

Although the identical duplex cannot be the structure of those types of DNA on which X-ray diffraction studies have been carried out<sup>3</sup> (e.g., sperm, bacteriophage, thymus), it is not excluded that this helix resembles the configuration of DNA or ribonucleic acid (RNA) at some stage of the biological reduplication process. It is interesting in this connection that Rich and Watson<sup>4</sup> concluded, from an X-ray diffraction study of rather poorly oriented RNA fibers, that (1) the bases are stacked roughly perpendicular to the fiber axis; (2) the repeat distance along the fiber axis is 25–28 Å; (3) the structure is helical; (4) the diameter of the fiber is 21–25 Å. Calculation of the Fourier transforms of the three possible identical duplex structures would allow a more quantitative evaluation of the fit to the X-ray data, but since really precise information concerning the expected dimensions and conformations of the various parts of a polynucleotide chain is lacking, the comparison of the results of such calculations with the X-ray data cannot be unequivocal at the present time. Nevertheless, we intend to make such comparisons as are possible in the near future.

It should be noted that the identical duplex permits the point-to-point alignment of two homologous macromolecules, a process which is presumed to be a prerequisite for genetic recombination. If the hereditary information of an organism resided in a single polynucleotide chain at that stage of its life-cycle at which an exchange of genetic factors occurs, then the union of two such chains into an identical duplex could be the mechanism by which corresponding genetic regions are brought into exact apposition. The significance of the identical duplex structure for the mechanism of DNA replication and genetic recombination will be discussed in more detail elsewhere.

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<sup>1</sup> J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737, 1953.

<sup>2</sup> J. Donohue, these PROCEEDINGS, **42**, 60, 1956.

<sup>3</sup> M. H. F. Wilkins, A. R. Stokes, and H. R. Wilson, *Nature*, **171**, 740, 1953; R. E. Franklin and R. G. Gosling, *Nature*, **171**, 746, 1953.

<sup>4</sup> A. Rich and J. D. Watson, these PROCEEDINGS, **40**, 759, 1954.

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## THE OPTICAL ROTATORY DISPERSION OF SIMPLE POLYPEPTIDES. II

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In the accompanying note<sup>1</sup> evidence has been adduced that simple polypeptides in the  $\alpha$ -helical configuration show anomalous optical rotatory dispersion of a specific kind. More particularly, it has been shown that the effective monomer rotations of poly- $\gamma$ -benzyl-L-glutamate and of poly- $\alpha$ -L-glutamic acid obey the law

$$[m'] = \frac{a_0\lambda_0^2}{(\lambda^2 - \lambda_0^2)} + \frac{b_0\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \quad (1)$$

where  $a_0$  depends on the nature of the side chain attached to the helical skeleton and also on the solvent, but where  $b_0$  ( $\approx -640^\circ$ ) and  $\lambda_0$  ( $\approx 2100 \text{ \AA}$ ) are the same for both polymers and substantially independent of the solvent. Before these experiments were undertaken, the importance of a structurally invariant term proportioned to  $(\lambda^2 - \lambda_0^2)^{-2}$  had been predicted on theoretical grounds; by means of provisional calculations, its order of magnitude had been correctly estimated.<sup>2</sup>

In this note a more detailed account of the spectrum of an  $\alpha$ -helix is given, and more thorough calculations of the optical activity are made. It is shown that the two prominent peptide bands at 1850 and 1500  $\text{\AA}$  can account quantitatively for the observed dispersive term involving  $b_0, \lambda_0$ . If, as seems plausible, this term does indeed owe its origin to the two peptide bands, the calculations show that poly- $\gamma$ -benzyl-L-glutamate and poly- $\alpha$ -L-glutamic acid exist as right-handed  $\alpha$ -helices in solvents like dimethyl formamide.

#### THE PEPTIDE CHROMOPHORE

The spectrum of formamide above 1220  $\text{\AA}$  shows two broad absorption bands, one with a maximum at 1717  $\text{\AA}$  ( $N \rightarrow V_1$ ) and the other at 1345  $\text{\AA}$  ( $N \rightarrow V_2$ ).<sup>3</sup> These systems appear at considerably longer wave lengths in N-dimethyl formamide, whose absorption curve above 1500  $\text{\AA}$  shows peaks at 1974  $\text{\AA}$  ( $N \rightarrow V_1$ ) and 1621  $\text{\AA}$  ( $N \rightarrow V_2$ ).<sup>3</sup> The peptides themselves have peaks at 1850  $\text{\AA}$  ( $N \rightarrow V_1$ ),<sup>4</sup> which correlate well with the 1717  $\text{\AA}$  maximum for the unsubstituted amide, on the one hand, and with the 1974  $\text{\AA}$  peak of the N-disubstituted amide, on the other.<sup>5</sup> Their spectra at shorter wave lengths do not appear to have been measured, but it may be anticipated that the only strong band systems above 1350  $\text{\AA}$  due to the peptide linkage occur near 1480  $\text{\AA}$  ( $N \rightarrow V_2$ ), by a similar interpolation. All these absorption bands are attributed to excitations which are predominantly

