

## On the Interpretation of Dielectric Results on Sodium Thymonucleate. II. The Dipole Association of Highly Polymerized Molecules

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Dielectric studies of sodium desoxyribonucleate (DNA) in aqueous solution have revealed that the interaction of the molecules plays a greater rôle than could be assumed *a priori* in such dilute solutions as a few hundredths per cent.

It is a generally valid fact that the association tendency increases with the dipole moment. As DNA in aqueous solution has a very high increment also implying a very high dipole moment, the intermolecular forces of attraction can therefore also be expected to be considerable. With many physico-chemical methods DNA evidences a number of deviations from the known rules, supposedly due to "association". The term "association" has a very wide connotation and will therefore be more closely defined later.

In dielectric studies DNA also shows anomalies<sup>1</sup>. In solutions with a concentration as low as 0.1 per cent, the anomalous dispersion takes place with a distinct deviation from the theoretical course. In measurements in still more dilute solutions, the dielectric dispersion appears at a lower frequency, *i.e.* it then corresponds to a longer relaxation time and a higher molecular weight. Also the dielectric increment per gram per litre increases as the concentration decreases. This fact indicates the presence of a non-polar association even in 0.1 per cent solution but that later — as expected — it decreases with dilution.

From another aspect as well DNA shows properties corresponding to association. The molecule can easily be disaggregated in extremely dilute aqueous solutions by, for example, the addition of a minute quantity of electrolytes. This takes place with the retention of the "specific" polarity of the molecule, *i.e.* the same dipole moment per mononucleotide is obtained, as opposed to

the conditions in higher salt concentrations where the polarity decreases appreciably. This aggregation-disaggregation corresponds to the variation in the polar "association". Such a preponderance of a purely polar association in extremely dilute solutions is surprising. The fact that an "association" and dipolar interaction occur to such an extent and in so distinctly different ways gave rise to this investigation.

Fürth's ellipsoid method<sup>2</sup> adapted for high frequency<sup>3,4</sup> was used for the determination of the dielectric constant (DC) of solutions with considerable electric conductivity. The method is not, however, suitable for experiments at, for example, varying temperatures nor does it permit, as would be desirable, a number of other investigations. In the present study therefore, the conditions will instead be considered theoretically and an attempt made, in conjunction with available experimental data, to understand the behaviour of such high polymerized dipoles (multipoles) as DNA in aqueous solution. The first question will then be to investigate the effect of the degree of polymerization of DNA, whose simple dipoles are the mononucleotides.

#### THE APPLICABILITY IN THE CALCULATION OF THE EXCHANGE ENERGY FOR DIPOLES IN SOLUTION

Referring to an earlier paper in the present series<sup>5</sup> the structural formulae for DNA can be replaced, from a dielectric point of view, by a simple model. DNA consists of a number of mononucleotides, packed closely above each other, with the purine and pyrimidine groups pointing in one direction and the phosphoric acid groups in the opposite direction. The mononucleotides can be interpreted as dipolar ions in which less important differences in the moment-bearing groups can be disregarded. We can therefore consider DNA as a polarly polymerized multipole in which the simple and similar dipoles lie side-by-side. The resulting transverse dipole moment,  $\mu$ , which caused the dielectric dispersion, will then be

$$\mu = \mu_0 \cdot n = n \cdot e \cdot l \quad (1)$$

where  $\mu_0$  is the dipole moment of the individual average mononucleotide,  $n$  is the number of mononucleotides, *i.e.* the degree of polymerization,  $e$  is the charge ( $+e$  and  $-e$  respectively) of the momentbearing groups and  $l$  is the distance between the positive and negative centres of gravity in each mononucleotide.

