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Superstructure in Polypeptide Films as Noted by Small Angle Light Scattering

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Abstract—Films of various synthetic polypeptides were investigated by use of low angle photographic light scattering. Some oriented films of poly-γ-benzyl-L-glutamate (PBLG) were prepared by casting them in the presence of a strong magnetic field. These were also investigated by both light scattering and by wide angle X-ray diffraction. Some light scattering experiments were carried out on films of reconstituted collagen in order to make some comparisons between the synthetic and natural peptides. It was found that, in general, a superstructure exists which can be interpreted as having the character of optically anisotropic rods. Speculation is made regarding the formation of these rod-like aggregates. Some electron electronmicroscopy was done to note the actual presence of these rod- or fibrillar-like bundles. It was found that these anisotropic bodies also exist in the magnetically oriented films but their long axis is found to lie perpendicular to the direction of the field while the molecular axis lies parallel to the field as noted from X-ray diffraction. No well-defined optical transitions were observed on either compression molded or chloroform cast films of PBLG when measured as a function of temperature.

1. Introduction

Since the discovery of liquid crystal behavior by Reinitzer⁽¹⁾ in 1888, a tremendous number of research investigations have been undertaken with an aim toward the understanding and utilization of these unique substances displaying mesophase behavior. Most efforts have been directed at materials which are monomeric, such as the cholesterol esters, various soaps and aromatic carboxylic acids. Most of these materials display a mesomorphism which is thermotropic in nature i.e. the various mesophase transitions or states arise as a result of a temperature change. Utilization of materials displaying thermotropic mesomorphism has resulted due to the drastic changes in their optical behavior at the various thermal transitions;

these may include color change, onset of optical rotation and optical anisotropy. It has been only recently that the occurrence of mesophase behavior has been noted in polymeric systems and particularly in the synthetic polypeptides (SP). Robinson(2) and Elliot and Ambrose(3) reported some of the earliest work on these SP systems in their microscopy studies of solutions of poly-γ-benzyl-L-glutamate (PBLG). A more recent study utilizing low angle light scattering on PBLG solutions has been carried out by Frenkel, Baranov and Volkov.(4)

These peptide materials differ in general from the majority of the monomeric mesophase systems in that the SP systems illustrate lyotropic mesomorphism rather than thermotropic. That is, the transitional behavior depends on solvent and the related solution parameters of pH, concentration, and solvent–solute interaction. The kinetics and structural behavior of these mesophases are also generally influenced by the presence of electromagnetic field gradients, by the surface volume ratio, and by the " type " of surface in contact with the mesophase system. The influence of electromagnetic fields on mesomorphism might, in fact, be denoted as electrotropic.

A number of investigations of SP systems and their lyotropic or electrotropic behavior have been reported. Robinson has been foremost in this area and has presented considerable microscopy evidence for the occurrence of cholesteric behavior of high molecular weight PBLG in helix forming solvents such as chloroform. Samulski(5) has extended these studies to consider the morphology of PBLG films formed by casting from different solvents which induce varying degrees of helicity of the PBLG backbone. He has presented some evidence based on X-ray diffraction measurements for the existence of a nematic mesophase in the solid state as well as in highly swollen or plasticized PBLG films. He has also considered the effects of magnetic fields (during casting) on the final film morphology. Others(6) have also studied the electrotropic effects on PBLG with respect to its fluid behavior. Characterization parameters used have been viscosity, dielectric anisotropy, birefringence, etc.

There now appears to be a surge of interest in the peptides of both synthetic and natural origin. This interest stems from the desire to simulate and/or utilize the behavior of these mesophase systems and from the new thrust of activity in the area of biomaterials. Several of the peptides can be fashioned into a material which *a priori* might be expected to be more compatible with the body than many of the other available synthetic polymers. Therefore there is a desire to learn about the dependence of morphology, and of mechanical, dielectric, and biological compatibility characteristics on type of peptide, methods of preparation and fabrication. A third root of interest is more basic in nature and is that desire to understand the behavior of these unique substances (all mesophase systems) which bridge the gap in behavioral characteristics between the ordered three-dimensional crystalline solid and the isotropic liquid. It is the purpose of this paper to shed additional light on the morphology and texture of peptide films. Synthetic peptides will be emphasized but for comparative purposes some data will be presented which concerns natural peptides—specifically reconstituted collagen. We will also indicate the effect of the variables of magnetic field strength and of temperature on some of the synthetic peptide systems. These morphological characteristics, as presented here, have been studied primarily by use of small angle photographic light scattering (LS) and some scanning electronmicroscopy studies. This information should broaden our present understanding of the polymeric peptides and help to stimulate further studies on these materials.

2. Scattering Considerations

GENERAL

Light scattering and its dependence upon molecular parameters has been utilized extensively for obtaining information on polymeric solutions and films. Little work, however, has been directed at mesophase systems, the SP systems in particular. For example, Wallach and Benoit(7) considered the scattering from dilute solutions of various peptides but there have been few applications of the scattering technique to these materials when in concentrated solutions or film form. Stein *et al.*(8) have shown that mesophase transitions and superstructural characteristics of various monomeric thermotropic cholesteryl compounds can be followed by the scattering technique. These characteristics were determined by the conventional photographic low angle light scattering system which has since been utilized often on synthetic polymeric solids.(9,10,11,12,13) Frenkel *et al.*(4,14) have also utilized low angle light scattering to study concentrated solutions of PBLG. The data presented indicates that the type of observed scattering pattern depends on solution age. Essentially no data however is given for solid films.

