3**, 97 (1965).
(17) S. F. Matushita and F. Ibuki, *Mem. Res. Inst. Food Sci., Kyoto Univ.*, **22**, 32 (1960).

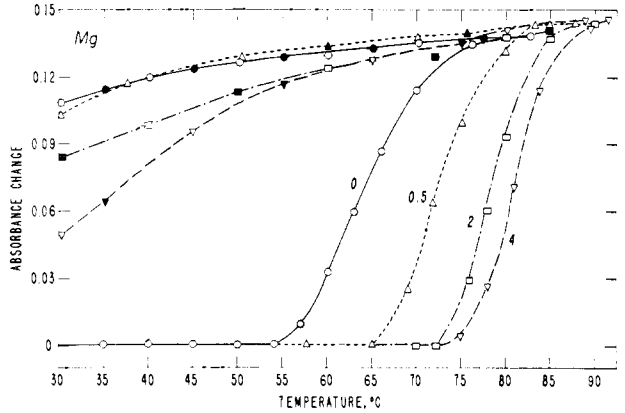


Figure 1. Melting behavior of solutions containing $5 \times 10^{-5}$ $M$ (P) DNA, $5 \times 10^{-3}$ $M$ sodium nitrate, and magnesium(II) nitrate in the following mole ratios to DNA(P): ○●, 0; △▲, 0.5; □■, 2; ▽▼, 4. The open symbols represent heating and the closed symbols cooling curves. Initial absorbances prior to heating were: ○, 0.347; △, 0.360; □, 0.362; ▽, 0.362.

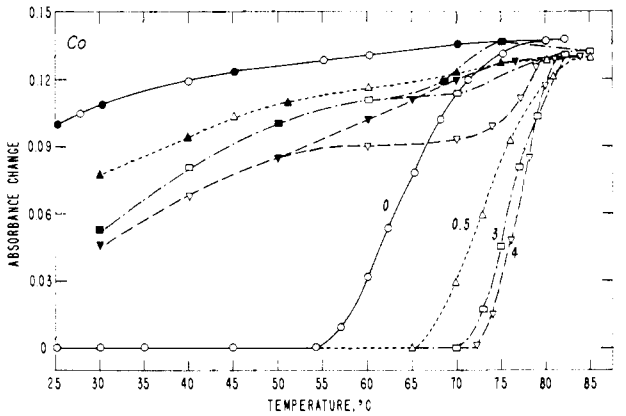


Figure 2. Melting behavior of solutions containing $5 \times 10^{-5}$ $M$ (P) DNA, $5 \times 10^{-3}$ $M$ sodium nitrate, and cobalt(II) nitrate in the following mole ratios to DNA(P): ○●, 0; △▲, 0.5; □■, 3; ▽▼, 4. The open symbols represent heating and the closed symbols cooling curves. Initial absorbances prior to heating were: ○, 0.347; △, 0.344; □, 0.357; ▽, 0.371.

ions prefer base sites. The difference was strikingly illustrated by the effect of magnesium and copper ions on the melting behavior of DNA.[2] Magnesium ions increase $T_m$ by binding phosphate and stabilizing the double helix, whereas copper ions decrease $T_m$ by binding to the bases and destabilizing the double helix. Thus magnesium and copper ions can be said to have opposite effects on DNA.

It has recently become clear that the choice of a metal ion for its binding site on polynucleotides is not an all-or-nothing proposition. Thus the copper ions that are so effective in base binding[18-29] also bind phosphate[20,21,25,28,30] and are therefore capable of cleaving phosphodiester links in polyribonucleotides.[16] On the other hand, zinc ions, which are so effective in degrading phosphodiester links due to phosphate binding,[16] have been demonstrated to bring about a temperature-reversible unwinding of DNA through binding to bases.[31] Thus it does not appear that metal ions can be readily placed into two categories, those that bind to phosphate and those that bind to bases.[32]

In the present paper we have studied the effect of seven divalent metal ions on DNA structure by the determination of melting curves as a function of metal concentration and examination of ultraviolet spectra. Metal ions in the first transition series and magnesium and cadmium have been compared. It is quite obvious from our results that these metals, some of which have been considered phosphate binders and others base binders, do not differ so much in the nature of their interaction with DNA as they do in the degree of interaction. The ability of the various metals to influence the melting temperature of DNA, to determine the extent of rewinding into the double helix after heat denaturation, and to perturb the ultraviolet spectrum can all be correlated by the relative affinities of these metals for phosphate and base sites on the DNA molecule. Thus the metal ions do not fall into a dichotomy; the phosphate binders simply can be found at one extreme and the base binders on the other extreme of a pattern of metal ion behavior.

