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Hypochromism and Other Spectral Properties of Helical Polynucleotides

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Exciton interaction in a double-stranded helical polymer, such as a homogeneous polynucleotide, leads to a splitting of each excited electronic energy level of the monomer into two exciton bands of energy levels. Consideration of the selection rules derived from zeroth-order functions show that for double-stranded polynucleotides $\pi \rightarrow \pi^*$ transitions are allowed to, at most, one exciton level of each band, both polarized perpendicularly to the helix axis; whereas, $n \rightarrow \pi^*$ transitions are allowed only to one level of the upper band, that being polarized parallel to the helix axis. Examination of the spectral properties of the four constituents of deoxyribonucleic acid (DNA) reveals that although exciton bands probably exist in regions of spectral overlap, there should be isolated energy levels lying below the main exciton bands. These levels may act as localized energy traps during excitation propagation through the helix. In order to treat the problem of hypochromism, it is necessary to use first-order functions. An equation (similar to one derived by Tinoco) is developed which takes into account the effect of exciton interaction and dispersion-force interaction on the oscillator strength of the helix. Application of this equation to a helical, double-stranded polyadenylic acid having the configuration of DNA indicates that perturbation resulting from dispersion-force interaction among the bases can account for the observed hypochromism.

Recently there has been considerable interest in the spectral properties of biological macromolecules such as nucleic acids and proteins. It has been known for some time that the spectral properties of these helical polymers differ considerably from those of the monomer residues. For example, in the transition from a random coil (whose spectral properties are similar to those of the monomeric chromophore) to a helical form, there are corresponding decreases in the oscillator strength (hypochromism) of certain electronic transitions and changes in the rotatory dispersion properties. The recent theoretical work of several investigators²⁻⁴ has been directed toward explaining these spectral changes which occur in the formation of an ordered structure, such as a helix.

The present paper will be concerned with a theoretical treatment of the spectral properties of deoxyribonucleic acid (DNA), which is a double-stranded helical polynucleotide, and of the related synthetic helical polynucleotides.

The spectral differences between the monomer residues and the polymer are due to the interaction among residues which are related to one another by a definite geometrical orientation. The ordered structure and non-conjugative interaction among the bases of helical polynucleotides make a description of electronic states amenable to the application of exciton theory. Moffitt applied exciton theory to the case of a single-stranded helix.² He found that zeroth-order exciton wave functions may be used to describe the polarizations of electronic transitions within the helix as well as the contribution of interaction within the helix to rotatory dispersion. On the other hand, zeroth-order exciton wave functions predict no change in the transition probability (*i.e.*, the square of the transition moment integral remains unchanged upon formation of the helix); yet, for DNA one of the most striking spectral properties is the hypochromism of the absorption band at 2600 Å.

More accurate, first-order wave functions for the helix may be obtained by further application of perturbation theory, which mixes exciton states corresponding to various electronic states of the residues. In effect, this takes into account the perturbation of each residue wave function as a result of interaction among the residues. Using this approach, Moffitt, *et al.*,³ derived an equation for the first-order transition moment integral, and Tinoco,⁴ using this equation, has derived an expression for the first-order oscillator strength by means of which he has calculated the hypochromism of DNA.

The present paper will be divided into two major parts. In the first part we shall discuss properties of double-stranded helical polynucleotides in terms of simple (zeroth-order) exciton wave functions. These include the splitting of excited state energy levels into exciton bands, polarizations and selection rules for optical transitions to energy levels of the exciton bands, and the transfer of electronic excitation energy. In the second part we shall show that the use of more complex (first-order) exciton wave functions can lead to a satisfactory explanation of hypochromism. Here, our results differ from those of Tinoco in that one of the principal terms in Tinoco's equation is shown to be cancelled identically by another term.

I. Properties Related to Zeroth-Order Exciton States.—Deoxyribonucleic acid (DNA) is a double-stranded, helical polynucleotide containing four types of nucleotides: adenylic, guanylic, cytidylic and thymidylic acids linked together by 3'-5' phospho-diester bonds of the deoxyribose moieties. We shall limit the description here to those structural features that have a bearing on spectral properties, referring to a more detailed description by Rich.⁵

The presently accepted configuration of DNA was proposed by Watson and Crick.⁶ The two polynucleotide strands form a double helix in which the ribose phosphate chains are on the outside, and the substituted purine and pyrimidine bases are directed inward with the base planes perpendicular to the axis of the helix. The bases

(1) American Cancer Society Post-doctorate Fellow, academic years 1958-1959 and 1960-1961. This work was supported in part by a grant from the Division of Biology and Medicine, U. S. Atomic Energy Commission.

(2) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).

(3) W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci., U. S. A.*, **43**, 723 (1957).

(4) I. Tinoco, *J. Am. Chem. Soc.*, **82**, 4785 (1960).

(5) A. Rich, *Revs. Modern Phys.*, **31**, 191 (1959).

(6) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

of one strand are hydrogen-bonded to those of the other strand in such a way that adenine is always paired with thymine and guanine with cytosine. Both strands are right-handed but are opposite in sense of direction. Consequently, the base of one member of each hydrogen-bonded pair is related to the other member by two operations (see Fig. 2): (1) rotation through the angle ω about the helix axis, and (2) rotation of 180° about an axis perpendicular to the helix axis. The angle ω was calculated from the radius of the helix and the distance between the two number one carbon atoms of the respective deoxypentoses⁷ and was found to be 104° . There are ten base pairs per complete turn, so that the angle of rotation between successive base pairs is 36° . The distance between base pairs is 3.4 \AA . along the helix axis.

Optical Properties of the Bases.—The ultraviolet absorption spectrum of each of the four bases in DNA shows a band having a peak near 2600 \AA . The maximum extinction coefficient of this band ranges from 5×10^3 for cytosine to 1.3×10^4 for adenine. Spectral studies of various substituted purines and pyrimidines indicate that the band is complex, containing both $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions.^{8,9}

Transitions of the $n \rightarrow \pi^*$ type are due to the non-bonding electron pairs of the O and N atoms of the bases. Since there are several kinds of non-bonding electron pairs, there is the likelihood of more than one type of $n \rightarrow \pi^*$ transition within the envelope of the 2600 \AA . band. Thus far, they have been observed only on the long wave length tail of the band.⁹ All $n \rightarrow \pi^*$ transitions are polarized perpendicularly to the base plane.

Transitions of the $\pi \rightarrow \pi^*$ type are polarized in plane of the base and make up the largest component of the 2600 \AA . band. For the pyrimidine type bases there is only one $\pi \rightarrow \pi^*$ transition in this region. However, for the purine type bases, solvent effects on the spectra indicate that there are two such transitions in this region, polarized perpendicularly to one another.¹⁰ The weaker transition appears as a shoulder in the neighborhood of 2400 \AA . and is apparently about one-half as intense as the main component near 2600 \AA .

In addition to the 2600 \AA . band each of the bases has a more intense $\pi \rightarrow \pi^*$ transition in the neighborhood of 2000 \AA . Because of the difficulties encountered in the vacuum ultraviolet region, the spectra at shorter wave lengths have not been measured.