Since theories of interpretation of the observed scattering patterns(15,16) have had little utilization by those researchers in the mesophase area, it seems worthwhile to present briefly some of the basic fundamentals that are particularly useful for examining the superstructure of mesophase systems. The degree of applicability will become apparent in the results.

Origins of Scattering

Light scattering arises primarily from four possible sources: (1) density fluctuations; (2) fluctuations in directional orientation of the optic axes in local anisotropic regions; (3) fluctuations in the local anisotropy but with no correlation between local regions; and (4) optical rotation. The first can arise in optically isotropic systems whereas the others require the presence of optical anisotropy. Since many mesophase systems give rise to optical rotation, the latter source can be of particular importance.

It is well known that in an isotropic medium the polarization of the scattered ray at small scattering angles is essentially identical to that of the incident beam if the Rayleigh–Gans treatment can be applied.† It therefore follows that no scattered radiation is transmitted through a second polarizer (analyzer) if it has its electric vector rotated at 90 degrees to the electric vector of the polarized incident beam. The presence of optical anisotropy, however, can give rise to the transmission of scattered light when the polarizer and analyzer are crossed. Interpretation of the observed or measured scattering leads to information regarding superstructure that arises due to preferential molecular packing, aggregation or crystallization. By rotation of the analyzer one can attempt to separate the isotropic

† Applicability of this approach is in order when the refractive index difference is small between the scattering object and the surroundings and, when the dimensions of the scattering object are of the order of the wavelength of the incident radiation. This treatment is the basis for the scattering theory discussed in this paper.

and anisotropic contributions. Quantitative interpretations can be undertaken either by the use of statistical approaches(17,18) or by the comparison of the scattering patterns calculated from definite geometrical models.(9,15) For our purposes here, the use of theory based on definite models will be sufficient although not completely satisfactory.

Scattering from Anisotropic Rods

Rhodes and Stein(20a) have calculated the H_v† and V_v† scattering pattern expected based on the scattering by independent infinitesimally thin anisotropic rods in various planar configurations i.e. oriented or random. The equations for the scattered V_v and H_v intensities per unit of scattering volume have been given as

$$I_{V_v} = \rho_0{}^2 N_0 L^2 \int_{\beta=0}^{\pi} (\epsilon^{-2}\cos^2\beta\,\epsilon^2\sin^2\beta)^{-1/2}(\delta\cos^2\beta' + \alpha_t)^2 \cdot \left[\frac{\sin(kaL/2)}{(kaL/2)}\right]^2 \mathrm{d}\beta \tag{1}$$

$$I_{H_v} = \rho_0{}^2 N_0{}^2 L^2 \int_{\beta=0}^{\pi} (\epsilon^{-2}\cos^2\beta + \epsilon^2\sin^2\beta)^{-1/2}\,\delta^2\sin^2\beta'\cos^2\beta' \cdot \left[\frac{\sin(kaL/2)}{(kaL/2)}\right]^2 \mathrm{d}\beta \tag{2}$$

where N_0, L and ρ_0 refer to the number, N_0, of rods per unit of volume each being of length L and having a scattering power per unit length of ρ_0. The optical anisotropy of the rod, δ, is defined to be

$$\delta \equiv \alpha_l' - \alpha_t' \tag{3}$$

where

$$\alpha_l' = \alpha_l - \alpha_s \tag{4}$$

$$\alpha_t' = \alpha_t - \alpha_s \tag{5}$$

the subscripts of l, t and s respectively refer to polarizability in the longitudinal and tangential direction of the rod, and, of the

