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LIQUID-CRYSTALLINE STRUCTURES IN POLYPEPTIDE SOLUTIONS

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INTRODUCTION

IN this paper the remarkable properties, including the very high optical rotatory power, of a liquid crystalline phase which is formed by certain polypeptide solutions will be discussed.

Although liquid crystals have attracted much interest since their discovery in 1888[1] (a recent review article contained as many as 475 references)[2] many physical chemists seem to be unfamiliar with the subject. It may therefore be helpful to state briefly what is meant by this rather unsatisfactory term.

Most crystals pass directly at their melting point from the crystalline state to an isotropic liquid, but many crystalline solids pass through one or more mesophases which, though birefringent, are more or less fluid. These mesophases have various degrees of organisation but most can be described by one or other of two terms introduced by Friedel: smectic and nematic.[3]

In the smectic structure the molecules are arranged in layers, which are themselves arranged in families of closed surfaces known as Dupin cyclides, which give rise to focal conics which are visible in a polarization microscope and can be used for the identification of such structures. These smectic structures and the microscopic patterns associated with them have been adequately described elsewhere.[3,4] The nematic structure, which is the only one of the two classes with which this paper is concerned, has a lower degree of organization that the smectic; there are no layers, but the molecules are arranged with their long axes parallel, while being free to move relative to one another in the direction of these axes. Friedel also described a third, though less familiar, type of structure, met with chiefly in esters and ethers of cholesterol, which is related to the nematic structure but which has a higher degree of organization. In what follows it will be found that these last have much in common with those which have been found to occur in certain polypeptide solutions.

A given material may form one or several mesophases each of which is stable between two well defined and reproducible transition temperatures and the temperature range over which any one is stable may cover a considerable number of degrees. These mesomorphic forms are not only produced on heating certain solids but with some substances (e.g. soaps, and the polypeptides to be discussed in what follows) on adding a limited amount of a suitable solvent. In these cases the mesophase may be stable over a considerable and reproducible range of *concentration*, which will, however, depend on the temperature. Such mesophases, whether formed by changes of temperature or addition of solvent, are generally called liquid crystals or liquid crystalline

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[1] F. Reinitzer, *Monatsh. Chem.* **9**, 421 (1888).
[2] G. H. Brown and W. G. Shaw, *Chem. Rev.* 57, 1050 (1957).
[3] G. Friedel, *Ann. Physique* **18**, 273 (1922).
[4] N. H. Hartshorne and A. Stuart, *Crystals and the Polarization Microscope* (3rd Ed.). London (1960).

structures. The term "liquid crystals" is somewhat inadequate and slightly misleading, but, as it is established by long usage, it will be used in this paper. It should be stressed that these mesophases are definite forms of matter, a knowledge of which is essential for the full understanding of any material which can give rise to them.

The liquid-crystalline phase in poly-γ-benzyl-L-glutamate

Solutions of poly-γ-benzyl-L-glutamate (PBLG) in certain solvents (e.g. dioxan, methylene chloride, chloroform, *m*-cresol) when above a certain concentration, A, separate into two liquid phases, the phase of higher concentration being spontaneously birefringent.[5,5a] If the concentration is above a still higher concentration, B, only the birefringent phase will exist. The values of A and B in a particular solvent depend on the molecular weight of the polymer, the birefringent phase appearing at a lower concentration with a higher molecular weight. The birefringent phase often first separates in the form of liquid spherulites, that is to say, birefringent liquid spheres showing a black maltese cross when observed between crossed polars.* On cooling or concentrating the solution these spherulites grow in size and eventually coalesce to form a continuous phase. Alternatively if the continuous phase is disturbed, long viscous streamers or filaments may separate from it which, as the disturbance ceases, break up into spherulites. The size and stability of the spherulites is very variable, depending as it does on numerous factors (density of solvent, interfacial tension, concentration, viscosity, purity, etc.).

The black maltese cross together with the sign of the birefringence indicate that the major refractive index, which here means the molecule, lies tangentially rather than radially. Much more remarkable, however, is that both the spherulite and the continuous birefringent phase show equi-spaced alternate dark and light lines (Fig. 1) which are visible even in natural light. The periodicity of these lines, S, depends on concentration, solvent, temperature, etc., but has a reproducible value, for any one set of conditions and may be as large as 100 μ or too small for the microscope to resolve. The periodicity is independent of the arrangement of the lines (e.g. whether parallel lines or concentric curves are observed), the optical system, or the shape of the vessel. Between crossed polars periodic retardation colours are seen, and, by using a quartz-wedge it can be shown that the retardation plotted against distance in a direction at right angles to the lines has an oscillating value and forms a smooth continuous curve.

Figs. 2 and 3 show a particularly large and regular spherulite. It will be seen that it shows one radial line of dislocation, or rather "disinclination", as Frank has called such lines in liquid crystals.[6] Each spherulite contains one, and only one, such radial line. The spherulites therefore, although fluid, have polarity. Similar lines. of disinclination are also to be seen in the continuous phase.

This description of the spherulites at once makes it apparent that we are concerned with a liquid crystalline structure of unusual interest. To understand the nature of this structure it will be simpler to consider the patterns that arise in a flat rectangular cell, say 1 cm square and 1 mm deep. After filling the cell families of equi-spaced lines gradually appear, those near the edge being parallel to the sides of the cell. Further

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* The word polar is used for either a polarizer or an analyser.

[5] C. Robinson, *Trans. Faraday Soc.* **52**, 571 (1955).

[5a] A. Elliott and E. J. Ambrose, *Disc. Faraday Soc.* **9**, 246 (1950).