With greater distances between the molecules, corresponding to the extremely dilute solutions that will mainly be studied, it can be anticipated that the electrostatic forces will essentially outweigh the induction and disper-

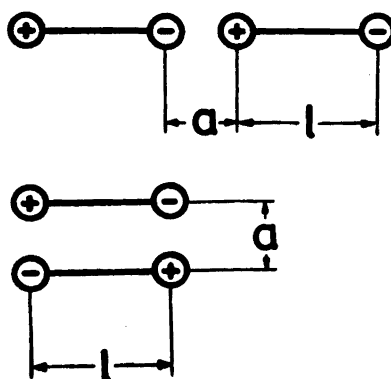


Fig. 1. Different forms of association in simple dipoles.

Upper figure: polar association.

Lower figure: non-polar association.

sion effects. In the following we shall therefore only deal with these Coulombic forces.

The mutual interaction between the dipoles is dependent on the size of the exchange energy. The greater the energy, the smaller is its activity and the smaller is the possibility for the dipoles of orientating freely in an electric field.

The attraction between two charges,  $+e$  and  $-e$ , corresponds to an exchange energy,  $E$  which according to Coulomb's law is

$$E = - \frac{e^2}{\epsilon \cdot r} \quad (2)$$

where  $\epsilon$  is the DC for the intermediate medium and  $r$  the distance between the charges.

The question is then the extent to which different values for  $E$  can be compared. The "effective" charges are considered to be equal. We can assume the DC should lie around 80 in the field outside the molecule. In the immediate vicinity of the charges, on the other hand, the difference must be considerable and approach the value *in vacuo*. We can also assume a low value for the internal field within the DNA molecule despite a large number of polar groups. In the case of the field strengths between solute dipoles in dilute solutions, it is, however, only with relatively small inter-molecular distances that the lines of the forces fail to enter the solvent. If we compare the exchange energy between dipoles at considerable distances, we can thus assume that the effect of the polar solvent is constant.  $\bar{\epsilon}$  is then the expression of the effective DC that can be envisaged to arise, taking into consideration the thickness of the layers with different dielectric constants.

When the exchange energy is to be compared under conditions that are fairly similar with the exception of the distance, we are thus relatively independent of minor variations in the effective charges and the resulting DC of the solvent. We can therefore write

$$E = - \frac{e^2}{\bar{\epsilon}} \cdot \frac{1}{r} \cong \text{const.} \cdot r^{-1} \quad (3)$$

A comparison of the different value for  $E$  thus shows that this mainly depends on the distance.

The main forms of association to be discussed in the following are the polar and the non-polar (with anti-parallel arrangement). As can be inferred from Fig. 1, the exchange energy for simple dipoles can be calculated from the following equations:

$$\overset{\rightarrow\rightarrow}{E} = - \frac{e^2}{\bar{\epsilon}} \left[ \frac{1}{a} + \frac{1}{a+2l} - \frac{2}{a+l} \right] = - \frac{e^2}{\bar{\epsilon}} \cdot \overset{\rightarrow\rightarrow}{P} \quad (4)$$

$$\overset{\rightleftharpoons}{E} = - \frac{e^2}{\bar{\epsilon}} \left[ \frac{2}{a} - \frac{2}{\sqrt{a^2+l^2}} \right] = - \frac{e^2}{\bar{\epsilon}} \cdot \overset{\rightleftharpoons}{P} \quad (5)$$

$\overset{\rightarrow\rightarrow}{E}$  is then the energy with polar and  $\overset{\rightleftharpoons}{E}$  with non-polar association,  $a$  the shortest distance between the dipoles and  $l$  the length of the dipole. The mean distance between the dipoles,  $r$ , is thus respectively  $a$  and  $(a+l)$ .

A definition of the function for the distance is introduced into equations (4) and (5). This *position function*,  $P$ , evidently determines the variation in the exchange energy. Moreover with our assumptions, the relation between the position functions is the same as that between the exchange energies.

