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OPTICAL ROTATION AND POLYPEPTIDE STRUCTURE*

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COMPARISON of experimental data on the optical rotation of polypeptides with an appropriate theoretical calculation can give a great deal of structural information about the polypeptide. However, there is at this time no theory which is known to be reliable. We would like to review what progress has been made along these lines.

The general quantum mechanical equation relating the average optical rotation to the electronic wavefunctions of a molecule was derived by Rosenfeld[1] in 1928.

$$[m'] = \left(\frac{3}{n^2+2}\right)\frac{[\alpha]M}{100}$$
$$[m'] = \frac{96\pi i N_0}{hc}\sum_n{}' \frac{\lambda_{0n}{}^2 \mu_{0n}\cdot \mathbf{m}_{0n}}{\lambda^2 - \lambda_{0n}{}^2} \qquad (1)$$

N_0 = Avogadro's number.
$i = \sqrt{-1}$
h = Planck's constant
c = speed of light
λ_{0n} = wave length corresponding to the transition from ground state 0 to excited state n.

The sum is over all excited states. The electric and magnetic dipole transition moments are defined as

$$\mu_{0n} = e\int \psi_0{}^* \sum_l \mathbf{r}_l\, \psi_n\, d\tau$$

$$\mathbf{m}_{0n} = \frac{e}{2mc}\int \psi_0{}^* \sum_l \mathbf{r}_l \times \mathbf{p}_l \psi_n\, d\tau$$

The wavefunctions refer to the entire molecule and the sums are over all the electrons in the molecule.

An approach to the calculation of the optical rotation can be made by considering the molecule to be an aggregate of discrete groups of electrons.[2a,b] To evaluate the transition moments the sum over electron is divided into sums over groups and the

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† National Science Foundation Fellow.

[1] L. Rosenfeld, *Z. Physik.* **52**, 161 (1928).
[2a] J. G. Kirkwood, *J. Chem. Phys.* **5**, 479 (1937).
[2b] W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 723 (1957).

molecular wavefunctions are written as products of group wavefunctions. The result can be written as

$$[m'] = \sum_{\text{groups}} \{g^0(V_{ij}, \boldsymbol{\mu}^i_{n_i 0}, \boldsymbol{\mu}^j_{n_j 0}, \mathbf{R}_{ij}) + g^1(V_{ij}, \boldsymbol{\mu}^i_{n_i 0}, \mathbf{m}^j_{n_j 0}, \mathbf{R}_{ij}) + [m_i']\} \qquad (2)$$

The potential V_{ij} is the interaction energy between the instantaneous charge distributions in groups i and j and $\mathbf{R}_{ij}$ is the distance between these groups. The electric and magnetic transition moments now refer to electrons, wavefunctions, and transitions in specific groups i and j. The optical rotation of group i is $[m'_i]$. It can be calculated from the effect of the time average fields of the other groups on group i. Moffitt[3] has shown that the value of g^1/g^0 is of order of magnitude $d/\mathbf{R}_{ij}$ where d is a particular distance within a group. That is, g^1 will be small relative to g^0 if the groups are small relative to the distance between them. The relative magnitude of $[m']$ to g^0 is difficult to assess. As the intensity of an absorption band is proportional to μ_{0n}^2, we expect strong bands to contribute to the rotation mainly through g^0 while weak bands contribute through $[m_i']$.

Calculations for polypeptides have been made considering only the g^0 term. This allows the optical rotation as a function of wave length to be obtained from the geometry of the molecule and from measurable optical properties (absorption coefficients or polarizabilities) of the groups. The key assumptions are that there is no electron exchange between groups and that the interactions between groups is purely electrostatic. The validity of these assumptions will obviously depend on the choice of groups.

The α-helix

Moffitt[3,4] attempted to find the contribution of the amide groups to the optical rotation of an α-helix. He felt that the above assumptions were valid only for these non-bonded groups. The calculation involves determining the electronic interaction of each amide group with other amides in the polypeptide and with the incident light. For the interaction with the light the transient charge distribution of the excited amide can be characterized by the transition electric dipole moment. However, for the interaction with the nearest neighbor amides, Moffitt felt that the dipole approximation was inadequate. He used instead the transition monopoles at the N, C, and O atoms of the amide group. Both the mono- and di-pole moments were obtained from the spectrum of the amide group as determined by Peterson and Simpson[5]. Only transitions at 1850 and 1480 A were considered. Moffitt's final numerical result was incorrect because of an error in the choice of electronic wavefunction.

