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Explanation of Ionic Sequences in Various Phenomena. V. Determination of the Structure of DNA and RNA

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SUMMARY

The Watson-Crick model for DNA has been modified to account for observed physical phenomena associated with DNA. The viscosity data obtained on both partially denatured DNA and on undenatured DNA in various aqueous salt solutions give rise to acidic-type cationic sequences. These results can only be explained if there exist additional bonds other than the hydrogen bonds between base pairs. Considering the experimental data, the only possible type of bond that could exist is an ionic bond between the negatively charged phosphate group and the polar or positively charged nitrogen atom attached to the C′-1 position of the deoxyribose unit. The formation of such an ionic bond produces a natural twist in the DNA strand. The model is also applicable to RNA, although steric factors affect the stability and structure of such an RNA. Values of ΔH for the formation of the DNA and RNA double helix substantiate the proposed models. The most plausible structure for the double-stranded DNA helix is a model where the base pairs are on the surface rather than on the interior of the helix. Hydrophobic bonds do not exist except possibly in the altered structure produced during preparation of the DNA for X-ray analyses. The viscosity results on undenatured DNA in various salt solutions are caused by two factors: a destruction of the ionic bond and a reversal of charge effect on the DNA. The results show that the charge on DNA must change from a negative to a positive charge with addition of salt. The proposed model is applied to replication of DNA and formation of RNA from DNA and associated phenomena.

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INTRODUCTION

The double-stranded helical model for DNA as proposed by Watson and Crick [1, 2] leaves many questions unanswered concerning the stability of this helical model. For example, Rawitscher et al.[3] observed that the values of ΔH and ΔS for the formation of the helix are both approximately three or four times greater than they should be. They concluded that other bonds are involved in stabilizing the helix. Baldwin [4] points out that the RNA:DNA hybrid can be formed when single-stranded DNA is used, but it fails to form when double-stranded DNA is used. His suggestion that this incongruity is due to the lower stability of the RNA:DNA base pair compared to that of DNA:DNA does not seem plausible. Another apparent paradox is why certain salts increase [5-7] the value of Tm (the midpoint of the "melting curve" of the hydrogen-bonded bases) but yet lower [8, 9] the viscosity of DNA at room temperature. The increase in Tm suggests that cations such as Mg^{2+} stabilize the helix; conversely, the decrease in $[\eta]$—which measures the conformation of the DNA molecule rather than the stability of the hydrogen bonds between bases as observed in Tm measurements—suggests that these same cations destroy the helix.

In this paper the type of bonds involved in stabilizing the DNA double-stranded helix will be examined on the basis of the effects of various salts. The criteria established in a previous paper [10] for determining the types of bonds in polymers will be applied. These criteria indicate that in addition to hydrogen bonds between base pairs as proposed by Watson and Crick [1, 2], the DNA helix is stabilized by bonds between the phosphate group and the nitrogen attached to the C′-1 carbon of the sugar unit. It should be emphasized that in the model proposed here, the hydrogen bonds between base pairs are still maintained. This model, however, explains the existence of other forces, as proposed by Rawitscher et al.[3].

Addition of salt such as $MgCl_2$ alters the conformation of DNA and can be attributed to the destruction of these ionic bonds. In the absence of sufficient amounts of such salts, it is proposed that the nitrogen attached to the C′-1 carbon atom of deoxyribose either becomes positively charged by interacting with H^+ in the medium or remains as a polar atom because of the unsymmetrical distribution of electrons. In other words, the nitrogen attached to the C′-1 position may take on a positive charge because of the electron-withdrawing properties of either the attached sugar molecule or possibly the other nitrogen atoms in its ring structure. This polar or positively charged nitrogen group then interacts with the negatively charged oxygen atoms of an adjacent phosphate group to form an ionic bond. No hydrophobic bonds are present. The phosphate-base ionic bonds stabilize the conformation of the helix while the base-base hydrogen bonds hold the two strands together.

It will also be seen that these ionic bonds between phosphate and base may prohibit the base pairs from being inside the helix. Thus specific hydrogen bonds exist between bases but, in contrast to the Watson-Crick model, the hydrogen-bonded bases may be on the outside rather than on the inside of the double-stranded DNA helix. The Watson-Crick model [1] is based on chemical analyses. Their proposed structure is partially substantiated by X-ray studies [2] of Li^+ or Na^+ salts of DNA, in which case the ionic bonds between phosphate and base would be destroyed. Consequently, as will be seen, the proposed model is in harmony with chemical analyses and X-ray data and also answers the above apparent incongruities as well as other phenomena.

Before correlating the experimental evidence, it is important to note that experimental data such as viscosity measurements have been obtained on both denatured and undenatured DNA. Most of the studies have been made by carrying out a series of heat-denaturation experiments in various aqueous salt solutions. Each solution of DNA is heated for a specific time at a stated temperature and then cooled rapidly to room temperature. The changes in the viscosity, etc., of the partially denatured DNA samples are then plotted versus the heating temperature to obtain the temperature at the midpoint of the curve ($T_{1/2}$ or Tm). The value of Tm is therefore the midpoint of complete denaturation. Other studies [9], such as those carried out by Scruggs and Ross [8] measure the viscosity of DNA in various aqueous salt solutions at room temperature without any prior heating, i.e., without denaturing the DNA. It must be emphasized that there is a definite distinction between these two types of experiments. In the heat-denaturation experiments, the hydrogen bonds between base pairs are destroyed. In the room-temperature experiments these hydrogen bonds are not destroyed. However, the reason there is a drop in viscosity is not known [8]. It will now be shown that these latter experiments deal with the destruction of the ionic bond between the negatively charged phosphate group and the positively charged nitrogen base attached to the C′-1 position of the sugar.

PROPOSED MODEL AND ITS RELATIONSHIP TO CHANGES IN VISCOSITY OF DNA AT 25°C WITH ADDITION OF SALT

The melting temperature (Tm) results of Hamaguchi and Geiduschek [5], of Eichhorn [6], and of Venner and Zimmer [7] show that the DNA helix is stabilized according to the sequences $Sr^{2+} > Ba^{2+} > Mg^{2+} > Ca^{2+} > Li^+ > Na^+$ when Cl^- salts are used. However, the exact reverse of this was obtained by Scruggs and Ross [8] for undenatured DNA. They observed an appreciable drop in viscosity with an increase in concentration of various chloride salts. Such a

drop in viscosity without prior heating indicates that the salt alters or destroys the original DNA structure. Figure 1 gives their data for the effect of $MgCl_2$. As shown in their [8] Fig. 4, two effects occur.

First, the extent of the minimum in $[\eta]$ occurs according to the acidic sequence $Mg^{2+} > Li^+ > Na^+ > K^+ > Cs^+$. That is, the Mg^{2+} ion lowers the viscosity more than the Li^+ ion, and the Li^+ ion gives a greater reduction in $[\eta]$ than the Na^+ ion and so on. The ability of a particular cation to lower the value of $[\eta]$ is given by the lines on the right side of Fig. 1. Spermine does not lower the value of $[\eta]$ because it stabilizes the helix structure by stretching from one phosphate group to another.

