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The Cholesteric Phase in Polypeptide Solutions and Biological Structures

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Introduction

In previous papers[1–5] the remarkable properties of a liquid birefringent phase that separates from certain solutions of polypeptides in organic solvents when a limiting concentration is exceeded have been described. This phase, first observed by Elliott and Ambrose[1] in solutions of poly-γ-benzyl-L-glutamate (PBLG) shows microscopically visible periodicities and a very high optical rotatory power.[2] The order of magnitude of the optical rotatory power is comparable to that exhibited by the well-known cholesteric liquid crystals[21, 22] formed by esters and ethers of cholesterol, and which also show visible periodicities (the Grandjean layers) although these are smaller than the periodicities in the PBLG solutions.

The optical rotation and the periodicities indicate a helical arrangement of the molecules in these two systems, the observed periodicity being equal to half the pitch of the helix. De Vries developed a theory, based on such a helical arrangement, to explain the optical properties of the cholesterol compounds and derived an equation relating the optical rotation to the pitch of the helix. Data from the cholesterol compounds were not available to allow him to verify this equation, but it has been possible to show that the equation holds[3, 5] for the PBLG solution where the periodicity is comparatively high. This agreement with his theory for these conditions made it reasonable to expect that if similar polypeptide solutions in which the periodicity was comparable to the wavelength of light could be prepared that these would show the "cholesteric" colours and selective reflection of circularly polarized light charac-

teristic of the cholesterol compounds when the periodicities are of this size. Later, the expected cholesteric colours were observed in solutions of poly-γ-ethyl-L-glutamate, but the circular polarization of the reflected light was not detected.[5]

Since the last of these publications the circular polarization has been observed with polypeptide solutions, so that all the optical properties of cholesteric liquid crystals have now been observed in this liquid birefringent phase of polypeptide solutions. It is therefore justified to call this the cholesteric phase of polypeptide solutions.

The polypeptide solutions differ from the cholesterol compounds in being two-component systems. As a consequence the birefringent phase can exist in equilibrium with a more dilute isotropic phase; and when the former is dispersed in the latter spherulites are formed having a characteristic pattern which is diagnostic of the twisted structure. The mechanism of the phase separation can be largely understood from Flory's theoretical treatment of suspension of rigid rods.[9]

In the present paper these new observations will be described and the properties of this cholesteric phase in polypeptide solutions will be summarized so that the significance of similar properties which have been detected in solutions of DNA, transfer RNA and the colour of certain scaleless beetles may be appreciated.

The Occurrence and Appearance of the Birefringent Phase

The birefringent phase has been observed in solutions of several polypeptides in solvents in which the polypeptide is known to retain the α-helical conformation. These polypeptides include poly-γ-benzyl-L-glutamate (PBLG), poly-γ-methyl-L-glutamate, poly-γ-ethyl-L-glutamate (PELG) and poly-β-benzyl-L-aspartate (PBLA). In all these solutions the birefringent phase begins to separate when a limiting concentration, A, is exceeded, and is the only phase present when a somewhat higher concentration, B, is exceeded. (These concentrations will be referred to later as the A and B points.) Between these two concentrations the isotropic

and the birefringent phases exist in equilibrium, the isotropic phase having the concentration A, and the birefringent phase the concentration B. The values of A and B are dependent on the axial ratio of the polypeptide, but are relatively independent of the solvent.

In all these birefrigent solutions the rod like polypeptide molecules assume a characteristic arrangement which gives rise to either microscopically visible periodicities, or, where the periodicities are too small to be visible in the microscope, iridescent colours, similar to those shown by cholesteric liquid crystals. The size of the periodicity, S, depends on concentration, solvent and temperature, but is relatively independent of molecular weight. The dependence of S on these and other factors has been most studied in PBLG solutions,[4] while the iridescent colours have been investigated in PELG solutions.[5]

When a concentration of (for example) PBLG in dioxan is observed at any concentration between the A and B points some of the birefringent phase may be seen dispersed in the isotropic phase as spherulites (Fig. 1). In both the spherulite and the undispersed birefringent phase, parallel, equidistant alternately dark and light bands are visible even in natural light. S, the periodic distance between these lines is proportional to approximately $1/C^2$, where C is the concentration of the birefrigent phase. Values of S from 2 to 100 μ have been observed microscopically. Between the A and B points, that is at concentrations where spherulites may be observed, S remains constant, since the concentration of the birefringent phase is constant. S is found to be independent of the shape of the vessel containing the solution, the thickness of the solution observed, the wavelengths of the light used, and the "texture" of the birefringent solution.

Between crossed polars, the polarization colours show local retardation maxima at the centre of each band (Fig. 2). Examination with a compensator shows that the retardation oscillates through a series of local maxima and minima[2] along a line at right angles to these bands.

The birefringent solution is quite fluid, and if it is allowed to flow the bands will tend to be oriented parallel to their direction of flow.

After flow has ceased there will be a slow reorientation of the structure, until some more or less stable arrangement is reached, which will depend in part on the shape of the cell into which the solutions have been introduced. If a flat circular or rectangular cell, having uniform depth of about 0.5 mm is filled with the birefringent phase and sealed to prevent solvent evaporation, it will at first be noticed that the bands near the walls of the cell will become parallel to them. After some time, which may be hours or even days,

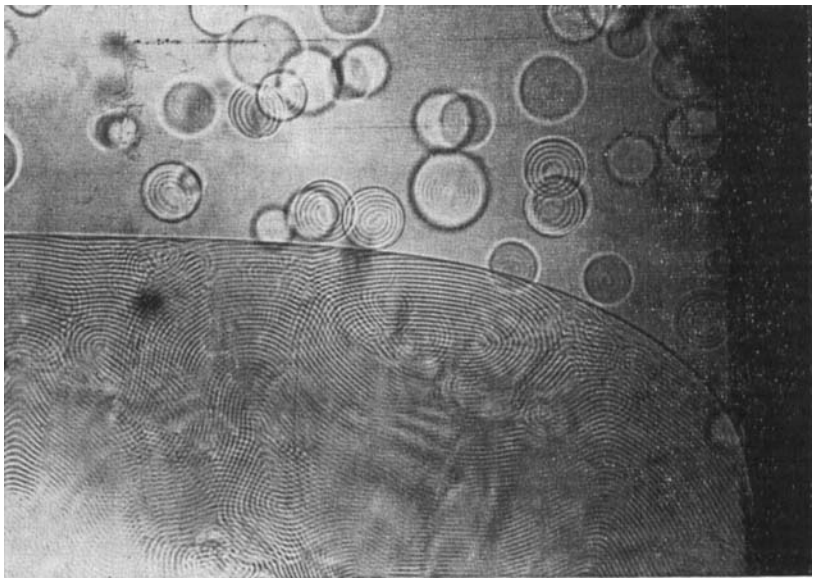

Figure 1. Two phase solution of PBLG in dioxan; $S = 14$. Natural light.