Fitts and Kirkwood[6] were willing to attempt a complete calculation of the optical rotatory dispersion of an α-helix with no side chains. They used the dipole approximation for the interaction between all groups, i.e., amide-amide, amide-CH, and CH—CH. However, they omitted interactions between adjacent atoms to approximately remove the contribution from asymmetric carbons. The necessary optical parameters were the transition dipole moments of the amide and the polarizabilities of the CH and the amide.

[3] W. Moffitt, *J. Chem. Phys.* **25**, 467 (1956).
[4] W. Moffitt, *Proc. Natl. Acad. Sci.* **42**, 736 (1956).
[5] D. L. Peterson and W. T. Simpson, *J. Amer. Chem. Soc.* **79**, 2375 (1957).
[6] D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 723 (1957).

Tinoco and Woody[7] extended the calculations of Fitts and Kirkwood to include polyglycine and polyalanine. They also calculated the optical activity for light incident parallel to the helix axis of the polypeptide besides the average rotation. In the Kirkwood approximation the general equation for the effective molecular rotation can be written as

$$[m'] = \sum_i \sum_{j \neq i} \frac{\alpha_i \alpha_j A_{ij}}{\lambda^2} \tag{3}$$

where α_i, α_j are the polarizabilities of groups of type i and j; A_{ij} is a constant depending mainly on the geometry of the molecule and the direction dependence of the polarizabilities. For a peptide with hydrocarbon side chains we write

$$\alpha_{\text{amide}} = \frac{A_1\lambda^2}{\lambda^2 - \lambda_1^2} - \frac{A_2\lambda^2}{\lambda^2 - \lambda_2^2} + A_3$$

$$\alpha_{\text{hydrocarbon}} = A_4$$

then

$$[m'] = \frac{a_{11}\lambda^2}{(\lambda^2 - \lambda_1^2)^2} + \frac{a_{12}\lambda^2}{(\lambda^2 - \lambda_1^2)(\lambda^2 - \lambda_2^2)} + \frac{a_{22}\lambda^2}{(\lambda^2 - \lambda_2^2)^2} + \frac{a_{10}}{\lambda^2 - \lambda_1^2} + \frac{a_{20}}{\lambda^2 - \lambda_2^2} + \frac{a_{00}}{\lambda^2} \tag{4}$$

The first three terms represent the mutual interactions of the 1850 A (λ_1) and 1480 A (λ_2) amide transitions. The next two terms include the interaction of these two special transitions with the rest of the polypeptide. The last term is the contribution from the constant polarizabilities. All six terms are of the same order of magnitude and can be positive or negative. There will obviously be considerable uncertainty in the magnitude of the final calculated rotation. The results are given as the solid lines in Figs. 1, 2, and 5.

The pure L *polymer.* Fig. 1 gives the calculated average rotation for (1) right-handed poly-L-alanine and (5) left-handed poly-L-alanine. The broken lines show experimental results for (2) polybenzyl-L-glutamate in ethylene dichloride,[8] (3) poly-L-glutamic acid in dioxane-H_2O,[8] (4) poly-L-leucine in benzene-*m*-cresol,[9] and (6) poly-L-alanine as solid film.[10] The general agreement between experiment and calculation for the left-handed α-helix is striking.

The D,L *copolymer.* Fig. 2 gives the calculated average rotation for (2) right-handed polyglycine omitting adjacent atoms,[11] (4) right-handed poly-DL-alanine and (5) left-handed polyglycine. The broken lines show experimental results for helices with the sense of the L isomers of (1) polybenzyl-DL-glutamate in dioxane[12] and (3) poly-DL-leucine in benzene-*m*-cresol.[9] The comparison in this figure favors right-handed helices, as previously concluded by Fitts and Kirkwood, but is in contradiction to our conclusion from Fig. 1. As the experimental results were found by various extrapolation procedures, it is well to consider these carefully.

[7] I. Tinoco, Jr. and R. W. Woody, *J. Chem. Phys.* **32**, 461 (1960).
[8] W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.* **42**, 596 (1956).
[9] A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* A **242**, 325 (1957).
[10] A. Elliott, W. E. Hanby and B. R. Malcolm, *Disc. Faraday Soc.* **25**, 167 (1958).
[11] This is the model used by Fitts and Kirkwood. Their calculated curve agrees within experimental error with curve (1).
[12] P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.* **43**, 213 (1957).