[6] F. C. Frank, *Disc. Faraday Soc.* **25**, 29 (1958).

from the sides the cell becomes filled with groups of equidistant lines grouped in complicated and irregular patterns. These groups of lines are seen to undergo gradual rearrangement towards a simpler and more uniform pattern. Sometimes sudden rearrangements take place, the movements being somewhat similar to those of bubbles coalescing in a foam. As time goes on small, clear, polygonal areas appear which contain no lines but which are framed in continuous lines which lie parallel to the sides of the polygon (the corners of the polygon may be rounded and in some cases the whole of a side may be curved) (Figs. 4 and 5). These uniform areas tend to grow in size, neighbouring areas coalescing to form a larger area. The appearance of the parallel lines at once strongly suggests that we are looking at layers seen edge on, and, if we imagine the clear uniform areas to be layers lying parallel to the cover glass, the whole arrangement suggests groups of boxes, one inside the other, and each such group packed closely among its neighbours (Fig. 6). Actually, as we shall see, it is not necessary to assume the existence of such *layers* as distinct from *planes* in which the preferred orientation of the molecules is uniform.

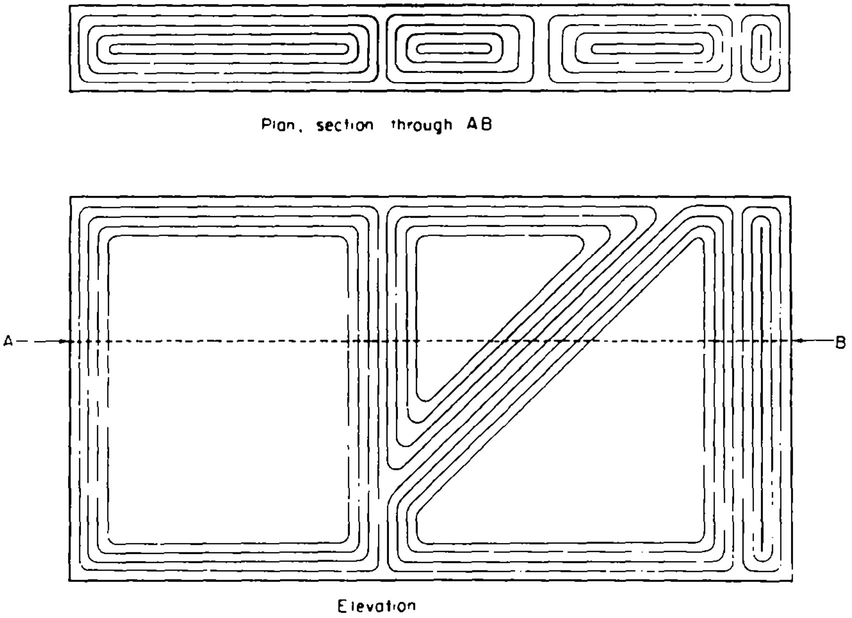

FIG. 6. An arrangement of layers in a cell which would result in the appearance of uniform areas.

The uniform areas when seen between crossed polars are generally brightly and uniformly coloured. On rotating the stage of the microscope this colour does not change, but if the analyser is rotated, the colour changes continuously. The colour, in fact, arises from a very high optical rotatory power with a corresponding optical dispersion, so that the extinction position for light of each colour differs considerably and consequently when using white light some colour is always subtracted whatever the position of the analyser. If the concentration was not too high, areas which were sufficiently large and uniform for quantitative observations were frequently obtained, but with higher concentration the experimental difficulties increased. With the specimens which gave these suitable areas the optical rotation could be measured correct to about 3° by using the polarization microscope as a polarimeter.

The structure in the liquid-crystalline phase

For the purpose of this general account of the phenomenon it will be convenient at this stage to describe the structure which we have put forward to explain these observations, rather than to give the detailed arguments which lead to its proposal and which can be found in the original papers.[6–8] Afterwards we shall consider the quantitative observations which confirm our belief in the existence of this structure.

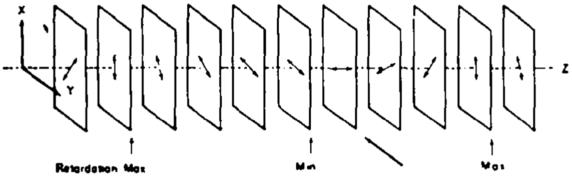

FIG. 7. Model of the twisted structure.

The structure may be looked upon as derived from a nematic structure by superimposing on it an axis of torsion of uniform pitch at right angles to the long axes of the molecules. Consequently if we consider planes at right angles to this axis, the preferred orientation of the molecules, though always at right angles to it, will change continuously along its length and the same orientation will be repeated at equal intervals. The model is shown in Fig. 7 where Z is the axis of torsion and X, Y, Z is a cartesian coordinate system. A number of equally spaced planes at right angles to Z and some arbitrary distance apart are shown . The long axes of the molecules lie in these planes and their orientation in each one is indicated by a double headed arrow. If a volume of solution is observed at right angles to the Z axis, maxima in the major refractive index and birefringence will be observed along parallel lines where the molecules are at right angles to the direction of observation and minima where the molecules are parallel to the direction of observation, so that the periodicity S, will equal half the pitch of the torsion. On the other hand, if the same volume is observed in a direction parallel to the axis of torsion, no periodicities will be seen, but a high degree of optical rotation will be observed. Although S is dependent on solvent, concentration and temperature the *structure* will be as shown in Fig. 6 in any solution in which these are uniform. However, the orientation of the structure may vary from place to place throughout the solution, and the three dimensional pattern thus produced may be called the *texture* of the solution (following the use of this word by Friedel[3] when describing liquid-crystals.) Thus we may have various *textures* but all will have the same *structure*. A special case is that of the spherulite where the nature of the texture arises from the spherical shape imposed by the interfacial tension. A very elegant explanation of the spherulitic pattern has been given by Pryce and Frank[8] and this is consistent with the structure of any small volume of the spherulite (small compared to the spherulitic pattern, but large enough to contain many molecules) being as shown in Fig. 7 except in the vicinity of the line of disinclination. Figs. 10 and 11 of reference 6 show two different textures, each of which has formed in a capillary.