localized among the four unsaturation ( $\pi$ ) electrons of the planar  $\begin{array}{c} \text{O} \\ || \\ \text{---C---NR}_1\text{R}_2 \end{array}$  groups.

The remaining, longer-wave-length transitions ( $>1350 \text{ \AA}$ ) in peptides are of three kinds. Some may arise from the chromophores, such as acid or ester functions, which belong to particular side chains. Others are due to Rydberg transitions, in which an unsaturation electron is promoted to an orbital of large radius, and to  $n_0 \rightarrow \pi^*$  excitations involving a nonbonding oxygen electron ( $n_0$ ) and an excited unsaturation orbital ( $\pi^*$ ). The Rydberg series are not very strong, but their sharpness in formamide enables them to be seen; the latter  $n_0 \rightarrow \pi$  transitions are very weak and completely overshadowed by the two prominent  $N \rightarrow V$  systems.<sup>3</sup>

For reasons that will appear, we shall attribute the values of the helical constants,  $b_0, \lambda_0$  to the two  $N \rightarrow V$  systems, at least in greater measure. It is therefore of some importance to characterize these more closely. Hunt and Simpson find that the oscillator strength of the  $N \rightarrow V_1$  system at 1974  $\text{\AA}$  in dimethyl formamide is 0.237.<sup>3</sup> It is apparently much the same for the analogous peptide band at 1850  $\text{\AA}$ , for which Ham and Platt give the value  $0.27 \pm 0.07$ .<sup>4</sup> For the 1620  $\text{\AA}$  band of the N-dimethyl formamide an oscillator strength of 0.209 is found,<sup>3</sup> which we may presume to characterize also the 1480  $\text{\AA}$  peptide band. The polarization of these

transitions is of great interest: Peterson and Simpson have studied the dichroism of sublimation flakes of myristamide in the region of the  $N \rightarrow V_1$  band.<sup>6</sup> From this they concluded that the most likely direction of polarization was inclined at an angle of  $9.1^\circ$  to the NO axis (in the sense that a parallel line through the N atom cuts the CO bond).<sup>7</sup> In the absence of any other pertinent data, we shall suppose the polarization of the  $1850 \text{ \AA}$  bands of peptides to be similarly directed. No information about the dichroism in the shorter-wave-length region is available at present. However, from the  $\pi$ -electronic origin of these bands it is clear that the  $N \rightarrow V_2$  system at  $1480 \text{ \AA}$  must also be polarized in the plane of the peptide bond and that its dipole vector is not far from orthogonal to that of the  $N \rightarrow V_1$  system.

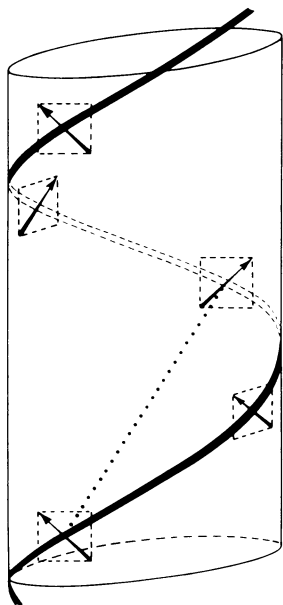
To summarize: From the available data on amides, we infer that the peptide grouping has only two strong bands above  $1350 \text{ \AA}$ . The one ( $N \rightarrow V_1$ ), with an oscillator strength of 0.237, lies at  $1850 \text{ \AA}$  and is polarized at an angle of  $9.1^\circ$  to the direction of the NO bond. The other ( $N \rightarrow V_2$ ) has its maximum at  $1480 \text{ \AA}$ , an oscillator strength of 0.209, and is also polarized in the plane of the peptide bond, but perpendicularly to the  $N \rightarrow V_1$  system.