The Zeroth-order Exciton Wave Functions and First-order Energy Levels.—The method of molecular exciton theory has been used successfully to describe many of the optical properties of crystals,¹¹ polymers² and dimers.¹² This theory is applicable to any system composed of an ordered array of

identical or nearly identical, molecules having no appreciable intermolecular conjugative interaction. An essential feature is the banding of excited states which results from splitting of the excitational degeneracy among the molecules of the system. Although DNA contains four kinds of bases, the overlap of the absorption bands near 2600 \AA . is appreciable. In these regions of common spectral absorption an excitational degeneracy exists, and therefore one might expect to find an exciton-type interaction.

In this section we shall consider exciton interaction in a double-stranded helical polynucleotide. Particular reference is made to the work of Moffitt² on single-stranded helical polymers. Although the details of the present development will differ considerably from his, the general features will be quite similar. For this reason we shall use the same notation wherever feasible.

In order to avoid complicating factors for the time being, let us assume that we are dealing with a double-stranded polynucleotide containing a single type of base, for example adenine, but having the configurational properties of native DNA. Later on we shall consider the DNA molecule as a variation of this idealized case.

In the double-stranded helix the bases of one strand are hydrogen-bonded to those of the other strand in a unique way to form a set of base pairs. Therefore, a double-stranded helix may be considered as a single-stranded helical array of base pairs. If the two strands are designated as a and b, then the electronic Hamiltonian for a given base pair may be written

$$H_n = (H_a + H_b + V_{ab})_n \quad (1)$$

where the index n labels the base pair of the helix, H_a and H_b are the electronic Hamiltonians for the isolated bases, and V_{ab} represents the potential energy of interaction between the bases. The ground and β^{th} excited state electron functions for the given base pair have the forms

$$\phi_{0n} = (\psi_{0a}\psi_{0b})_n \quad (2)$$

and

$$\phi_{\beta n} = 2^{-1/2} (\psi_{\beta a}\psi_{0b} \pm \psi_{0a}\psi_{\beta b}) \quad (3)$$

respectively. The electrons in the ground and β^{th} excited state of base a are represented by the functions ψ_{0a} and $\psi_{\beta a}$, respectively, and similarly for base b. Here and throughout the remainder of the paper it is assumed that overlap of the functions and, consequently, electron exchange between bases are negligible. It will be noticed from (3) that account is taken of the degeneracy of the excited state β represented by $\psi_{\beta a}\psi_{0b}$ and $\psi_{0a}\psi_{\beta b}$. The properly symmetrized zeroth-order base pair functions are therefore given by the linear combinations, where the indexes (\pm) on the left denote the corresponding phase relationships on the right. The energy levels of the ground and excited states of the base pair are then given by the stationary state equations

$$H_n \phi_{0n} = E_{0,\beta} \phi_{0n} \quad (4)$$

where

$$E_{0,\beta} = 2E_0 + \int \psi_{0a}^{\dagger} V_{ab} \psi_{0b}^{\dagger} \psi_{0b} \psi_{0a} \, d\tau \quad (5)$$

(7) L. Pauling and R. B. Corey, *Arch. Biochem. Biophys.*, **65**, 164 (1956).

(8) M. Kasha, in "The Nature and Significance of $n \rightarrow \pi^*$ Transitions," "Symposium on Light and Life," The Johns Hopkins Press, Baltimore, Md., 1961, p. 31.

(9) S. F. Mason, *J. Chem. Soc.*, 1240 (1959).

(10) S. F. Mason, *ibid.*, 2071 (1954).

(11) A. S. Davydov, *J. Exptl. Theoret. Phys.*, **18**, 210 (1948).

(12) G. S. Levinson, W. T. Simpson and W. Curtis, *J. Am. Chem. Soc.*, **79**, 4314 (1957).

and

$$H_n \phi_{\beta n \pm} = E_{\beta \pm} \phi_{\beta n \pm} \quad (6)$$

where

$$E_{\beta \pm} = E_{\beta} + E_0 + \int |\psi_{\beta a}|^2 V_{a,b} |\psi_{\beta b}|^2 d\tau \pm \int \psi_{\beta a}^* \psi_{\beta b} V_{a,b} \psi_{\beta a} \psi_{\beta b} d\tau \quad (7)$$

The ground and excited state energies of an isolated base are denoted by E_0 and E_{β} respectively and the interaction energy of the two bases of the pair is given by the integrals in (5) and (7). Integration is to be taken over position and spin coordinates of all electrons of the base pair.

An important consequence of intra-base pair interaction is the splitting of the excited state degenerate level, which, as indicated by the second integral in (7), results from excitation resonance within the base pair. Hereafter, in order to distinguish from what is to follow, we shall refer to this splitting as the *base-pair splitting* and the resulting energy levels as the *plus* and *minus* branches.

We are now in a position to consider exciton interaction within the helix. Let us assume that the helix contains N base pairs, M complete turns and P base pairs per turn. The Hamiltonian of the helix in terms of the base-pair Hamiltonians is

$$H = \sum_n H_n + V \quad (8)$$

where

$$V = 1/2 \sum_m \sum_{n \neq m} \mathcal{U}_{mn} \quad (9)$$

is the total potential energy of interaction of all the base pairs. The zeroth-order ground state function for the helix is a product of the N base pair functions, namely

$$\Phi_0^{(0)} = \prod_{n=1}^N \phi_{0n} \quad (10)$$

where the superscript refers to the order of approximation. Using the Born-von Karman boundary conditions,^{13,14} we can write the zeroth-order excited state function of the helix as the linear combination

$$\Phi_{\beta, K \pm}^{(0)} = N^{-1/2} \sum_n \exp\left(\frac{i2\pi K n}{N}\right) \phi_{\beta n \pm} \quad (11)$$

where

$$\phi_{\beta n \pm} = \Phi_{\beta}^{(n)}(\phi_{0n}) \quad (12)$$

is that excited state of the helix in which the excitation is localized on the n^{th} base pair. The energy levels represented by (12) are N -fold degenerate. Interaction among the base pairs splits this degeneracy into a band of energy levels, each of which corresponds to a particular value of K in (11). The indexes \pm refer, as before, to the two

branches of the base pair splitting. Thus, as a result of exciton interaction in the helix, the plus and minus branches are each split into a band, the levels of which, correct to first order, have the values

$$E_{\beta, K \pm} = \int \Phi_{\beta, K \pm}^{(0)*} H \Phi_{\beta, K \pm}^{(0)} d\tau = E_{\beta \pm} + (N-1)E_{0, \beta} + D_{\beta} + \xi_{\beta, K \pm} \quad (13)$$

where D_{β} is one of the first-order energy terms, namely

$$D_{\beta} = \sum_{j(\neq n)} \int \Phi_{\beta n \pm}^* \mathcal{U}_{nj} \Phi_{\beta n \pm} d\tau + 1/2 \sum_{\substack{i,j \\ i \neq j \neq n}} \int \Phi_{\beta n \pm}^* \mathcal{U}_{ij} \Phi_{\beta n \pm} d\tau \quad (14)$$

and $\xi_{\beta, K \pm}$ denotes the first-order excitation resonance (exciton interaction) term, more specifically

$$\xi_{\beta, K \pm} = \frac{1}{N} \sum_m \sum_{n(\neq m)} \exp\left[\frac{i2\pi K(m-n)}{N}\right] \int \Phi_{\beta n \pm}^* \mathcal{U}_{mn} \Phi_{\beta m \pm} d\tau \quad (15)$$