In the case of simple dipoles, Duncken<sup>6</sup> set up an equation for the relation,  $\Phi$ , between the energies with these different forms of association

$$\Phi = \frac{\overset{\rightleftharpoons}{E}}{\overset{\rightarrow\rightarrow}{E}} = \frac{\overset{\rightleftharpoons}{P}}{\overset{\rightarrow\rightarrow}{P}} = \frac{2 \left( \frac{1}{\sqrt{1+l^2/a^2}} - 1 \right)}{\frac{2}{1+l/a} - \frac{1}{1+2l/a} - 1} \quad (6)$$

The value of  $\Phi$  at different distances between the dipoles is given in Fig. 4 (p. 1412). It gives the impression that in the case of simple dipoles it is the relative dipole length, *i.e.*  $l/a$ , that is decisive. It can be seen that the polar association predominates at greater distances.

In order to simplify the problem, the calculations are made only for those values applicable to DNA. Thus, for the dipole length, 10 Å is taken as the most probable value for the distance between the charges within the mononucleotides. Although it is not essential for the calculations to fix this figure it is important to bear in mind that a *relatively great dipole length* must be counted on for DNA. Only in the case of extremely large distances between the dipoles, of an order of magnitude in which it is not as a rule necessary to take the exchange energy between the molecules into account, can the dipole length in relation to the distance be disregarded. For very strong dipoles such as DNA, these conditions are, however, of great interest. Simpler calculations are then very useful. We can recall that with parallel dipoles with dipole moments  $\mu_1$ , and  $\mu_2$  at a distance of  $r$ , the bound energy is obtained by means of

$$E = \frac{\mu_1 \cdot \mu_2}{\epsilon \cdot r^3} (2 \cos \varphi_1 \cdot \cos \varphi_2 - \sin \varphi_1 \cdot \sin \varphi_2) \quad (7)$$

where  $\varphi_1$ , and  $\varphi_2$  are the angles between the dipole axis and the straight line between the centres. In extreme cases with polar and non-polar association,

$$\varphi_2 = 180^\circ + \varphi_1 (= \alpha)$$

and the following simple relations are obtained

$$\begin{aligned} \overrightarrow{E} &= - \frac{\mu_1 \cdot \mu_2}{r^3} \\ \overleftrightarrow{E} &= - \frac{2 \cdot \mu_1 \cdot \mu_2}{r^3} \end{aligned} \quad (8a, b)$$

In the study of the effect of the degree of polymerization to be made in the following, the distance,  $d$ , between the dipoles is also required. This has been established from X-ray data to be approximately 3.4 Å<sup>7</sup>.

#### THE EXCHANGE ENERGY BETWEEN POLARLY POLYMERIZED MULTIPOLES SUCH AS DNA

It is necessary, for the calculation of the exchange energy between two molecules,  $X$  and  $Y$ , to know the position functions for the individual dipoles in relation to the dipoles in the other molecule. The association forms are seen schematically in Fig. 2. The position function,  $P$ , for each pair of simple dipoles is obtained as follows.  $P_{1,1}$  denotes the position function for the terminal simple dipoles in each molecule, and  $P_{1,2}$  the position function for

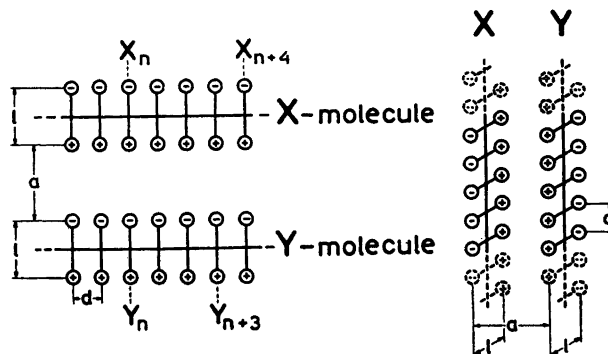


Fig 2. Schematic representation of polar (on the left) and non-polar association of two multipoles with the same structure as DNA.