#### THE SPECTRUM OF THE HELIX

The spectrum of an  $\alpha$ -helix will closely resemble that of the simpler peptides, with the absorption systems of the side-chain chromophores superposed on the bands enumerated above. However, the structure of the  $N \rightarrow V_1$  and  $N \rightarrow V_2$  systems will now be more complex. Owing to the interaction of the instantaneous electric moments of one peptide bond with those of its neighbors, these systems are both composite. The  $N \rightarrow V_1$  spectrum, for example, consists of one component which is polarized parallel to the screw axis, and a degenerate pair whose polarization is perpendicular to this. In the upper state of the former, the instantaneous moments of the chromophores are all in phase. In the excited states of the latter, however, the phase difference between the electronic motions in adjacent groups is given by the angle  $2\pi/P$ , where  $P = 3.7$  is the number of residues per unit turn of the  $\alpha$ -helix. States in which the phase differences are different also exist—indeed, for a helix of  $N$  residues, there are  $(N - 3)$  of these. But they do not appear as the upper states of band systems, since transitions to them are electronically forbidden: the parallel and perpendicular systems account for the total intensity.

For illustrative purposes only, we show these two states for the simpler helix, with  $P = 4$ , in Figures 1 and 2. The electric moments are drawn as the diagonal arrows in squares representing the plane of the peptide bond. The actual moments for the  $N \rightarrow V_1$  system bear a close geometrical resemblance to these diagrams. For example, the planes of the peptide bonds in the  $\alpha$ -helix are also parallel to the screw axis. The moments for the  $N \rightarrow V_2$  system may be pictured by drawing the arrows along the other diagonals of their respective squares, maintaining their mutual phase relationships. Only one component of the degenerate pair of states is shown (Fig. 2); the other would be represented by turning the screw through one-quarter of its pitch.

It is clear, on comparing the two diagrams, that the energies of the respective excited states differ because of the electrostatic interactions between the different



THE UPPER STATE AND POLARIZATION PROPERTIES OF THE PARALLEL BAND

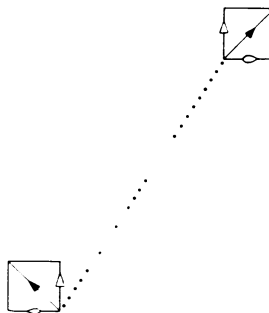
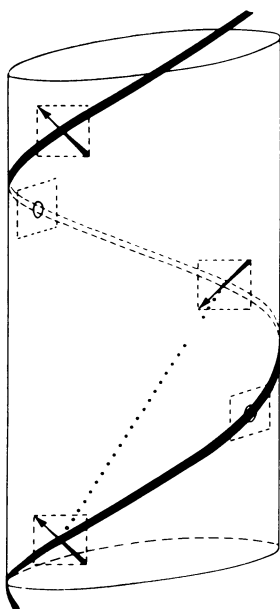


FIG. 1.



THE UPPER STATE AND POLARIZATION PROPERTIES OF THE PERPENDICULAR BAND

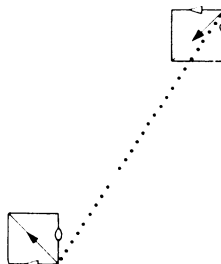


FIG. 2.

residue moments. Explicitly, if  $\nu_{\parallel i}$ ,  $\nu_{\perp i}$  represent the excitation energies of the upper states for the parallel and perpendicular components of the  $i$ th band system ( $N \rightarrow V_1$ , say), it is easily seen that

$$\Delta\nu_i = \nu_{\parallel i} - \nu_{\perp i} = \sum_l \left[ 1 - \cos\left(\frac{2\pi l}{P}\right) \right] \frac{V^i_l}{hc} \quad (2)$$

where the summation is taken over all neighbors  $l$  of a given residue;  $V^i_l$  is the energy of interaction between the instantaneous moments of this residue with those of the  $l$ th; it is assumed that the helix is long, and  $\Delta\nu_i$  is expressed in wave numbers.<sup>1</sup>

The instantaneous residue moments are not point dipoles but, rather, extended over the OCN atoms composing this particular chromophore—for it is along this path that the chromophoric electrons may be supposed to move. In so far as they interact with electromagnetic waves, they may be treated as point dipoles, but their multipolar character will manifest itself in their interactions with near neighbors.