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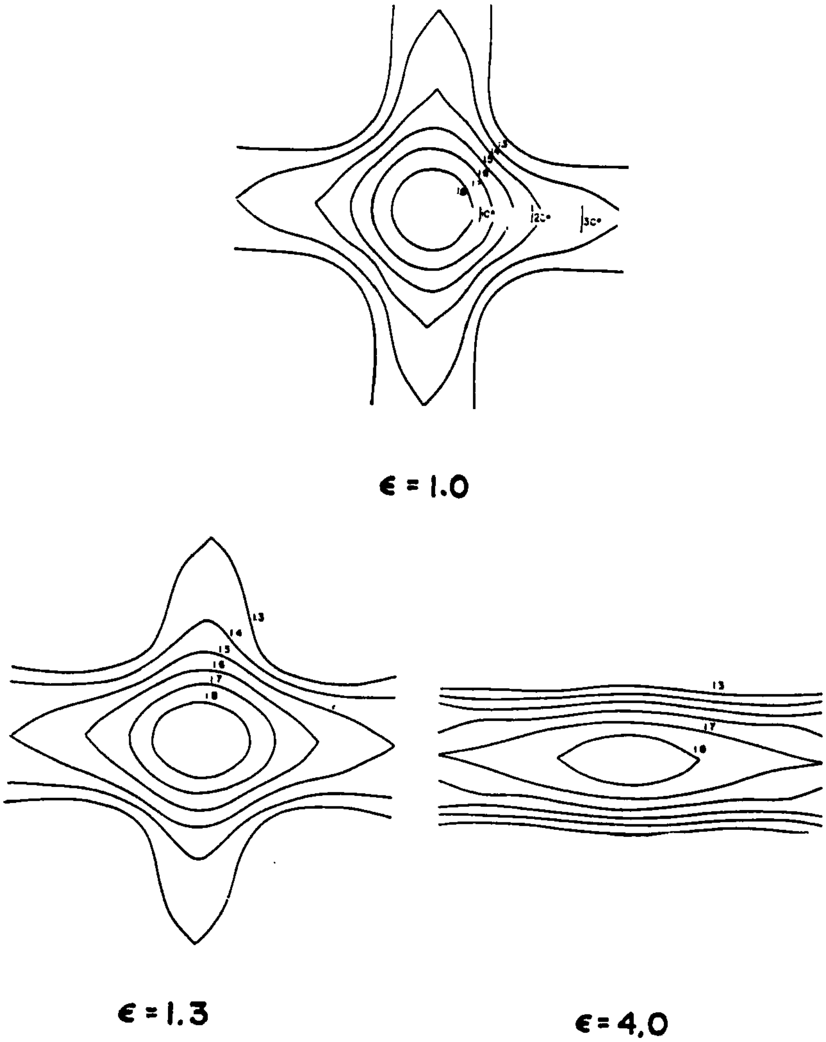
† H_v refers to a horizontal (H) and vertical (v) alignment of the electric vectors of the analyzer and polarizer respectively. If a stretch axis is present, it would be aligned along the v direction. Similarly a V_v orientation refers to both electric vectors being aligned in a vertical direction. The stretch axis would also be parallel to these vectors. In an H_h position, the stretch axis would be perpendicular to both analyzer and polarizer vectors.