The term D_{β} , together with the first integral in (7), represents the van der Waals interaction energy of the excited state of the helix and results in a shift relative to the zeroth-order energy. The banding of the energy is due to the N values of $\xi_{\beta, K \pm}$ for each branch of the base-pair splitting. Most of these energy levels are doubly degenerate, since $\xi_{\beta, K \pm} = \xi_{\beta, N-K \pm}$. Because of the fact that helical interaction terms depend on the sign relation of base pair functions, the banding pattern of the plus branch is not necessarily identical with that of the minus branch, *i.e.*, $\xi_{\beta, K^+} \neq \xi_{\beta, K^-}$. Consequently, the two bands do not necessarily have the same width, depending on the geometry of the helix and the polarizations of the transition in each base.

In summary, exciton interaction in a double-stranded helical polynucleotide results in a double band of energy levels corresponding to each excited state of an isolated base. The fact that two bands occur is a reflection of intra-base-pair exciton interaction, whereas the bands themselves are produced by helical exciton interaction among all the base pairs. Most of the energy levels of each band are doubly degenerate. Although reference is made specifically to polynucleotides, the results are quite general and may be applied to any system containing two sets of molecules in which the molecules of each set are related to one another by a simple symmetry operation such as a translation or a screw axis.

Selection Rules and Polarizations of Optical Transitions.—In this section we shall develop the selection rules, polarizations and intensities of radiationally induced transitions as predicted by zeroth-order exciton functions. These properties will then be considered for the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in helical polynucleotides.

The matrix elements specifying transition probabilities may be expressed in terms of either momentum or position coordinates of the electrons. For simplicity only the position coordinate representation will be used here. The vector quantity repre-

(13) M. Born, *Proc. Phys. Soc. (London)*, **54**, 362 (1942).

(14) As was pointed out in detail by Moffitt, *et al.*,³ the use of Born-von Karman cyclic boundary conditions is not rigorously correct for polymers of finite length. Although their use introduces an appreciable error into the rotational strength of the helix, the error in the transition moment integral is relatively small, being of order $N^{-1/2}$. Since we are interested here only in absorption properties, it is assumed that cyclic boundary conditions gives results that are at least qualitatively correct for helices having large N .

sending the total electric-dipole operator of all the optical electrons in the helix is

$$\mu = e \sum_i \mathbf{q}_i \quad (16)$$

where e is the charge of the electron and \mathbf{q}_i is the position coördinate of the i th electron. We wish to evaluate the matrix elements for transitions to the various exciton levels of the electronic state β , namely

$$\begin{aligned} \mu_{\beta, \kappa \pm}^{(0)} &= \int \Phi_0^{(0)*} \mu \Phi_{\beta, \kappa \pm}^{(0)} d\tau \\ &= N^{-1/2} \sum_m \exp\left(\frac{i2\pi n K}{N}\right) \mu_{\beta, n \pm} \end{aligned} \quad (17)$$

The latter term is a vector summation over the transition moment dipoles of the individual base pairs in which each is modulated by an exciton coefficient. Because of the dependence of $\mu_{\beta, n \pm}$ on n , the value of the summation for any particular K depends on the geometry of the helix.

Let us choose a Cartesian coördinate system such that the z axis coincides with the axis of the helix. The x and y axes then lie in a plane perpendicular to the axis of the helix, but their directions in this plane are arbitrary to the extent that we may express the components of $\mu_{\beta, n \pm}$ as

$$\begin{aligned} (\mu_{\beta, n \pm})_x &= |\mu_{\beta \pm}| \sin \hat{\phi} \pm \cos \eta_n \pm \\ (\mu_{\beta, n \pm})_y &= |\mu_{\beta \pm}| \sin \hat{\phi} \pm \sin \eta_n \pm \\ &= |\mu_{\beta \pm}| \sin \hat{\phi} \pm \cos(\eta_n \pm - \pi/2) \\ (\mu_{\beta, n \pm})_z &= |\mu_{\beta \pm}| \cos \hat{\phi} \pm \end{aligned} \quad (18)$$

The angle between $\mu_{\beta, n \pm}$ and the z axis is designated as $\hat{\phi} \pm$ and is independent of n . The angle between the perpendicular component of $\mu_{\beta, n \pm}$ and the x axis is $\eta_n \pm$ and is a function of n . More specifically

$$\begin{aligned} \eta_n \pm &= 2\pi n/P + \zeta \pm \\ &= 2\pi nM/N + \zeta \pm \end{aligned} \quad (19)$$

where the angle $\zeta \pm$ reflects the arbitrariness in our choice of the direction of the x axis and P is the number of base pairs per turn.

By using the Euler formulas to express $\cos \eta_n \pm$ in complex form and by substituting (18) into (17), we get

$$\begin{aligned} \mu_{\beta, \kappa \pm}^{(0)} &= N^{-1/2} |\mu_{\beta \pm}| \sum_n \left\{ \frac{1}{2} \sin \hat{\phi} \pm \times \right. \\ &\left. \left(\exp i \left[\frac{2\pi n(K-M)}{N} - \zeta \pm \right] + \exp i \left[\frac{2\pi n(K+M)}{N} + \zeta \pm \right] \right) i + \frac{1}{2} i \sin \hat{\phi} \pm \left(\exp i \left[\frac{2\pi n(K-M)}{N} - \zeta \pm \right] - \right. \right. \\ &\quad \left. \left. \exp i \left[\frac{2\pi n(K+M)}{N} + \zeta \pm \right] \right) j + \right. \\ &\quad \left. \cos \hat{\phi} \pm \exp i \left[\frac{2\pi n K}{N} \right] k \right\} \end{aligned} \quad (20)$$

It can be seen readily that this summation is identically zero unless K has one of the values M , $N-M$ or N . These selection rules for K give us the following allowed components of the exciton bands

$$\begin{aligned} \mu_{\beta, M \pm}^{(0)} &= \frac{1}{2} N^{1/2} |\mu_{\beta \pm}| \sin \hat{\phi} \pm e^{-i\zeta \pm} (i + ij) \\ \mu_{\beta, N-M \pm}^{(0)} &= \frac{1}{2} N^{1/2} |\mu_{\beta \pm}| \sin \hat{\phi} \pm e^{i\zeta \pm} (i - ij) \\ \mu_{\beta, N}^{(0)} &= N^{1/2} |\mu_{\beta \pm}| \cos \hat{\phi} \pm k \end{aligned} \quad (21)$$

Transitions to energy levels of the exciton band corresponding to $K = M$ and $N - M$ are polarized perpendicularly to the helix axis. These levels are degenerate, since $E_{\beta, M \pm} = E_{\beta, M - N \pm}$. On the other hand, transitions to the non-degenerate level corresponding to $K = N$ are polarized parallel to the helix axis.