The diagrams are useful not only for picturing the origin of the terms separating the different upper states within a given composite band system of this kind. Vector addition of the arrows, treated now as point dipoles, is easily shown to represent, both in magnitude and direction, the dipole vector whose interaction with radiation gives rise to the transitions: in Figure 1 it is obvious that the electric vector of the light must be parallel to the screw axis, and, similarly, transitions to the state pictured in Figure 2 must be perpendicularly polarized. (It is because these vector sums vanish by cancellation that states, whose residue moments have different phase relationships, do not appear in the spectrum.) Again, when the electrons are accelerated by radiation near the resonant frequency, their resultant momentum at any instant may be represented by the same vector sum. We see, in particular, that perpendicularly polarized light will induce electronic motions with a net angular momentum parallel to the polarization vector and, *mutatis mutandis*, that when the electric vector vibrates along the screw axis, it will give rise to fluctuating electronic angular momenta about this axis. (This is more obvious, in both figures, if the vectors are compounded in pairs—that of the first residue with that of the third, and that of the second with that of the fourth.) These angular momenta are of course associated with magnetic moments, by Ampère's law.

Now the condition that a given electronic transition be optically active is just that there should be a nonvanishing magnetic moment parallel to the electronic displacements induced by the radiation. Both the parallel and perpendicular components of such composite band systems satisfy this criterion in first order. Neglecting, for the moment, any other sources of asymmetry that these chromophores may have, it may be shown that their rotational strengths are given by<sup>1</sup>

$$r_{\parallel i} = \frac{3e^2\hbar}{8\pi mc} f_i \rho_i e_{it} e_{iv} = -r_{\perp i}, \quad (3)$$

where  $f_i$  is the oscillator strength of the complete band, per residue,  $\rho_i$  is the distance from the screw axis of the moment vector, whose tangential and vertical direction cosines are  $e_{it}$ ,  $e_{iv}$ , respectively. (This applies to a right-handed helix;

the sign of the second term is reversed for a left-handed helix. The terms, vertical and tangential, refer to a cylindrical co-ordinate system, in which the outward radial vector, the tangential vector, and the vertical direction of the advancing screw form a right-handed Cartesian set of axes.) These are both exceedingly large, but opposed. Their contributions to the residue rotation at wave lengths far from resonance cancel apart from terms proportional to the separation  $\Delta\nu_i$  of the two component systems in the  $i$ th band. (However, if one could arrange for the incident light to propagate along the screw axis alone, the electric vector would always be perpendicularly polarized, and the observed rotations would be very high.) In terms of the quantities  $b_i$  introduced in the accompanying note, we see that they are, however, responsible for the abnormally dispersing term  $b_i\nu_i^2\nu^2/(\nu_i^2 - \nu^2)^2$ , where  $\nu_i = 1/2(\nu_{\parallel i} + \nu_{\perp i})$  and

$$b_i = -\frac{72Ne^2}{mc^2} f_i \rho_i e_{it} e_{iv} \left( \frac{\Delta\nu_i}{\nu_i} \right). \quad (4)$$

This angle will be appreciable only if the moment vector for the appropriate transition has nonvanishing components along and tangentially to the screw axis, for otherwise  $e_{it}e_{iv}$  vanishes. Since  $\Delta\nu_i$ , a measure of the interactions between the residue moments, is large only when the associated transition is strong,  $b_i$  is roughly proportional to the square of the oscillator strength  $f_i$ . For the left-handed helix the sign is naturally reversed.

The  $n_0 \rightarrow \pi^*$  bands will show no detectable dispersion of this sort, since their oscillator strengths will be less than 0.001. On similar grounds, the Rydberg transitions may also be neglected in this respect. The side-chain chromophores may absorb intensely but will probably rotate more or less freely in solution, which tends to average out the terms contributing to  $\Delta\nu_i$ ; in addition, these are a long way apart from each other, so that they will interact but feebly on this score also. Thus, of the bands at wave lengths greater than 1350 Å, only the  $N \rightarrow V_1$  and  $N \rightarrow V_2$  systems should show complex dispersion of this kind. The other bands will be optically active, but their partial rotations are represented by the simple dispersive terms  $a_i\nu^2/(\nu_i^2 - \nu^2)$  and do not contain additional terms involving  $b_i$ 's of any size. Since the successive peptide chromophores, of which the basic  $\alpha$ -helical skeleton is composed, are essentially unaffected by variations in solvent, temperature, and side chain, provided only that they do not destroy the  $\alpha$ -helix, we see that the only important  $b_i$  terms should also be independent of such variations. This is not true, of course, for the  $a_i$  terms.