the terminal dipole to the neighbour of the corresponding dipole in the other molecule, etc. We then obtain the following expression

$$\begin{aligned} \vec{P}_{1,1} &= \frac{1}{a} + \frac{1}{a+2l} - \frac{2}{a+l} \\ \vec{P}_{1,2} &= \frac{1}{\sqrt{a^2+d^2}} + \frac{1}{\sqrt{(a+2l)^2+d^2}} - \frac{2}{\sqrt{(a+l)^2+d^2}} \\ \vec{P}_{1,3} &= \frac{1}{\sqrt{a^2+4d^2}} + \frac{1}{\sqrt{(a+2l)^2+4d^2}} - \frac{2}{\sqrt{(a+l)^2+4d^2}} \end{aligned}$$

As a generalization, this therefore gives the following for *polar* association

$$\vec{P}_{1,n} = \frac{1}{\sqrt{a^2+(n-1)^2d^2}} + \frac{1}{\sqrt{(a+2l)^2+(n-1)^2d^2}} - \frac{2}{\sqrt{(a+l)^2+(n-1)^2d^2}} \quad (9)$$

↔ In the same way, the following general expression for the position function,  $P$ , is deduced for *non-polar* association

$$P = \frac{2}{\sqrt{a^2+(n-1)^2d^2}} - \frac{2}{\sqrt{a^2+l^2+(n-1)^2d^2}} \quad (10)$$

It can easily be seen that the exchange energy,  $E_n$ , for two multipoles with the degree of polymerization  $n$  for the simple dipoles is obtained from the position functions

$$-\frac{\epsilon}{e^2} \cdot E_n = n \cdot P_{1,1} + 2(n-1) \cdot P_{1,2} + 2(n-2) \cdot P_{1,3} + \dots + 2 \cdot P_{1,n} \quad (11)$$

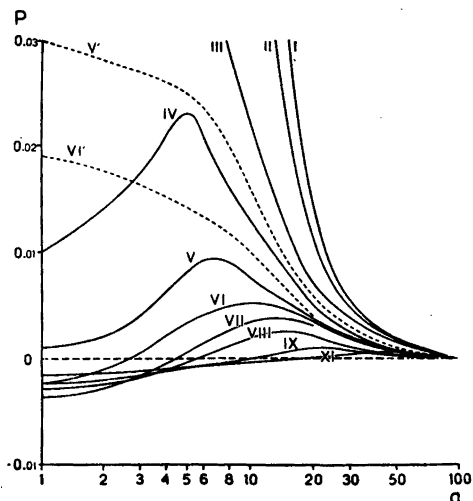


Fig. 3. The variation in the polar position functions for different distances between multipoles (see text). By way of comparison the non-polar position functions  $P_{1,5}$  and  $P_{1,6}$  are included (dotted lines).

No difficulties are encountered in the calculation of the position functions although it is a laborious procedure. Since the component variables are of the same order of magnitude and must be varied within a very wide range, it is necessary to calculate a large number of position functions and thence to obtain a relative value for the exchange energy.

It is convenient to use substitution with trigonometrical functions. It is then found that

$$\vec{P} = t \cdot [\cos \alpha + \cos \beta - 2 \cos \gamma] \quad (12)$$

$$\vec{P} = 2 t \cdot [\cos \alpha - \cos \delta] \quad (13)$$

where

$$\tan \alpha = a \cdot t; \tan \beta = (a + 2l) \cdot t; \tan \gamma = (a + l) \cdot t; \tan \delta = t \cdot \sqrt{a^2 + l^2} \text{ and } t = \frac{1}{(n-1) \cdot d}$$

The cosine functions can then be obtained directly from the tangent values with suitable tables.

The series of which the exchange energy is composed converges slowly and a relatively large number of terms must be included and estimated. It is however, possible to calculate the first and most important terms and then to estimate the remainder by means of the integral calculus. The error in the sum of the integrals is then low.