Since the selection rules hold for both branches of the base-pair splitting, there will be at most, for any double-stranded helix, two sets of doubly degenerate perpendicular transitions and two non-degenerate parallel transitions. These transitions determine the total intensity in zeroth-order of a given electronic band of the helix. This can be seen from the fact that

$$|\mu_{\beta, \pm}^{(0)}|^2_{\text{Helix}} = \sum_K |\mu_{\beta, \kappa \pm}^{(0)}|^2 = N |\mu_{\beta \pm}|^2 \quad (22)$$

which means that the transition probability for the helix is just N times that of an isolated base pair. In a similar manner it can be shown easily from the relation

$$\mu_{\beta \pm} = 2^{-1/2} (\mu_a \pm \mu_b) \quad (23)$$

that the transition probability of an isolated base pair is twice that for a single base. Therefore, use of zeroth-order functions predicts no change in transition probability upon formation of a helix, which is not in accord with observation. This problem will be taken up in Part II.

It should be pointed out that the selection rules for K as derived above are not rigorously correct, because of the fact that we have neglected the phase of the light wave in evaluating the transition moment integral. For small molecules, it is a valid approximation to assume that the vector potential of the light wave is constant over the length of the molecule. However, for molecules such as DNA, where the length is of the same order of magnitude as the wave length of the light, this approximation is no longer suitable. Since the z axis of the helix is the only direction for which this variation in phase is important, the appropriate phase factor is $\exp(i2\pi z/\lambda)$. Here z is direction of propagation of the light wave and can be taken to be a discontinuous function; namely, $z = nL/N$ where L is the length of the helix, N is the total number of base pairs and n labels the base pair. Since the vector potential of the light wave is perpendicular to the direction of propagation, the phase factor will contribute to only the perpendicular components of the band, *i.e.*, to the first two components of (20). Thus, we get the more exact selection rules: $K = M - (L/\lambda)$, $N - M - (L/\lambda)$, and N . Due to the fact that L/λ is small compared to M , the effect of such correction on the selection rules and, consequently, on the optical properties of the helix are negligible. However, to give integral values of K , L/λ must also be an integer. This raises a question concerning the proper boundary conditions to be used in the exciton method of treating crystals as well as macromolecules.

Whether or not transitions to both branches of the base-pair splitting are optically allowed as electric-dipole transitions depends on the geometry of the base pair and polarizations of the electronic

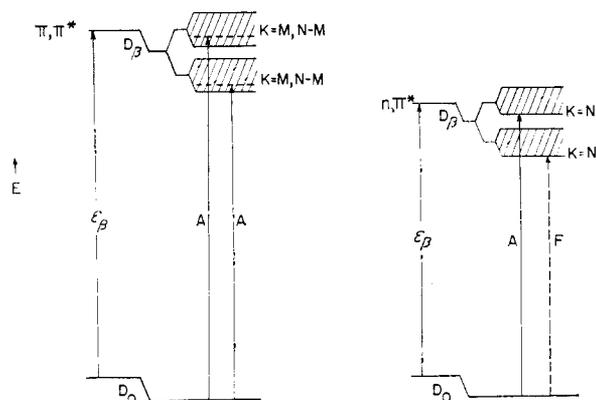


Fig. 1.—A schematic representation of exciton bands in a double-stranded, helical polynucleotide. The magnitudes of the splittings are exaggerated, and in reality the bands of the upper and lower branches overlap. The energies of the indicated transitions in the monomer are represented by ϵ_β ; D_0 and D_β represent van der Waals interaction in the ground and excited states, respectively; and A and F indicate, respectively, optically allowed and forbidden components.

transition in each base. We shall now consider some of the properties of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in helical polynucleotides. A schematic energy level diagram showing some of the features of these transitions in such a DNA-like molecule is given in Fig. 1.

$\pi \rightarrow \pi^*$ Bands.—Since all $\pi \rightarrow \pi^*$ transitions are polarized in plane, the transition moment dipoles of each base pair are perpendicular to the helix axis. Thus, the parallel components of the exciton band are zero [note that the angles ϕ^\pm in (20) have the value $\pi/2$], and all of the intensity of the band lies in the perpendicular components.

The apportionment of intensity to the branches of the base-pair splitting depends on the polarization direction in each base. The latter is not known explicitly but can be described in terms of the angle θ between the transition moment dipole and the radial vector, as shown in Fig. 2. The relative intensities of the two branches is a function of the angle between the transition moment dipoles of the base pair, which has the value $(\omega - \theta) - \theta = 104^\circ - 2\theta$. As can be seen from (23), if the transition moment dipoles are aligned parallel, transitions are allowed only to one branch. This is the case if $\theta = 52^\circ + k\pi/2$, where k is zero or an integer. On the other hand, if the transitions are aligned perpendicularly (*i.e.*, if $\theta = 7^\circ + k\pi/2$), transitions to the two branches will be equally allowed.

The work of Mason¹⁰ suggests that the main component of the 2600 Å. band is long-axis polarized in base. If this is the case, θ for this transition should have a value between the aforementioned extremes; and, as depicted in Fig. 1, the intensity should be divided (somewhat unequally) between the two branches.

$n \rightarrow \pi^*$ Bands.—For transitions of the $n \rightarrow \pi^*$ type the transition moment dipoles of each base pair are parallel to the helix axis. Hence ϕ^\pm in (20) is zero, meaning that only the parallel com-

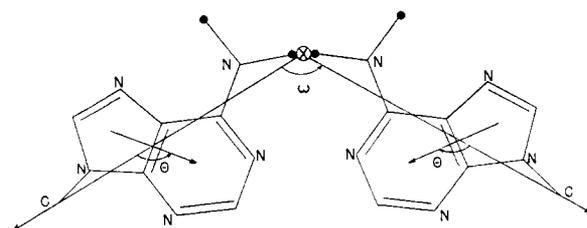


Fig. 2.—A hypothetical adenine-adenine base pair having the geometrical arrangement of the base pairs in DNA. In the real case of DNA one of the bases is replaced by thymine and stabilization by hydrogen bonding exists.

ponent of the transition moment corresponding to $K = N$ is electric-dipole allowed.

With respect to base-pair splitting, on the other hand, transitions to one of the branches are optically forbidden. This corresponds to the one for which the transition moment dipoles in (23) are out of phase, and in the dipole-dipole approximation is the lower-energy branch. Thus the only allowed $n \rightarrow \pi^*$ component is a parallel polarized transition to the upper-branch level for which $K = N$. Evaluation of (15) in the dipole-dipole approximation shows this level to be on the lower boundary of the upper-branch band, as depicted in Fig. 1.