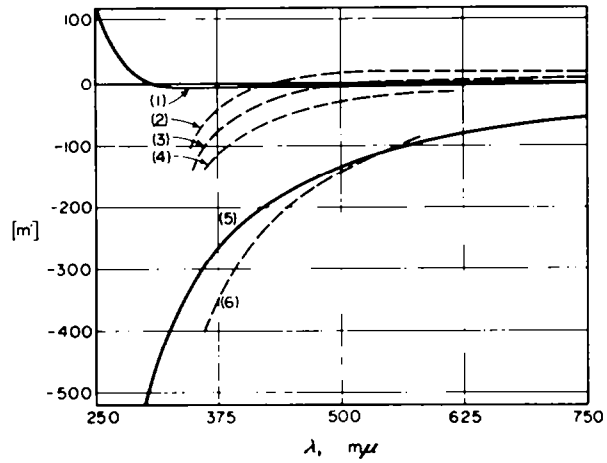


FIG. 1. The average optical rotation vs. wavelength. The solid curves are calculated: (1) right-handed poly-L-alanine, (5) left-handed poly-L-alanine. The broken curves are experimental: (2) polybenzyl-L-glutamate in ethylene dichloride,[8] (3) poly-L-glutamic acid in dioxane-water,[8] (4) poly-L-leucine in benzene-*m*-cresol,[9] (6) poly-L-alanine as solid film.[10]

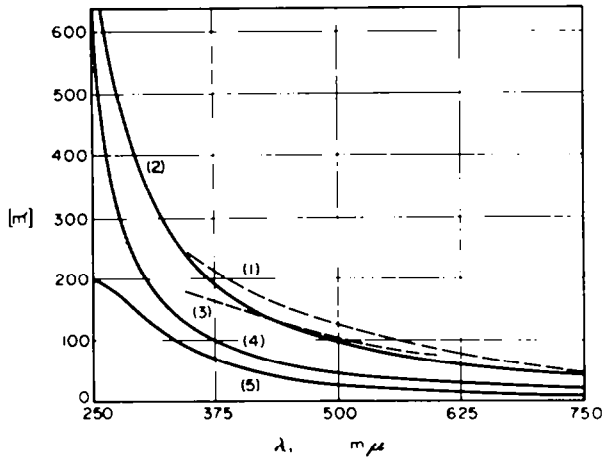


FIG. 2. The average optical rotation vs. wave length. The solid curves are calculated: (2) right-handed polyglycine (omitting adjacent atoms), (4) right-handed poly-DL-alanine, (5) left-handed polyglycine. The broken curves are experimental: (1) polybenzyl-DL-glutamate in dioxane,[12] (3) poly-DL-leucine in benzene-*m*-cresol.[9] These latter curves are for helices with the sense of the poly-L-isomer.

Measurements of the optical rotation of D,L polypeptides have been made in an attempt to determine the contribution of the α-helix to the rotation.[13] A typical experiment[9] is shown in Fig. 3 where the observed rotation for a polypeptide in a helix solvent is plotted vs. f_D, the mole fraction of D monomers in the polymerizing system. By extrapolation of the initial linear portion of curve to $f_D = 0{\cdot}5$, one obtains the "rotation of the α-helix with racemic residues." Let us examine what this means.

All theories of optical rotation agree that the rotation is due to the interaction between groups. We can distinguish between groups in the covalently bonded backbone

[13] An alternative experiment might be to form co-polymers with glycine and extrapolate to 100% polyglycine

BB and those in the side chains L or D and therefore write quite generally for the rotation:

$$[m'] = (BB) + f_L(L - BB) + f_D(D - BB) + f_D^2(D - D) + f_L^2(L - L) + 2f_Df_L(D - L) \quad (5)$$

The contribution from the backbone is (BB); (L − BB) is the contribution from the interaction between L side chains and backbone, etc. We can think of equation (5) as