H_V

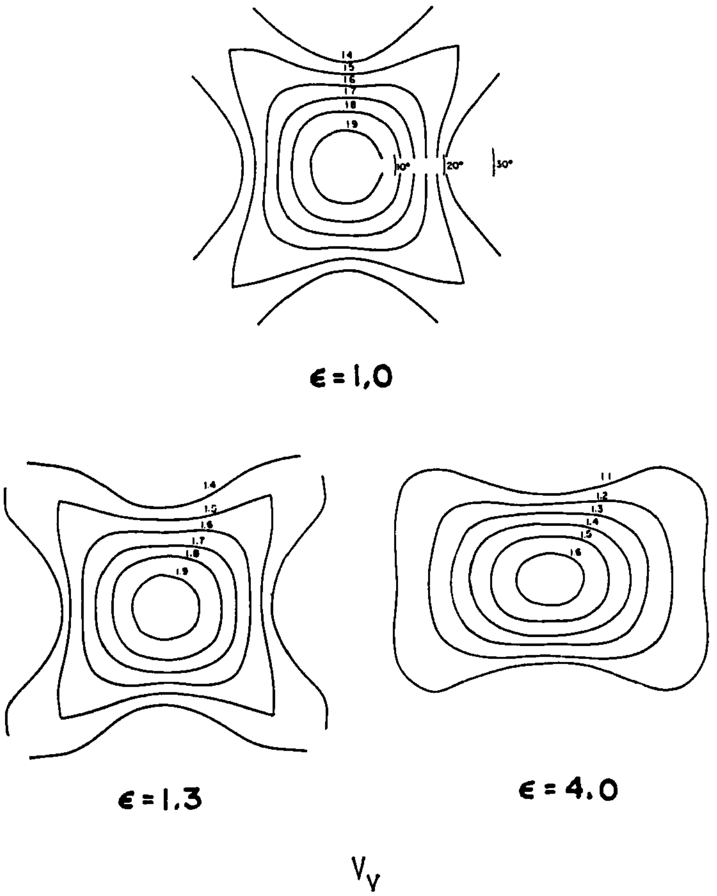


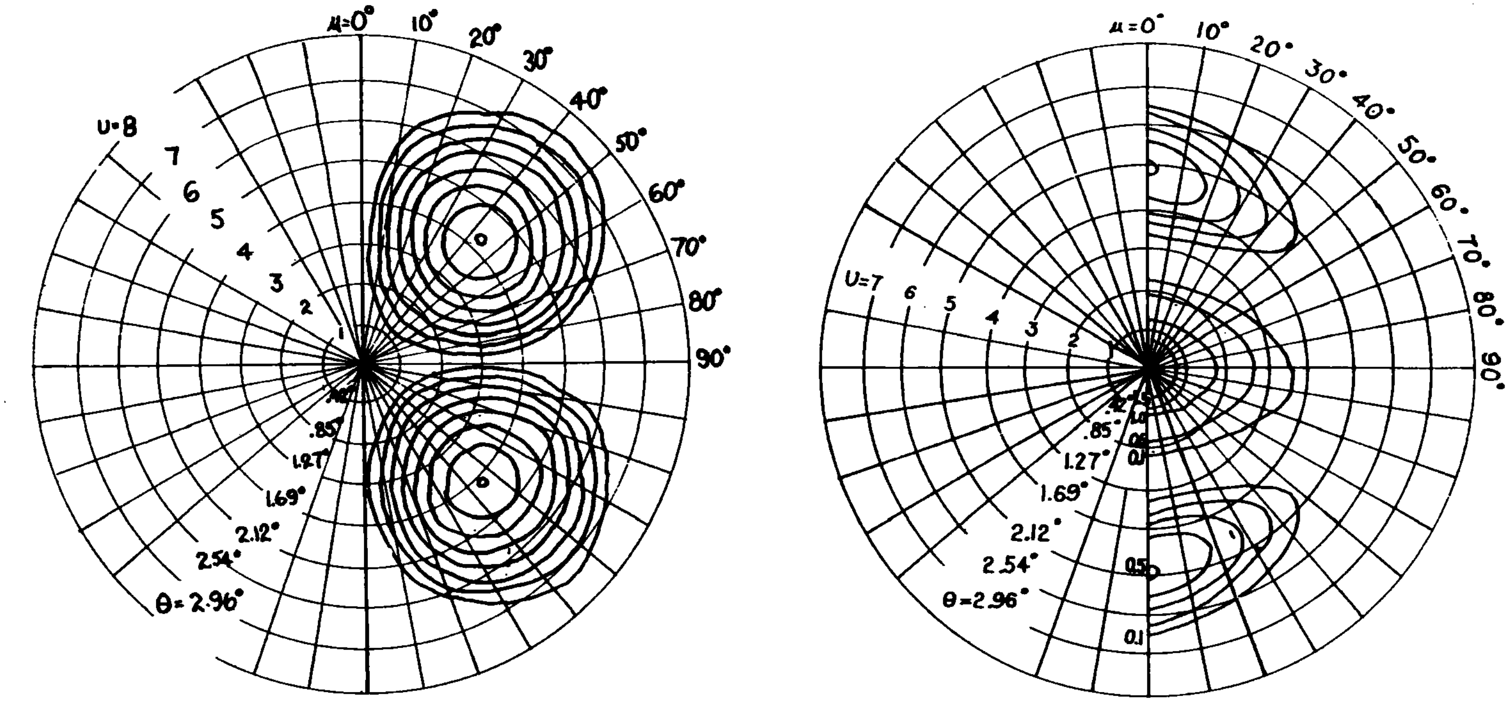
Figure 1. Contour plots of the scattered intensity (arbitrary units) for H_v and V_v patterns as predicted from Eqs. (1) and (2). Plots show different values of the orientation parameter $\epsilon(\omega = 45°,\ \delta = 4,\ b_t = -1)$. The stretch axis is vertical. [Courtesy of R. S. Stein.]

surroundings. The parameter k is $2\pi/\lambda$ where λ is the wavelength of radiation in the medium. The first term in parentheses in each equation is the form of an elliptical orientation distribution function where ϵ refers to an orientation parameter. If ϵ is unity, the distribution of rods is circularly symmetric. It is assumed in the theory that all the rods lie in a plane which is normal to the incident beam. This assumption as applied to cast SP films seems reasonable based upon the X-ray and swelling data of Samulski(5) as measured on films of PBLG. We have also noted similar planar orientation in cast reconstituted collagen films. The angle β is the angle that the rod axis makes with the vertical axis while β' is equal to $\beta + \omega$ where ω is the angle that the *optic* axis of the rod makes with the *rod* axis. The final parameter a is a function of the radial and azimuthal scattering angles as well as being dependent upon β. Further comments on these equations and their limitations will follow shortly. It is in order to mention that a more general theory of scattering from rods has been presented by Van Aartsen.(20b)

Typical H_v and V_v scattering contour plots as calculated from use of Eqs. (1) and (2) are shown in Fig. 1 where three different values of the orientation parameter, ϵ, have been used. The various values of the other mathematical parameters are given in the figure caption. It should be stated here that patterns 1a and 1d can be rotated 45° if the optic axis parameter (ω) is allowed to lie along the rod axis rather than at 45°. Patterns have been observed from films of synthetic polymers which display both types of scattering behavior.(20,21) Further applicability of this model has been illustrated by Samuels(21) in his study of hydroxypropylcellulose in which he clearly showed that by using an appropriate orientation distribution function for the rods, the scattering behavior from oriented films agreed excellently with the theory.

SCATTERING FROM ANISOTROPIC SPHERES

Polymers, in general, tend to form spherulites during the onset of crystallization. This leads typically to the formation of a spherical anisotropic superstructure with a characteristic radius ranging from a fraction of a micron to hundreds of microns. The exact size depends on various molecular parameters and on the crystallization kinetics. The size, anisotropy and nature of the spherulite, can be



A) One-half the calculated scattered intensity contour for H_V scattering.

B) One-half the calculated scattered intensity contour for V_v scattering.