#### THE CALCULATION OF $\Delta\nu_1$ AND $\Delta\nu_2$

We shall now evaluate the quantities on the right-hand side of equation (4) for the two strong peptide bands, using the assignments discussed above. By elementary geometry,

$$\begin{aligned} \rho_1 &= 1.59 \text{ \AA} = \rho_2, \\ e_{1t}e_{1v} &= -0.493 = -e_{2t}e_{2v}, \\ f_1 &= 0.237, \quad f_2 = 0.209, \\ \nu_1 &= 54,050 \text{ cm}^{-1}, \quad \nu_2 = 67,570 \text{ cm}^{-1}, \end{aligned} \quad (5)$$

so that we only have to evaluate  $\Delta\nu_1$  and  $\Delta\nu_2$ . (The subscripts 1 and 2 refer, respectively, to the  $N \rightarrow V_1$  and  $N \rightarrow V_2$  systems.)

Consider one particular term  $V_i^i$  of equation (2), which represents the interaction of the electric moments induced by the  $i$ th band in the  $l$ th residue and in a given residue,  $m$ , say. More completely,

$$V_{im}^i = \int \int \psi_{ii}^* \psi_{0m} \left( \sum_s \sum_t \frac{e^2}{r_{l_s m_t}} \right) \psi_{0i} \psi_{im} d\tau_l d\tau_m, \quad (6)$$

where  $e^2/r_{l_s m_t}$  represents the electrostatic repulsion between an electron  $l_s$  of the  $l$ th residue and another  $m_t$  of the  $m$ th;  $\psi_{0i}$  and  $\psi_{ii}$  are the wave functions of the former electrons in their ground and  $i$ th excited states, respectively. The evaluation of this integral is standard if we are prepared to replace the electric moments by point dipoles. However, since the extension of a particular (conjugated) chromophore is of the same order of magnitude as its separation from its immediate neighbors, this approximation is invalid (it leads to errors of about  $\pm 50$  per cent). In its place we adopt a method of calculation which is based on approximations suggested by current electronic theories of spectra.

We regard the chromophoric electrons of a given residue as represented by wave functions made up from antisymmetrized products of atomic orbitals. It is unnecessary to specify in what linear combinations these occur; they may be constructed as molecular orbital functions with configurational interaction, for example. We shall also suppose the four chromophoric electrons to belong only to the three conjugated atoms of the peptide bond, the overlap between whose  $\pi$ -orbitals we neglect. (It has been shown, for example by Parr,<sup>3</sup> that the latter approximation is very good so far as electrostatic interactions of this kind are concerned.) Now consider the representation of an arbitrary "one-electron" operator

$$\Theta_i = \sum_s \Theta_{is} \quad (7)$$

in this scheme, where  $\Theta_{is}$  acts on the co-ordinates of electron  $l_s$  but on no others of the  $l$ th residue. It is readily demonstrated that

$$\int \psi_{ii}^* \Theta_i \psi_{0i} d\tau_l = C_O^i \omega_{O_i} + C_C^i \omega_{C_i} + C_N^i \omega_{N_i}, \quad (8)$$

$$\omega_{R_i} = \int \phi_{R_i}^*(l_s) \Theta_{is} \phi_{R_i}(l_s) d\tau_{l_s}, \quad (R = O, C, N),$$

$\phi_{R_i}$  being a normalized  $2p\pi$  orbital of atom  $R$  in the  $l$ th residue. The coefficients  $C_R^i$  are independent of the specific nature of the operator  $\Theta$ . In particular, setting  $\Theta_i$  equal to the identity operator, we find that

$$C_O^i + C_C^i + C_N^i = 0, \quad (9)$$

since  $\psi_{ii}$ ,  $\psi_{0i}$  are orthogonal. Again, setting  $\Theta_i = \vec{\mu}_i$ , the electric dipole vector for the electrons of the  $l$ th chromophore corresponding to this transition, we have

$$\vec{\mu}_{ii} = e(C_O^i \vec{r}_{O_i} + C_C^i \vec{r}_{C_i} + C_N^i \vec{r}_{N_i}), \quad (10)$$

$\vec{r}_{R_i}$  being the position vector of atom  $R_i$ . Now  $\vec{\mu}_{ii}$  is determined, apart from a phase factor, by dichroic studies. Thus, since the three conjugated atoms are not colinear, equations (9) and (10) together determine  $C_O$ ,  $C_C$ , and  $C_N$  apart from a

common phase factor (whose choice is in any case immaterial); for convenience we suppose the  $C_R$  to be real. We now set  $\Theta_{i_s} = e^2/r_{i_s m_i}$  and apply the same reasoning to the  $m$ th residue, finally finding that

$$V_{im}^i = \sum_R \sum_S C_R^i C_S^i \int \int |\phi_{R_i}(l_i)|^2 \frac{e^2}{r_{i_s m_i}} |\phi_{S_m}(m_i)|^2 d\tau_{i_s} d\tau_{m_i}. \quad (11)$$