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[7] C. Robinson and J. C. Ward, *Nature, Lond.* **180**, 1183 (1957).

[8] C. Robinson, J. C. Ward and R. B. Beevers, *Disc. Faraday Soc.* **25**, 29 (1958).

De Vries' theory of cholesteric liquid crystals

A similar twisted structure has been proposed by de Vries[9] to explain the optical rotatory power of some esters and ethers of cholesterol. These materials show a very high form-optical-rotation which is associated with periodicities, smaller than those we have so far described as occurring in the polypeptides, but which can be made visible as bright and dark lines by filling a wedge-shaped space in cleft mica with the substance. These have been interpreted by some authors, including Friedel, as intersections of planes (Grandjean planes) in the liquid crystal by the boundaries. De Vries derives an equation relating the optical rotatory power to the pitch of the torsion, the wavelength of the light and the birefringence of what may be called the untwisted medium. Data were not available for him to verify this relaionship for cholesteryl compounds, but the polypeptide solutions with their larger spacings, the size of which can be varied over a wide range by altering the concentration or the solvent, are more suitable for the necessary measurements. When the spacings in the polypeptide solutions are large enough, a simplified form of de Vries' relation may be used, as has been shown by Dr. Ward of this laboratory. The conditions under which this simplified equation holds may be explained as follow. (For a full understanding of de Vries' theory his original paper should be consulted).

A major difficulty in developing a theory of the twisted structure (Fig. 7) is that the dielectric "constants" are here a function of Z, the axis of torsion. De Vries deals with this by transforming to new axes ξ, η which twist round with the structure, so that in these axes the dielectric constants do not vary with Z. These are related to the stationary X and Y axes by

$$X = \xi \cos \frac{2\pi Z}{P} - \eta \sin \frac{2\pi Z}{P}$$

$$Y = \xi \sin \frac{2\pi Z}{P} + \eta \cos \frac{2\pi Z}{P}.$$

The formula which de Vries gives for the optical rotation is

$$\Theta \left(= \frac{d\psi}{dZ} \text{ in de Vries} \right) = - \frac{2\pi}{P} \frac{\alpha^2}{8\lambda'^2(1 - \lambda'^2)} \tag{1}$$

where $\Theta = \frac{d\psi}{dZ}$ is the optical rotation of a uniform area per unit thickness, $\alpha = \frac{\varepsilon_2 - \varepsilon_1}{2\varepsilon}$ (where ε_1 and ε_2 are the principal dielectric constants along the two axes ξ and η which twist round with the structure, $\varepsilon = \frac{1}{2}(\varepsilon_1 + \varepsilon_2)$, P is the pitch of the helix and $\lambda' = \frac{\lambda}{P\sqrt{\varepsilon}}$.

The two principal refractive indices of the untwisted material are then $n_1 = \sqrt{\varepsilon_1}$ and $n_2 = \sqrt{\varepsilon_2}$. Then the birefringence of what here will be called the untwisted material may be denoted by $n = n_2 - n_1$ (where $n_2 > n_1$) and its average refractive index, $\frac{1}{2}(n_1 + n_2)$, by N. In order to compare the theoretical conclusions with our experiments a relation between α and n is required.

$$\alpha = \frac{\varepsilon_2 - \varepsilon_1}{2\varepsilon} = \frac{n_2^2 - n_1^2}{n_2^2 + n_1^2} = \frac{2nN}{2N^2 + n^2/2}$$

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[9] H. de Vries, *Acta Cryst.* 4, 219 (1951).

In practice n is always small compared with N, so that $n^2/2$ may be neglected compared with $2N^2$, so we may write

$$\alpha = \frac{n}{N}$$

and hence de Vries' equation (1) may be written

$$\Theta = -\frac{2\pi n^2 \mathrm{P}}{8\lambda^2(1 - \lambda^2/\mathrm{P}^2N^2)} \tag{2}$$

When $\frac{\lambda^2}{\mathrm{P}^2N^2}$ is small compared with unity the formula reduces to

$$\Theta = -\frac{\pi n^2 \mathrm{P}}{4\lambda^2} \quad \text{radians/micron}$$

or

$$\Theta = \frac{-n^2\mathrm{P}}{\lambda^2} \times 4{\cdot}5 \times 10^4 \text{ degrees/micron} \tag{3}$$

where P and λ are in microns. The negative sign indicates that the optical rotation is in the opposite sense to that of the helical twist of the torsion. This is an important point since it means that the sense of the twist can be determined simply from the sign of the optical rotation. (This formula is identical with that derived by Maugin[10] for a pile of birefringent plates arranged helically by using the Poincaré sphere and rolling cone, to explain the optical rotation which is produced by rotating the coverglass over a nematic liquid crystal (azoxyphenetol) through an angle. His treatment neglects reflections at the interfaces between the plates and assumes that the thickness of the plates are large compared to the wavelength of light. The formula gives results which agree with experimental observations in cases where the pitch is low, but, as will be shown later, the more exact equation derived as in de Vries' theory by using the electro-magnetic theory and assuming a continuous twist is needed to explain the results when the pitch is small).

Verification of the simplified de Vries' equation

Even after considerable experience the formation of satisfactory uniform areas still remained largely a matter of chance, depending no doubt on factors which are difficult to control, such as the condition of the glass surface, and chance impurities which may act as nuclei. It was sometimes necessary to let the cell stand for several weeks before suitable areas appeared. The difficulties increased as the size of the periodicities decreased and it has not yet been found possible to obtain values of Θ corresponding to the lower values of S.