Figure 2. Contour plots of the scattered intensity (arbitrary units) for H_v and V_v patterns as predicted from an undeformed anisotropic sphere (Eqs. (3) and (4)). (a) H_v; (b) V_v. The stretch axis is vertical. [Courtesy of R. S. Stein.]

studied utilizing well established light scattering theory.(9,22) Examples of theoretical H_v and V_v spherulite scattering patterns are given in Fig. 2 as calculated from Eqs. (3) and (4) given below.

$$I_{V_v} = A V_0^2 \left(\frac{3}{U^3}\right)^2 [(\alpha_t - \alpha_s)(2 \sin U - U \cos U - \mathrm{Si}U) + (\alpha_r - \alpha_s)(\mathrm{Si}U - \sin U) - (\alpha_t - \alpha_r)(\cos^2 \left(\frac{\theta}{2}\right) \cos \mu \cdot (4 \sin U - U \cos U - 3\mathrm{Si}U)]^2 \tag{6}$$

$$I_{H_v} = A V_0^2 \left(\frac{3}{U^3}\right)^2 [\alpha_t - \alpha_r) \cos^2 \left(\frac{\theta}{2}\right) \sin \mu \cos \mu \cdot (4 \sin U - U \cos U - 3\mathrm{Si}U)]^2 \tag{7}$$

where

$$\mathrm{Si}U = \int_0^U \frac{\sin x}{x} \mathrm{d}x \tag{8}$$

and

$$U = \frac{4\pi R}{\lambda} \sin \left(\frac{\theta}{2}\right) \tag{9}$$

In the above α_t and α_r represent the respective tangential and radial polarizabilities of the anisotropic sphere of radius R imbedded in an isotropic matrix having a uniform polarizability of α_s. Parameters A, V_0, μ and θ are an instrumental constant, the volume of the sphere, and the azimuthal and radial scattering angles. The wavelength in the medium is λ.

It is noted that in both Eqs. (2) and (7) the H_v intensity depends only on an anisotropy term $(\alpha_t - \alpha_r)$ or δ whereas the V_v intensity is composed of both isotropic and anisotropic contributions. In the spherulite model the surrounding matrix has been treated as having a uniform polarizability of α_s. This assumption could plausibly be in error. Rather more realistic is that there is a distribution of α_s's caused by local density fluctuations. These density fluctuations which also give rise to V_v scattering are not accounted for by the model. If there is a significant contribution to the total V_v intensity by such a distribution, then the V_v pattern predicted by the *model* may be buried in the intensity caused by the unaccounted for matrix density fluctuations. This means that an H_v pattern may be nearly in agreement with the model calculations as given here but at the same time, the V_v pattern may not display the appropriate symmetry.

In the rod model a similar situation exists in that a uniform matrix polarizability, α_s, is assumed. Again, this does not consider any effects of other density fluctuations on the total V_v scattering. Hence, as in the spherulitic case, one may experimentally observe a "good" H_v rod pattern but not a "good" V_v pattern. These considerations should be kept in mind by the reader for they are useful in interpreting the results presented. It might be added that the rod model or the anisotropic sphere model, as presented here, do not consider interparticle effects. Stein and Picot(23) have shown that interparticle interference effects do not drastically alter the scattering behavior for the sphere model. Although we are not aware of similar calculations for the rod system we anticipate that the effects would be of a secondary nature.

In their studies on cholesteryl esters, Stein and Rhodes(9) show that several of the mesophase systems give scattering patterns in agreement with Fig. 2 a and b. Robinson(2) presents photomicrographs of spherulitic structures in aged chloroform solutions of PBLG with his data supported by the scattering patterns observed by Frenkel *et al.*(14) Ishikawa(24) offers similar microscopy evidence for poly-γ-methyl-L-glutamate (PMLG) in chloroform. He indicates that the resulting spherulitic form (optically positive or negative) that is induced depends largely on the age of the solution. Elliott and Ambrose(3) indicate that in their studies of PBLG cast from chloroform similar liquid spherulites are noted in solution but that upon evaporation of solvent, the film structure tends to be fibrillar or rodlike in texture. Their photomicrographs, in fact, tend to be somewhat indicative of localized nematic structure. These same authors also observed similar structures in films of poly-L-glutamic acid (PLGA) and of poly-γ-methyl-L-glutamate (PMLG) when cast from *m*-cresol. In light of these previous data we have attempted to apply photographic scattering techniques to further elucidate the morphology and texture in some of the peptide systems with the emphasis in this paper on those of synthetic origin.

MATERIALS

All synthetic polypeptides, with the exception of poly-γ-methyl-L-glutamate (PMG), were obtained from Pilot Chemicals (Boston, Mass.). The PMG was obtained from Johnson and Johnson and came in film form. The films of reconstituted collagen were supplied through the courtesy of Biomedix Laboratories, Inc. (Princeton, N.J.).