The distance between two atoms in different residues is large enough, with respect to their respective radii, that we now replace these integrals by their asymptotic values, namely,  $e^2/r_{R_i S_m}$ , the energy of repulsion between a charge  $e$  on nucleus  $R_i$  and a like charge situated at nucleus  $S_m$ . If the directions of polarization as well as the oscillator strengths of transitions are known, the procedure we use has the merit of dispensing with the dipole approximation, without requiring a knowledge of those higher multipolar terms that spectroscopic studies do not yield.

The method outlined was followed in order to estimate the interaction of a given residue with its six successors and six predecessors in the helical sequence. The usual dipole approximation is valid thereafter and was used for the next eight residues in both directions. The contribution of more distant terms was estimated using an asymptotic integral. (It was found that the first three neighbors on either side of a given residue account for 70 per cent or more of the final sums.) The values we obtained were

$$-\Delta\nu_1 = 2800 \text{ cm}^{-1}, \quad -\Delta\nu_2 = 3130 \text{ cm}^{-1}. \quad (12)$$

Combining these with equation (5) and substituting in equation (4), we obtain the angles

$$b_1 = -1774^\circ, \quad b_2 = +926^\circ, \quad (13)$$

for a right-handed  $\alpha$ -helix. These are the best values that we could obtain on the basis of the available spectroscopic information.

#### THE OPTICAL ROTATORY DISPERSION

It should be emphasized that the  $b_i$ 's we have just calculated are both almost maximal. The oscillator strengths of the bands are high, the residue chromophores interact strongly, and the directions of polarization give orientational factors  $|e_i e_j|$  close to  $1/2$ , which they cannot, of course, exceed. The concomitant bands are also the only systems above 1350 Å showing these features. This suggests that these two terms dominate the dispersive form of the optical rotatory power. Bands at shorter wave lengths are not expected to influence the observed rotations between 7000 and 3000 Å to anything like the same extent (see below). Accordingly, we shall attribute the values of  $b_0$  and  $\lambda_0$  in equation (1) to the contributions from  $b_1$ ,  $b_2$  and  $\lambda_1$ ,  $\lambda_2$  alone:

$$b_0 \lambda_0^6 = b_1 \lambda_1^6 + b_2 \lambda_2^6, \quad b_0 \lambda_0^4 = b_1 \lambda_1^4 + b_2 \lambda_2^4. \quad (14)$$

Some empirical support is found for this assumption, inasmuch as  $b_0$ ,  $\lambda_0$  are apparently helical invariants, whereas  $a_0$  is not. This is most easily rationalized on the basis of equations (11) of the preceding note; if the terms containing  $a_i$  are relatively small in the second and third of these, they reduce to their successors (12).



In the latter,  $b_0$ ,  $\lambda_0$  are now determined by the  $b_i$ ,  $\lambda_i$  alone, and, as we have shown, these should depend only on the nature of the helical skeleton. The parameter  $a_0$  is then solely responsible for the variable dispersive features, as observed.

Substituting the values we have computed, it is found that

$$b_0 = -580^\circ, \quad \lambda_0 = 2000 \text{ \AA}, \quad (15)$$

for a right-handed  $\alpha$ -helix. This is embarrassingly close to the observed values of about  $-640^\circ$  and  $2100 \text{ \AA}$ , respectively. For a left-handed  $\alpha$ -helix, the predicted sign for  $b_0$  is simply reversed. It is tempting, therefore, to regard this correspondence as meaningful and to conclude that both the helices we have studied are right-handed. Before so doing, however, it is well to review the assumptions underlying this comparison.