## Results and Discussion

**Melting Curves.** The comparison of the melting curves of DNA in the presence of different metal ions, all at the same single concentration, could lead to the interpretation that there is a dichotomy of the type discussed in the introductory section. When melting curves are obtained at different metal ion concentration, this dichotomy disappears.

We begin with the melting curves of magnesium DNA solutions. The $T_m$ of DNA alone under the experimental conditions of Figure 1 is 63°. One-half mole of magnesium(II) per DNA(P) increases the melting temperature about 10°. The melting temperature of magnesium(II) DNA solutions continues to increase as more magnesium ions are added. Thus magnesium ions behave in the manner to be expected for metal ions that bind exclusively to phosphate. The negative charges on the phosphate groups of native DNA repel each other and tend to unwind the molecule unless counterions are present. The more such counterions there are, the lower the tendency to unwind. Thus the higher the magnesium concentration, the higher the melting temperature of the DNA.

The higher the magnesium ion concentration, the greater also is the decrease in absorbance on cooling the solution. This phenomenon has been explained as being due to randomly formed stacks of nucleotides.[33]

(18) A. Albert, *Biochem. J.*, **54**, 646 (1953).
(19) E. Frieden and J. Alles, *J. Biol. Chem.*, **230**, 797 (1958).
(20) M. Cohn and T. R. Hughes, Jr., *ibid.*, 273, 176 (1962).
(21) G. L. Eichhorn and P. Clark, *Proc. Natl. Acad. Sci. U. S.*, **53**, 586 (1965).
(22) S. Hiai, *J. Mol. Biol.*, **11**, 672 (1965).
(23) J. H. Coates, D. O. Jordan, and V. K. Strivastava, *Biochim. Biophys. Acta*, **20**, 611 (1965).
(24) A. M. Fiskin and M. Beer, *Biochemistry*, 4, 1289 (1965).
(25) (a) J. Eisinger, R. G. Shulman, and B. M. Szymanski, *J. Chem. Phys.*, **36**, 1721 (1962); (b) C. Ropars and R. Viovy, *J. Chim. Phys.*, **62**, 408 (1965).
(26) D. Bach and I. R. Miller, *Biopolymers*, **5**, 161 (1967).
(27) H. Venner and C. Zimmer, *ibid.*, **4**, 321 (1966).
(28) H. Brintzinger, *Biochim. Biophys. Acta*, 77, 343 (1963).
(29) S. E. Bryan and E. Frieden, *Biochemistry*, **6**, 2728 (1967).
(30) H. Moll, P. W. Schneider, and H. Brintzinger, *Helv. Chim. Acta*, **47**, 1837 (1964).
(31) Y. A. Shin and G. L. Eichhorn, *Biochemistry*, 7, 1026 (1968).
(32) Recent nmr studies with the complexes of ATP with manganese(II), nickel(II), and cobalt(II) have also indicated binding of the metals to both phosphate and base sites of this molecule: R. G. Shulman and H. Sternlicht, *J. Mol. Biol.*, **13**, 952 (1965).
(33) P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn, and M. Litt, *Proc. Natl. Acad. Sci. U. S.*, **45**, 482 (1959).