The fact that the lower branch is optically forbidden may have an effect on the excited state properties of the helix, since the exciton band corresponding thereto represents a potentially metastable state. For example, radiationless transitions from the allowed level to these forbidden levels could enhance photochemical reactivity or intersystem crossing to lower-lying triplet levels with subsequent phosphorescence. Unfortunately, lack of experimental results limits discussion of this point to mere speculation. Suffice it to mention that the enhanced phosphorescence of some dye molecules upon polymerization is a well established phenomenon.¹⁵

Recently, Rich and Kasha¹⁶ measured the polarizations of the transitions in oriented films of polynucleotides. Their results, consistent with the conclusions drawn here, show that the $\pi \rightarrow \pi^*$ component of the 2600 Å. band is perpendicularly polarized, whereas the region of the band containing $n \rightarrow \pi^*$ transitions is polarized parallel to the helix axis.

As far as DNA is concerned, exciton interaction should be similar to, but not exactly the same as, that in a synthetic, homogeneous polynucleotide. The base pairs, adenine-thymine and guanine-cytosine, are distributed along the helix in a presently indeterminate manner. For our purposes we might consider the sequence to be random. The absorption spectra of the four kinds of bases vary significantly in the neighborhood of the common, main absorption band of 2600 Å. Near 2600 Å., where there is large spectral overlap of all four bases, interaction leads to the formation of exciton states extending over the entire helix. However, the spectra of both guanine and cytosine extend farther to the red than do those of adenine and

(15) E. G. McRae and M. Kasha, *J. Chem. Phys.*, **28**, 721 (1958).

(16) A. Rich and M. Kasha, *J. Am. Chem. Soc.*, **82**, 6197 (1960).

thymine, that of guanine extending the farthest. Thus on the extreme long wave length side of the spectrum, corresponding to absorption by guanine alone, the only exciton interaction possible is between guanine residues. This will be significant only in those regions of the helix where guanine-cytosine pairs are in proximity. Consequently, the lowest excited levels of DNA are isolated from the main exciton band and correspond, at most, to localized exciton states of the helix.

The problem of energy transfer in DNA is an important one in the study of radiation genetic damage. In an exciton state, the excitation migrates over the helix with a velocity dependent on the exciton interaction energy, $\xi_{\beta, \kappa}^{\pm}$. Energy transfer in DNA has not been observed spectroscopically, but studies on radiation genetic damage¹⁷ show that certain genetic sites are much more susceptible to damage than others. This suggests that the excitation may be transferred over the helix until it becomes trapped at a localized site where "photochemical change" occurs with concomitant genetic damage expected. The fact that guanine has localized states below the main exciton band denotes the possibility of its functioning in this capacity.

II. First-order Exciton States, Hypochromism.—

One of the most pronounced spectral changes accompanying the transition from random coil to helix is the marked decrease in absorption intensity of the 2600 Å. band. Such hypochromism, which may be as large as 40%, is a generally occurring phenomenon among both nucleic acids and synthetic polynucleotides. In addition to the large hypochromism of the 2600 Å. band as a whole, however, there is a small, but significant, hyperchromic shift on the long wave length side in the region of $n \rightarrow \pi^*$ transitions.¹⁸

As indicated by (22), zeroth-order exciton functions imply a conservation of transition probability upon helix formation. However, the intensity is governed by the oscillator strength, which may be expressed in terms of the transition probability as

$$\begin{aligned} \epsilon_{\beta, \kappa}^{\pm(0)} &= \frac{8\pi^2 m_0}{3\hbar^2 e^2} \epsilon_{\beta, \kappa}^{\pm} |\mathbf{u}_{\beta, \kappa}^{\pm(0)}|^2 \\ &= \frac{8\pi^2 m_0}{3\hbar^2 e^2} |\mathbf{u}_{\beta, \kappa}^{\pm(0)}|^2 (\epsilon_{\beta} + \Delta D_{\beta} + \xi_{\beta, \kappa}^{\pm}) \quad (24) \end{aligned}$$

where $\epsilon_{\beta, \kappa}^{\pm}$ is the transition energy of the helix, ϵ_{β} is the transition energy of an isolated base and ΔD_{β} is the change in non-resonance interaction energy upon excitation. The intensity of the band, therefore, is changed in zeroth-order by an amount which is proportioned to the change in excitation energy, a quantity which is much too small to account for the observed hypochromism. Thus, we shall proceed to first-order exciton states and determine the resulting effect on the oscillator strength.

The First-order Oscillator Strength.—Further application of perturbation theory in a straightforward manner takes into account the effect of base-base interaction on the electron distribution in each base. Since the interaction potential has

(17) L. S. Lerman and L. J. Tolmach, *Biochim. et Biophys. Acta*, **33**, 871 (1959).

the symmetry of the helix, it is convenient to express its effect in terms of the helix functions. The perturbed functions of the helix, correct to first order, then have the general form

$$\Phi_{\beta, \kappa}^{\pm(1)} = \sum_{\alpha, L, \pm} C_{\beta, \kappa}^{\pm \alpha, L} \Phi_{\alpha, L}^{\pm(0)} \quad (25)$$

The problem at hand is to establish the nature of the C 's and to determine which of the electronic states, α , contribute to the transition moment integral.

In view of the general form of the functions in (25), the complexity of notation to be encountered in its expansion can be anticipated. Thus, in order to facilitate the handling of the resulting equations, we shall make the following simplifications: (1) The equations will be derived for a molecular aggregate in which all molecules are related by one type of symmetry operation. In our case this is a single-stranded helix, in which the bases are related by simple screw operations. Thus the requirement of a double set of indexes is avoided. Since the base-pair coefficients have the same orthonormality properties as the helix exciton coefficients, it can be shown easily that the results obtained are qualitatively the same as for a double-stranded helix. In order to evaluate the final equation, the double indexes will be inserted. (2) The dipole-dipole approximation will be used to put the perturbation coefficients in (25) into a usable form. Hereby, interaction terms of the types dipole-induced dipole and induced dipole-induced dipole are obtained. Since the dipole moments of the bases are not known, all terms involving these will be omitted. This amounts to considering the effect only of London dispersion forces.