The only data we have used in these calculations are those concerning the dichroism of the two peptide bands. We can justly claim that the numbers we derived from the available information were the only sensible ones. Thus our values for  $b_1$ ,  $b_2$ ,  $\lambda_1$ , and  $\lambda_2$  are likely to be as accurate as the data on which they are based. However, it would be preferable to use spectral studies of the  $\alpha$ -helix itself (polyalanine would be the most suitable material). Working with oriented samples, it should be possible to obtain  $\Delta\nu_1$ ,  $\Delta\nu_2$  and  $|e_{1\parallel} e_{1\perp}|$ ,  $|e_{2\parallel} e_{2\perp}|$  and  $f_1$ ,  $f_2$  directly. These would enable us to calculate  $b_1$ ,  $b_2$  with the least uncertainty, and, at the same time, they afford an internal check of our method for computing  $\Delta\nu_1$ ,  $\Delta\nu_2$ . But, owing to the diffuse nature of the bands, it is unlikely that the latter can be measured very accurately.

Certainly the most serious assumption we have made is that  $b_0$ ,  $\lambda_0$  are determined by  $b_1$ ,  $\lambda_1$  and  $b_2$ ,  $\lambda_2$  through equations (14). This can be justified only heuristically. If further measurements on simple polypeptides with different side chains confirm the constancy of  $b_0$ ,  $\lambda_0$ , but the variability of  $a_0$ , we shall be on firmer ground. For this will show, on empirical grounds, that equations (12) of the preceding note are at least approximately valid. It then only remains to justify our neglect of the bands lying at shorter wave lengths than  $1350 \text{ \AA}$ . In this connection we note that, although the values of  $b_0$ ,  $\lambda_0$  which we have calculated differ appreciably from  $b_1$ ,  $\lambda_1$ , the actual rotations at important wave lengths (e.g.,  $5000$ – $3500 \text{ \AA}$ ), calculated with both sets of parameters, do not differ nearly as much. This means that the presence of the  $1480 \text{ \AA}$  band modifies, but does not greatly affect, the abnormal dispersion due to the  $1850 \text{ \AA}$  system. Although the  $1480 \text{ \AA}$  system has a value of  $b$  that is absolutely large—one that is unlikely to be exceeded by the shorter-wave-length systems, which correspond to more localized, if more highly excited oscillators—the adverse  $\lambda_i$  dependence reduces its importance very considerably. On these grounds, the influence of the shorter-wave-length systems is likely to be yet smaller; its neglect should not seriously affect our conclusions.

Our analysis is therefore only tentative. However, it suggests the following experiment. If it were possible to observe the optical rotation for light around  $3500 \text{ \AA}$ , propagating entirely along the length of the helices, we should expect them to be strongly dextrorotatory on the basis of equation (3), since the electric vector is now always perpendicular to the screw axis. The measurement is hardly straightforward. For the molecules need to be well aligned in a medium of similar

refractive index, which permits the side chains to rotate unless these have no strong chromophores of their own.

#### DISCUSSION

It will be noticed that we have not attempted to calculate the actual rotation at any wave length, for this involves  $a_0$  in addition to  $b_0, \lambda_0$ . We do not believe that it is possible at the present time to make reliable computations of  $a_0$ . Indeed, our less ambitious identification of  $b_0, \lambda_0$ , through equations (14), with  $b_1, \lambda_1$  and  $b_2, \lambda_2$  can be only tentative until it is confirmed by more direct dichroic studies of the polypeptides themselves. Fitts and Kirkwood are more optimistic and have recently claimed to assess the rotation itself, due to a right-handed  $\alpha$ -helix with no asymmetric  $\alpha$  carbon atoms ("polyglycine").<sup>9</sup> Even more recently,<sup>10</sup> they have compared their results with data on the differences in specific rotation between the  $\alpha$ -helical and randomly coiled forms of poly-L-peptides. It seems appropriate, therefore, to discuss the difficulties that we consider to be inherent in their work. In order to avoid a more general discussion of optical activity, we shall confine our remarks to their application of Kirkwood's own polarizability theory to the present problem.<sup>11, 12</sup>

1. *The  $\alpha$ -Helix Is Treated as a Continuous Uniform Thread.*—Since there are only 3.7 operative units—whose intrinsic properties are highly unsymmetrical—per turn of the helix, their model cannot at best be other than a most crude facsimile of a real polypeptide.

2. *The Thread Is Regarded as Cylindrically Symmetric, Locally.*—The polarizability tensors of its infinitesimal sections are assumed to be ellipsoids of revolution, with their principal axes along the respective directions of the thread. If a continuum model of the successive peptide linkages is to be adopted at all, surely it must be acknowledged that the polarizability tensor has much lower symmetry than this. The planes of the peptide bonds are parallel to the screw axis and contain the segments of the helical thread. Fitts and Kirkwood imply that peptides are no more polarizable along the conjugated NCO path, in a direction almost coincident with the polarization of the first strong  $N \rightarrow V_1$  band, than they are along an axis perpendicular to this plane. All measurements on the anisotropies of conjugated systems refute this assumption. Indeed, it would be much more reasonable to assume that, if the individual segments were to be described by polarizability tensors with this symmetry, their principal axes were normal to the thread directions and to the outward radius vectors from the screw axis.