Second, the ability to produce this minimum occurs according to another acidic sequence $Mg^{2+} > Cs^+ > K^+ > Na^+ > Li^+$. That is, the minimum occurs at a lower salt concentration with KCl (0.5 M) than with LiCl (1.5 M). It will be shown below that this latter effect

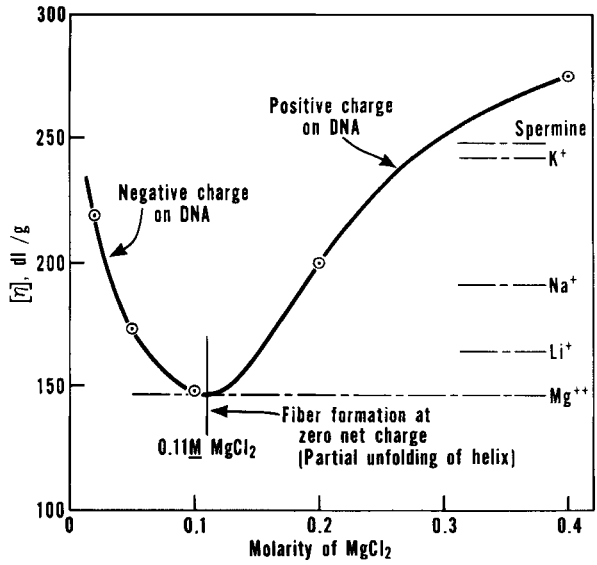

Fig. 1. Intrinsic viscosity of T4 DNA versus molarity of $MgCl_2$ at 25°C. Data obtained from Table 1 and Fig. 5 of Scruggs and Ross [8]. The minimum values of $[\eta]$ for the Cl^- salt solutions of K^+, Na^+, Li^+, and spermine are designated by the lines on the right side of the figure. The magnitude of the minimum in $[\eta]$ gives the acidic sequence $Mg^{2+} > Li^+ > Na^+ > K^+ >$ spermine. The minimum is caused by a combination of the destruction of ionic bonds and a reversal-of-charge phenomenon on the DNA (see the text).

is due to the reversal of charge phenomenon. Thus, as depicted in Fig. 1, the DNA is initially negatively charged. As Mg^{2+} ions are added, the DNA reverses its charge and becomes positively charged. At zero net charge fiber formation occurs.

It will now be shown that the first effect, i.e., the ability to produce the lowest minimum in $[\eta]$, is due to the interaction of the phosphate group with a positively charged base to form an insoluble salt.

As noted, the sequence $Mg^{2+} > Li^+ > Na^+ > K^+ > Cs^+$ for the minimum in values, as depicted in Fig. 1, is an acidic sequence [11-13]. The same acidic sequence is obtained for the insolubility of the positively hydrated phosphate group [11]. For example, the Mg^{2+} salt of phosphate is more insoluble that the Li^+ salt, which in turn is more insoluble than the Na^+ salt, and so on. As noted in Table 1 of a previous paper [10], the greater the insolubility of the cation, the greater will be its effectiveness in destroying salt bonds. Consequently, the same acidic sequence for the drop in viscosity implies that a bond involving the negatively charged phosphate group is being destroyed. The effect is not due either to reduction in repulsive forces from the negatively charged phosphate groups or to the destruction of hydrogen bonds between the hydroxyl groups or deoxyribose, because in both cases the Li^+ ion would be the least and the Cs^+ ion would be the most effective in destroying the helical structure. Such a sequence is the reverse of that observed. Moreover, the drop in $[\eta]$ is not due to destruction of hydrophobic bonding or hydrogen bonding between uncharged nitrogen bases because the sequence is acidic and not basic or polar (see below for further discussion of hydrophobic bond). Consequently, the results eliminate all possibilities except a bond involving the negatively charged phosphate group. These conclusions are also applied below to the acidic sequence obtained for the increase in the value of Tm.

The negatively charged phosphate groups are the only groups on DNA containing a negative charge, and hence these groups are the ones that must be participating in some type of bond. The question is, with which groups do the negatively charged phosphate react? They cannot react with the deoxyribose units because these sugars exist in a furanose structure [1, 2] and therefore no hydroxyl groups are available. The only other possible groups are the nitrogen groups of the bases. But to obtain such an ionic sequence, a strong interaction must exist between the phosphate and these nitrogen groups. If such an interaction exists, it would destroy the hydrogen bonds between base pairs—which it does not.

Another factor against any possible strong interaction between the phosphate and these nitrogen bases is the distance between these groups. Cavalieri et al. [14, 15] in their model must assume that several double helices have to be intertwined in tight aggregates in order to have the phosphate even approach the amino groups on ad-

jacent strands. Consequently, interaction of the phosphate group with these nitrogen bases can be ruled out because of the distance between phosphate and amino groups, the observation that such groups are uncharged, and the fact that strong interaction of phosphate groups with nitrogen bases would destroy hydrogen bonds between base pairs.

An examination of the structure of DNA shows that the four pyrimidine and purine bases are attached to the C′-1 position of the deoxyribose by means of a neutral nitrogen atom. Considering the above elimination of other groups and the close proximity of this nitrogen to the charged phosphate group, the formation of an ionic bond between the phosphate group and any other group is limited to this nitrogen atom. Now there are two possibilities for the formation of an ionic bond between the phosphate and the nitrogen attached to the C′-1 carbon. First, this neutral nitrogen is a polar atom because of the electron-withdrawing properties of either (or both) the attached sugar unit or the nitrogens of the ring structure as mentioned above. Hence this positive nitrogen atom could interact with the negative oxygen atom of the phosphate group to produce an ionic bond. Second, or alternatively, this nitrogen group could pick up a hydronium ion from the aqueous medium and could thus change from a dipolar to a positively charged group. In other words, in the vicinity of a strong negative charge such as that on the phosphate group, the pK of the C′-1 nitrogen atom may be either increased or decreased. Consequently, if it is increased, the hydronium ion attracted to the vicinity of the phosphate group could become attached to the purine or pyrimidine nitrogen atom. The resulting positive charge could form a salt bond with the phosphate group. The model is depicted in Fig. 2. In this model the negative charge on the phosphate group is shared between two oxygen atoms. The interaction of the polar nitrogen group with the phosphate ion would produce a similar model. Addition of ions such as Mg^{2+} to DNA solutions will destroy this ionic bond between the phosphate and the polar or positively charge nitrogen by canceling the negative charge on the phosphate group. In the case of the positively charge nitrogen, this interaction would most likely release the H^+ from the nitrogen atom. Also, if there are no preferential interactions of the salt bond with the anions and cations in the medium, the latter model will be neutral. But DNA is negatively charged. Consequently, the polar nitrogen may be the correct model, but further experimentation is necessary to verify which model is correct.

Thus the proposed model explains the acidic sequence obtained for the ability of a cation to lower the initial viscosity of DNA. Moreover, as noted above, spermine [$H3N^+$—CH_2—$(CH_2)_2$—NH_2^+—$(CH_2)_4$—NH^+—$(CH_2)_2$—CH_2—NH_3^+] does not lower the value of $[\eta]$ because it is able to stretch from one phosphate group to another. This stabilization is further proof that the salt bond between phos-

phate and the C′-1 nitrogen base is involved in stabilizing the helical structure of DNA.