areas appear nearer the centre of the cell in which no parallel bands are seen and which have a uniform colour between crossed polars. The colour of these "uniform areas" does not change on rotating the microscope stage, but does change continuously on rotating the analyser, showing that it arises from the dispersion of the high optical rotation produced by these areas. These areas grow in size and tend to coalesce, producing polygonal areas which are surrounded by the periodic bands parallel to their sides (Fig. 2). The optical

rotation of the uniform area can be determined if the polarization microscope is used as a polarimeter, and specific rotations of from 20,000 to 140,000° have been measured. (The rotation is therefore so high that the contribution from the ordinary rotation of the polypeptide solution may be neglected.) This high optical rotatory power is at once reminiscent of that given by the homeotropic texture of cholesteric liquid crystals.

The appearance of the cell at this stage suggests sets of transparent boxes packed one inside the other like the familiar child's toys, those in each set having the same-sized sections, while each set is so shaped that the various sets may be packed together, like the pieces of a jig-saw puzzle, to fill the whole cell. (See Figs. 12 and 14 of Ref. 2). In this conception the parallel bands represent the sides of the boxes seen edge on, while the uniform areas represent the section of the smallest box in a particular set seen through the transparent lids and bottoms of the larger boxes in that set.

Superficial examination, therefore, suggests we are looking at an arrangement of parallel equidistant layers. Further, the sign of the birefringence, together with the fact that the highest refractive index of PBLG is parallel to the long axes of the molecules, shows that their long axes lie in, or nearly in, these "layers". However, if observations are made using a single polar only, either as analyser or polarizer, these portions of the bands which are normal to the electric vector are no longer visible, showing that their visibility in natural light or between crossed polars is due to differences in the orientation of the molecules rather than in their concentration. Thus, there is no evidence of layers in the sense of those in smectic liquid crystals, and when we see a line of constant retardation we are looking at a plane, edge on, in which the long axes of the molecules lie, and in which they have a certain preferred orientation.

These observations may be explained if the rod-shaped polypeptide molecules are assumed to be arranged as follows. In any small element of volume (except in the immediate neighbourhood of the lines of disinclination, which will be referred to later) the long axes of the molecules are parallel, or approximately parallel, but superimposed on this arrangement, is an axis of torsion having a

uniform pitch, large compared to the distance between the molecules, and which is at right angles to the long axes of the molecules. The preferred orientation of the molecules, while always remaining at right angles to this axis, changes continuously along its length in such a way as to be repeated at intervals equal to half the pitch. The molecular arrangement is therefore helecoidal. (Thus, if from a number of points along this axis, lines of equal length are drawn perpendicular to it, each in the direction of the preferred orientation at that point, then the ends of these lines will all lie on a helix.)

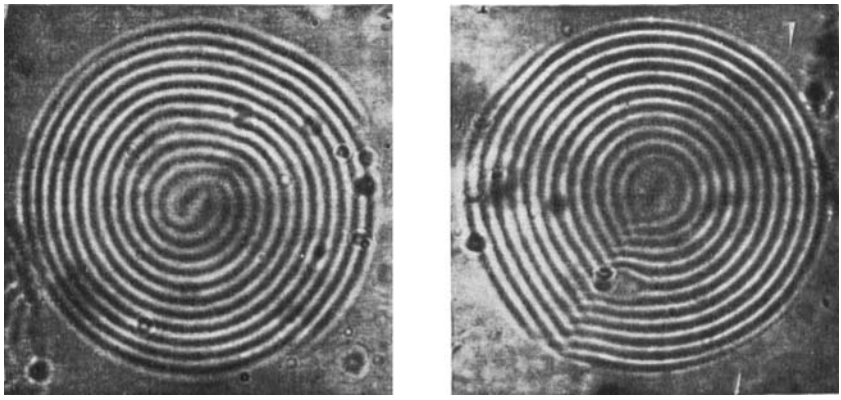

Figure 3. Two views of the same spherulite in natural light: (a) showing double spiral; (b) showing radial line of disinclination. (The photographs were processed so as to greatly increase the contrast.)

Let us consider two special cases. Firstly, an arrangement where the axes of torsion are everywhere parallel (as in the uniform areas, or in a region where straight equidistant bands are seen), and secondly, in the spherulite structure (Fig. 3), where the axes of torsion are radial.

In the first case, where a uniform area is seen we are looking in a direction parallel to the axis of torsion, and this torsion accounts for the observed optical rotation. On the other hand, where straight parallel bands are seen, with an oscillating value of the retardation along a line at right angles to them, the axis of torsion is at right angles to the direction of observation and to the bands. Hence the

observed retardation changes continuously along the direction of the axis and local maxima and minima are observed at intervals equal to half the pitch of the torsion, where the preferred orientation is either at right angles or parallel to the direction of observation.

In the second case, it is necessary to explain the double spiral of equispaced convolutions and the single radial "fault" (or line of *disinclination*, as Frank[6] has more accurately named it in analogy with *dislocations* in crystals) which are observed in these spherulites. Here the molecules are arranged on concentric spherical surfaces instead of parallel planes. This has been explained by Pryce and Frank.[7] Their explanation is briefly as follows:

Consider a succession of concentric spheres numbered $n = 1, 2, 3 \ldots$, of radius $r_0 + nc$, each sphere having a singular point P lying on the same common radius OP. If we consider the family of circles and one great circle on one of these spheres, all tangential to a line PQ, itself tangential to the sphere at P, then the molecules on that spheres are everywhere arranged parallel to the direction of these circles. A similar family of circles may be constructed on the other sphere of the family, but for each sphere the point P moves out along the radius to OP and the line PQ' to which the circles on each sphere are tangential is inclined so as to make an angle $n\alpha$ with the line PQ. The molecules on each concentric sphere are arranged parallel to the directions of the appropriate family of circles. Then every molecule is nearly parallel to its neighbours on the same sphere (except near to the singular radius OP, which corresponds to the observed line of disinclination), and will be inclined at an angle α to those on the same radius in neighbouring shells. Pryce and Frank also point out that on any section of the model, except through the singular radius, the locus of the line on which the molecule makes any given constant angle to the plane of the section is a double spiral.