The position functions obviously decrease with increasing distances but this change takes place in different ways. With non-polar association, the

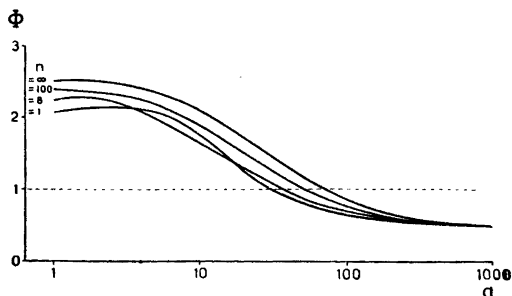


Fig 4. The relation between the interaction with non-polar and polar association ( $\Phi$ ) and distance between the dipoles ( $a$ ) with different degrees of polymerization ( $n$ ).

decrease in  $\bar{P}$  is regular. For  $\bar{P}$ , on the other hand, even  $\bar{P}_{1,4}$  reaches a distinct maximum. From  $\bar{P}_{1,6}$  and onwards, a resulting repulsion instead of attraction takes place at short distances between the multipoles. The repulsion is maximal for  $\bar{P}_{1,7}$  and  $\bar{P}_{1,8}$  depending on the distance between the multipoles but then decreases rapidly. Fig. 3 shows this variation in the position functions. It should, however, be noted that the absolute values for the exchange energy are not directly comparable for small distances between the multipoles. They have been included in order to follow the course in principle and are only of qualitative significance.

The numerical values for some  $\bar{P}$  and  $\bar{P}$  at various distances and degrees of polymerization are given in Table 1. It is found, as anticipated, that the position functions at distances that are very large in comparison with the dipole length vary very insignificantly with moderate degrees of polymerization. This makes it possible to give a simple approximate expression for the dependence on the degree of polymerization. If we insert in equation (11) the value for the position function  $\bar{P}$  as constant and equal to  $\bar{P}$ , we obtain

$$-\frac{\epsilon}{e^2} \cdot E = \bar{P} [n + 2(n-1) + \dots + 2] = \bar{P} \cdot n^2 \quad (14)$$

We can write, as a generalization,

$$E = -const. \cdot n^x \quad (15)$$

in which the exponent  $x$  deviates little from the value 1 with a low  $n$  and a but rapidly approaches the borderline value 2 at greater distances. We can therefore conclude that *the degree of polymerization has a relatively greater effect at larger distances*, a fact that is of importance for the understanding of DNA in very dilute aqueous solutions. It then also follows that the exchange energy for molecules such as DNA can be very considerable as a result of high

degree of polymerization, even at distances where the dipolar exchange energy can usually be disregarded.

The relation between the exchange energies in non-polar and polar associations is of particular interest. This is shown in Fig. 4. As seen, the effect of the degree of polymerization throughout is such that the non-polar association can be expected to predominate. This effect is greatest at moderate distances and only at distances of 100 Å and more is the effect of the degree of polymerization less. It is interesting to note that in comparison with the conditions for simple dipoles, as calculated by Dunken, the distance at which equilibrium is attained, from the energy view point is displaced from approximately 30 Å to approximately 100 Å. This implies that at all distances at which a firmer and actual association combination occurs, this always has a decided tendency to be non-polar.

#### DISCUSSION AND CONCLUSIONS

The result of the calculations of the exchange energy with polar and non-polar association has a number of consequences for the conception of DNA. A very strong tendency to non-polar arrangement must be assumed with short distances between the dipoles. On the other hand, the dielectric experiments show the occurrence of molecular aggregates whose formation is entirely polar. Results obtained with other methods show in addition a pronounced thread shape. It is necessary to reconcile these findings. In addition to the explanation by means of molecules assembled end-to-end, we can envisage the formation of aggregates by a polar linking of projecting parts. Such a molecule would be approximately thread-shaped and correspond to all available data. The foregoing calculations nevertheless definitely indicate that such an interpretation is incorrect. We must therefore conclude that the formation of larger aggregates takes place end-to-end despite the fact that considerable electrostatic forces oppose such a polar polymerization.