The PMG film was dissolved in chloroform and films were cast on a glass substrate. A similar procedure was used for the PBLG fibrous material. The polylysine (HBr) was cast from a 20 weight % aqueous solution on glass. Polyglutamic acid was prepared from a 30 weight % DMF solution. This was allowed to dry between two glass substrates. The procedures for preparing the collagen films have been reported elsewhere.(25,26)

RESULTS AND DISCUSSION

Figure 3 shows the H_v and V_v patterns taken on PBLG films cast from chloroform solutions that were not older than one month. Fresh and one-year-old solutions gave identical results; the patterns suggest rod-like scattering (compare with Figs. 1 a and d) rather than that of spherulitic character. The V_v pattern is not as "perfect" as Fig. 1d but this may be due to the superposition of background scattering from matrix density fluctuations. Figure 4 shows H_v and V_v patterns taken from the same film but at a different location. The differences between these patterns and those of Fig. 3 are believed to be due to the effect of local orientation rather than to the effect of optical rotation. In fact, the comparison of Fig. 3 to

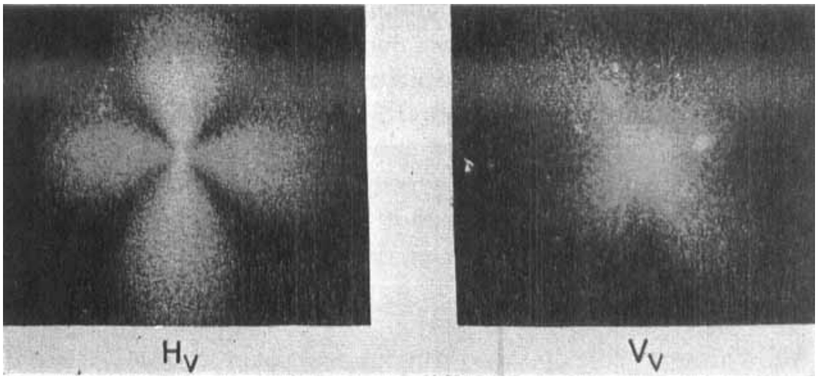


Figure 3. H_v and V_v scattering patterns for chloroform cast films of PBLG on glass. Sample to film distance (STF) was 9 in.

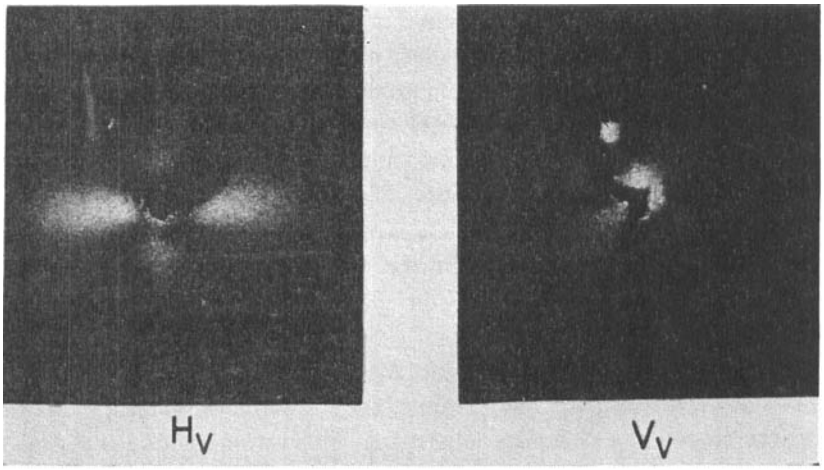


Figure 4. H_v and V_v scattering patterns obtained from the same PBLG film as used for Fig. 3. This pattern was from a different location in the film. STF = 5 in.

Figure 5. H_v scattering pattern obtained from a " pool " of PBLG solution placed on a glass substrate. STF = 5 in.

Fig. 1 b and e supports this speculation. This local orientation may well result from the manual spreading of solution on the glass substrate. The H_v pattern shown in Fig. 5 is taken from a " pool " of the PBLG solution placed on a glass slide. That the H_v 0°–90° rod-like pattern is still apparent is significant and along with the smaller pattern (relative to Fig. 3a) implies there is a larger superstructure responsible for the scattering† or that there is simply a lower concentration of scattering elements. Most likely, the second speculation is the more reasonable since it is really only the top surface layer of the pool that is responsible for the scattering. After a pool of the clear solution was placed on the glass substrate, a whitish cast appeared on the upper surface in a very short time and only when this turbid layer was present could the cross H_v pattern be obtained. The scattered intensity has, in fact, been enhanced when a draft of air was passed over the glass substrate. This implies that the pattern difference between Fig. 5 and Fig. 3a is due to a concentration effect of scattering elements.

In light of the data, we investigated films cast from solvent-peptide systems of PGA-dioxane, PGA-DMF-H_2O, PMG-chloroform and polylysine-HBr-H_2O [(PL)-water]. Figure 6 shows the H_v and V_v patterns for the PMG films. Figure 7 shows only the H_v pattern for the PL system. The V_v pattern, although observed, was so highly buried in background scatter that a suitable photograph of the pattern could not be obtained. Figure 8 presents the H_v patterns found from PGA dioxane solution cast material. Two distinctly different cross patterns are obtained from this PGA system; one is a 0°–90° cross pattern while the other is a ± 45° cross pattern. These two separate patterns, however, existed in the same sample preparation.

In this specific case the film preparation technique led to slow

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† The reader recalls that scattering angle and scattering element " size " are reciprocally related. Based on the sample film distances used for Figs. 3, 4 and 5 it is clear that smaller scattering angles occur in Fig. 5. Caution must be taken, however, when trying to compare " sizes " of scattering elements where rod scattering is involved. This is due to the fact that monotonic drop off in scattered intensity exists for all rod sizes and clearly the size of the pattern is influenced by exposure time. A case where average size of scattering element can be determined from scattering pattern is that of the spherulitic structure.