We shall proceed to develop (25) more explicitly, in a manner similar to that of Moffitt, *et al.*³ In view of the second simplification given above, the only perturbation terms in the ground state of the helix which contribute to transition moments are those in which two bases are simultaneously excited. Likewise, the only perturbation terms of importance in the excited electronic state, β , are those in which a single base is excited to the state α . Therefore, the first-order states of the helix may be written

$$\Phi_{\beta, \kappa}^{(1)} = \Phi_{\beta}^{(0)} - \sum_{\alpha, \alpha'} \sum_L \frac{\int \Phi_{\alpha, L}^{(0)*} V \Phi_{\beta}^{(0)} d\tau}{\epsilon_{\alpha} + \epsilon_{\alpha'}} \Phi_{\alpha, L}^{(0)} \quad (26)$$

and

$$\Phi_{\beta, \kappa}^{(1)} = \Phi_{\beta, \kappa}^{(0)} - \sum_{\alpha (\neq \beta)} \sum_L \frac{\int \Phi_{\alpha, L}^{(0)*} V \Phi_{\beta, \kappa}^{(1)} d\tau}{\epsilon_{\alpha} - \epsilon_{\beta}} \Phi_{\alpha, L}^{(0)}$$

The first-order transition moment integral

$$\mu_{\beta, \kappa}^{(1)} = \int \Phi_{\beta}^{(1)*} \mu \Phi_{\beta, \kappa}^{(1)} d\tau$$

may be expanded by substitution of (26). By making use of the orthonormality of the exciton coefficients and by neglecting all terms in second order, we get

$$\begin{aligned} \mu_{\beta, \kappa}^{(1)} &= \sum_n A_{n\kappa} \left\{ \mu_{\beta, n} - \sum_{m (\neq n)}^N \frac{V_{\beta n, \beta m}}{2\epsilon_{\beta}} \mu_{\beta m} - \right. \\ &\quad \left. 2 \sum_{\alpha (\neq \beta)} \frac{\epsilon_{\alpha}}{\epsilon_{\alpha}^2 - \epsilon_{\beta}^2} \sum_{m (\neq \alpha)}^N V_{\beta n, \alpha m} \mu_{\alpha m} \right\} \quad (27) \end{aligned}$$

where we have used the notation

$$V_{\beta n, \alpha m} = \int \Phi_{\beta n, \alpha m}^{(0)*} V \Phi_0^{(0)} d\tau, \text{ etc.}$$

Here the A_{nK} represent the normalized exciton coefficients. The last two terms on the right are the first-order components.

The transition probability, correct to first order, for the exciton level K is readily found to be

$$|\mu_{\beta, K}^{(1)}|^2 = \sum_{nn'} A_{n'K}^* A_{nK} \left\{ \mu_{\beta n'} \cdot \mu_{\beta n} - \sum_m \frac{V_{\beta n, \beta m}}{\epsilon_\beta} \mu_{\beta m} \cdot \mu_{\beta n'} - 4 \sum_{\alpha(\neq\beta)} \frac{\epsilon_\alpha}{\epsilon_\alpha^2 - \epsilon_\beta^2} \sum_m V_{\beta n, \alpha m} \mu_{\alpha m} \cdot \mu_{\beta n'} \right\} \quad (28)$$

To determine the first-order oscillator strength, for the entire exciton band of the state β , we substitute (28) into (24) and sum over all exciton levels, K . Thus, to first-order

$$f_{\beta, H}^{(1)} = \sum_K f_{\beta, K}^{(1)} = Nf_\beta + \frac{8\pi^2 m_e}{3\hbar^2 e^2} \sum_n \left\{ \sum_K \sum_{n'} A_{n'K}^* A_{nK} \xi_{\beta, K} \mu_{\beta n'} \cdot \mu_{\beta n} - \sum_{m(\neq n)} V_{\beta n, \beta m} \mu_{\beta m} \cdot \mu_{\beta n} - 4 \sum_{\alpha(\neq\beta)} \frac{\epsilon_\alpha \epsilon_\beta}{\epsilon_\alpha^2 - \epsilon_\beta^2} \sum_{m(\neq n)} V_{\beta n, \alpha m} \mu_{\alpha m} \cdot \mu_{\beta n} \right\} \quad (29)$$

where f_β is the oscillator strength of an isolated base. It should be noted that the permanent dipole term, ΔD_β in (24), has been omitted and that the orthonormality of the exciton coefficients

$$\sum_K A_{n'K}^* A_{nK} = \delta_{n'n}$$

requires that $n' = n$ in all but the second term.

The second term in (29) was not included in Tinoco's expression,⁴ and it is in this respect that the derivation here differs from his. This term may be simplified by substituting the value of $\xi_{\beta, K}$, which in accordance with (15) is

$$\xi_{\beta, K} = \sum_j \sum_{j'(\neq j)} A_{jK}^* A_{j'K} V_{\beta j', \beta j} \quad (30)$$

Summation over K gives the selection rules: $j = n$ and $j' = n'$. Therefore, the second term reduces to

$$\frac{8\pi^2 m_e}{3\hbar^2 e^2} \sum_n \sum_{n'(\neq n)} V_{\beta n', \beta n} \mu_{\beta n'} \cdot \mu_{\beta n}$$

which is equal in absolute value, but opposite in sign, to the third term. Thus, the two terms cancel¹⁸ identically, which means that there is no contribution to the oscillator strength from the exciton band of the state β . The oscillator strength of the helix now takes on a more simplified form, namely

$$f_{\beta, H}^{(1)} = Nf_\beta - \frac{32\pi^2 m_e}{3\hbar^2 e^2} \sum_{\alpha(\neq\beta)} \frac{\epsilon_\alpha \epsilon_\beta}{\epsilon_\alpha^2 - \epsilon_\beta^2} \times \sum_n \sum_{m(\neq n)} V_{\beta n, \alpha m} \mu_{\alpha m} \cdot \mu_{\beta n} \quad (31)$$

(18) Since the acceptance of this manuscript for publication Tinoco has pointed out the cancellation of these terms (cf. I. Tinoco, *J. Chem. Phys.*, **34**, 1087 (1961)).

The problem remains to express (31) in a form which can be evaluated. In the point-dipole approximation the perturbation matrix elements become

$$V_{\beta n, \alpha m} = |\mu_\beta| |\mu_\alpha| \left[\frac{\mathbf{e}_{\beta n} \cdot \mathbf{e}_{\alpha m}}{R_{mn}^3} - 3 \frac{(\mathbf{e}_{\beta n} \cdot \mathbf{R}_{mn})(\mathbf{e}_{\alpha m} \cdot \mathbf{R}_{mn})}{R_{mn}^5} \right] \equiv |\mu_\beta| |\mu_\alpha| G_{\beta n, \alpha m} \quad (32)$$

where $\mathbf{e}_{\beta n}$ and $\mathbf{e}_{\alpha m}$ are unit vectors having the directions of the indicated transition moment dipoles, and \mathbf{R}_{mn} is the vector between the optical centers of the bases. Furthermore, if we neglect end effects (*i.e.*, assume that every base has an identical environment), the summation over n in (31) may be replaced by the factor N . We may then write (31) in the form

$$\frac{f_{\beta, H}^{(1)}}{Nf_\beta} = 1 - 4 \sum_{\alpha(\neq\beta)} \frac{\epsilon_\alpha}{\epsilon_\alpha^2 - \epsilon_\beta^2} |\mu_\alpha|^2 \sum_{m(\neq n)} G_{\beta n, \alpha m} \mathbf{e}_{\alpha m} \cdot \mathbf{e}_{\beta n} \quad (33)$$