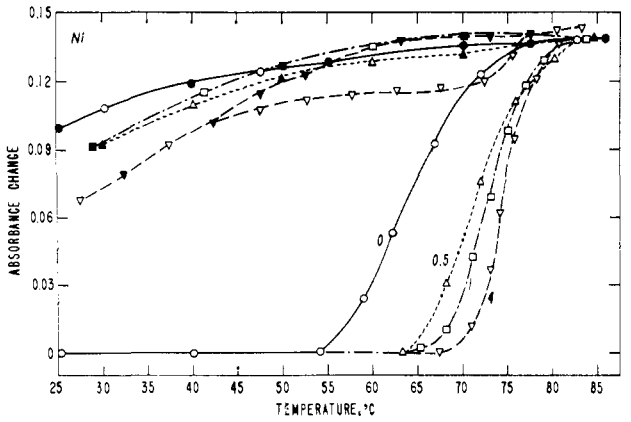


Figure 3. Melting behavior of solutions containing $5 \times 10^{-5}$ $M$ (P) DNA, $5 \times 10^{-3}$ $M$ sodium nitrate, and nickel(II) nitrate in the following mole ratios to DNA(P): ○●, 0; △▲, 0.5; □■, 1; ▽▼, 4. The open symbols represent heating and the closed symbols cooling curves. Initial absorbances prior to heating were: ○, 0.347; △, 0.360; □, 0.356; ▽, 0.341.

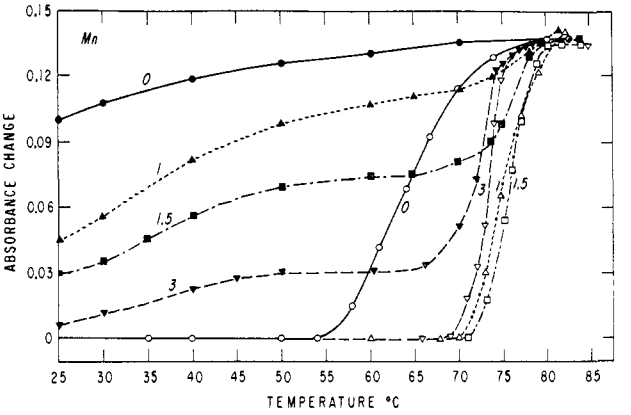


Figure 4. Melting behavior of solutions containing $5 \times 10^{-5}$ $M$ (P) DNA, $5 \times 10^{-3}$ $M$ sodium nitrate, and manganese(II) nitrate in the following mole ratios to DNA(P): ○●, 0; △▲, 1; □■, 1.5; ▽▼, 3. The open symbols represent initial heating and the closed symbols reheating curves. Initial absorbances prior to heating were: ○, 0.347; △, 0.359; □, 0.357; ▽, 0.360.

Figure 1 shows that this random stacking is completely reversible, since the reheating curves are completely superimposable upon the cooling curves.

Figures 2 and 3 show the heating, cooling, and reheating curves for cobalt(II) DNA and nickel(II) DNA solutions, respectively. Both ions again reveal the same tendency of increasing $T_m$ with increasing metal concentrations as magnesium. A very significant change enters the picture when one examines the curves of 4 mol of metal/mol of DNA(P). The cooling curve is no longer superimposable on the reheating curve. The reheating curve is clearly a two-step process, the second step of which represents a fairly cooperative melting with a $T_m$ similar to that of the initial heating curve. Thus the addition of sufficient nickel(II) or cobalt(II) seems to allow some of the metal to hold complementary bases in register during heating, so that rewinding can occur on cooling. However, this binding to the bases affects only a small portion of the DNA. Most of the DNA is obviously permanently unwound during the first heating with 4 mol of metal; cooling produces some random stacking which is melted out during the first step of the reheating, before the small amount of double helix is melted out in the second step.

Thus, even though the $T_m$ of nickel(II) and cobalt(II) DNA solutions continues to increase with increasing metal ion concentration, indicating relatively high phosphate affinity, the reheating curve gives an indication that perhaps a small portion of the metal also binds to the bases.

We have thus witnessed what appears to be a rather significant difference in behavior between magnesium(II) on the one hand and nickel(II) and cobalt(II) on the other. Another great change in behavior occurs with manganese (Figure 4). The $T_m$ of all manganese(II) DNA solutions is still higher than the $T_m$ of DNA alone but now for the first time the $T_m$ does not continue to increase with increasing metal ion concentration. The maximum value of $T_m$ occurs at a manganese/DNA(P) ratio of 1.5. A solution containing 3 mol of manganese(II)/DNA(P) melts below a solution containing only 1 mol of manganese. Thus the melting behavior suggests that manganese binds DNA at two sites. Relatively low concentrations of manganese(II) affect the $T_m$ as expected from phosphate binding, *i.e.*, the $T_m$ is increased. But higher manganese(II) concentrations give lower values of $T_m$, indicating that another reaction counteracts the effects of the binding of manganese to phosphate. This other reaction would appear to be binding to bases.