In any considerable volume of the birefringent phase which is observed, the arrangement will in general be more complicated than in either of these two cases, since the direction of the axis of torsion will change from place to place, but the basic structure will remain as described. Continuity of the structure between lengths of

straight parallel bands (i.e. at the sides of uniform areas or of the rectangular cell) is maintained by lengths of curved parallel bands, while some lines of disinclination are also present which, as in the spherulites, are necessary for the continuity of the structure in the three dimensional arrangement.

The structure of these solutions may therefore be looked upon as derived from a nematic liquid crystalline arrangement by imposing on it an axis of torsion at right angles to the long axis of the solute molecules; and since the pitch of the helix is large compared to the distance between the molecules, the molecules in any small unit of volume will be nearly parallel. Confirmation of this model is obtained as follows:

(1) X-ray diffraction shows that the molecules are approximately parallel in any small volume.
(2) The concentration at which phase separation takes place is given by Flory's calculations for a suspension of parallel rods.
(3) In suitable solvent mixtures the structure untwists to give a nematic structure.
(4) When the pitch is large compared to the wavelength of light, the value of the optical rotatory power agrees with that calculated from a theory of de Vries which he based on essentially the same model in order to explain the properties of cholesteric liquid-crystals.
(5) The properties predicted by de Vries' theory, when the pitch is small, are in fact found in PELG solutions.

The evidence from these five sources will be discussed in what follows.

X-ray Evidence

That the polypeptide molecules are approximately parallel in any small unit of volume is shown by X-ray observations. The X-ray pictures showed only a single ring, the diameter of which decreased with increasing concentration, but the diameter of the ring was consistent with the rod-shaped molecules being arranged parallel either in a two-dimensional hexagonal array or in a rather more random two-dimensional array.[4]

Phase Separation

The polypeptides in which this twisted structure has been observed are all in the α-helical conformation. The molecules therefore may be looked upon as rods having considerable rigidity, since any appreciable bending of the helix involves the breaking of at least three hydrogen bonds. Flory[8] has pointed out that the formation of an orderly phase in which the molecules are approximately parallel is to be expected from a solution of rigid rod-like molecules when a certain concentration is exceeded, since a random distribution of molecules is uneconomical of space. He showed by a statistical-mechanical approach that, where the interaction between the solute molecules can be neglected, the concentration at which phase separation occurs is given approximately by

$$V = 8/x(1 - 2/x)$$

where x is the axial ratio of the molecule. Flory[9] determined the axial ratio of the samples of PBLG, for which we had determined the A and B points, from the degrees of polymerization and the known dimensions of the α-helix and calculated the A and B points which would be expected from his theory.

The calculated and experimental results are shown for comparison in Fig. 4. Flory remarks that "the correspondence between theory and experiment is remarkably good, bearing in mind the idealizations employed and the probable latitude of inaccuracy in the experiments". Although for the lower axial ratios the experimentally determined values show a considerable difference from Flory's calculated values (a discrepancy which Flory suggests may be due to the polydispersity of the samples used), for high axial ratio the agreement is good. On the other hand, the ratio of the concentrations of the two phases for a given axial ratio are in good agreement with theory, and further, extrapolation of the experimental values indicates that phase separation would not take place with axial ratio of less than 6 or 8, which is also in agreement with theory.

More recently Flory and Leonard[10] have measured the dependence of solvent vapour pressure against volume fraction of solvent,

ϕ_1, in solutions of PBLG in pyridine and in 1,2-dichloroethane, and of poly-γ-benzyl-L-aspartate (PBLA) in chloroform, for values of ϕ_1, from 0 to 0.4. In all these cases the solvent activities are insensitive to the molecular weight of the polymer and increase *smoothly* with solvent concentration. The latter observation strongly

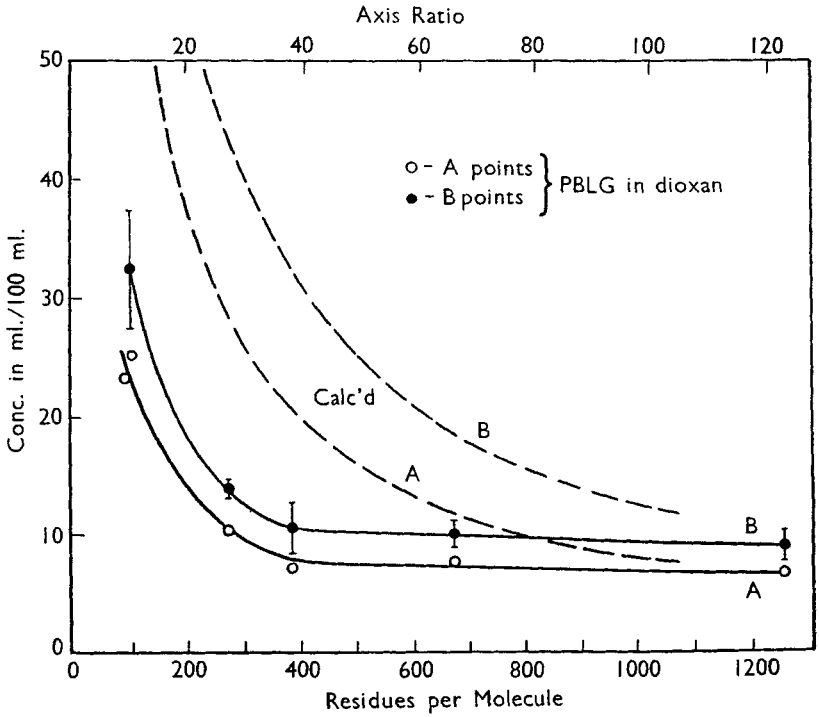

Figure 4. *A* and *B* as calculated by Flory (broken curves), and as determined by Robinson and coworkers (solid curves), plotted against the axis ratio. (Reproduced from Ref. 9.)

suggests that the α-helical conformation persists throughout this concentration range, contrary to the conclusion of Luzatti and coworkers[11] that with PBLG on dilution exceeding $\phi_1 = 0.6$ the α-helix is transformed into the 3/10 helix. The Henry's law slope of the curve at $\phi_1 = 0$ is much greater than that predicted by Flory's theoretical treatment of a system of rigid rods, but Flory and

Leonard showed both this and the general shape of the curves could be accounted for by a modified theory in which it is recognized that the polypeptide molecule has an outer "soft shell" composed of the flexible side chains and that the initial process of solution is exclusively mixing of the solvent in the side chain domain. The driving force for this mixing is primarily entropic and results in a repulsive force between the solute molecules. This conception, Flory and Leonard emphasize, is of importance in understanding the solubility of polypeptides. Thus, it would seem that PBLG and PBLA can dissolve as α-helices in a variety of solvents as a result of their bulky side chains (amounting to some two-thirds of the volume in the case of PBLG), while the shorter flexible side chains of poly-L-phenylalanine and poly-L-leucine account for these polypeptides being for the most part insoluble except in those solvents which cause disruption of the α-helix.