Using the proposed structure given in Fig. 2 or the similar model made from the polar nitrogen, two structural models can be made for the DNA strand. In both models the formation of this salt bond produces a natural twist in the DNA chain. Model I is depicted in Fig. 3. This model retains the Watson and Crick proposal that the bases are on the inside of the helical strand. In model II (Fig. 4) the bases can still be paired by hydrogen bonds to an adjacent strand, but these base pairs will now be parallel with the helix and therefore will be on the outside or surface of the helix.

It might be supposed that either model does not agree with X-ray studies and therefore both are incorrect. For example, Fig. 3 shows that the bases must be parallel with the fiber axis and not perpendicular as in the Watson and Crick model [1, 2]. Furthermore, Fig. 4 shows that the bases are outside and not inside the helix, which contradicts studies by X-ray analyses [1, 2]. However, in obtaining an X-ray pattern a high concentration of a particular cation is always employed [16]. The presence of concentrated amounts of cation would destroy the ionic bond. This destruction would enable the DNA double-stranded helix to reorientate into a different position.

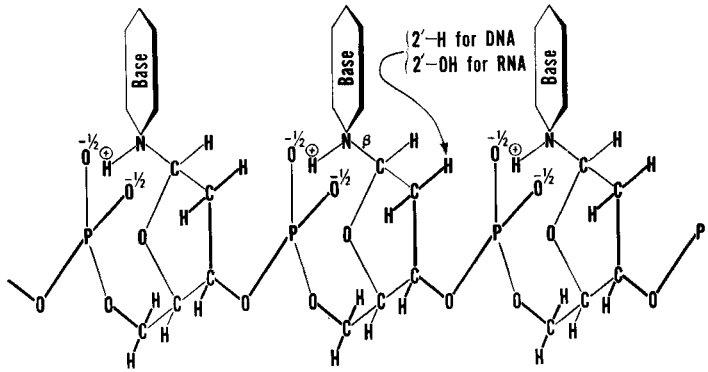

Fig. 2. Schematic drawing of proposed structure for DNA and RNA. As an alternative structure, the positive charge on the C′-1 nitrogen could be caused by the electron drawing properties of the attached sugar and/or base rather than by an attachment of a hydronium ion as in the above case. However, both structures produce the same helical conformation.

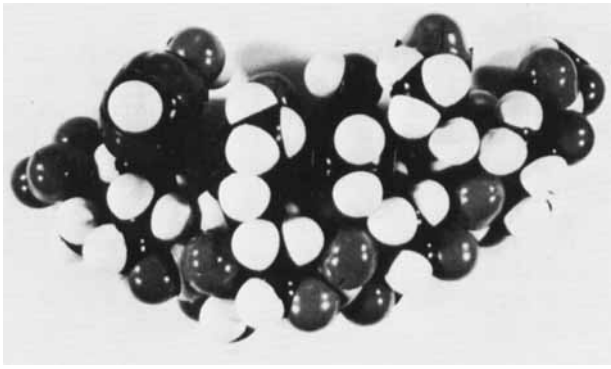

Fig. 3. Model I of the proposed DNA structure. In both models I and II the ionic bonds produce a natural curvature to the DNA strand and are formed as depicted in Fig. 2. The bases are on the inside of the helix and their planes are parallel to the fiber axis, which is in contrast to the Watson-Crick model [1, 2].

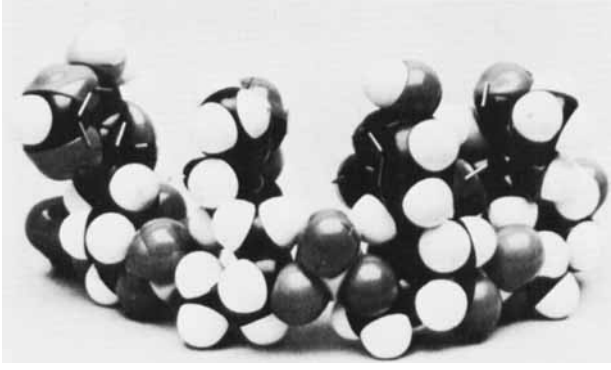

Fig. 4. Model II of the proposed DNA structure. The large Stuart atomic models depict the ionic bonds between the phosphate and C′-1 nitrogen base. Note that adjacent bases are still piled almost flat one to another and are in a definite arrangement just as in the Watson-Crick model. However, the bases for the double-stranded helix of this model would be on the surface of the helix rather than on the inside.

Such reorientations are not unusual for polymers. For example, amylose helices containing seven glucose units per helical turn can be made if the complexing agent is sufficiently large [17]. These crystals of amylose can be readily changed from seven glucose units per turn to crystals having six glucose units per helical turn by washing the crystals with a smaller complexing agent. As noted by Yamashita and Hirai [17], this large variation in structure takes place without altering the external form of the crystals. In other words, the transition from one type of helix to another occurs by thermal movement of molecules in the chain direction in the solid state [17].

In a similar manner, large changes in the helical structure of DNA could occur during preparation of the sample for X-ray analysis. Studies by Cooper and Hamilton [16] show that when sufficient moisture (humidity) is present, the crystallized A patterns of Na^+ or Li^+ salts of DNA are changed to the semicrystalline B patterns. They interpret this change as being due to a destruction of the helix by the now hydrated cations. Yet studies of DNA in 4 M NaCl or 4 M LiCl show [5] that heat must be applied to these concentrated salt solutions to destroy the DNA helix. Consequently, the conclusions made by Cooper and Hamilton [16] that parts of the helix are being destroyed by hydrated cations does not sound reasonable. A more plausible interpretation is that the added water allows the helix to partially return to its original structure. This partial change in structure would therefore change the crystalline A pattern to the semicrystalline B pattern. Consequently, the results on amylose and the formation of the semicrystalline B pattern illustrate that the structure of DNA in solution may be quite different from that in the crystalline salt form as studied by X-ray diffraction patterns.

Model II, where the bases are on the face of the helix (Fig. 4), is favored because steric hindrance between bases occurs in the other model. This steric hindrance in model I can be seen by examining Fig. 3. Furthermore, the external position of the bases in model II allows these bases to be more available for the synthesis of RNA. Consequently, model II is favored, but verification of its existence cannot be given at this time. In either model, hydrogen bonding must still occur between specific base pairs as originally proposed by Watson and Crick [1, 2] because of the differences in size and structure of these bases.

Model II is depicted on the left side of Fig. 5. Here the steel band represents the two strands of DNA joined together by hydrogen bonds between base pairs. The conformation of the helix is stabilized by the hydrogen bonds between the base units. Addition of a salt such as $MgCl_2$ partially destroys or, if sufficient interaction occurs, completely destroys the salt bond between the phosphate and C′-1 nitrogen base. The formation of this more random polymer is depicted on the right side of Fig. 5.