That it actually consists of binding to bases is demonstrated by the reheating (after cooling) curves of manganese(II) DNA. With manganese(II) the presence of only 1.5 mol of metal/DNA(P) causes a significant portion of the DNA to melt cooperatively at the same $T_m$ as in the initial heating. The presence of 3 mol of metal causes most of the DNA to melt cooperatively at this high temperature. Thus manganese ions are capable of base interaction to such an extent that a large proportion of double-stranded DNA appears to be regenerated on cooling.

The melting behavior of zinc(II) DNA has been discussed in the preceding paper of this series;[31] Figure 5 presents the salient features in a manner comparable to Figure 1–4. The maximum $T_m$ for zinc DNA is at 1 mol of zinc(II)/DNA(P); 2 mol decreases the $T_m$, although the $T_m$ of zinc DNA solutions is never below the $T_m$ in the absence of zinc. Most of the DNA melts out cooperatively with a high $T_m$ at a mole ratio of 1 zinc/DNA(P), and all of the DNA melts out cooperatively when this mole ratio is 2. As the preceding paper has shown, these characteristics of the melting profile of zinc(II) DNA solutions indicate that the presence of zinc during "denaturation" holds the two chains in close enough proximity so that all of the double helix is regenerated on cooling.

Another dramatic change in melting patterns is observed on passing from zinc(II) to cadmium(II). The latter metal is the first that we have encountered that produces a lowering of the $T_m$ observed in the absence of added metal (Figure 6). The maximum $T_m$ is reached at 0.5 mol of cadmium(II)/DNA(P); 3 mol of cadmium(II) gives an almost 10° decrease in $T_m$. Thus cadmium binds even more strongly than zinc to the bases. A second important change between zinc and cadmium will be noticed in the cooling curves. No decrease in absorbance occurs on cooling a heated

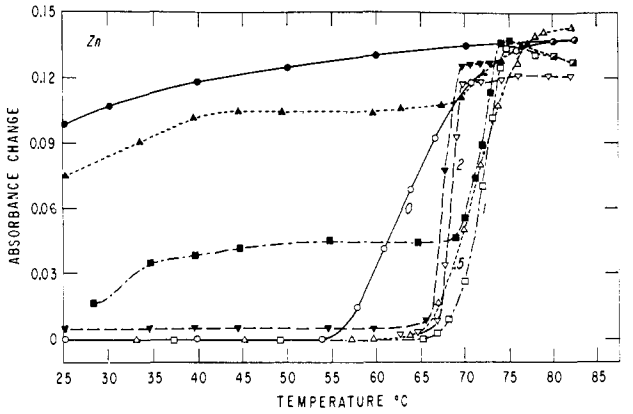


Figure 5. Melting behavior of solutions containing $5 \times 10^{-5}$ $M$ (P) DNA, $5 \times 10^{-3}$ $M$ sodium nitrate, and zinc(II) nitrate in the following mole ratios to DNA(P): ○●, 0; △▲, 0.5; □■, 1; ▽▼, 2. The open symbols represent initial heating and the closed symbols reheating curves. Initial absorbances prior to heating were: ○, 0.347; △, 0.363; □, 0.367; ▽, 0.368.

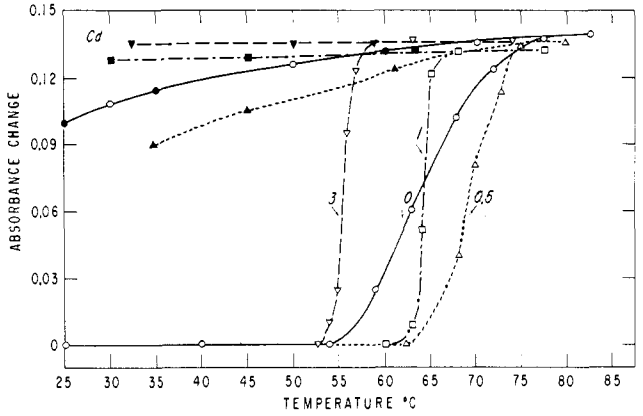


Figure 6. Melting behavior of solutions containing $5 \times 10^{-5}$ $M$ (P) DNA, $5 \times 10^{-3}$ $M$ sodium nitrate, and cadmium(II) nitrate in the following mole ratios to DNA(P): ○●, 0; △▲, 0.5; □■, 1; ▽▼ 3. The open symbols represent heating and the closed symbols cooling curves. Initial absorbances prior to heating were: ○, 0.347; △, 0.347; □, 0.353; ▽, 0.367.