It is therefore interesting to note that while PBLG and PBLA have respectively six and five flexible links in their side chains, the other polypeptides, PELG and PMLG, which we have described as giving the cholesteric structure, have six and five links respectively. There seems little doubt that these considerations of Flory and Leonard will be useful in indicating other molecules which might be expected to give lyotropic cholesteric phases.

It is seen therefore that the predominant factor in determining the phase separation is a purely geometrical one. Flory does not discuss how the twist in the polypeptide structure arises but it would seem clear that this superimposed effect arises out of the asymmetric distribution of the dipoles on the α-helices producing a force between the molecules which tends to force them slightly out of the parallel position. But it follows from the considerations leading to his equation that the resulting arrangement must still be consistent with the need to fill the space available.

The de Vries' Theory of Cholesteric Liquid Crystals

In 1915 de Vries,[12] to explain the properties of cholesteric liquid crystals, proposed a twisted structure that is essentially the same as the one we have put forward for the birefringent phase formed by

polypeptide solutions. He derived an equation which Ward has shown may be rewritten

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

where Θ is the optical rotation per unit thickness, n is the birefringence of what may be called the untwisted material, N is its average refractive index, P is the pitch of the helix in microns, and λ the wavelength of light *in vacuo* in microns.

In the results here discussed we are concerned with two special cases. Firstly, where λ^2/P^2N^2 is small compared to unity the equation reduces to

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

or

$$\Theta = -\frac{n^2 P}{\lambda^2} \times 4.5 \times 10^4 \text{ degrees/micron} \tag{2}$$

Since Θ and P can be directly measured, it should be possible to verify the validity of the equation for these polypeptide solutions if the corresponding untwisted structure can be shown to exist and its birefringence measured to give the value of n. The negative sign of the equation shows that the optical rotation is of opposite sense to the twist of the helical torsion, but the fact that n appears in the equation as its square means that where, as here, the rotation is proportional to λ, a change in sign of the optical rotation can only be caused by a change in the sense of the twist. Consequently, since the optical rotation of a birefringent solution of PBLG in dioxan was found to be negative, and that of its solution in methylene chloride positive, the sense of the twist in these solutions must be opposite. It follows that there must be some mixture of these two solvents in which the structure is untwisted and in which the molecules are arranged parallel without the superimposed twist. This, in fact, was found to be so in a solution of PBLG in a mixture of dioxan and methylene chloride in which the volume of concentration of dioxan was 0.2. As this concentration was approached from either side, S was observed to increase continuously until its size

made it difficult to measure, but the value at which it became infinite could be accurately determined by interpolation after plotting $1/S$ against the volume concentration.[5] The solution of this composition, instead of the periodic spacings, showed the characteristic "threads" of a nematic liquid crystal. This was therefore the untwisted structure and a value for n was obtained by measuring its birefringence.

A solution of similar appearance was obtained by mixing equal proportions of PBLG and PBDG, to give a total volume fraction of polypeptide of 0.208. In a cell of uniform thickness this gave discreet areas of orientation in which the major refractive index lay parallel to the glass. The birefringence of these areas was found to be 0.026.

This was then compared to a solution containing the same volume fraction (0.208) of PBLG only. The optical rotation of the uniform areas of this solution was measured for five different wavelengths, and found to be inversely proportional to λ. The value S was also determined and the calculated value of n was then found to be 0.025. The experimental error was perhaps as large as 10%, but the good agreement leaves little doubt of the validity of de Vries' equation.

The preparation of specimens having large enough uniform areas suitable for measuring the optical rotation was difficult and there were many failures. However, measurement of the optical rotation, with corresponding measurement of S, was made on fourteen solutions of PBLG and one of PBDG, having several concentrations in several different solvents, and using PBLG of two different molecular weights. All the measurements were made in a room thermostated at 25° C. In all cases the optical rotation was inversely proportional to λ throughout the visible spectrum. Direct measurements of n on a racemic solution of poly-γ-benzyl-glutamate were not made except in the case of dioxan, since no considerable amount of PBDG was available, but n/v_1 where v is the volume fraction of polymer was calculated from the equation for each solution. Fourteen of these results were within $\pm$ 20% of a mean value of 0.0248 (the fifteenth result was 0.0400 for a solution

of PBLG in m-cresol) although the specific rotation varied from 20,000° to 140,000° and the microscopic spacing from 5 to 100 μ. The six values of n obtained for solutions in dioxan of volumes fraction from 0.140 to 0.292 were all within 12% of a mean value of 0.0248.

The results, therefore, provide convincing evidence of the validity of de Vries' equation. It should be remembered that, without the equation, even the order of magnitude of the optical rotation could not have been foreseen. No significant dependence on either concentration or degree of polymerization can be deduced from these few results. Calculation showed that the error introduced by using the simplified form of de Vries' equation instead of his exact expression is less than 4% in all cases and less than 1% in all.

The Condition when S is Small

It can also be seen from de Vries' theory that although there are no layers in the sense of those found is smectic liquid crystals, that when S is comparable to the wavelength of the incident light there will be a kind of Bragg reflection, so that for the first order reflections

$$\frac{\lambda}{N} = 2\sin\theta = P\sin\theta \tag{3}$$

where θ is the angle made by the incident or reflected light with the planes at right angles to the axis of torsion in which the long axes of the molecules lie. Therefore, when $\theta = 90°$,

$$\frac{\lambda}{N} = P \tag{4}$$

so λ^2/P^2N^2 in Eq. (1) passes through unity at this point and the optical rotation consequently changes sign. This is the second special case referred to in the previous section.

De Vries shows that for a band of wavelengths in this region the reflected light will be circularly polarized, one circular component of the incident light being reflected and the other passing through unchanged. The sense of the reflected component depends on the sense of the twist of the torsion. If the incident light is circularly polarized in this sense the reflected light will be circularly polarized

in the same sense, in contrast to reflection of circularly polarized light from ordinary surfaces, where the sense of the circular polarization is always reversed.

These properties are all found in the cholesteric liquid crystals which de Vries' theory was designed to explain; in these the brilliant "cholesteric" colours are reflected at the Bragg angle and the distance between the Grandjean planes takes the place of S in the polypeptide solutions. It was therefore to be expected that since the observations with polypeptide solutions having large values of P were consistent with the theory, that these additional properties would be found in polypeptide solution if sufficiently small values of P could be obtained.