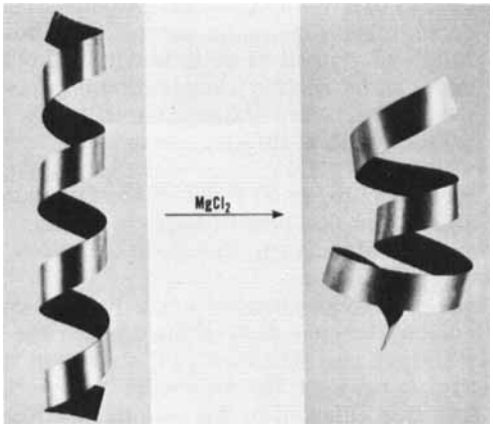

Fig. 5. Destruction of ionic bonds by addition of $MgCl_2$ to DNA solutions. The band represents two DNA strands held together by hydrogen bonds between base pairs. At room temperature the $MgCl_2$ is capable of destroying only the ionic bonds between the phosphate and C′-1 nitrogen base units. This model shows how the intrinsic viscosity of DNA is lowered in the presence of $MgCl_2$ (Fig. 1). The double strands would have a structure as depicted in Fig. 4, where the bases are on the surface of the DNA helix.

If a model of RNA is made instead of DNA using the large Stuart-type atomic models as shown in Figs. 3 and 4, the same type of double-stranded helix cannot be produced. In other words, a scale model of RNA shows that the introduction of the C′-2 hydroxyl group in going from deoxyribose to ribose produces steric hindrances for the formation of the proposed ionic bond. Consequently, a double-stranded RNA helix cannot be as readily formed. If it is formed, it should have a different helical structure or contour than the double-stranded DNA. Furthermore, there are indications [18] that the hydroxyl group of the ribose may interact with the phosphate group. Such an interaction may compete with the ionic bond between the phosphate and polar nitrogen group.

CORRELATION OF MODEL WITH ΔH VALUES

Rawitscher et al. [3] have shown that for both DNA and RNA, the values of ΔH for the formation of these double-stranded helices is too large based on the Watson-Crick model. Both DNA and RNA can form the proposed ionic bonds between their phosphate and C′-1

nitrogen base groups. However, as noted above, the steric hindrance involved in the formation of this ionic bond in RNA will produce a different helical conformation than that of the DNA. Furthermore, the bond in RNA may be between the C′-2 hydroxyl and the phosphate oxygen ions [18]. Thus the increase in the heat of reaction over that expected can be readily explained by the formation of such ionic bonds. Hence, the hydroxyl-phosphate interaction may account for these ΔH values for RNA, but there are presently no other possibilities other than the above model that can be used to account for these ΔH values for DNA.

In their experiments on RNA, Rawitscher et al. [3] examined the heat of interaction of polyriboadenylic acid (poly A) and polyribouridylic acid (poly U). At 25°C the value of the corrected ΔH for the heat of interaction of poly A and poly U is $\Delta H = -6970 \pm 170$ cal/mole for each base pair [3]. For two hydrogen bonds per base pair, this value of ΔH gives $\Delta H = -3485$ cal for each hydrogen bond, which is much too large. That is, the value of ΔH should be similar to that for the formation of hydrogen bonds between water molecules ($\Delta H = -1500$ cal/mole). In other words, the formation of hydrogen bonds in polynucleotides should release approximately 1 to 2 kcal/bond [3]. Consequently, Rawitscher et al. [3] concluded that forces other than those due to hydrogen bonds between base pairs must be involved.

According to the proposed model for RNA, a total of two ionic bonds per base pair would be formed when going from the poly A plus poly U random coils to the helix structure. Using the value of $\Delta H = -6970$ cal/mole of base pair, then the ΔH for the formation of these two ionic bonds would be $\Delta H = -6970 + 3000 = -3970$ cal or approximately -2000 cal for each ionic bond. Consequently, the other bonds predicted by Rawitscher et al. [3] can readily be explained by incorporating the ionic bonds between the phosphate groups and either the C′-1 nitrogens or the C′-2 hydroxyls.

It was also pointed out by Rawitscher et al. [3] that the formation of the DNA helix at pH 7 gives an exceptionally high enthalpy value ($\Delta H = -8260$ cal/mole of base pair). Using their value of 59.2% adenine-thymine base pairs in salmon testes DNA, then there are 2.4 hydrogen bonds per base pair because each adenine-thymine pair has two and each cytosine-guanine pair has three hydrogen bonds. The average value of ΔH for each hydrogen bond is therefore $\Delta H = -3442$ cal, assuming no other types of bonds. Again as in the case of poly A plus poly U, the value of ΔH suggests the presence of other bonds. Adopting the proposed model for DNA, the two ionic bonds per base pair should give $\Delta H = -8260 + 3600 = -4660$ cal or approximately -2300 cal for each ionic bond, assuming each hydrogen bond has $\Delta H = -1500$ cal/mole. Consequently, the ionic bond in DNA may be more stable than that in RNA, which is in agreement with the proposed models. The values of ΔS observed by

them [3] would also be more reasonable if the proposed ionic bonds are considered.

The experiments of Rawitscher et al. [3] in determining ΔH and ΔS values are therefore further support for the proposed models of DNA and RNA depicted in Figs. 3 and 4. It should also be noted that they determined their ΔH values in KCl solutions. As seen in Fig. 2, the intrinsic viscosity of DNA in KCl solutions is only slightly lower than that obtained with the peptide spermine. Because spermine stabilizes the DNA helix just as do proteins, the similar viscosity for the KCl solutions suggests that KCl does not appreciably disrupt the ionic bonds between phosphate groups and the C′-1 nitrogen bases on RNA or DNA. Consequently, such ionic bonds could be formed in the studies made by Rawitscher et al. [3]. It might be added that much lower values of ΔH and ΔS should be obtained in 0.1 M or more $MgCl_2$, 1.5 M or more LiCl, or in 1.0 M or more NaCl, because in these salt solutions some or most of the ionic bonds between the phosphate and C′-1 nitrogen base have been destroyed.

DESTRUCTION AND STABILIZATION OF HYDROGEN BONDS IN DNA BY ADDITION OF SALT

As discussed above, the values of Tm represent the ability of DNA to be denatured by heat in specific salt solutions. The anionic and cationic sequences resulting from such experiments will now be examined in light of the above proposed model for DNA and in light of previous results on the structure of hydrated ions. It will be shown that the anion will lower the value of Tm if its value of D_- is greater than that of water. The greater the value of the effective dielectric constant of the anion, the greater will be its ability to destroy the hydrogen bonds between bases [10, 13], i.e., the greater will be its effect in lowering the value of Tm. Because the phosphate group stimulates the formation of the ionic bond by interacting with the C′-1 nitrogen base, the cation can have two effects: It is capable of destroying both the hydrogen bonds between base pairs and the ionic bond between the phosphate and C′-1 nitrogen base. The greater the insolubility of the cation with the phosphate group, the greater will be its ability to destroy this ionic bond.