In order to evaluate the right side of (33), however, it is convenient to express $|\mu_\alpha|^2$ in terms of the experimentally measurable oscillator strength, f_α , and the transition energies in terms of wave numbers, $\bar{\nu}$. Thus, the ratio of the helix absorption to base absorption becomes

$$\frac{f_{\beta, H}^{(1)}}{Nf_\beta} = 1 - 4K \sum_{\alpha(\neq\beta)} \frac{f_\alpha}{\bar{\nu}_\alpha^2 - \bar{\nu}_\beta^2} \sum_{m(\neq n)} G_{\beta n, \alpha m} \mathbf{e}_{\alpha m} \cdot \mathbf{e}_{\beta n} \quad (34)$$

where

$$K = \frac{3e^2}{8\pi^2 c^2 m_e} = 1.07 \times 10^{10} \text{ \AA}^3 \text{ cm.}^{-2}$$

The term involving summation over m is purely geometrical in nature, since it depends only on the orientation of bases in the helix and the directions of the transition moment dipoles in each base. The first-order contribution to the absorption intensity of the helix depends, therefore, on the product of geometry-dependent and polarizability terms. The dependence of a change in oscillator strength on the ordering of the structure of the polymer becomes apparent, since for a random orientation, such as the random-coil form of DNA, the geometry-dependent term averages to zero.

One physical interpretation of the first-order effect is rather straightforward. Electromagnetic radiation of frequency $\bar{\nu}_\beta$, in addition to being absorbed by the bases, induces a polarization of the electrons in each base. The contribution of each electronic transition, the $\alpha \leftarrow 0$ state transition, represents a polarization dipole which is proportional to $f_\alpha / \bar{\nu}_\alpha^2 - \bar{\nu}_\beta^2$ and has the directional property of μ_α . The polarization dipoles oscillate with the frequency $\bar{\nu}_\beta$ and are either in phase or 180° out of phase with μ_β , depending on the relative magnitudes of $\bar{\nu}_\alpha$ and $\bar{\nu}_\beta$. There is an interaction between the transition moment dipole of one base [for example base n in (34)] and the polarization dipoles of all other bases of the helix. Whether this interaction effects an increase or a decrease in f_β depends on the relative orientations of the bases (*i.e.*, on the geometry term) and the above-mentioned phase relation.

Interaction of the transition dipoles associated with electronic bands is reciprocal. That is, if the state transition $\alpha \leftarrow 0$ causes a decrease in

f_β , the transition $\beta \leftarrow 0$, in turn, causes a corresponding increase in f_α . This can be seen from the fact that the perturbation term in (34) is anti-symmetric to permutation of α and β . Therefore, one band steals intensity from the other; consequently, in this approximation *the total intensity of all electronic transitions should be unaffected by helix formation.*

Calculation of Magnitude of Hypochromism.—In this section we shall show that polarizability effects alone, as described by (34), can account for the observed hypochromism of the 2600 Å. band. The double-stranded polyadenylic acid model mentioned earlier will be used, since inclusion of more than one type of base would make the calculations extremely complex. Nevertheless, in view of the large hypochromism observed in synthetic polynucleotides containing homogeneous strands, the calculations should have significance.

Since we are dealing with a double-stranded helix, it is necessary to use two indexes to locate each base in the helix. To denote the bases in the geometry terms of (34), we shall let r and s label the strand and m and n label the base pairs. Furthermore, it will be helpful to let the symbol $T_{\alpha\beta}$ designate the geometry-dependent term; thus

$$T_{\alpha\beta} = \sum_r \sum_{\substack{m \\ (rm \neq sn)}}^N e_{\alpha,rm} \cdot e_{\beta,sn} \left\{ \frac{e_{\alpha,rm} \cdot e_{\beta,sn}}{R_{rm,sn}^3} - \frac{3}{R_{rm,sn}^5} (e_{\alpha,rm} \cdot R_{rm,sn})(e_{\beta,sn} \cdot R_{rm,sn}) \right\} \quad (35)$$

where summation is taken over bases rm with respect to a fixed base sn in the helix. Let us choose a coordinate system with reference to base sn , which in Fig. 2 will be taken to be the one on the left. The unit vectors i and j are parallel and perpendicular, respectively, to the radial vector of base sn . With the unit vector k directed upward from the plane of the paper and parallel to the helix axis, the unit vectors form a right-handed set. The vector joining base centers then becomes

$$\begin{aligned} R_{rm,sn} &= \rho \{ \cos[2\pi(m-n)/P + (r-s)\omega] - 1 \} i + \\ &\rho \{ \sin[2\pi(m-n)/P + (r-s)\omega] \} j + \\ &3.4(m-n)k \end{aligned} \quad (36)$$

where ρ is the distance from the helix axis to the base center. From bond lengths and the known radius of the helix, ρ was calculated to be approximately 4 Å.

Because of the existence of numerous electronic transitions, there are several types of geometry-dependent terms to consider. One simplification, however, results from the fact that transitions which are perpendicularly polarized within the helix do not interact to change oscillator strengths. Hence $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions do not interact in this sense and may be treated separately.

The following types of geometry terms appear.

I. n, π^* Terms.—Since the transition moment dipoles of all the bases are directed parallel to the helix axis, we have $e_{\beta,sn} = e_{\alpha,rm} = k$. Substitution into (35) gives

$$T_{n,\pi^*} = -2.0 \times 10^{-3} \quad (37)$$

Here, we have included only nearest neighbor interactions; *i.e.*, summation is taken over bases

$(r-s, m-n) = (0, 1); (0, -1); (1, 0); (1, 1);$ and $(1, -1)$.

II. π, π^* Terms.—Evidence indicates that these in-plane electronic transitions are polarized along the long and short axes of the base.¹⁰ Since the directions are not known explicitly, we shall describe the unit vectors in terms of θ , as defined in Fig. 2; thus

$$e_{\beta,sn} = \cos \theta i + \sin \theta j \quad (38)$$

If we assume that all $\pi \rightarrow \pi^*$ transition moment dipoles of an individual base either are parallel or are perpendicular to one another, only two kinds of π, π^* terms occur: (a) In those cases where the state transition $\alpha \leftarrow 0$ is polarized parallel to the state transition $\beta \leftarrow 0$, we have

$$\begin{aligned} e_{\alpha,rm} &= \cos [2\pi(m-n)/P + (r-s)\omega + (-1)^{r-s}\theta] i + \\ &\sin [2\pi(m-n)/P + (r-s)\omega + (-1)^{r-s}\theta] j \end{aligned} \quad (39)$$

where the factor $(-1)^{r-s}$ takes into account the fact that bases on opposite strands are related by a 180° rotation about the radial vector. Substitution of (36), (38) and (39) into (35) and summation over nearest neighbors gives

$$\begin{aligned} T_{\parallel}(\theta) &= (-2.83 + 14.88 \cos^2 \theta + 5.92 \cos^4 \theta + \\ &20.32 \cos \theta \sin \theta + 5.70 \cos^3 \theta \sin \theta) \times 10^{-3} \end{aligned} \quad (40)$$