cadmium(II) DNA solution containing 1 mol or more of cadmium/DNA(P). The cadmium is bound strongly enough to the DNA bases that the bonds remain intact on cooling, without regenerating the double helix, as with zinc, or producing a certain amount of random stacking, as with, *e.g.*, magnesium(II). The addition of solid salt, *e.g.*, sodium nitrate, to the cooled cadmium DNA solution results in rewinding the DNA, as indicated by the reversion to the original absorbance of the solution. The lowering of absorbance is practically instantaneous.[34]

Cadmium(II) ions thus behave very much like the previously studied copper(II) ions in their reaction with DNA;[21,22,27] melting curves similar to those in Figures 1–6 are presented for copper(II) DNA in Figure 7. The maximum $T_m$ occurs at only 0.1 $M$ copper(II)/

(34) It may be pointed out that sodium nitrate was routinely added to the cooled metal–DNA solutions. With manganese(II) DNA, this salt addition resulted in reversion to the initial absorbance after failure to reach this absorbance by cooling alone. With zinc(II), salt addition resulted in complete reversion at concentrations of zinc insufficient to produce renaturation by cooling. With magnesium(II), cobalt(II), and nickel(II), salt addition produced further, but incomplete, lowering of the absorbance.

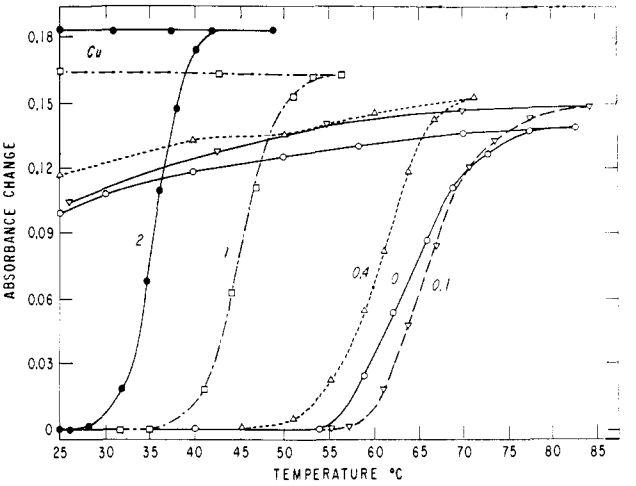


Figure 7. Melting behavior of solutions containing $5 \times 10^{-5}$ $M$ (P) DNA, $5 \times 10^{-3}$ $M$ sodium nitrate, and copper(II) nitrate in the following mole ratios to DNA(P): ○, 0; ▽, 0.1; △, 0.4; □, 1; ●, 2. Initial absorbances prior to heating were: ○, 0.347, ▽, 0.354; △, 0.355; □, 0.355; ●, 0.358.

DNA(P), and almost a 30° decrease in $T_m$ occurs in the presence of 2 mol of copper(II). The lack of absorbance change on cooling in the presence of 1 mol or more of copper(II) has been previously observed; so also has the "renaturation" of the DNA by addition of salt. However, whereas the electrolyte enhancement "renatures" DNA immediately in the presence of cadmium(II), the reaction in the presence of copper(II) takes 5 hr to go to completion.[21] This phenomenon is in line with copper(II) being much more firmly bound than cadmium(II) to the DNA bases and therefore much less readily dislodged from the H-bonding sites.

One aspect of the results of the melting curves shown in Figures 1–7 is demonstrated in Figure 8, which compares the $T_m$ of DNA in the presence of the various metal ions as a function of metal ion concentration. It can be seen that the qualitative differences in the interaction of metal ions with polynucleotides arise from fundamentally quantitative differences, as the family of curves in Figure 8 would indicate. There is a continuous change in the pattern from magnesium(II) at one extreme to copper(II) at the other. The dichotomy between ions such as magnesium(II) and copper(II) that had been previously inferred[2,3] really does exist, but the comparison of a variety of metals reveals that these extremes are part of a pattern, into which other metals fall as intermediates, and each metal behaves a little differently from every other metal.