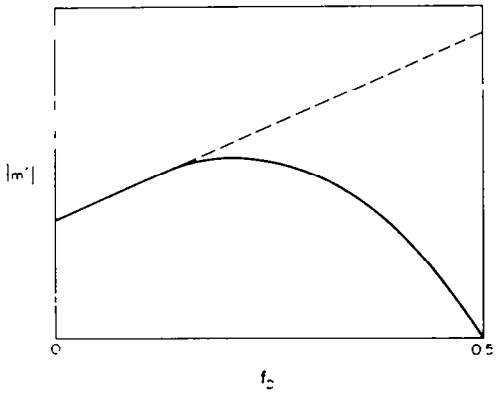


FIG. 3. The optical rotation for a polypeptide in a helix solvent is plotted vs. f_D, the mole fraction of D monomers in the polymerizing system. By extrapolation (broken line) of the initial linear portion of the curve to $f_D = 0{\cdot}5$, one obtains the "rotation of the α-helix with racemic residues."

representing the solid line in Fig. 3. The equation for the broken line used in extrapolation can then be written

$$[m']^{DL} = [m']^{L} + \left(\frac{\partial[m']}{\partial f_D}\right)_{f_D \to 0} f_D \quad (6)$$

The form of this equation (and therefore the extrapolated value $[m']^{DL}_{f_D=0.5}$) will depend on the assumptions made about the effect of D substitution on the backbone. These could be:

(1) Helix backbone is unchanged by slight substitution of D residues. The extrapolated value for the helix with racemic side chains becomes

$$[m']^{DL}_{f_D=0.5} = (BB_L) + 0{\cdot}5(D - BB_L) + 0{\cdot}5(L - BB_L) + (D - L) \quad (7)$$

The subscript L on BB means that the backbone rotation is that of the pure L isomer.

(2) Helix sense is directly proportional to the mole fraction of each isomer.

$$[m']^{DL}_{f_D=0.5} = (BB_L) - (D - L) \quad (8)$$

(3) Helix backbone is distorted by slight substitution of D residues. This means we must add the following terms to equation (7)

$$0{\cdot}5\left[\frac{\partial(BB_L)}{\partial f_D} + \frac{\partial(L - BB_L)}{\partial f_D} + \frac{\partial(L - L)}{\partial f_D}\right] f_D \to 0$$

These terms represent the linear change in rotation due to the distortion of the helix by the addition of D residues.

It is clear that the significance of the extrapolated value depends strongly on what actually happens to the backbone helix when D residues are added. The experimental workers have assumed that the helix backbone is unaffected by slight D substitution, therefore they should favor equation (7). As (D — BB_L), the contribution of the interaction of D side chains with the backbone helix corresponding to the L isomer, is *not* equal to minus (L — BB_L), this extrapolation does *not* give the rotation of a helix without side chains. For polyalanine we have calculated (D — BB_L) to have the same sign and order of magnitude as (L — BB_L). The contribution (D — BB_L) is of equal magnitude and *opposite* sign to (L — BB_D). This is why assumption (2) (the inversion of the helix sense is directly proportional to D concentration) is necessary to give an extrapolated value which does not contain side chain-backbone interactions. Both cases (1) and (2) have contributions, which may be small, from the interaction of the side chains with each other, (D — L). The most reasonable assumption is probably (3), i.e. some helix distortion occurs every time a D residue is introduced. Models show that substitution of a D for an L residue in an α-helix creates enough steric hindrance to block completely rotation of the β carbons in both the D and interacting L side chains. In Fig. 4 is a picture of left-handed poly-L-alanine containing one D residue. The methyls in this model can barely rotate, but for all larger side chains rotation is not possible. The increase in enthalpy and decrease in entropy which this would cause could be offset by distortion and/or disruption of the helix.

The conclusion we draw from this discussion is that the optical rotation of D,L polymers is very difficult to interpret. Curve (4) in Fig. 2 is calculated for right-handed poly-DL-alanine on the basis of equation (7). Curve (5) for left-handed polyglycine is essentially equal to (BB) and so it is roughly a comparison to experiment based on equation (8). If assumption (3) is correct then neither of these comparisons is valid and Fig. 2 does not give us any information about the helix sense.

The oriented polymer. Fig. 5 gives the calculated rotation for light incident along the helix axis for (1) left-handed polyglycine, (2) left-handed polyglycine omitting adjacent atoms, (3) left-handed poly-DL-alanine, (5) left-handed poly-L-alanine, and (6) right-handed poly-L-alanine. The dotted lines show experimental results for polybenzyl-L-glutamate in (4a) dioxane[14] and (4b) ethylene dichloride.[15] We notice that all the calculated curves for left-handed helices are qualitatively the same and that they are in general agreement with the experiments.