(b) If the state transitions $\alpha \leftarrow 0$ and $\beta \leftarrow 0$ are perpendicularly polarized, $e_{\alpha,rm}$ has the same form as (39) except that θ is replaced by $(\theta + \pi/2)$. Thus we get

$$\begin{aligned} T_{\perp}(\theta) &= (2.52 + 5.71 \cos^2 \theta - 5.71 \cos^4 \theta + \\ &2.80 \cos \theta \sin \theta - 5.60 \cos^3 \theta \sin \theta) 10^{-3} \end{aligned} \quad (41)$$

We are now in a position to evaluate the oscillator strength ratio (34) for the 2600 Å. band. The oscillator strength of the $n \rightarrow \pi^*$ transition on the long wave length side of the 2600 Å. band is perturbed only by other $n \rightarrow \pi^*$ transitions. The negative value of T_{n,π^*} indicates that the intensity of this transition should be increased by interaction with higher-lying $n \rightarrow \pi^*$ bands. This may account for the slight hyperchromism in the long wave length region reported by Rich and Kasha.¹⁶ However, since neither the oscillator strengths nor the frequencies of these transitions are known, it is not possible to make numerical calculations. Nevertheless, in view of the small oscillator strengths of $n \rightarrow \pi^*$ transitions, one would not expect to find a significant effect by far-removed transitions; consequently, the over-all intensity of the 2600 Å. band should be changed but slightly by such interactions.

The intensities of both $\pi \rightarrow \pi^*$ components of the 2600 Å. band are perturbed by the higher-energy $\pi \rightarrow \pi^*$ bands. However, due to restrictions in spectral measurements below 2000 Å., we are limited to the inclusion of only the 2000 Å. transition in (34). In conformity with (34) we shall let α designate the upper state for the 2000 Å. band, $\beta 1$ designate the upper state for the larger band component near 2600 Å., and $\beta 2$ designate the upper state for the weaker component near 2400 Å. The oscillator strengths of the bands in adenine were calculated from the relation $f = 4.32 \times 10^{-9} \int \epsilon d\nu$ and found to be $f_{\beta 1} + f_{\beta 2} = 0.31$ and $f_{\alpha} \approx 0.61$. Resolution of $\beta 1$ and $\beta 2$ is not possible

for adenine; but the approximate values 0.21 and 0.10, respectively, were estimated from the absorption curve determined for purine in hydrocarbon solvent.

By using (34) to calculate $f_{\beta,H}/2Nf_{\beta} = f_{\beta_1,H} + f_{\beta_2,H}/2N(f_{\beta_1} + f_{\beta_2})$, we obtain the effect of helix formation on the over-all intensity of the 2600 Å. band. The results are shown in Fig. 3 as a function of θ , which in this case specifies the angle of polarization of the transition to state β_1 . The polarizations of transitions to states β_1 and β_2 are mutually perpendicular. However, the polarization of the transition to state α , relative to these, is unknown. Therefore, we have made calculations using both possible polarizations of transitions to state α . The first case (I) assumes that transitions to states α and β_1 have parallel polarizations, whereas the second case (II) assumes that the polarizations are mutually perpendicular. As can be seen in Fig. 3, the curves for the two sets of polarizations are very similar, the one for parallel polarizations showing greater effects for most values of θ .

For values of θ in the range 0 to 45° both cases give results in good agreement with experiments as far as the hypochromism of the entire 2600 Å. band is concerned. However, it should be pointed out that the data in Fig. 3 show nothing about the relative effects on bands corresponding to upper states β_1 and β_2 for the two cases. Case II predicts most of the hypochromism to be in the band for β_2 excitation, with very little effect on the band for β_1 excitation. Contrary to observation,¹⁶ one would expect, therefore, a pronounced change in band shape and small decrease in the extinction coefficient at 2600 Å. upon helix formation. Case I, on the other hand, attributes most of the effect to the band for β_1 excitation. Thus for parallel polarizations of transitions to states α and β_1 , the theory is in better agreement with observation.

The over-all intensity of the 2600 Å. band is not affected, of course, by internal interaction of transitions within the band. Nevertheless, one of the two $\pi \rightarrow \pi^*$ transitions can steal intensity from the other. This results in a change of band shape, the degree of which depends on θ . Calculations show that at most these internal effects are rather small.

One interesting point which arises is the fact that the values of θ which predict maximum hypochromicity do not correspond to the polarizations suggested by substitution effects,¹⁰ namely, that the polarization of the transition to state β_1 is along the long axis. On the contrary, as may be seen in Fig. 2, the results obtained here would indicate that this transition is short-axis polarized. However, because of the approximations introduced into our calculations, no unqualified conclusions may be made concerning the polarizations of the electronic transitions.

It should be emphasized that the basis of the theory developed here is the same as that of the

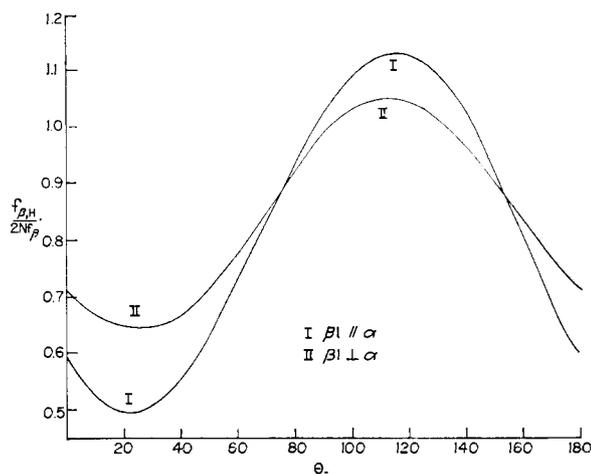


Fig. 3.—Calculated hypochromism of a double-stranded, helical polyadenylic acid having the configuration of DNA. The angle θ denotes the polarization of the transition to the state β_1 .

theory presented earlier by Tinoco.⁴ The essential difference is that it has been shown here that exciton interaction does not contribute to hypochromism. Nevertheless, the results of the present calculations for a double-stranded helix are qualitatively very similar to those obtained by Tinoco, the difference being that the changes in oscillator strength calculated here are more pronounced for most values of θ . In both treatments hypochromism is attributed mainly to dispersion force interaction among the bases, and in this regard calculations have been limited to the inclusion only of the effects of the transition near 2000 Å. Undoubtedly, higher-energy electric-dipole transitions have a significant effect. Thus the results are by no means a complete assay of the hypochromicity but can serve only as an indication that dispersion force interaction can account for the phenomenon.

Although we have applied (34) specifically to polynucleotides, the equation is applicable to any system containing an ordered array of molecules. For example, one might expect to find changes in the oscillator strength of proteins upon denaturation. In this regard, Imahori and Tanaka¹⁹ recently have observed hypochromism in the 1900 Å. transition of poly-L-glutamic acid concomitant with the random coil-to-helix transformation. It is not yet possible to make calculations for this case, however, since very little is known about the higher-lying electric-dipole transitions.

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(19) K. Imahori and J. Tanaka, *J. Mol. Biol.* **1** 359 (1959).