According to Hamaguchi and Geiduschek [5], the value of Tm for sea urchin DNA in water, pH 7.1, is 89.9°C, whereas for 4 M solutions of NaCl, NaBr, NaI, and NaSCN the values of Tm were found to be 90, 89.1, 76.4, and 68°C, respectively. Thus the ability of the anion to destroy hydrogen bonds in DNA follows the sequence $Cl^- < Br^- < I^- < SCN^-$. This sequence is what one would expect based on the values of the effective dielectric constant, D_- [10]. For Cl^-, $D_- < D_1$ and for Br^-, $D_- \leq D_1$, where D_1 is the dielectric constant of unassociated water [10]. On the other hand, $D_- > D_1$

for I^- and SCN^-. Consequently, the absence or only slight effects of the Cl^- and Br^- ions are due to their low values of D_-, and the large effects of the I^- and SCN^- ions are due to their large values of D_-. The destruction of hydrogen bonds in DNA by anions is therefore readily explainable using values of D_-.

As discussed previously [11], certain polyatomic anions such as the carboxylate and sulfate ions can form chelate structures with cations. Such chelate structures would explain the low values of Tm obtained with the Na^+ salt of $CCl_3CO_2^-$ (Tm < 25°C) and $CF_3CO_2^-$ (Tm = 63°C). In other words, the chelation of the anion to a base would increase its ability to destroy the hydrogen bonds.

The cationic sequence for Tm values of DNA is a product of two different effects and, consequently, is not as apparent as the above anionic sequence. One effect involves a stabilization and the other a destruction of the hydrogen bonds between base pairs. Let us consider the destructive effect first. As shown previously [10], the A region of a cation will increase the ability of the B region to destroy a hydrogen bond if that hydrogen bond contains a base. Consequently, K^+ ions are less effective than Na^+ ions in destroying hydrogen bonds involving bases even though the value of D_+ for K^+ is greater than for Na^+. Thus the sequence $K^+ < Na^+, Li^+$ should be obtained. The results of Hamaguchi and Geiduschek [5] show that K^+ salts given larger values of Tm than either Li^+ or Na^+ salts. Thus for the Tm values for the 4 M salt solutions we have NaSCN < KSCN: 68°C < 76°C and LiI < KI: 78.8°C < 81.8°C. Consequently, $K^+ < Na^+$ and $K^+ < Li^+$ in its ability to destroy hydrogen bonds. These results are expected based on previously proposed models.

Concerning the second effect, the model presented above for DNA shows that the hydrogen bonds between base pairs are strained. Therefore, if this strain is removed, the strength of the hydrogen bonds should increase. But the strain is caused by the ionic bond between the phosphate and C′-1 nitrogen group. Consequently, if this ionic bond is destroyed, the strength of the hydrogen bonds should be increased. But the stronger the A region of the cation, the greater will be its ability to destroy this ionic group because the phosphate group is positively hydrated [11]. In other words, the stronger the A region of the cation, the more insoluble will be the cation-phosphate interaction and the greater will be the cation's ability to destroy the ionic bond. Consequently, $Li^+ > Na^+$ in its ability to destroy the ionic bond. This stabilization effect of such cations can best be seen when the anion, e.g., Cl^-, has a value of $D_- < D_1$ and is therefore unable to destroy hydrogen bonds. Thus the Tm value for 4 M LiCl is 91.6°C as compared to 89.9°C for water [5]. In other words, in 4 M LiCl, the value of Tm is increased, showing a stabilization effect. Moreover, Tm values show that $Li^+ > Na^+$, stabilizing the DNA helix, i.e., LiCl > NaCl > H_2O:

91.6°C > 90°C ≥ 89.9°C for 4 M salt solutions. Consequently, these results show the stabilization effect on the hydrogen bonds when the ionic bond is destroyed. This stabilization effect has been further verified by the results of Eichhorn [6], who showed that Mg^{2+} ions increase the value of Tm of calf thymus DNA from 63°C in water to 80°C in 10^{-4} M Mg^{2+} ion.

Considering both effects, the observed sequence of $K^+ < Li^+ < Na^+$ for lowering Tm values using 4 M bromide salts [5] is now explainable. This sequence considered alone cannot be correlated with the hydrophobic ($Na^+ < K^+ < Li^+$), the basic ($K^+ < Na^+ < Li^+$), or the acidic ($Li^+ < Na^+ < K^+$) sequences for destroying secondary bonds [11, 12]. However, when both effects are considered, the results showing an intermediate sequence are understandable.

The divalent cation sequence substantiates these conclusions concerning the destruction of the proposed ionic bonds for DNA. Venner and Zimmer [7] observed that for the Cl^- salts that cations increased the value of Tm of Proteus mirabilis DNA according to the sequence $Sr^{2+} > Ba^{2+} > Ca^{2+}$. The divalent cation sequence for the insolubility of the phosphate salts of DNA should also be $Sr^{2+} > Ba^{2+} > Ca^{2+}$ [11]. This sequence is the same as the cationic sequence for the increase in the value of Tm. In other words, Sr^{2+} forms the most insoluble salt with the phosphate group of DNA, and therefore it is the most capable in destroying the ionic bond between the phosphate and C′-1 nitrogen base. Thus, for both mono- and divalent cations, the greater the insolubility of the phosphate-cation salt bond, the greater will be the increase in the value of Tm, that is, the greater will be the stabilization of the hydrogen bonds between the base pairs of the DNA double-stranded complex.

But as noted in the reversal-of-charge phenomenon [12], the greatest effect for reducing charge repulsion should occur with the most soluble salt and not with the most insoluble salt. Reduction in charge repulsion between phosphate groups is therefore not a factor in stabilizing the helix. This contradicts the common assumption made by many [4]. But the conclusion is reasonable because the distances between phosphate groups on adjacent chains is quite large.

A further effect is encountered when the anion is capable of chelating as in the case of ClO_4^-. Such anions appear to destroy or lower the ability of the Li^+ ion to interact with the ionic bond [5]. Moreover, such chelation or complexing effects can also occur with cations. The low values of Tm obtained with Cu^{2+} ions [6] indicate that the destruction effect in this case is greater. Thus the strong ability of Cu^{2+} ions to form complexes with bases most likely destroys the hydrogen bonds. Such an effect is absent in Mg^{2+} ions.

ABSENCE OF HYDROPHOBIC BONDING

As pointed out by Howard et al. [19], a number of scientists have proposed that hydrophobic bonding exists as a stabilizing force for the DNA helix. However, the above acidic sequence for increasing the value of Tm together with the acidic cationic sequence for changes in $[\eta]$ at 25°C (see discussion given above for Fig. 1) indicates that hydrophobic bonding is absent. Moreover, the fact that the DNA helix is stable in 15.8 M $(CH_3)_4NCl$ further suggests [10] that hydrophobic bonds are not involved. That is, previous results [10] show that hydrophobic bonds should not be stable in concentrated solutions of $(CH_3)_4NCl$. Also the $(CH_3)_4N^+$ is ineffective in destroying salt bonds [10]. Consequently, it is concluded that hydrophobic bonds do not exist in DNA. However, there is a possibility that hydrophobic bonds could be formed after the ionic bonds have been destroyed with salt such as Mg^{2+} in preparing the DNA for X-ray analysis. At present there are no data to eliminate or substantiate this conclusion.