In general Θ was measured for six different wavelengths (λ = 436, 509, 546, 578, 589 and 644 mμ) in each experiment. Since the rotation was often considerably greater than 180°, to obtain the absolute value it was necessary to take readings on several areas of different thickness which had been allowed to form in a wedge-shaped cell (this procedure also showed that the rotation was proportional to the thickness, or very nearly so, which otherwise could not have been assumed). In all the solutions whose optical rotation has so far been measured, which had values of S lying between 5 and

[10] M. C. Maugin, *Bull. Soc. Fr. Min.* 34, 6, 71 (1911).

100 μ, on plotting Θ against $1/\lambda^2$ a straight line was obtained which cut the abscissa near the origin ($1/\lambda^2 \simeq 0{\cdot}5$, where λ is in μ). When this had been established it was found possible to dispense with the use of a wedge-shaped cell to determine the sign and the absolute value of the rotation. Fig. 8 shows the dependence of Θ upon $1/\lambda^2$ in a typical experiment.

Except in the higher concentrations, where the periodicities become difficult or impossible to resolve, S, that is P/2, can generally be measured without difficulty by direct microscopic observation (with methylene chloride however the structure seemed unstable over a considerable range of concentration within which consistent results were not obtained). Fig. 9 shows the dependence of S upon concentration for solutions in dioxan. Here approximately $S = k_1/C^2$ where k_1 is a constant and C is the concentration in ml/100 ml. A similar concentration dependence was found in the other solvents. No significant dependence upon molecular weight was found.

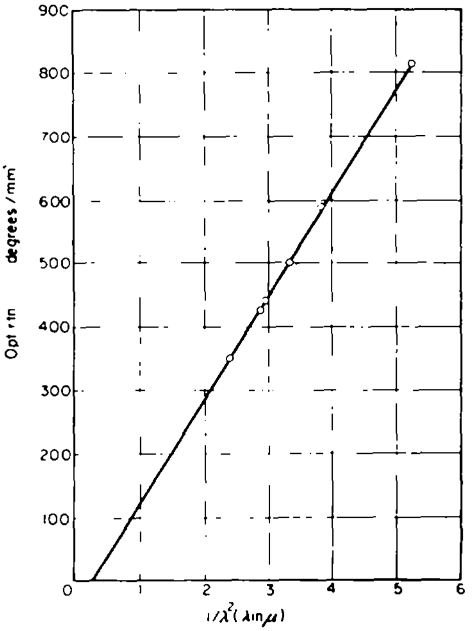

FIG. 8. Dependence of optical rotation on wavelength. PBLG in chloroform. 18 g/100 g. S = 25 μ.

Table 1 shows 15 results on several preparations of PBLG and PBDG covering a range of molecular weights, concentrations and solvents. R9 and R5 were specimens of PBLG in which the molecules contained approximately 670 and 270 residues respectively. D1 was a specimen of PBDG of similar, but undertermined, molecular weight. The addition of 10 per cent ethanol in three of the experiments not only reduced the size of the spacing but reduced the time required to obtain suitable uniform areas. In column 6 are given the values of n, the birefringence of the untwisted structure, calculated from the de Vries equation and divided by the volume fraction. All these results, except for a single result for meta-cresol and one obtained with chloroform and ethanol as solvent, fall within 0·212 and 0·308. (Calculations showed that using the simplified

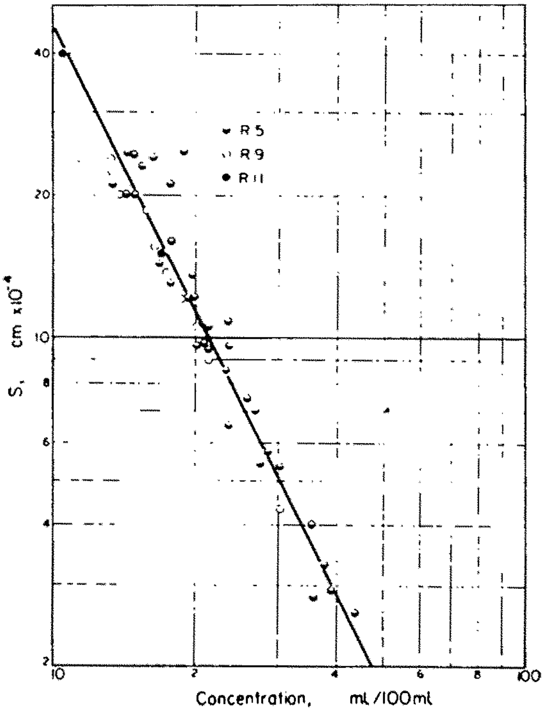

FIG. 9. Double logarithmic plot of S against concentration. PBLG in dioxan.

TABLE 1. BIREFRINGENCE OF THE UNTWISTED MEDIUM AS CALCULATED FROM THE OPTICAL ROTATION AND THE MICROSCOPIC SPACING

Solvent and polymer	Volume fraction	Spacing (μ)	$\frac{\text{Opt. rot.}}{\text{mm}}$	$\frac{\text{Opt. rot/mm}}{\text{vol. fracn.}}$	$\frac{\text{Calc. bir.}}{\text{vol. fracn.}}$
CH_2Cl_2, R9	0·114	100	−157	−1408	212
CH_2Cl_2, R5	0·238	24	−255	−708	277
CH_2Cl_2, *R5	0·184	20	92	383	260
$CHCl_3$ R5	0·276	25	499	1265	308
$CHCl_3$, R5	0·309	18	310	770	259
$CHCl_3$, *R5	0·275	9	92	257	236
$CHCl_3$, *R5	0·350	4·8	74	162	177
Dioxan, R5	0·140	20	59	329	235
Dioxan, R5	0·210	11	56	204	218
Dioxan, R5	0·274	6·5	75	210	250
Dioxan, R5	0·292	5	90	236	266
Dioxan, R9	0·251	6·4	82	251	284
Dioxan, D1	0·211	6·5	−58	−212	246
D.C.A, R9	0·330	11·8	158	370	239
				average	248
m-Cresol, R5	0·242	6·7	90	353	400

* = +10% Ethyl alcohol

rather than the exact de Vries equation would not be expected to introduce an error of more than 4 per cent in any of these cases.)