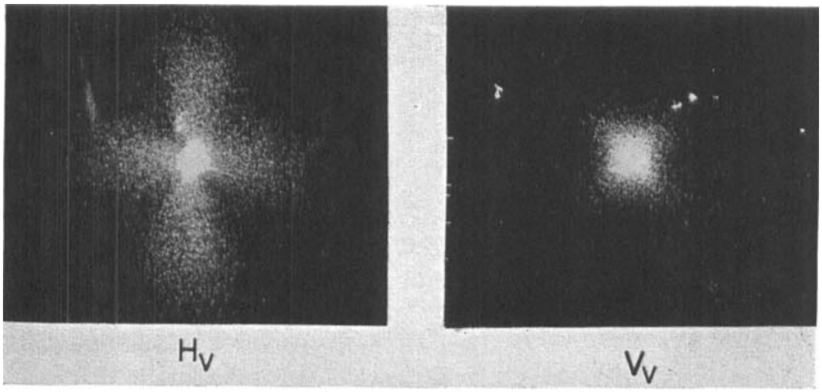

Figure 6. H_v and V_v scattering patterns obtained from a chloroform cast of PMG.

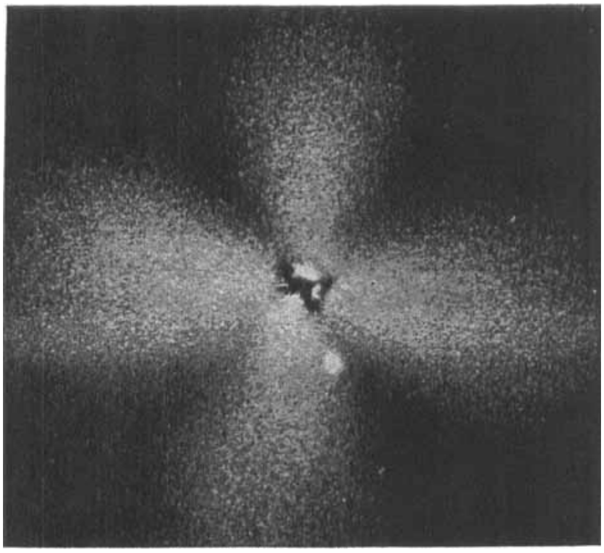

Figure 7. H_v scattering pattern obtained from the polylysine preparation cast on glass. STF = 5 in.

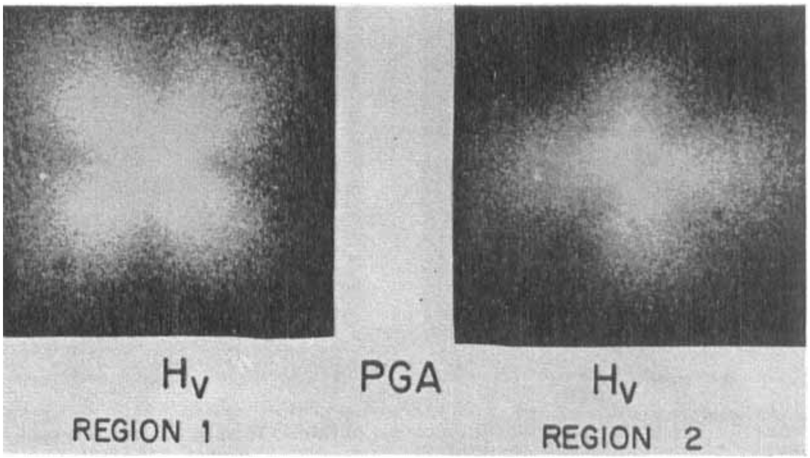

Figure 8. H_v scattering patterns obtained on the PGA-DMF preparation cast between two glass slides. Region 1—still contains solvent; Region 2—" dry ".

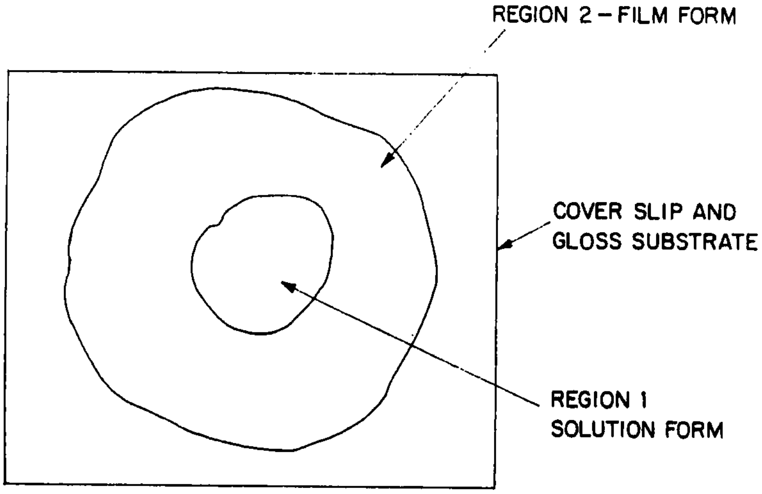

Figure 9. Illustration showing Regions 1 and 2 of the PGA-DMF preparation.

evaporation of solvent under the glass cover slip, resulting in two regions being formed as schematically illustrated in Fig. 9. Patterns 8 a and b come from regions 1 and 2 respectively.