Other experimental results show either that hydrophobic bonds are absent or that the results can be explained by mechanisms which do not require hydrophobic bonds. Thus Howard et al. [19] point out that the ability of free bases to destroy the hydrogen bonds between bases in DNA is due to the competition of the free bases for these hydrogen bonds. Again the results are explainable without invoking the possibility of hydrophobic bonds as major forces.

The results of Herskovits [20] on the ability of alcoholic salt solutions to decrease the optical rotation and slightly increase the absorbancy can also be explained without assuming hydrophobic bonds. The displacement of water with the hydrophobic alcohols increases the strength of ionic bonds between the phosphate ion and the added salt. By insolubilizing the phosphate ion, the ionic bond between the phosphate unit and the C′-1 nitrogen base is destroyed. Hence the value of the optical rotation of DNA would decrease. Consequently, hydrophobic bonding does not have to be invoked to explain these experimental results.

Further proof is seen in the reversibility of alcoholic salt solutions. That is, the effect of the alcoholic salt solutions is reversible, whereas the effect of dimethyl sulfoxide (DMSO) or heat is irreversible [20]. Consequently, this reversibility in alcoholic salt solutions suggests that the ionic bonds between phosphate groups and the C′-1 nitrogen bases are destroyed but that the bonds between the base pairs are not destroyed. If hydrophobic bonds between base pairs exist, destruction of these bonds would also destroy the associated hydrogen bonds between base pairs. Thus destruction of either would produce an irreversible conformational change in DNA. Consequently, the reversible denaturation of DNA in alcoholic salt solutions indicates that some other type of bond is being destroyed.

On the other hand, hydrogen bonds as well as any possible hydrophobic bonds would be destroyed in DMSO or upon heating. Therefore, the reversibility of the alcoholic solvent action on DNA shows that the accompanying increase in absorbancy or decrease in optical rotation cannot be due to the destruction of hydrogen or any possible hydrophobic bonds between bases.

REVERSAL OF CHARGE PHENOMENA ON DNA

The acidic sequence $Mg^{2+} > Li^+ > Na^+ > K^+ > Cs^+$ is obtained with respect to the cation's ability to lower the value of $[\eta]$ of DNA at 25°C. This sequence is due to the destruction of ionic bonds between phosphate and C′-1 nitrogen bases on DNA as seen above. The data of Scruggs and Ross [8] show the presence of another sequence. They observed that the molarity at which this minimum occurs in the values of $[\eta]$ depends on the type of salt. Thus the minimum occurs at 0.10 M $MgCl_2$, 0.5 M KCl, 1.0 M NaCl, and 1.5 M LiCl. In addition, they [8] report that CsCl solutions give a minimum in $[\eta]$ for DNA at salt concentrations slightly lower than that of the 0.5 M KCl solution. The sequence is therefore $Li^+ < Na^+ < K^+ < Cs^+ < Mg^{2+}$ with respect to the ability of the cation to produce the minimum value of $[\eta]$. This sequence is the reverse of the one above for the ability of the cation to give the lowest minimum value of $[\eta]$.

The above acidic sequence $Li^+ < Na^+ < K^+ < Cs^+$ cannot be due to destruction of hydrogen bonds between bases (basic sequence) or between hydrophobic groups (nonpolar sequence). It cannot be due to destruction of hydrogen bonds between deoxyribose units because these units are too far apart. Moreover, this sequence is not due to the destruction of the ionic bond between the electrostatically charged phosphate group and the C′-1 nitrogen base because in this case the most insoluble cation (Li^+) would give the greatest effect. Thus the only possibility remaining is that the sequence is due to a reversal-of-charge phenomenon (a reduction in electrostatic repulsive forces). The more soluble the cation-phosphate salt, the greater will be the reversal-of-charge phenomenon [12], i.e., the greater will be the ability of the cation to reduce the negative charge on DNA.

The position of the Mg^{2+} ion in the above sequence also substantiates that this is a reversal-of-charge phenomenon. The Mg^{2+} ion gives a less soluble phosphate salt that the above monovalent cations. Therefore, if this sequence were due to destruction of ionic bonds between phosphate and the C′-1 base units, then the Mg^{2+} ion would be positioned before the Li^+ ion instead of after the Cs^+ ion. Because the divalent cation such as Mg^{2+} is capable of retaining a +1

charge after interaction with the phosphate unit, the sequence for reversal of charge is greatest for the most insoluble divalent cation [10, 12]. Consequently, the position of the Mg^{2+} in the monovalent sequence verifies the conclusion that the phenomenon is due to a reversal of charge on the DNA molecules.

The above reversal-of-charge phenomenon exemplifies itself in electrophoretic mobility. Previously Ross and Scruggs [21] had examined the change in mobility of DNA with an increase in concentration of salt. As shown in their Fig. 1, the electrophoretic mobility is reduced considerably as the concentration of salt is increased. Ross and Scruggs [21] examined these phenomena only to concentrations of salt up to 0.4 M NaCl or LiCl. If, however, the concentration of salt had been increased above the concentrations obtained in observing minimum values in $[\eta]$, then a reversal of charge could have been detected by electrophoretic mobility. Such an experiment would completely prove the above conclusions. Nevertheless, the fact that the mobility of DNA is reduced with an increase in salt concentration adds further verification that a reversal of charge on DNA is occurring.

The reversal-of-charge phenomenon is manifested in the value of the viscosity coefficient k′. As shown by Scruggs and Ross [8], the value of k′ for DNA in NaCl solutions increases as the concentration of NaCl increases until a maximum value is obtained at 1.0 M NaCl and thereafter decreases with an increase in NaCl concentration. This maximum in k′ occurs at a point where the value of $[\eta]$ reaches a minimum. Apparently, the same correlation between maximum k′ value and minimum $[\eta]$ is obtained for the other salt solutions [8]. A maximum in k′ would be due to less electrostatic repulsive forces between DNA molecules. Hence, at the maximum value of k′ a minimum in $[\eta]$ would also occur because the electrostatic charge on DNA is going through a minimum value. Thus as shown by electrophoresis [21], the decrease in k′ in going from 0.2 M to 1.0 M NaCl is due to a decrease in the negative charge on the DNA. This decrease in electrostatic charge allows the DNA molecules to interact with each other. Hence the value of k′ increases. Likewise, the decrease in k′ in going from 1.0 M to 3.0 M NaCl must be due to an increase in the positive charge of the DNA (reversal-of-charge). The increase in positive charge again inhibits the interaction of one DNA double-stranded molecule with another.

Thus while the ability to lower the value of $[\eta]$ depends on the insolubility of the cation-phosphate interaction, the ability to attain this minimum in $[\eta]$ will depend on the electrostatic charge of the DNA double-stranded helix. The minimum value in $[\eta]$ is not obtained until the charge is zero. This inability may be due to a stiffness of the randomly coiled DNA molecule caused by charge repulsion between phosphate groups on the same DNA molecule. In other

words, even though more ionic bonds between phosphate and C′-1 nitrogen base units are broken in 0.5 M LiCl than in 0.5 M KCl solutions, the values of $[\eta]$ for both solutions are approximately the same because the greater intramolecular electrostatic repulsion in 0.5 M LiCl reduces the effect of a greater destruction of ionic bonds in this solvent. Thus two opposing effects occur upon addition of salt to DNA solutions: (1) The salt destroys the ionic bond and thereby decreases the stiffness of the DNA polymer, and (2) the addition of salt past the zero net charge on DNA increases the stiffness of the DNA polymer by producing a positive charge on the DNA (reversal-of-charge phenomenon).