Although S decreases rapidly with concentration no solution of PBLG was obtained in which S was less than about $2\,\mu$, since further concentration resulted in opaque solutions and in no case were iridescent reflections observed with this polypeptide (except in one case where a solution had dried to a solid glass by very slow evaporation in a small stoppered flask over a period of months). More recently Dr. J. Watson prepared solutions of poly-γ-ethyl-L-glutamate (PELG) in ethyl acetate which showed brilliant iridescent colours when suitably concentrated. One of the solutions, containing 29.0 g/100 g, and showing considerable iridescence, was further concentrated until a colour near the red end of the spectrum was reflected back parallel to the incident white light. After evaporation the resulting viscous solution was thoroughly mixed by slow and prolonged rotation of the flask, until the uniformity of the colours showed the solution to be homogeneous. (The long time found to be required was instructive in showing the difficulty in reaching homogeneity in concentrated polymer solutions.)

This solution was then introduced into a glass tube of 0.5 mm uniform internal diameter, which after sealing at both ends was placed in a large thermostated beaker of water. When the tube was then illuminated with a parallel beam of white light a continuous spectrum of colour was seen if the reflected light was allowed to fall on a white surface (Fig. 5).

If the temperature of the beaker was raised, the angle, ϕ which

the reflected light makes with the incident light increased, so that the whole spectrum moved round to a new position, but returned to the original position when the original temperature was restored.

Measurements of ϕ were made at several wavelengths at 20°, 30° and 40° C, and finally repeated at 20° C. The results were consistent with the solution containing equidistant reflecting planes and with there being groups of these reflecting planes oriented at every angle to the incident light, so that the light was reflected at the Bragg angle and the experiment was the optical analogue of an X-ray powder diagram. Assuming that the reflections are in accordance with Eq. (3), where $N = 1.4$ (the approximate refractive index of the solution), $\theta = 90° - \phi/2$.

TABLE 1 S Calculated from ϕ, Determined at Different Temperatures

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

The observed values of ϕ and the calculated values of S are shown in Table 1. The value of S increased from 0.19 μ at 20°C to 0.39 μ at 40° C, the results being independent of λ. This effect of temperature is comparable to that on the microscopic spacings in a solution of PBLG in dioxan, which Bevers[4] found increased from 8 to 13 μ between 25°C and 45° C.

The results were found to be reproducible when the experiment was repeated with the same sealed tube after several months.

It follows that the optical rotation will change sign when

$\lambda = 1.4 \times (0.19\mu \times 2) = 532$ mμ and that the reflected light for wavelengths near to this value would be expected to be circularly polarized.

Arrangement for Detecting Circular Polarization

Circularly polarized light may be produced by allowing the plane polarized light transmitted by a sheet of polaroid to pass through a quarter plate, having its slow direction at 45° to the electric vector

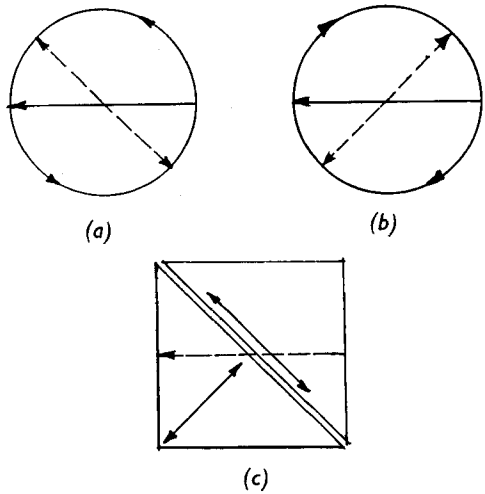

Figure 6. (a) Left circular polarizer; (b) Right circular polarizer; (c) The arrangements (a) and (b) inverted and mounted adjacently, as used for detecting the sense of the circular polarization. (Single-headed arrows indicate slow direction of quarter-wave plate. Double-headed arrows indicate electric vector of polaroid. Diagrams are as seen looking towards the light source, solid lines being in a plane nearer to the observer than broken lines.)

of the polaroid. The sense of the circular polarization will be right- or left-handed, according to whether the 45° angle is arranged clockwise or anticlockwise, as observed when looking towards the light source (see Fig. 6a and b). If either of these arrangements is inverted, so as to be used as an analyser, with the polaroid between the observer and the quarter-wave plate, it will extinguish the circularly polarized light produced by the opposite arrangement.

Therefore, to detect the presence and the sense of the circular polarization, two triangular pieces of polaroid were mounted adjacently, as shown in Fig. 6c, on top of a quarter-wave plate, with their slow directions at right angles to one another, but making an angle of $+45°$ and $-45°$ with the slow direction of the former.

However, when the light reflected from the solution in the 0.5 mm tube was observed through this arrangement, no diminution in its intensity was detected. This was also the case when the solution was observed in flat cells, even when their thickness was as little as 0.1 mm.

Eventually, however, the effect was detected in a 2 cm diameter stoppered test tube containing about 2 ml of the solution. After this solution had been inverted several times it was noticed that in some places there was left adhering to the wall of the tube a thin film which showed iridescent reflections. This, when observed through the arrangement of Fig. 6c appeared colourless through the triangle on the left, thus indicating that the reflected light was right-hand circularly polarized.

The stoppered flask containing a solid PBLG solution previously referred to (p. 481) was subsequently examined and a similar small patch of weakly iridescent film was found adhering to the inside wall. The reflected light from this was also found to be right-hand circularly polarized, although there was no indication of circular polarization in the light reflected from the bulk of the solid PBLG in the flask.

It was therefore decided to construct a very thin cell, having a uniform thickness of 0.01 mm, in which a suitably thin layer of solution could be observed. This presented certain difficulties since it had to be filled with the viscous solution after it had been carefully mixed to give a uniform concentration and evaporation had to be prevented during the mixing.