The effect of side chains. All the calculations have been made for the simplest polypeptides, glycine and alanine, while most of the experiments have been done on more complicated polymers. The effect of the side chains on the optical rotation is difficult to calculate. One problem is in not knowing the configuration of the side chains. Two extreme examples are shown in Fig. 6. Fig. 6a shows left-handed poly-benzyl-L-glutamate with the side chains arranged randomly, while Fig. 6b shows that an extremely non-random arrangement of the benzene rings is sterically possible. In spite of these difficulties it will probably be possible to estimate the effect of the various side chains on the optical rotation.

The effect of solvent. Calculations of the optical rotation have of course omitted

[14] K. Yamaoka, unpublished experiments.
[15] I. Tinoco, Jr., *J. Amer. Chem. Soc.* **81**, 1540 (1959).

solvent interactions. Theoretically we know they exist and there is experimental proof for the change is optical rotation in various helix solvents.[16] For our comparisons we can either use solvents which interact weakly and randomly with the polymer or else try to obtain a parameter which is independent of solvent. By making measurements near the amide absorption band one may find such a parameter.

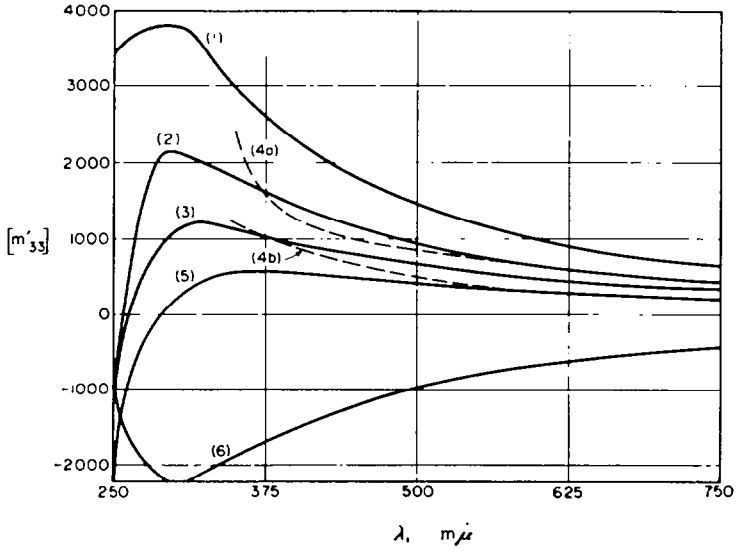


FIG. 5. The optical rotation for light incident along the helix axis vs. wave length. The solid curves are calculated: (1) left-handed polyglycine, (2) left-handed polyglycine (omitting adjacent atoms), (3) left-handed poly-DL-alanine, (5) left-handed poly-L-alanine, (6) right-handed poly-L-alanine. The broken curves are experimental: polybenzyl-L-glutamate in (4a) dioxane[14] and (4b) ethylene dichloride.[15]

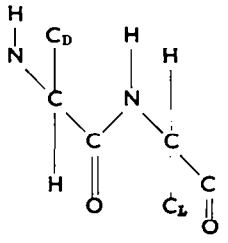
Other measures of the sense of the helix. X-ray scattering would seem to be the definitive method of determining the sense of the helix. However, recent x-ray work has been contradictory. Arndt and Riley[17] made an extensive determination of x-ray powder diagrams of proteins and synthetic polypeptides. They claimed that in an amorphous solid protein, most of the detectable scattering would come from intramolecular distances. Therefore, they need not assume a particular intermolecular packing to interpret their results. They concluded that there were large amounts of left-handed α-helices (but not right-handed helices) in all their samples. Calculations have been made by Brown and Trotter[18] and Elliott and Malcolm[19] to compare with the same x-ray data on poly-L-alanine fibers.

Their conclusions have depended on the assumption on interchain packing. Brown and Trotter favored a left-handed helix with poor agreement between observed and calculated data, but Elliott and Malcolm with much better agreement definitely chose a right-handed helix. With the uncertainty in the packing still to be resolved we feel

[16] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[17] U. V. Arndt and D. P. Riley, *Phil. Trans. Roy. Soc.* A **247**, 409 (1955).
[18] L. Brown and I. F. Trotter, *Trans. Faraday Soc.* **52**, 537 (1956).
[19] A. Elliott and B. R. Malcolm, *Proc. Roy. Soc.* A **249**, 30 (1959).

further evidence is desirable. It may be that the work of Kendrew *et al.*[20] on single crystals will provide this evidence.