It remained to obtain an experimental value for n. The model proposed for the structure suggests that it might be possible to obtain this from maxima in the periodic retardation in the twisted structure. In favourable cases values could be obtained in this way, but, because of the small periodicity and other factors the results were very rough. However the values obtained fell within the limits of those calculated in Table 1. It should be remembered that without the de Vries relationship there would have been no way of forecasting even the order of magnitude of the optical rotation, so that even these rough values were of interest. A more reliable value, however, was obtained from a racemic mixture of the D and L enantiomorphs (e.g. a solution containing equal quantities of PBLG and PBDG). This forms a birefringent solution which show no periodicities, but properties characteristic of a nematic liquid-crystalline material, including the "threads" from which the name is derived. When such a solution containing 20·8 ml/100 ml of total polymer was introduced into a capillary it became oriented spontaneously and had a birefringence that was uniform throughout the capillary. The birefringence divided by the volume concentration was found to be 0·029 and this value was reproducible. In a flat cell, 1 mm deep, a value of 0·026 was given by discrete areas in the same solution. The birefringence divided by the concentration was then calculated from the values of Θ and S given by a solution of the same concentration containing PBLG only. This value was also 0·026. It seems, therefore, justifiable to assume that the nematic structure observed in the racemic mixture corresponds to the untwisted form of the structure formed in solutions of the optically active polypeptide. The good agreement between the calculated and observed values, therefore, not only show that de Vries equation holds for the structure proposed for PBLG, but demonstrates quantitatively that this may be looked upon as a twisted structure derived from the nematic structure, this twist arising from either the right- or left-handedness of an L or D enantiomorph. How much the scatter of the results in Table 1 is due to the value of n being dependent on concentration and solvent and how much due to experimental errors remain, a matter of conjecture. (In some of the experiments the determination of concentration introduced a considerable error.)

The phenomena when S *is small*

So far we have only considered cases where P is large enough to make λ^2/P^2N^2 small compared to unity, so that de Vries' equation may be written in the simplified form of equation (3). However we see from equation (2) that if P is progressively decreased so that λ^2/P^2N^2 passes through unity the optical rotation will change sign. This non-linear relationship and change of sign of the optical rotation in passing through a narrow band of wavelengths is what is found in the liquid crystals of the cholesteryl compounds and which is there associated with the small periodicity of the Grandjean planes. A very striking characteristic of these compounds is their property of reflecting bright iridescent colours when illuminated with white light. The colours are reflected in accordance with the Bragg equation

$$n\lambda = 2\,\mathrm{S}\sin\theta$$

where n is the order of the reflection, λ is the wavelength of the light, S is the distance between the planes at which reflection occurs and θ is the angle between the planes and the direction of the incident light. Since we are concerned with visible light and not X-rays, λ will here be the wavelength in the *medium*, that is to say the wavelength of the light *in vacuo* divided by the refractive index of the solution. (Compare Oster's treatment of iridescent bodies formed by tobacco mosaic virus.[11]) The value of S obtained from this relationship agrees with that determined by microscopic observation of the Grandjean planes. When $S = \lambda/2$, θ will be 90° and therefore visible light will be reflected normally when S is between approximately 0·4 and 0·2 μ. De Vries' theory of the twisted structure is successful in explaining these properties and also the fact (observed in the cholesteric liquid crystals) that, in the region of reflection, one circularly polarized component of the incident light is totally reflected while the other passes through unchanged and, further, that the rotation of the electric vector of the reflected light is, contrary to what is found in normal substances, in the opposite sense to that of the incident light.

If the twisted structure given by the polypeptide solutions only differs quantitatively from that found in the esters and ethers of cholesterol, one would expect the iridescent colours to be shown by the polypeptide solutions when the value of S was of suitable size. Iridescent colours had in fact been observed with solid films slowly evaporated from a solution of PBLG. Recently however the phenomenon has been found in solutions of poly-γ-ethyl-L-glutamate (PELG) in ethyl acetate where very small spacings can be obtained in solutions which are still sufficiently fluid to allow them to be transferred easily from one vessel to another.

These solutions, if the concentration is suitably adjusted, reflect brilliant colours which depend on the direction of the incident light and of observation. When such a solution is introduced into a capillary tube and is illuminated with a parallel beam of white light a continuous spectrum of colour is observed if the reflected light is allowed to fall on a white surface. If corresponding observations are made with monochromatic light it is found that each wavelength is reflected at the angle which would be expected if it is assumed that the solution contains reflecting planes which are all equally spaced and that groups of these planes are oriented at every angle to the incident light (the experiment in fact being the optical analogue of an X-ray powder diagram.) That the planes in which the molecules lie and have equal orientation are so oriented is to be expected from the microscopic observations on the PBLG solutions of higher periodicities. The nature of these reflections is well illustrated in the following experiment. PELG was dissolved in ethyl acetate to give a concentration of 29·0 g in 100 g of solution. The solution was then somewhat further concentrated until, on illuminating with white light a colour towards the red end of the spectrum was reflected back parallel to the direction of the incident light. The solution was mixed on a very slow stirrer until the reflected colours showed the concentration to be uniform (this takes some days due to the high viscosity, low diffusion coefficient and large dependence of S on the concentration) and was then introduced into a glass tube, 0·5 mm in diameter, which was afterwards sealed at both ends and immersed in a large thermostated beaker of water. When the tube was illuminated at 20° only first order reflections were seen. The angle ϕ which the reflected light made with the incident light was measured for six wavelengths. This could be read correct to ±2°. On raising the temperature to 30°, the value of ϕ increased considerably for each wavelength and some second order reflections appeared with lower values of ϕ. After making corresponding