The cell was constructed by cementing together a large cover glass and microscope slide, in between which was a 0.01 mm thick aluminium foil spacer. The aluminium foil was cut so as that the internal area of the cell was approximately 4×2 cm and a 2 mm gap was left at the top and bottom of the cell. A glass tube, fitted at one

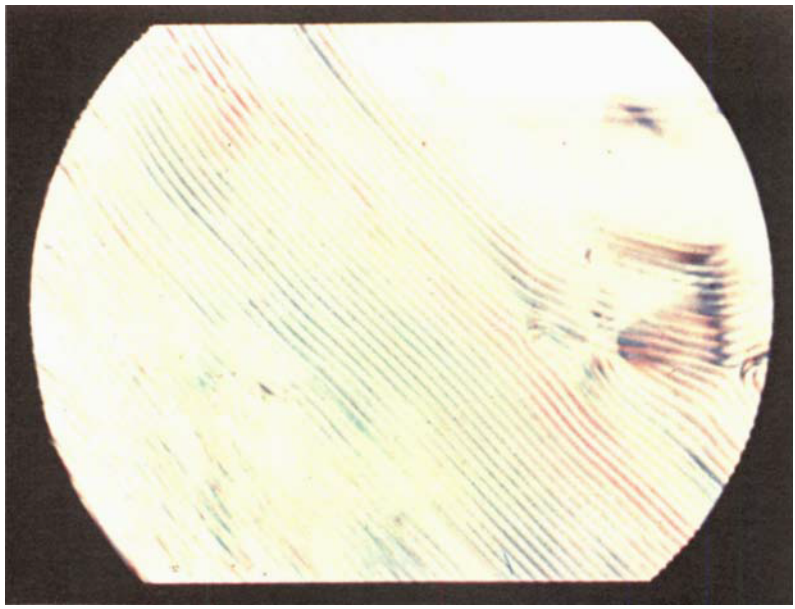

Figure 2. Solution of PBLG in dioxan, between crossed polars, showing a portion of a uniform area surrounded by equidistant bands.

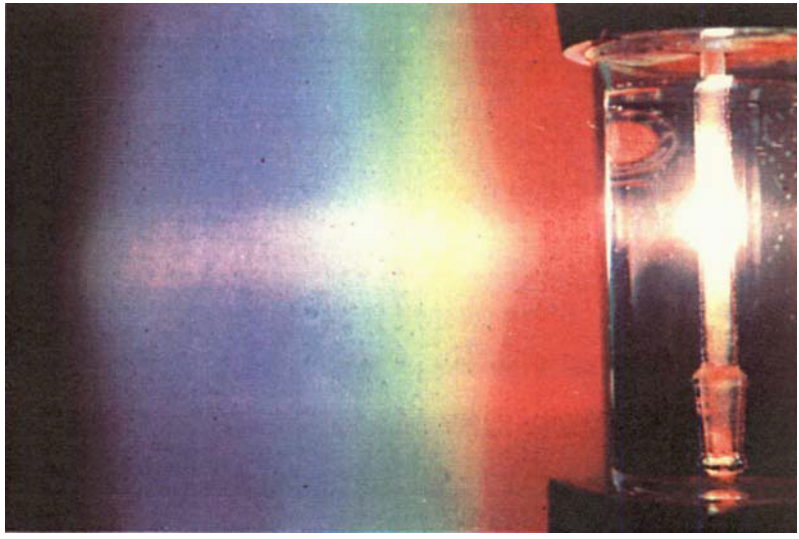

Figure 5. Solution of PELG illuminated with beam of white light.

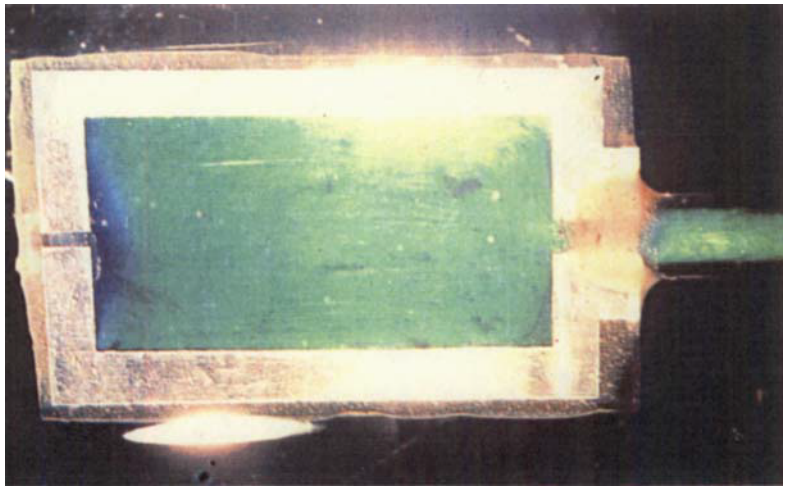

Figure 8. Solution of PELG in thin cell illuminated with natural white light. The green reflected light was circularly polarized.

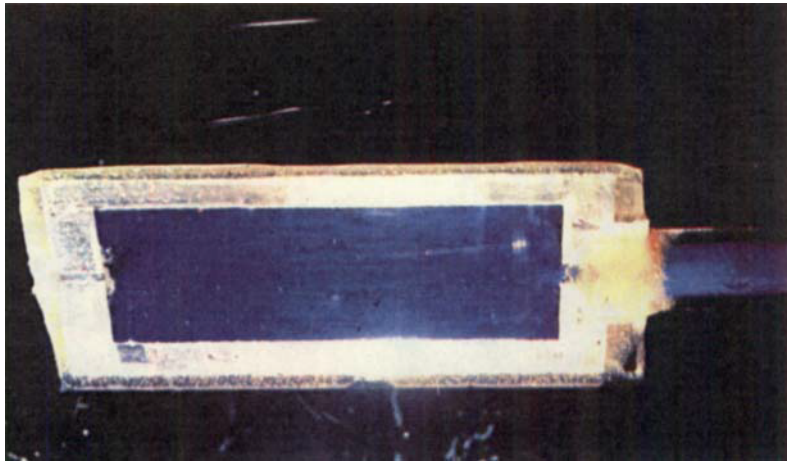

Figure 9. As Fig. 8, but photographed at another angle.

end with a ground glass stopper, had a groove cut in the other end so that it could be cemented over the gap at the top of the cell as shown in Fig. 7. The other gap was left open to act as an air outlet during the filling.

To fill the cell, it was inverted and the tube connected to the solution by a ground glass connection. The solution was then forced upward into the cell by compressed air. It was found necessary to regulate the flow so that the miniscus in the cell remained horizontal

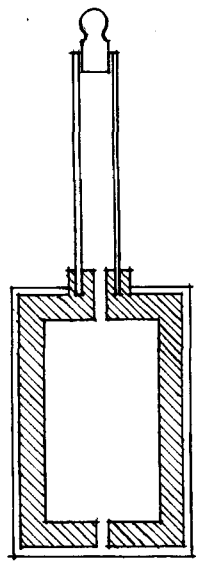

Figure 7. Construction of thin cell, 0.01 mm thick. Hatched area = aluminium foil.

to prevent air bubbles being trapped in the viscous liquid. The cell took 30 min. to fill and even during the filling the solution appeared a fairly uniform bright red with the ordinary room lighting when observed in a direction nearly normal to the cell. When full, the air outlet was sealed, the stopper replaced, and the cell again inverted.