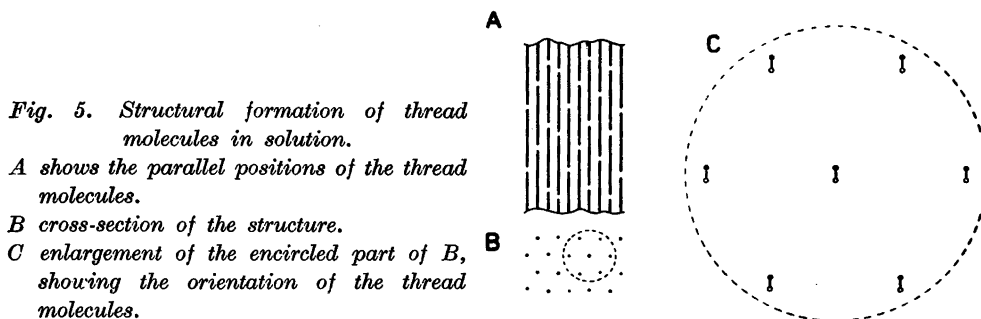
As a result, we must envisage other forms of linking between the molecules to form a larger aggregate. We must assume that the union between two basic molecules is brought about by relatively strong short-distance forces. Since the slight disaggregation with electrolytes likewise indicates that it is predominantly electrostatic, the nature of this linking is fairly well defined. This problem will be treated in a later paper.

The effect of electrolytes on DNA is reversible so that the same aggregate weight is obtained after dialysis<sup>3</sup>. It could be expected that the aggregation, under the influence of the strongly dipolar interaction would not regain a high degree of polar structure. The reversibility therefore appears to require another mechanism in addition to the specific connecting forces.

Table 1. Some values of the position functions ( $P_{n,n+m}$ ).

Distance, $a$ (in Å)	1	3	5	8	10	20	50	100	1000
<b>Position Functions</b>									
<i>Polar</i>									
<b>Arrangement</b>									
$P_{1,1}$	.866	.222	.107	.050	.033	.009	$95 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	$19 \cdot 10^{-8}$
$P_{1,2}$	.173	.123	.079	.042	.029	.008	$95 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	
$P_{1,3}$	.045	.044	.040	.028	.022	.007	$92 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	
$P_{1,4}$	.010	.017	.023	.016	.013	.005	$87 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	
$P_{1,5}$	.001	.004	.008	.009	.008	.005	$82 \cdot 10^{-5}$	$14 \cdot 10^{-5}$	
$P_{1,6}$	-.002	.000	.003	.004	.005	.004	$76 \cdot 10^{-5}$	$14 \cdot 10^{-5}$	
$P_{1,7}$	-.004	-.001	.000	.002	.002	.003	$68 \cdot 10^{-5}$	$13 \cdot 10^{-5}$	
$P_{1,8}$	-.003	-.002	-.001	.000	.002	.002	$61 \cdot 10^{-5}$	$13 \cdot 10^{-5}$	
$P_{1,9}$	-.002	-.002	-.001	.000	.000	.001	$53 \cdot 10^{-5}$	$13 \cdot 10^{-5}$	
$P_{1,10}$	-.002	-.001	-.001	-.001	.000	.000	$45 \cdot 10^{-5}$	$12 \cdot 10^{-5}$	$19 \cdot 10^{-8}$
<b>Non-polar</b>									
<b>Arrangement</b>									
$P_{1,1}$	1.801	.475	.221	.094	.059	.011	$78 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	$10 \cdot 10^{-8}$
$P_{1,2}$	.383	.260	.161	.074	.052	.009	$78 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,3}$	.130	.112	.090	.049	.040	.007	$76 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,4}$	.057	.053	.046	.033	.026	.006	$73 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,5}$	.030	.027	.027	.020	.017	.004	$70 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,6}$	.018	.016	.014	.012	.010	.004	$66 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,7}$	.011	.011	.010	.009	.009	.004	$62 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	
$P_{1,8}$	.007	.007	.007	.007	.006	.004	$58 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	
$P_{1,9}$	.006	.006	.005	.005	.004	.002	$53 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	
$P_{1,10}$	.004	.004	.004	.003	.003	.002	$48 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	$10 \cdot 10^{-8}$