It is interesting to note that the consequences of this alternative assumption expose the weaknesses of the continuum model very strikingly. In place of the  $f(\theta)$  obtained by Fitts and Kirkwood—a function which behaves like  $\frac{1}{6}\theta(1 + \gamma^2)^{-1/2}$  for small values of  $\theta$  and is therefore integrable between 0 and  $\theta_L$ —we now find a function which behaves like  $\theta^{-1}(1 + \gamma^2)^{1/2}$  near the origin: the optical activity becomes logarithmically infinite. And this singularity can be removed only by adding an artificial cutoff, which is foreign to the premises of the model.

3. *The Anisotropy Ratio  $\beta$  Is Taken To Be about  $1/3$  for the Glycine Residue.*—We can find no basis for this supposition. A glycine residue has an approximate plane of symmetry, coinciding with that of the peptide bond, but no other elements of symmetry. Thus the orientation of the principal axes of the polarizability tensor

for such a group is frequency dependent, in the first place, and, as we have remarked, there is no reason why two of the axes should be equivalent:  $\beta$  is not a well defined scalar at all, far less equal to  $1/3$ , and yet the results of Fitts and Kirkwood are directly proportional to its square.

4. *It Is Assumed that the Contribution to the Rotation from the Asymmetric  $\alpha$  Carbon Atoms Is the Same Both for the  $\alpha$ -Helical and the Randomly Coiled Configuration.*—It is on this basis that Fitts and Kirkwood compare their calculations with experimental data. If the groups directly attached to the  $\alpha$  carbon atoms were rotating freely, the optical activity of the randomly coiled forms would vanish identically. Indeed, it is to the restricted rotation about bonds linking the various groups to asymmetric carbon atoms that Kirkwood, in his 1937 paper, attributes the rotatory power of such systems.<sup>11</sup> The assumption made by Fitts and Kirkwood therefore implies that the conditions for restricted rotation about the bonds to the asymmetric  $\alpha$  carbon atoms are identical both in the randomly coiled and in the  $\alpha$ -helical forms of the polypeptides. But it is in just this respect that, by definition, the two configurations differ. The comparison which they make between theory and experiment is without significance.

5. *The Dispersion Described by Fitts and Kirkwood Is Contradicted by the Observed Behavior.*—Fitts and Kirkwood remark that their model also leads to an abnormal frequency dependence of the form we had previously predicted. On the grounds already stated, we cannot admit that their formulation of the dispersive behavior is well founded. Their result requires that the helical contribution, both to  $a_0$  and to  $b_0$ , should have the same sign, whereas they are observed to differ.

<sup>1</sup> W. Moffitt and J. T. Yang, these PROCEEDINGS, 42, 596–603, 1956.

<sup>2</sup> W. Moffitt, *J. Chem. Phys.*, 24, August, 1956.

<sup>3</sup> H. D. Hunt and W. T. Simpson, *J. Am. Chem. Soc.*, 75, 4540, 1953.

<sup>4</sup> J. S. Ham and J. R. Platt, *J. Chem. Phys.*, 20, 335, 1952.

<sup>5</sup> R. B. Woodward, *J. Am. Chem. Soc.*, 63, 1123, 1941.

<sup>6</sup> D. L. Peterson and W. T. Simpson, *J. Am. Chem. Soc.*, 77, 3929, 1955.

<sup>7</sup> Their measurements do not exclude another assignment, in which the dipole vector makes an angle of  $26.7^\circ$  with the NO axis in the opposite sense. Peterson and Simpson are, however, inclined to rule out this possibility on theoretical grounds.

<sup>8</sup> R. G. Parr, *J. Chem. Phys.*, 20, 1499, 1952.

<sup>9</sup> D. D. Fitts and J. G. Kirkwood, these PROCEEDINGS, 42, 33, 1956.

<sup>10</sup> D. D. Fitts and J. G. Kirkwood, *J. Am. Chem. Soc.*, 78, 2650, 1956.

<sup>11</sup> J. G. Kirkwood, *J. Chem. Phys.*, 5, 479, 1937.

<sup>12</sup> Although our work has much in common with the polarizability theory, we do not agree with certain important premises of the latter.