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[11] G. Oster, *J. Gen. Physiol.* 33, 445 (1950).

observations at 40° the temperature was again reduced to 20° and the readings at this temperature repeated. θ, the angle which the incident light makes with the set of parallel planes from which reflection takes place, is given by $\theta = 90° - \phi/2$. λ was obtained by dividing λ_v, the wavelength *in vacuo*, by 1·4, the approximate refractive index of the solution. The values of S, calculated from the Bragg equation are given in Table 2. Within the experimental error, which arose chiefly from the crudeness of the optical system, the same value was obtained for each wavelength and also on returning to the original temperature. The value in this and similar sealed tubes was found to be unchanged after several weeks. It will be seen that the value of S nearly doubled on increasing the temperature by 20°. (Beevers in this laboratory had previously found by microscopic observation that in a solution of PBLG in dioxan S increased from about 8 to 13 μ between 25° and 45°. With cholesteryl compounds, according to Friedel,[3] the temperature coefficient of S is positive for some and negative for others.)

TABLE 2. S, CALCULATED FROM ϕ AT DIFFERENT TEMPERATURES

	λ_v (in mμ)						Average: all wavelengths
	436	480	509	546	589	644	
ϕ, in degrees at 20°	70	65	40	13	—	—	
ϕ, in degrees at 30°	115	110	95	95	90	70	
ϕ, in degrees at 40°	135	130	120	120	110	105	
ϕ, in degrees at 20°	70	55	20	15	—	—	
S × 10^2, in μ at 20°	19	19	19	20	—	—	19
S × 10^2, in μ at 30°	29	30	27	29	30	28	29
S × 10^2, in μ at 40°	41	41	36	39	37	38	39
S × 10^2, in μ at 20°	19	19	18	20	—	—	19

The optical rotation in the region where these reflected colours are obtained would be expected to show the reversal of sign observed with the cholesterol compounds. This had not up to the present been verified since uniform areas suitable for the observations have not been obtained in these highly viscous solutions. Nor, has it yet been possible to show that the reflected light is circularly polarized in the expected sense, since this requires the planes to be more uniformly oriented.

We may here point out an important difference between the nature of the optical rotation of normal substances and of cholesteric liquid crystals. While the more familiar cases of optical rotation, including that produced by the α-helix, have their origin in the selective *absorption* of one circularly polarized component of the light, the "form"-optical-rotation of the twisted structure in polypeptides and in esters and ethers of cholesterol originates in the selective *reflection* of one circularly-polarized component of the light. In the first class we have the Cotton effect for wavelengths in the neighbourhood of the absorption band, while in the second class iridescent colours are observed in the neighbourhood of the reflection band and there is a change of sign of the optical rotation on crossing this band. Further, while in the first class the wavelengths of the absorption band is determined by the chemical constitution of the material, in the second class the wavelengths of the *reflection* band is entirely determined by the pitch of the torsion in the twisted structure and the birefringence of the untwisted medium.

Since the specific rotation of the twisted structure in the PBLG solutions which were investigated was 20,000° to 140,000°, any contribution directly due to the α-helices could safely be neglected.

Reversal of the sense of the twist

It will be seen from Table 1 that while the form optical rotation given by the PBLG solution in most solvents was positive, in methylene chloride it was negative. This cannot be due to the solvent affecting the sign of the birefringence of the untwisted material, since *n* appears in the equation as its square. It would seem, therefore, if the equation is valid, that there must be a change in the sense of the twist of the torsion in passing from, say, dioxan to methylene chloride. That this is in fact the case was shown by observing solutions in a series of mixtures of these two solvents. Instead of the optical rotation, the more easily measured quantity, S, was determined. The results are shown in Fig. 10 where $\frac{1}{S}$ is plotted against the percentage of dioxan in mixtures of this and methylene chloride. For convenience, the value of S in methylene chloride is plotted as a positive value and that in dioxan as a negative value. It will be seen that the curve cuts the abscissa at 0·8 volume fraction of dioxan at which point S may be considered infinite. Observations in the immediate neighbourhood of this point showing no periodicities but only nematic threads, as would be expected with a completely untwisted structure. The twist therefore, becomes progressively looser as

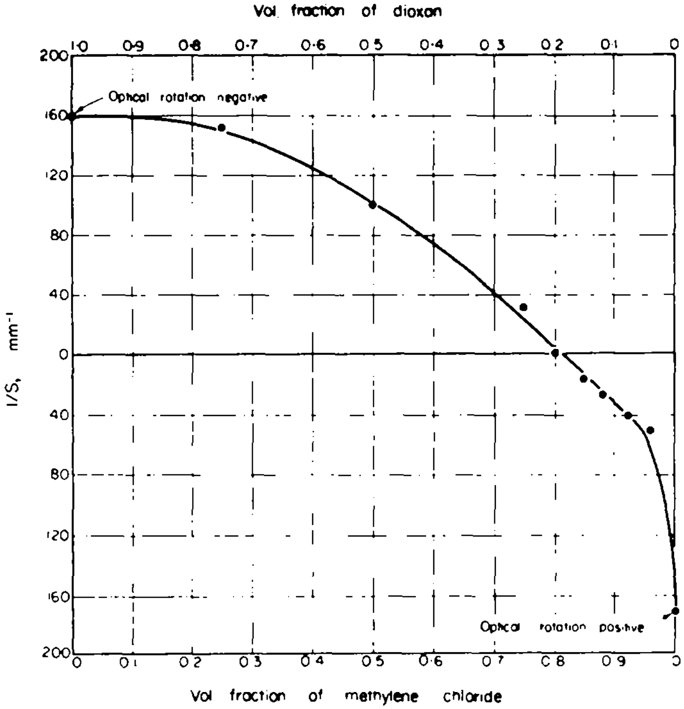

FIG. 10. Dependence of S on solvent composition for mixtures of methylene chloride and dioxan.

this point is approached from either side and, after it is reached, the structure again becomes progressively more highly twisted, but in the opposite sense.