It is indeed possible to categorize the metal ions into 3 divisions on the basis of Figure 8. Magnesium(II), cobalt(II), and nickel(II) exhibit increasing $T_m$ with increasing metal ion concentration. Manganese(II) and zinc(II) have maximum $T_m$ at intermediate metal ion concentrations, with $T_m$ always remaining above the $T_m$ of DNA in the absence of the metals. Cadmium(II) and copper(II) give maximum $T_m$ at low metal concentrations and with these metals $T_m$ values below that of DNA are attained. While these categories are real, and of significance in terms of the metal–DNA interactions, they are also arbitrary. Thus it can be supposed that if solutions with a high enough magnesium/DNA(P) ratio could be prepared, the

magnesium curve in Figure 8 would eventually reach a downward trend, producing a maximum $T_m$. Similarly, if the zinc curve could be extended, it can be imagined that a $T_m$ value below that of DNA without metal could be attained.

**Spectra of the Metal–DNA Complexes.** It could be anticipated that metal–base binding should be correlated with a change in the ultraviolet spectrum of DNA. Since this metal interaction is likely to occur after the DNA strands have been separated, the spectral changes should be observable at elevated temperature. In Table I the effect of 3 mol of metal on the absorption maxima of DNA is shown at 30 and 80°. It is apparent that the metals at the top of Figure 8 show no discernible change in absorption maximum, whereas the metals at the bottom of Figure 8 do show a significant displacement of the maximum. The maxima are broad and cannot, therefore, be read with greater precision than that given in the table. The trend, however, is clear. We note in particular that magnesium(II), cobalt(II), and nickel(II), which give increasing $T_m$ of DNA with increasing metal concentration, have little or no effect on the DNA absorbance, whereas the other metals, which give $T_m$ maxima in Figure 8, produce absorption changes in Table I. Thus the melting curves and absorption spectra of metal DNA complexes are readily correlated.

Table I. Effect of Metal Ions and Heat on the Absorption Maximum of DNA

| DNA + $M^{2+}$ | $\lambda_{max}$, mμ 30° | $\lambda_{max}$, mμ 80° | $\Delta\lambda_{max}$ |
|---|---|---|---|
| No metal | 259 | 259 | 0 |
| Mg | 259 | 259 | 0 |
| Co | 259 | 259 | 0 |
| Ni | 259 | 260 | 1 |
| Mn | 258 | 261 | 3 |
| Zn | 259 | 262 | 3 |
| Cd | 258 | 262 | 4 |
| Cu | 258 | 262 | 4 |

**Unwinding and Rewinding of Double-Helical DNA.** The three categories of metal ions determined by examination of Figure 8 can also be approximately correlated with the cooling and reheating behavior depicted in Figure 1–7. According to this behavior these metals can be classified and their behavior explained as follows.

I. **Magnesium(II).** The metal ions appear to bind primarily to phosphate. There is, therefore, no tendency to hold chains in proximity in the unwound form after heating, and cooling does not regenerate the double helix.

II. **Cobalt(II), Nickel(II), Manganese(II), and Zinc(II).** These metal ions apparently exhibit increasing affinity for the bases. Therefore, complementary bases are held in proximity during the unwound stage, amounting to a small amount of the total with cobalt(II) and nickel(II) and practically 100% of it with zinc(II). These metals also exhibit considerable affinity for phosphate. Thus, when the solutions are cooled, the phosphate-binding ability of these metal ions is capable of regenerating the double helix. These metal ions can therefore be used to unwind and rewind DNA reversibly by heating and cooling.