We consider that a satisfactory explanation is provided by according greater significance to the dipole directive forces discussed in this paper. It is only necessary to complement the views put forward by Bernal and Fankuchen<sup>8</sup> and Ferry<sup>9</sup> and others by stressing the *orientation* of the molecules. We then interpret DNA in dilute aqueous solutions as a quasi-crystalline structure. (*cf.* Girard and Abadie<sup>10</sup>). For it is characteristic of thread molecules that they have a tendency in solution to form longitudinal parallel streaks with a hexagonal formation in the cross-section. Fig. 5 shows how this conception is envisaged. It must be emphasized that the stability of this structure in solution is essentially dependent on the very large transverse dipole moment. Such an interpretation of the conditions in DNA appears to explain the



reversibility. It is the orientating effect of the surrounding molecules that permits the smaller units to be polarly — end-to-end — joined on re-polymerization and to overcome the Coulombic forces emanating from the basic molecules. The fact that if disaggregation is very advanced it becomes more difficult to obtain reversibility is thereby explained. The lower units cannot form a sufficiently pronounced quasi-crystalline structure to permit a spontaneous reformation of larger aggregates.

In DNA, disaggregation and aggregation appear to be the expression of an unusually pronounced *one-dimensional reactivity* (v. Hammarsten's discussion<sup>11</sup>). This property can be thought to appear under physiological conditions. *In vivo*, the dipole orientation by, for example, high polar proteins may correspond to the orientating effect of the neighbouring molecules *in vitro*.

In analogy to the other dielectric measuring results, the conception of the quasi-crystalline structure of DNA in dilute aqueous solutions explains the fact that the anomalous dispersion range is displaced towards higher frequencies at rising concentrations. The smaller the intermolecular distance becomes, the more is the free orientation prevented as a result of the dipolar exchange effect. Such an explanation for aqueous solutions is extremely schematic but nevertheless appears warranted.

Finally, the term "association" must be more closely defined. In DNA, as in similar thread molecules, nucleohistone and possibly in viruses and many other substances both a true molecular association between individual molecules and a dipolar interaction at greater distances must presumably occur. These phenomena are observed in the same way in dielectric measurements as a relatively too low value for the dielectric increment. In the case of high-molecular and high-polar substances, the conditions in solution are often hard to define and DNA, for example, often shows different kinds of association simultaneously. Even if it is not considered possible in extreme cases to differentiate clearly between these forms, it is nevertheless suitable for

practical purposes to make use of association as a common term. It is thought to be incorrect to deny the existence of associated aggregates of large molecules. The conditions found in DNA are almost certainly to be encountered in other high-molecular substances as well. Briefly, we prefer to use the term association, although it is applied in its widest connotation.

#### SUMMARY

A calculation is made of the exchange energy between associated polymeric multipoles with the probable dimensions of sodium thymonucleate (DNA). Strictly speaking, such a calculation is only valid for the conditions *in vacuo*. It is, however, found that, with considerable distances between the dipoles, comparable values are also obtained for solutions in polar media.

The exchange energy increases with the degree of polymerization. This increase is most rapid when the distance between the dipoles is large, and thus under conditions that most nearly correspond to extremely dilute solutions. Presumably, only multipoles with very high transverse dipole moments show interaction at such large distances between the dipoles as are usually found in dilute solutions.

The relation between the exchange energy in polar and non-polar association, calculated for simple dipoles by Dunker, is displaced on polymerization to the advantage of non-polar association. At short distances between the dipoles, a considerable predominance of the non-polar aggregate of association can be anticipated.

The results are discussed and lead to the postulation of a quasi-crystalline structure for solutions of thread molecules. The importance of large transverse dipole moments is stressed. Certain effects in dielectric measurements of DNA are explained on the basis of the structural formation.

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Received October 25, 1951.