These results show clearly that there is no simple relationship between the sense of the twist of the macrostructure and the sense of the α-helix since the latter is the same in both solvents, as is shown by the value of b_0 in the Moffit equation for the optical dispersion being much the same for each solvent.[12]

The molecular arrangement in the structure

The agreement of the results with the de Vries equation and other evidence which has been discussed here and in the original papers shows that we may have some confidence in the model correctly representing the distribution of molecular *orientations* within the structure. It remains to discuss how the *molecules* are distributed and how their distribution varies with concentration.

X-ray photographs of the birefringent phase showed a ring indicating a spacing, d, which increased continuously with concentration from 18 to 29 Å over a concentration range of 16·6 to 48·5 ml/100 ml. As the concentration increased this ring became less diffuse. If the preparations became oriented in the process of filling the capillaries which were used for the x-ray photographs, two more or less pronounced equatorial spots or arcs appeared on the ring. In no case, with the exposures used, were any other reflections observed which varied with concentration. In the liquid crystalline structure formed by tobacco mosaic virus (TMV) Bernal and Fankuchen[13] obtained in the more concentrated systems (their dried gels) four equatorial reflections corresponding to the spacings d, $d\sqrt{3}$, $d\sqrt{4}$, $d\sqrt{7}$, which is consistent with the long shaped particles forming a two-dimensional hexagonal array. On dilution the spacings increased in size but the relationship was maintained as long as the diminished intensity of the lower reflections allows them to be observed; thus in the *solutions* seldom more than the first two reflections were seen. Although only one such relection was observed in the PBLG, it still seemed reasonable to expect that the structure might be a similar two dimensional hexagonal array, with the addition of an axis of torsion at right angles to the long axes of the molecules (the higher reflections not being observed probably because of the greater fluidity of the system). The amount of torsion per molecule required to give the observed values of S is very small, so that its effect on the structure of a small region which is nevertheless large enough to give a sharp X-ray reflection, would be expected to be negligible.

The theoretical value of d for the hexagonal array may be calculated from the equation

$$C = \frac{W\sqrt{3} \times 10^{24}}{2Nd^2q\rho,} = \frac{1\cdot 6 \times 10^4}{d^2}$$

where C is the concentration in ml/100 ml; W = 219, the residual weight; N = Avogadro's number; d is the distance between the $(10\bar{1}0)$ planes in Å; q = 1·5 Å, the length of the projection of one residue on the axis of the α-helix; and $\rho = 1\cdot 3$, the assumed density of the polymer. In deriving this equation, dilution in a direction parallel to the long axes of the molecule has been neglected; such dilution will however be small owing to the high axial ratio of the molecules. In Fig. 11 the experimental

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[12] A. R. Downie, private communication.

[13] J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.* **25**, 111 (1941).

values are shown in a double logarithmic plot and the theoretical curve obtained from this equation is shown as a broken line. The agreement between the calculated and experimental values suggests that the structure consists of parallel rods having, in a plane at right angles to their length, something between a two dimensional hexagonal arrangement and a random arrangement, the arcs in the X-ray photographs showing, however, that the arrangement is far from random. Further the absolute value of d obtained shows beyond doubt that the structure is composed of discrete single molecules and not of complexes consisting of two or more molecules.

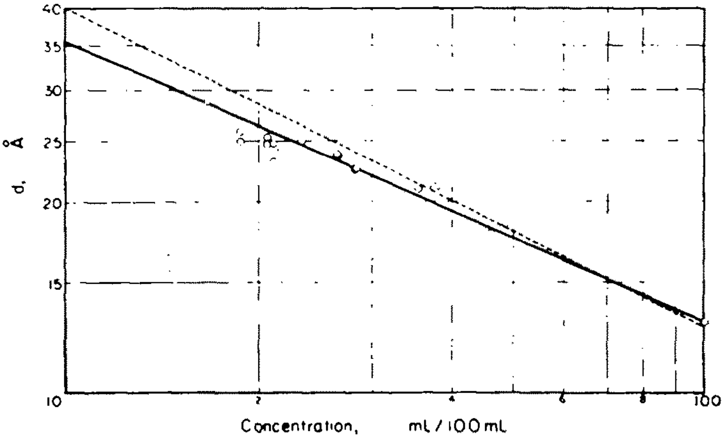

FIG. 11. Double logarithmic plot of d against concentration PBLG in dioxan.