Figures 8 and 9 show photographs of the cell taken from two different angles, while illuminated with a beam of white light making

an angle of incidence of 40°. The photographs were taken the day after filling.

As will be seen, the colour observed from any angle was remarkably uniform, although interspersed with dark streaks. It was found that the reflected colours from red to green were completely extinguished by a left circular polarizer, and even with the blue colour the intensity was considerably reduced.

Figure 10 shows a photograph of a portion of the cell taken through left and right circular polarizers mounted as already described. The angle of observation was such that reflected light was near the red end of the spectrum. The photograph shows the

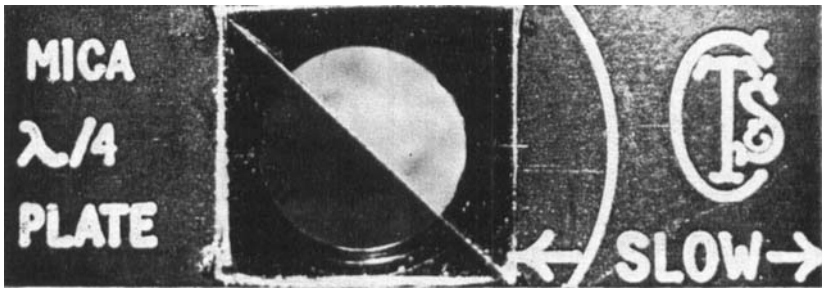

Figure 10. Portion of cell shown in Fig. 8, photographed through arrangement shown in Fig. 6c. The camera was focused on the polaroid.

complete extinction of the light produced by the arrangement on the left compared to the light intensity passing through that on the right.

The experiment therefore shows that for a considerable band of wavelengths the light reflected was right-hand circularly polarized. (The transmitted light was found, as expected, to be circularly polarized in the opposite sense.) It follows that the pitch of the torsion must be such that the optical rotation changes sign for a wavelength within this band, and therefore the optical rotation near to that wavelength cannot be measured. Hence, to obtain experimental evidence of the reversal of the sense of rotation it would be necessary to prepare solutions where the pitch was even smaller. Unfortunately, this was not found possible, since attempts to reduce it

sufficiently either by concentrating the solution or lowering its temperature resulted in the solution becoming opaque. There remains, however, little doubt that the optical properties of these polypeptide solutions, as well as being similar to those of cholesteric liquid crystals, are consistent with de Vries' theory.

Biological Structures

The results obtained with polypeptide solutions suggest that solutions of other optically active rod-like molecules might be found to form a cholesteric liquid-crystalline phase. Such a suggestion was made by Robinson, Ward and Bevers[4] in 1957, who wrote as follows, before the circular polarization of the reflected light had been recorded:

"Although more work is needed before the structure is fully understood, its general nature and its relationship to the nematic or untwisted structure now seems to be clear.

"The twisted structure is more highly organized than other liquid crystalline systems which have been described. It combines a high degree of organization with a left- or right-handed twist which is characteristic of its composition and environment. The solutions may nevertheless be surprisingly fluid and may dissolve other components without the qualitative nature of the structure being changed. It is conceivable that such highly organized, yet reproducible, liquids may play a role in chemical reactions involving some of the highly specific, optically active molecules present in biological systems. However, a periodicity of the same order as that found in PBLG solutions would easily be overlooked in biological units having a diameter not much greater than S, the repeat distance, while the form optical rotation would also be overlooked in a thin specimen. It might therefore be rewarding to re-examine some biological systems with these points in mind."

Solutions of DNA and RNA

Subsequently Robinson[5] examined solutions of DNA in aqueous NaCl. This seemed a likely material in which to find the pheno-

menon, since the double helix givies rigidity to the DNA molecules in the same way as the α-helical conformation does in the polypeptide molecules. Patterns resembling those observed in PBLG solutions were obtained in these solutions, having equidistant dark and light bands with a constant spacing of about 1 μ, the size of this spacing decreasing with concentration. Areas were also observed which resembled the uniform areas of the polypeptide solutions, but only in preparations which were so thin (0.01 mm) that no optical rotatory power could be detected.

Still more convincing evidence of the formation of a cholesteric liquid crystalling phase was obtained more recently by Spencer, Fuller, Wilkins and Brown[13] from solutions of transfer RNA which at certain concentrations formed a birefringent and an isotropic phase in equilibrium. In this they observed spherulites which showed the several features which we have described as being characteristic of polypeptide spherulites, including the dark and light bands forming a double helix of equispaced convolutions and the single radial line of disinclination.

The photographs of these spherulites are, in fact, difficult to distinguish from those obtained from PBLG solutions. When one considers the explanation of this spherulite pattern given by Pryce and Frank, it seems reasonable to accept its presence in the transfer RNA solutions as diagnostic of the occurrence of the same twisted structure in these solutions.

The Selective Reflection of Circularly Polarized Light from Beetles

In 1911, A. A. M. Michelson,[14] in a paper "On the metallic colouring of birds and insects", refers to the exceptional properties of a beetle, *Plusiotis resplendens*. He describes its appearance as follows: "Its whole covering appears as if covered with an electrolytic deposit of metal, with a lustre resembling brass." On examination with a Babinet compensator he found the light reflected from it was circularly polarized, whether the incident light was plane polarized or natural. He concluded: "The effect must therefore be due to a screw structure of ultra-microscopic, probably

molecular, dimensions." This remarkable observation acquires a new significance now that this same property has been observed in a concentrated polymer solution.

In 1924, Gaubert,[15] who, by his previous researches was aware of the properties of cholesteric liquid crystals, examined a number of beetles with a circular polarizer, and found that certain species reflected circularly polarized light (except at the red end of the spectrum where the sense was right-handed, for *Chrysena amena*). Gaubert found that the circular polarization only disappeared completely at 300° C.

In 1937, Mathieu and Farragi[16] gave the names of eight beetles which have this property, and made a quantitative study of two of these, *Potosia speciosissima*, and *Calcothea affinis*. On treating the teguments of the first of these with nitric acid, they became sufficiently transparent, without losing the brilliance of the reflected colour, to allow the transmitted light to be studied. This made it possible to determine the optical rotation and the ellipticity for different wavelengths. The curves obtained showed that the light is circularly polarized at the wavelength at which the sense of the optical rotation is reversed.