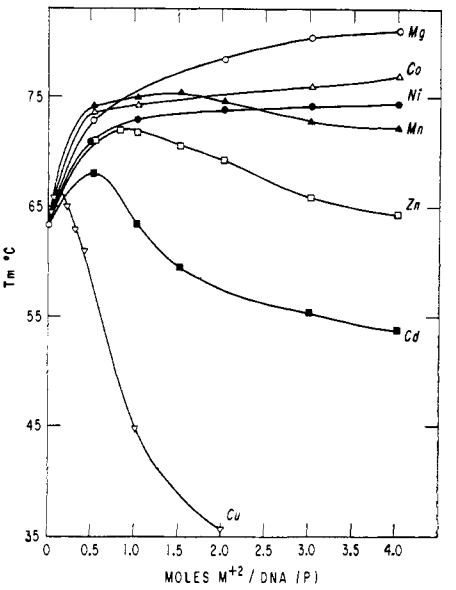


Figure 8. Variations of $T_m$ of solutions of DNA as a function of divalent metal ion concentration. Data are taken from Figures 1–7.

III. **Cadmium(II) and Copper(II).** These metal ions exhibit the greatest affinity for base among the ions studied here. They hold the bases of unwound DNA in register, and because they have such relatively high affinity for base, they continue to bind to the bases on cooling, preventing rewinding. The addition of sufficient solid electrolyte to these solutions to stabilize the double helix brings about rewinding. These metals can therefore be used to unwind and rewind DNA reversibly by heating and then cooling and adding electrolyte.

**The Apparent Relative Affinity of the Various Metal Ions for Phosphate and Base Sites on DNA.** These studies suggest that these metal ions can be placed in an order of base-binding ability/phosphate-binding ability, as follows: Mg(II) < Co(II), Ni(II) < Mn(II) < Zn(II) < Cd(II) < Cu(II). This order is in agreement with these criteria: (1) the cooling–reheating behavior exhibited in Figures 1–7 and summarized in the preceding section, (2) the $T_m$ *vs.* metal concentration effect of Figure 8, and (3) the absorption displacement of Table I. We have not definitely distinguished between nickel(II) and cobalt(II) in this series since criterion 1 would place nickel(II) slightly ahead of cobalt(II), whereas criteria 2 and 3 would reverse this order. The behavior of these metals, considering all three criteria, is so similar that they can probably not be differentiated by them.

It should be pointed out here that criteria 1 and 2 reflect only the *ratios* of base/phosphate binding affinities of the metals. Criterion 3 is related to the base-binding ability alone. Attention may be called to the fact that nothing in this paper indicates, *e.g.*, that magnesium(II) binds more strongly than copper(II) to phosphate. What is indicated is that the relative affinity of magnesium(II) for phosphate *vs.* base is much stronger than that of copper(II). It should also be emphasized that the present studies do not constitute a direct measure of phosphate and base affinities, but that the relative affinities can be inferred from

the effects on DNA structure and are generally in line with the binding studies cited in the literature.

**Other Metal Ions.** Considerable effort has been extended in the study of the reactions of silver(I)[35-38] and mercury(II)[39-46] with DNA. These metals bind very strongly to the bases and react readily with native DNA, producing an ordered structure in which the metal ions are strongly held between the two helices, which do not appear to be unwound. There is no evidence at all implicating these metals in binding to the phosphate of DNA. These metals cannot be directly compared with those studied here by melting techniques at low ionic strength, since the same base-bound complex species is stable throughout the temperature range of Figures 1–7. The metals unquestionably belong below copper(II) in the above series, as the effect on the absorbance maximum would demonstrate.[35,41] If mercury(II) and silver(I) were incorporated in the above series, it would look like this: Mg(II) < Co(II), Ni(II) < Mn(II) < Zn(II) < Cd(II) < Cu(II) < Ag(I) < Hg(II).

**The Importance of These Relative Affinities.** It is apparent from the correlations presented in this paper that the relative affinity of the metal ions for the two different sites on DNA is a significant factor in determining which of several effects on nucleic acid structure a given metal will produce under given conditions. Since metal ions are very important in both *in vivo* and *in vitro* reactions of DNA and other polynucleotides, this order must undoubtedly be considered in the understanding of metal ion participation in reactions of DNA and RNA as well as in the choice of metal to be used to bring about a desired effect on polynucleotides. When qualitative differences in metal ion effects are involved, the "lines" between them will vary with the effect under consideration. As we have seen, Figure 8 leads to "lines" between nickel(II) and manganese(II) and between zinc(II) and cadmium(II). The unwinding–rewinding phenomenon leads to "lines" between magnesium(II) and cobalt(II) and again between zinc(II) and cadmium(II).