These two opposing effects explain the leveling off of intrinsic viscosity values of DNA with addition of more salt. (See Fig. 4 of Scruggs and Ross [8].) Thus an increase in KCl concentration above 0.5 M KCl results in a leveling off of $[\eta]$ values because now intramolecular electrostatic repulsion forces again increase. This increase in stiffness due to an increase in the positive charge on DNA appears to exactly compensate the corresponding decrease in stiffness of the DNA due to a decrease in the number of ionic bonds. Consequently, the values of $[\eta]$ for LiCl solutions continue to decrease as the molarity increases from 0.5 M LiCl to 1.5 M LiCl because the electrostatic charge on the DNA is still decreasing. The leveling off of the $[\eta]$ values above 1.5 M LiCl is due to the exact compensation of these opposing effects, just as in the case of the KCl or NaCl solutions.

The intrinsic viscosity results obtained on DNA in $MgCl_2$ solutions as shown in Fig. 1 are now readily explainable. In the case of Mg^{2+} the minimum in $[\eta]$ values occurs earlier than in KCl solutions because $MgCl_2$ is more capable of reversing the electrostatic charge of the DNA molecule. As noted in Fig. 1, the values of $[\eta]$ decrease until approximately 0.11 M $MgCl_2$. The values of $[\eta]$ for 0.2 and 0.4 M $MgCl_2$ were obtained from Fig. 5 of Scruggs and Ross [8] by extrapolating their linear points to zero DNA concentration. The other three points were obtained from their [8] Table 1. These 0.2 and 0.4 M $MgCl_2$ points show that the intrinsic viscosity of DNA does not level off as in the case of the monovalent cations. But this minimum is to be expected solely on the bases of the two opposing effects cited above. In other words, the divalent Mg^{2+} ion is capable of increasing the positive charge on DNA more rapidly than the monovalent cations. Thus in going from 0.11 M $MgCl_2$ to higher molarities, there is an increase in stiffness of the randomly coiled DNA molecule due to an increase in its positive charge on DNA. This increase in stiffness more than compensates for the decrease in stiffness caused by destruction of ionic bonds. Thus contrary to the conclusions of Scruggs and Ross [8], the increase in η_{sp}/C at infinitely dilute DNA concentration, i.e., the increase in $[\eta]$, is not due to a cross linking of DNA molecules caused by

Mg^{2+} and phosphate groups. Rather, the increase in $[\eta]$ is due to a reversal-of-charge phenomenon.

The above conclusions explain why fibers of DNA can form in 0.11 M $MgCl_2$ solutions and not at higher molarities of $MgCl_2$ [8]. It is at this concentration of salt that the electrostatic charge of DNA is zero. Moreover, in 0.11 M $MgCl_2$ a sufficient number of hydrogen bonds between phosphate and C'-1 nitrogen units is destroyed in order to increase the flexibility of the DNA double-stranded molecule. Consequently, in 0.11 M $MgCl_2$ solutions the flexible DNA molecules are capable of intertwining with each other to form fibers. Transparent gels form at high concentrations of $MgCl_2$ under centrifugal force [8] because of the presence of repulsive forces due to the positive charge on the DNA molecules.

INFLUENCE OF REVERSAL-OF-CHARGE PHENOMENON AND DESTRUCTION OF IONIC BONDS ON THE VALUE OF k'

The above conclusions also explain the change in cationic sequences for the values of the viscosity coefficient k'. As stated above, the value of k' will depend on the ability of the salt to destroy ionic bonds as well as reduce or increase the electrostatic charge on the DNA. At 0.2 M salt solutions, the net charge on DNA is negative for all salt solutions given in Table 1. But the sequence obtained is

Table 1. Values of k' Obtained from Viscosity Studies on T4 B DNA in Various Salt Solutions after Extensive Dialysis[a]

	M^+, moles/liter		
	0.2	0.5	1.0
K^+	0.81	0.82	0.71
Na^+	0.76	1.07	2.42
Li^+	0.71	1.07	2.97
Cs^+	0.45		

[a]Data obtained from Table 1 of Scruggs and Ross [8]. The k' is obtained from the relationship $\eta_{sp}/C = [\eta] + k'[\eta]^2C$. The Cl^- ion was used as the counterion in all these experiments, and consequently hydrogen bonds between bases were not destroyed (see the text).

$K^+ > Na^+ > Li^+ > Cs^+$. This sequence is basic, as seen in Table 1 of a previous paper [10]. However, the sequence cannot be due to destruction of hydrogen bonds between the bases of DNA because the DNA solutions were not heated. If the phosphate ion on DNA did not have an A region but rather had only a B region with $D_- > D_1$, a basic sequence should occur [11, 12]. However, the pK of the phosphate ion on DNA according to Steiner and Beers [22] is below pH 1. Thus this result coupled with previous results [10] indicates that the phosphate group on DNA is a strong acid and contains A regions. Hence, if the ability of a cation to reverse the electrostatic charge is considered, the sequence should be acidic, not basic. Moreover, the Cs^+ ion should give the largest value of k′ because, as noted above, the value of k′ increases as the charge on DNA decreases to zero. On the other hand, if the main effect is due to destruction of ionic bonds and not to reversal of charge, the Cs^+ ion should give the lowest value of k′, which it does. The basic sequence at 0.2 M salt may therefore be the result of these two competing mechanisms: destruction of ionic bonds and reversal-of-charge phenomenon.

In 1.0 M salt the sequence is acidic: $Li^+ > Na^+ > K^+$. The greatest value of k′ is obtained with the Li^+ ion because the Li^+ ion is most effective in destroying the ionic bonds. The k′ value for 1.0 M KCl is exceptionally lower than that for 1.0 M NaCl or LiCl (0.71 compared to 2.42 and 2.97) because in 1.0 M KCl the DNA is taking on a positive charge. Consequently, the larger positive charge on DNA in 1.0 M KCl than in 1.0 M NaCl, plus the K^+ ions inefficiency in destroying these ionic bonds produces a value of k′ in 1.0 M KCl solutions, which is quite low.

The extensive dialysis done by Scruggs and Ross [8] may have also altered the values of k′. That is, the most insoluble phosphate salt has the least Donnan effect because it produces the lowest electrostatic charge on the DNA molecule. Thus, if a DNA solution is extensively dialyzed against different salt solutions of the same molarity, the concentration of salt inside the dialysis bag will be greatest for that salt solution which has the most insoluble DNA phosphate-cation salt complex [12]. For the 0.2 M solutions, the greater the salt concentration, the greater will be the value of k′. In Table 1 the basic sequence for the 0.2 M solutions is caused by the low value of k′ for the LiCl solution. If the Donnan effect is the reason for the basic sequence, one would expect the 0.2 M LiCl solution to give larger values of k′ instead of smaller because the Li^+ ion forms the most insoluble salt and hence would increase the salt concentration inside the dialysis bag. Consequently, the salt effects of Table 1 cannot be due to the Donnan effect, although such effects may have diminished the results. It is therefore concluded that the value of k′ is a function of a salt's ability to destroy the ionic bonds and to reverse the electrostatic charge of DNA molecules. Because these effects occur at different molarities for the

different chloride salts, the cationic sequence for values of k′ is basic at low salt concentrations and acidic at high salt concentrations.