Another approach to the problem of the sense of the helix is by consideration of the intramolecular forces which determine whether the L isomer forms a right or left-handed helix. Huggins[21] proposed that short range repulsive forces were dominant. This can be illustrated by the following sequence for a left-handed helix.



For a right-handed sequence the C_L and C_D would be interchanged. The distance from the β carbon in the L side chain C_L to the adjacent carbonyl oxygen is 2·7 A. Huggins thought this distance too short and therefore favored the C_D position; that is he thought that the L isomer should form right-handed helices. Donohue[22] has pointed out that identical distances between non-bonded O and C have been found in various amino acid crystals and thus rejects the argument.

We have been looking at the attractive forces between non-bonded atoms in the polypeptide. The important forces determining the relative stability of the right- and left-handed helix are permanent dipole forces and London forces between side chains and backbone. The dipole forces vary as r^{-3}, but can be attractive or repulsive, while the London force is always attractive and varies as r^{-6}. Our calculation for polyalanine which is still in progress, is analogous to one made by Pitzer and Catalano[23] to determine the relative heats of formation of isomeric hydrocarbons.

If Huggins' view is correct that steric repulsion of the β carbon determines the sense of the α-helix then all α-amino acid must form the same sense helix. However, if attractive forces are the main factor then each side chain can determine its preferred configuration. There is good evidence that not all polypeptides do have the same sense helix.[24a,b]

Other polypeptide structures

Very few calculations of optical rotation have been made for other polypeptide structures besides the α-helix. Murakami[25] has made calculations for oligopeptides of polyalanine. The random coil has not been explicitly considered. Zimm *et al.*[26] have used the continuous polarizability model of Fitts and Kirkwood[27] to show that the

[20] J. C. Kendrew in *Biophysical Science–A Study Program* (Edited by J. L. Oncley) p. 95. John Wiley, New York (1959). A personal communication from P. Doty has informed us that Kendrew has indeed found 70% right-handed helices in myoglobin.
[21] M. Huggins, *J. Amer. Chem. Soc.* **74**, 3963 (1952).
[22] J. Donohue, *Proc. Natl. Acad. Sci.* **39**, 470 (1953).
[23] K. S. Pitzer and E. Catalano, *J. Amer. Chem. Soc.* **78**, 4844 (1956).
[24a] E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.* **80**, 4631 (1958).
[24b] E. R. Blout, *Tetrahedron* **13**, 123 (1961).
[25] H. Murakami, *Bull. Chem. Soc. Japan* **27**, 246 (1954); **28**, 583 (1955).
[26] B. H. Zimm, P. Doty and K. Iso, *Proc. Natl. Acad. Sci.* **45**, 1601 (1959).
[27] D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **42**, 33 (1956).

optical rotation of a molecule containing short regions of helix and random coil is a linear function of the amount of each. Using the present, more realistic model of Fitts and Kirkwood[6] we find that the calculated optical rotation is a more slowly converging function of distance. Therefore more work is still needed to establish how many turns of a helix are essentially equivalent to an infinite helix.

Other types of helices and intermolecularly bonded structures have not been quantitatively considered although there is experimental evidence for the latter.

Conclusions

The goal of being able to determine the conformation of a polypeptide from its optical rotation has not yet been realized. There is a great deal of empirical knowledge about certain polypeptides, but the ability to generalize is not yet present. Current theories of optical rotation must be carefully tested; more calculations for polypeptides in various conformations and with various side chains are needed. For comparison we need measurements on simple polypeptides of known conformation. These measurements should be made in non-interacting solvents and particularly, at low wave lengths.

Note added in proof: An IBM 704 has been used to recalculate the results given as the solid lines in the figures. Numerical errors have thus been found in the original calculation (Ref. 7). The solid lines in Fig. 1 are incorrect; comparison of the new results with experiment do not lead to a choice of the sense of the helix. The remaining figures are not qualitatively changed by the recalculation.

Kendrew *et al.* [J. C. Kendrew, R. E. Dickerson, B. E. Strandberg, R. G. Hart and D. R. Davies, *Nature, Lond.* **185,** 422 (1960)] have conclusively found that polypeptides with the rotatory dispersion shown in Fig. 1 are right handed.

Acknowledgement—We wish to thank Mr. John Clark for making and photographing the molecular model shown in Figs. 4 and 6.