They also found that while the sense of the reflected light was left-handed, that of the transmitted was right-handed. They therefore concluded that the optical rotation was due to the selective reflection of one circular component of the incident light, the phenomenon thus being similar to that observed in cholesteric liquid crystals. (Mathieu and Farragi[17] also made a detailed quantitative study of the relation between optical rotatory power and the circular polarization of cholesteryl propionate and cinnamate.)

Since 1937, however, this peculiar characteristic of the colour of certain beetles seems to have been largely neglected, if not forgotten, in spite of the growing interest in helical structures in polymers and biological materials, and it would seem that both physicists and biologists are largely ignorant of it.

A recent excellent book on animal colour[18] makes no reference to circularly polarized light. But now that we know that these optical properties are qualitatively the same as those shown not only by

certain compounds of cholesterol, but by concentrated solutions of rigid, optically active polymer molecules, the subject acquires a broader interest.

To obtain more knowledge of the phenomenon as shown by beetles, I approached Dr. E. B. Britton, then of the Entomology Department of the British Museum, who kindly allowed me to examine their extensive collection of beetles.

To detect circular polarization, a left- and a right-hand circular polarizer, arranged adjacently as shown in Fig. 6c, but in which the mica quarter-wave plate was replaced by a 4×3 cm sheet of plastic of uniform quarter-wave retardation.

By passing this device over trays of beetles containing the numerous species, and observing them through the circular polarizer in ordinary daylight, it was possible in a short time to detect those in which the colour was either extinguished when observed through the polarizer of one or other sense. In no case did the appearance of the beetle change when observed through a left-hand circular polarizer, but many species, when seen through a right-hand circular polarizer either appeared black or a dull red-brown colour, while in other species some portion (e.g. the thorax) showed this effect. The sense of the circular polarization observed was therefore always left-handed, but there were many species of bright iridescent beetles which showed no change when so observed.

In a few species single individuals were noticed which instead of being iridescent had the dull red appearance of an iridescent specimen as seen through the left circular polarizer. Details of the history of these specimens was not obtainable, but the appearance was as if something, such as a solvent, had destroyed the helical structure without otherwise damaging the specimen.

The following observations on particular species illustrate the phenomenon:

Anoplognathus chloropyros has bright golden elytra and thorax which show green and blue iridescence according to the angle of incidence, while the legs are dull red and are not iridescent. Seen through the polarizer the whole surface of the beetle appears dull red, and not iridescent.

Plusiotis resplendens (the original species observed by Michelson) —the golden colour was greatly diminished but did not disappear completely. Other species of *Plusiotis* showed the effect more strongly. One, having a uniform appearance of burnished silver, completely lost this appearance when observed through the polarizer,

Phaneus imperator has areas of gold, blue, green and red gold, all of which appeared black through the polarizer.

Cetonia aurata (the common rosechafer). This species, though generally green, is remarkable in that individuals occur which have other colours. A tray containing over a hundred specimens was examined which contained black, red, blue and green individuals. All were uniformly coloured but iridescent. Seen through the polarizer all appeared black. This is of interest since it suggests that the pitch of the helix can vary in different individuals although the colour of any individual remains unchanged after years in a museum.

Subsequently Dr. Britton made a more complete survey of the collection, using the same device for detecting the circular polarization, and reported as follows (Private communication, April 1962):

"After a rather extensive examination covering all the metallic coloured beetles known to me, I can say that circularly polarized light is reflected only in the family Scarabaeidae and in this family in the sub-families Coprinae, Melolonthenae, Phaenomerinae, Rutelinae, Cetoniinae, Trichiinae.

"The sense of the circular polarization is the same in all cases. The phenomenon is commonest in the Rutelinae. There are one or two surprising anomalies (genera which give a negative result while closely related genera are positive), but these are rare."

Onslow [19] in an extensive research "On a periodic structure in many insect scales and the cause of their iridescent colours" also considered the origin of the colour of scaleless beetles. Although he makes no reference to circularly polarized light, he made observations relevant to the problem.

Careful sectioning and microscopic examination showed no indication of periodic structures that could account for the colour

of these beetles. While the colour of the insect scales were found to disappear or become greatly changed on immersion in liquid having a refraction near to 1.5, the refraction index of chitin, by contrast the colour of these beetles on immersion became even brighter. The beetles he examined included three species already referred to in the previous paragraphs as showing circular polarization. He found that when, for instance, the wing case of *Plusiotis resplendens* is carefully polished with the finest carborundum at first there is no change in the colour, but later the golden colour changes suddenly to magenta, to be followed rapidly by a change to black. The suddenness of the change he found "strongly impresses the observer with the difficulty of attributing the phenomenon to interference". Similarly the green *Cetonia aurata* on polishing changed to a fiery red, while as with other iridescent beetles the sections showed no difference from ordinary non-iridescence beetles. Sections of *Anoplognathus aureus*, which he mentions as closely resembling *Plusiotis resplendens* are also described. He concludes:

"Finally on morphological grounds it is easier to conceive of the development of a layer of *Schillerstoff* than a periodic structure. Schulze[20] has shown that the surface layer of a stag beetle (*Lucanus cervus*, Linn.) is not formed gradually from the hypodermal cells, as in the case of skin or scales, but is due to a fluid secreted from special glandular cells, which is ejected upon the surface of the wing at a late period in pupal development, where it rapidly hardens. This beetle is not iridescent, but presumably iridescent cuticles would be formed in the same way. It is difficult to see how a periodic structure can arise in a homogeneous medium after it has been excreted, except perhaps by a process analogous to crystallization."

Such a process, "analogous to crystallization", resulting in a periodic structure having the optical properties characteristic of the elytra of these iridescent beetles is what has been described in this paper when a solution of PELG is extruded; and it has also been explained how the same properties of this solution may well occur with solutions of other asymmetrical polymer molecules. Since a subsequent process of hardening which left the nature of the

structure unchanged is far from inconceivable, the process is no longer difficult to imagine, at least from the physical chemist's point of view.

It would seem therefore that further research into the nature and origin of these iridescent elytra would be repaying, since, even if it was found that the circular polarization of the reflected light had some quite different origin to what is here suggested, an alternate explanation would scarcely be less interesting. It would also be of interest to consider what survival value can account for the occurrence of this most unusual property in so many species.

The experiment demonstrating the circular polarization of the light reflected from the PELG solutions, and some of the observations on beetles, were carried out at Courtaulds' Laboratory, Maidenhead, England, prior to the closing of that laboratory in 1962, as was the work on the liquid crystalline phase of polypeptide solutions previously described.[1–5]

Thanks are due to Dr. J. Watson of Courtaulds Limited for providing the PELG solutions, the iridescent colours of which he was the first to observe; and to Mr. J. Hetherington for the photography and technical assistance.

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