APPLICATION OF PROPOSED MODEL TO DNA SELF-REPLICATION AND DNA-DIRECTED SYNTHESIS OF RNA

From the proposed model and the experimental evidence presented in the literature, the following conclusions are made:

1. In the production of messenger or ribosomal RNA by DNA, the hydrogen bonds between the bases on the double-stranded DNA helix are broken but the ionic bonds between the phosphate and C′-1 nitrogen base units are not.
2. In the self-replication of DNA, both types of bonds, i.e., hydrogen bonds between bases and ionic bonds between phosphate and C′-1 nitrogens, are broken in order to separate the two strands.
3. The strain imposed by the ionic bonds on the hydrogen bonds between the base pairs increases the selectivity for replication of correct base sequence.

The above conclusions answer some perplexing questions. First, it is known that when double-stranded DNA is used as a template for RNA synthesis, no RNA-DNA hybrid is formed [4]. Rather, the double-stranded DNA is not destroyed but remains intact. Conversely, a stable RNA-DNA hybrid is formed if a single-stranded DNA is used as a template. A remarkable characteristic of this hybrid is that the hybrid continues to function as a template even though the original RNA in the hybrid remains attached to or coiled around the DNA [4]. A significant difference occurs between the DNA-RNA hybrid and the double-stranded DNA. The RNA attached to the DNA is usually—but not always—displaced by the newly synthesized RNA. On the other hand, the DNA-DNA complex is never replaced by the synthesized RNA.

Baldwin [4] attempted to explain this anomaly by stating that the RNA-DNA hybrid is somewhat less stable than the DNA-DNA complex because of weaker hydrogen bonds between base pairs. Consequently, the RNA-DNA hybrid is not formed if double-stranded DNA is used as a template. However, this greater stability cannot be attributed to hydrogen bonding because in both cases almost the same number of hydrogen bonds is formed. The greater stability of the DNA double-stranded helix must be due to the greater stability of ionic bonds in DNA because of the steric effects of the C′-2 hydroxyl group on RNA (see above). Moreover, if hydrogen bonds between base pairs were the only factor involved, then there should be at least a small exchange between the newly produced RNA and the DNA strand. In other words, because a large exchange

occurs between DNA-RNA and RNA, then based on the strength of the hydrogen bonds involved, there should also occur at least some exchange between DNA-DNA and RNA. The fact that no such exchange occurs indicates that the ionic bonds are not broken during synthesis of RNA even though the hydrogen bonds between base pairs are broken.

The above conclusions also point to an interesting fact. During the production of RNA, all the hydrogen bonds of the bases involved in this synthesis must be broken at the same time. If they are not, the newly synthesized RNA could not compete so successfully with the RNA-DNA hybrid. That is, if only a few are broken at a time, then the more tightly held DNA which was originally bound would be in a better position to successfully compete with the more loosely, partly synthesized RNA. Consequently, the chromosome puffs seen during RNA synthesis must be due to the breaking of all hydrogen bonds between base pairs for a particular gene but retaining the stability or stiffness of the DNA strands by means of ionic bonds.

The production of RNA may therefore occur as follows. A positively charged protein protects the double-stranded DNA from interacting with the basic nucleotides in the surrounding aqueous medium. When this histone is removed, the nucleotides can interact competitively with the base on the DNA to form a three-stranded molecule or segment. Such a three-stranded helix has recently been shown by Howard et al. [19] to occur with synthetic polynucleotides and mononucleotides. The three-stranded helix would produce the chromosome puff. In other words, upon release of the histone the purine nucleotides would break the hydrogen bonds between the two strands of DNA by competitive hydrogen bonding. This slight separation of the two strands would allow the less hydrogen-bonded pyrimidine nucleotides to also interact with one of the DNA strands. The RNA would then be synthesized by means of the polymerase.

The selectivity of the DNA for a particular nucleotide must be due to factors other than the hydrogen bonds between the bases. Baldwin [4, p. 347] suggests that steric factors may be the decisive criteria in accounting for the extraordinary accuracy of the DNA polymerase in pairing the bases. As seen above, the increase in the value of Tm of DNA by addition of Mg^{2+}, Li^{+}, or Na^{+} can be explained on the basis of a removal of a strain on the hydrogen bonds between bases. This strain is removed by destroying the ionic bonds. In the synthesis of RNA the hydrogen bonds between bases are destroyed but ionic bonds most likely remain intact. Consequently, a strain could also be imposed on the hydrogen bonds between nucleotide and DNA. This strain could produce the "steric factors" suggested by Baldwin [4].

While the ionic bonds remain stable during DNA-directed synthe-

sis of RNA, this must not be the case for DNA replication. During such replication, it is known that the DNA strands separate, whereas during RNA synthesis they do not. This observation suggests that the DNA structure is additionally weakened at the time of DNA replication by destruction of bonds other than those between bases. Such bonds could be the ionic bonds between the electrostatically charged phosphate groups and the nitrogens attached to the C′-1 deoxyribose units. In other words, by destroying these ionic bonds, the DNA-DNA complex becomes even weaker than the RNA-DNA complex cited above. Such bonds could be broken by Mg^{2+} ions as noted in the above discussion of the viscosity studies of Scruggs and Ross [8].

As pointed out by Baldwin [4], the replication of DNA requires Mg^{2+} ions, whereas the synthesis of RNA by DNA requires either Mg^{2+} or Mn^{2+}. The Mn^{2+} ion cannot replace the Mg^{2+} ion in DNA replication. The solubilities of the phosphates for these cations are not known. However, the fluoride ion, which also has an A region as in the case of the phosphate ion of DNA, can be used to approximate the solubilities of phosphate salts [11]. The F^- salts give maximum solubilities of 1.29×10^{-1} M MnF_2 and 1.23×10^{-3} M MgF_2 at 20°C [23]. Consequently, with respect to the F^- ion, the solubility of the Mn^{2+} ion is about 100 times greater than that of the Mg^{2+} ion. If such a relationship holds for the phosphate ion, the Mn^{2+} ion would not have the capabilities of the Mg^{2+} ion is destroying the salt bonds. Rather its capabilities of insolubilizing the phosphate group must be approximately equal to that of the Li^+ ion, since both have about the same solubility for their fluoride salts (0.104 M LiF and 0.129 M MnF_2). But the data given in Fig. 1 show that the value of $[\eta]$ for DNA is not reduced as much by Li^+ ions as by Mg^{2+}. Consequently, the inability of Mn^{2+} to act as a catalyst in DNA replication may be due to its inability to effectively destroy the ionic bonds between phosphate and the C′-1 nitrogen base. In order to produce the correct steric factors, these ionic bonds most likely reform after the two strands have been separated.

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