It follows from these considerations that if we want to calculate the angle of twist per molecule in the Z direction and the distance between the molecules at different concentrations it will be instructive to base these calculations on the assumption of a two dimensional hexagonal array. The number of molecules which the Z axis passes through in a distance equal to the periodicity will be given by S/d and in the more dilute solutions of, for instance, PBLG in dioxan, this can be as high as 10,000. Since θ, the angle per molecule in the Z direction, is equal to $d/S \times 180°$, it will in this case be of the order of 1 minute. Since for the concentration of dioxan solutions which have been investigated $S \propto \frac{1}{C^2}$ and $d \propto \frac{1}{C^{1/2}}$, we see that $\theta \propto C^{3/2}$. So we find that the angle of deviation from the parallel position is not only very small even in the higher concentrations, but decreases on dilution. The polypeptide solutions which have been found to give the twisted structure all have their molecules in the α-helical configuration. Each molecule presents a helical arrangement of dipoles and, since all the helices in a solution are of the same sense, we may expect the intermoleuclar attractions arising out of the dipoles to impose a uniderectional twist on the array of parallel molecules.* The smallness of the angle and the fact that it decreases continuously on dilution suggests that it arises from a dynamic equilibrium, but it is at present not easy to see what factors determine its value.

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* The sense of the twist could conceivably be either the same as, or opposite to, the sense of that the α-helices from which the twisted structure is built up, since although in a left-handed helix the 1st, 2nd, 3rd, 4th, etc. residues form a left-handed helix, if we consider the 1st, 4th, 9th, 13th, etc. residues, they form a right-handed helix. This may partly explain the experimental observation that changing the solvent may alter the sense of the twist without altering the sense of the α-helix.

From models and the known dimensions of the α-helix it is found that the diameter of the cylinder described by the PBLG molecule with side-chains fully extended when rotated about its long axis is 28·2 Å. By subtracting this value from the intermolecular distance, $2D/\sqrt{3}$ we obtaine D, the distance of nearest approach of two side-chains in neighbouring parallel molecules at a given concentration.[8] This value of D for any given molecular weight will be greatest at the B point where the birefringent phase is at its lowest concentration. In the molecular weight range studied, it varied from 14·4 to −3·8 Å, the negative values showing that the side chains could overlap, in which case very slight movements of the side chain would bring adjacent parallel molecules into contact. But even where D has a positive value, since the length of the α-helix is always many times greater than D, the angle which its long axes must rotate through in order to make contact with a neighbouring molecule will always be very small (less than 1·5° in the cases investigated).[8] Very slight oscillations will consequently produce periodic contacts. It therefore does not seem necessary to assume that long range forces play a part in the formation of the structure, but, on the other hand, one can easily imagine how periodic contacts between the molecules in the model of Fig. 7 would result in a structure having the nature of a delicate three-dimensional network. Such a network would account for the fact that if a polypeptide solution containing visible periodicities is observed under the microscope, it is seen that the visible pattern shows elastic recovery under very slight shearing forces and that a slight but definite yield value must be exceeded before irreversible flow takes place.

The twisted structure in other systems

Qualitatively similar structures to those which have been described in detail for PBLG have been found in solutions of several other polypeptides in organic solvents, including PELG, PEDG and poly-γ-methyl-L-glutamate. In all these solutions the polypeptide molecules are known to form the α-helix configuration. This is evidently so even for solutions of PBLG in dichloracetic acid, for although Yang and Doty[14] found the random coil configuration in comparatively dilute isotropic solutions in this solvent, Downie *et al.*[15] found from the optical rotation that on heating a birefringent solution until it became isotropic that it was evidently in the α-helical configuration.

It has been shown[16,17] that poly-β-benzyl-L-aspartate forms an α-helix of the opposite sense to that of poly-γ-benzyl-L-glutamate. It was therefore of interest to find that a solution of a copolymer of equal proportions of β-benzyl-L-aspartic acid and γ-benzyl D-glutamic acid in chloroform, formed the twisted structure.

It seems probable that the rigidity of the molecule when in the α-helix configuration is an important factor in the formation of the birefringent phase, while the arrangement of the dipoles in a left- or right-handed helix gives rise to the twist characteristic of the structure. Robinson *et al.*[8] suggested that it might be possible to find in biological systems similar twisted structures which had previously been overlooked. Since then, Robinson (unpublished) has examined concentrated aqueous solutions of deoxyribonucleic acid [DNA) where again we have long-shaped, optically active, helical molecules. A solution containing about 6 per cent of DNA dissolved in 0·1 M NaCl

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[14] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).

[15] A. R. Downie, A. Elliot, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* A **242**, 325 (1957).

[16] A. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).

[17] E. M. Bradbury, A. R. Downie, A. Elliott and W. E. Hanby, *Nature, Lond.* **187**, 321 (1960); *Proc. Roy. Soc.* A **259**, 110 (1960).

(the solution was diluted under microscopic conditions, and the concentration was only very roughly estimated) when introduced into a cell 0·1 mm deep showed periodic lines after standing for several days. These lines (Fig. 12), whether observed in natural light or between crossed polars, resembled those in the polypeptide solutions. The value of S was in the neighbourhood of 1μ but increased appreciable on dilution. The concentration range over which these structures were observed was, however, small compared to that for the polypeptide solutions. So far it has not been possible to obtain uniform areas in a cell of this thickness, but in a 0·01 mm cell, areas which contained no lines but which were surrounded by periodic lines appeared (Fig. 13). Although the general appearance of these areas suggested that they were the uniform areas of the twisted structure, they were almost black between cross polars and the optical rotation, if any, was too small to measure, which would, however, be expected in a cell of this thickness unless the rotation was more than about 200° or 300° per millimetre of solution.

These experiments with DNA were of a preliminary nature and it is possible that the material was partly denatured when the structures described were observed. It seems nevertheless very probable that we were observing another example of the twisted structure, although this well not be proved until measurements of the optical rotatory power are obtained. It would however be of considerable interest if the existence of the twisted phase in biological systems could be substantiated. It is tempting to think that this highly organized liquid-crystalline phase, which can dissolve other components without its qualitative nature being changed, may be found to play a part in providing orientation in chemical reactions involving some of the highly specific, optically active molecules found in nature.

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