The observations made here with regard to metal ion affinity to DNA are of course also applicable to metal ion reactions with polyribonucleotides. We have recently discovered that the degradation of polyribonucleotides with zinc(II) can be inhibited by some metal ions, but not by others. In this case the "line" is drawn between cadmium(II) and copper(II). This phenomenon will be discussed in detail elsewhere.

**The Order of Metal-Binding Affinities.** The order in which the metals are placed by what is presumed to be their relative phosphate–base binding ability does not conform to any apparent "natural" order. The metals that have been studied here, if placed in the usual order of affinity for ligands, will follow the sequence: Mg(II) < Mn(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) > Cd(II).[47-49] Thus the positions of manganese(II) and cadmium(II) in particular cannot be reconciled with the more usual sequence. We can readily explain this departure from the usual order by the fact that we are here concerned with the ratio of two ligand affinities. One of these, the affinity to base, varies considerably among the metals, whereas the other, the phosphate affinity, is much less different from metal to metal.[50,51]

## Experimental Section

The DNA stock solution was prepared as previously from Sigma Type I calf thymus sodium salt by shaking gently for 4 days.[21] Metal nitrates and other inorganic chemicals were reagent grade.

Heating and cooling curves were observed with the Gilford Model 2000 recording spectrophotometer, as previously described;[31] $5 \times 10^{-3}$ *M* sodium nitrate solution was used as a blank.

The spectra were determined by manual manipulation of the Gilford spectrophotometer. The DNA was $5 \times 10^{-5}$ *M* (P), and the divalent metal ions were added as $1 \times 10^{-4}$ *M* nitrates. Room-temperature spectra were checked with those obtained with a Cary Model 14 spectrophotometer.

The pH of the solutions used in all of the experiments was obtained with a Radiometer Model 25 pH meter. The pH ranged from 6.3 to 6.5 for solutions with all metals except copper(II); solutions containing 2 mol of copper(II)/DNA(P) had a pH of 5.8.

Renaturation by addition of salt after heating and cooling DNA solutions in the presence of cadmium(II) or copper(II) was performed by addition of solid sodium nitrate (or a concentrated solution) in the amount required to produce a 0.1 *M* solution (actually, 0.105 *M*, when the sodium nitrate already present is considered). This procedure was as previously described.[21]

(35) T. Yamane and N. Davidson, *Biochim. Biophys. Acta*, **55**, 609, 780 (1962).
(36) R. H. Jensen and N. Davidson, *Biopolymers*, **4**, 17 (1966).
(37) M. Duane, C. A. Dekker, and H. K. Schachman, *ibid.*, **4**, 51 (1966).
(38) G. L. Eichhorn, J. J. Butzow, P. Clark, and E. Tarien, *ibid.*, **5**, 283 (1967).
(39) S. Katz, *J. Am. Chem. Soc.*, **74**, 2238 (1952).
(40) C. A. Thomas, *ibid.*, **76**, 6032 (1954).
(41) T. Yamane and N. Davidson, *ibid.*, **83**, 2599 (1961).
(42) K. Gillen, R. Jensen, and N. Davidson, *ibid.*, **86**, 2792 (1964).
(43) N. Davidson, J. Widholm, U. S. Nandi, R. Jensen, B. M. Olivera, and J. C. Wang, *Proc. Natl. Acad. Sci. U. S.*, **53**, 111 (1965).
(44) U. S. Nandi, J. C. Wang, and N. Davidson, *Biochemistry*, **4**, 1687 (1965).
(45) G. L. Eichhorn and P. Clark, *J. Am. Chem. Soc.*, **85**, 4020 (1963).
(46) R. B. Simpson, *ibid.*, **86**, 2059 (1964).
(47) D. P. Mellor and L. Maley, *Nature*, **159**, 370 (1948).
(48) H. Irwin and R. J. P. Williams, *ibid.*, **162**, 746 (1948).
(49) M. Calvin and N. C. Melchior, *J. Am. Chem. Soc.*, **70**, 3270 (1948).
(50) L. G. Sillén and A. E. Martell, Ed., "Stability Constants of Metal-Ion Complexes," The Chemical Society, London, 1964.
(51) S. Chabarek and A. E. Martell, "Organic Sequestering Agents," John Wiley & Sons, Inc., New York, N. Y., 1959, pp 511–585.