The results presented in Figs. 3–8 indicate that a rodlike texture exists in all of the SP systems after a solid or *dry* film has formed. Where solvent still remains (PGA-region 1), the morphological texture as noted by the light scattering patterns, is somewhat in question as to whether spherulitic or rodlike structure exists.

Rodlike or threadlike elements are typical of the nematic and smectic mesophases. Each of these mesophases requires a principal optic axis, yet the light scattering patterns imply a random orientation of anisotropic rods. Since the laser beam " sees " a multitude of these oriented regions each of which may have its optic axis oriented along a different direction, the apparent discrepancy is explainable. That is, we view the texture as being composed of randomly oriented anisotropic rods where each " rod " is itself composed of an oriented aggregate of peptide molecules. This structure is shown schematically in Fig. 10a. Such a structure, however, cannot explain the existence of the ± 45° cross H_v pattern of Fig. 8. It can, however, be rationalized in terms of Fig. 10b where the " rods " or swarms are now composed of oriented bundles of peptides with the optic axes aligned parallel (or perpendicular)† to the " rod " axis.‡ These proposed structures are reasonable in view of the photomicrograph of PBLG films cast from chloroform by Elliot and Ambrose.(3) Their photomicrographs, however, are of localized regions and hence it is not known if random orientation occurs over the cross section of the film. Our data offer further support for the presence of nematic structure in the solid film. The smectic phase has presently been ruled out based on X-ray diffraction studies by Samulski on similar films.(5)

The formation of anisotropic systems both in solution and in film form is not unexpected. Anisotropic phase development in the

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† The H_v patterns cannot separate these two cases of parallel or perpendicularly oriented optic axes. To do so, it is necessary to make use of the V_v patterns. Since these were poor for the peptide films this separation of kinds of orientation could not be made based on these data.

‡ Both types of " rod " models do not eliminate the possibility of folding of the chains, rather, only the postulation is made concerning the orientation of the optic axes of the chains.

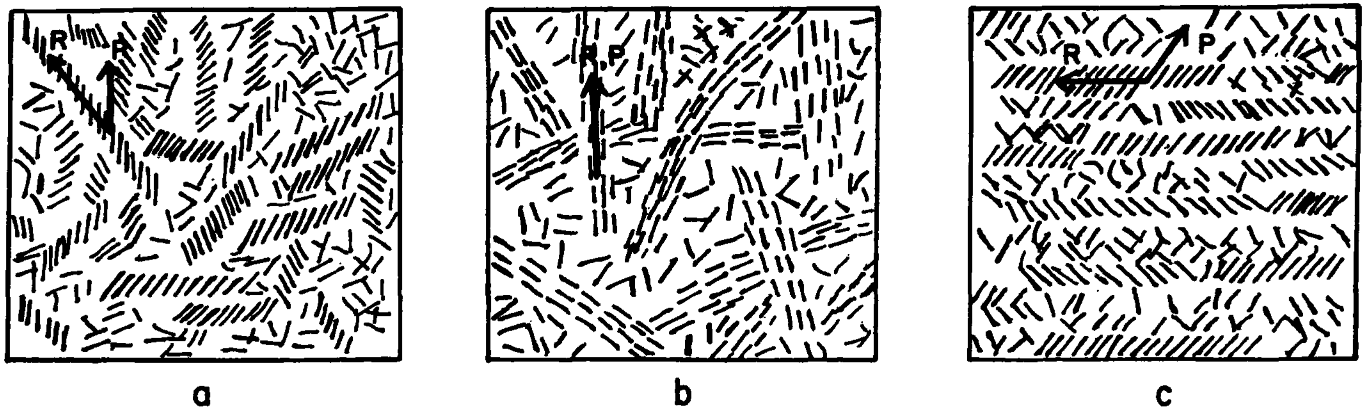

Figure 10. Sketch of rod-like models that would explain the observed light scattering patterns obtained from the polypeptides studied: (a) optic axis, P, is at 45° to the rod axis, R (random distribution of rods); (b) optic axis and rod axis are parallel (random distribution of rods); (c) as (a) except rods are preferentially aligned perpendicular to the magnetic field which lies along the vertical.

liquid can arise through the onset of solution crystallization or, by ordered packing as discussed in the theories developed by Onsager,(27) Isihara(28) and Flory.(29) Flory's treatment is particularly straightforward and is based on equilibrium thermodynamics. His approach considers minimization of the free energy of a solution of rodlike solute particles. In such a solution, the development of an anisotropic phase results due to the decrease in molecular competition in the anisotropic phase for arrays of " lattice " sites suitable for placement of the solute particle in the lattice. In the case of highly anisotropic particles (high axial ratio) this decrease in competitive search for contiguous lattice locations lowers the system free energy more than does the increase caused by loss of configuration entropy. The consequences of this theory, although pleasing, should be applied with caution in our case since there is most likely considerable departure from equilibrium conditions i.e., rapid loss of solvent evaporation during film preparation. This is particularly true where highly volatile solvents are used without a controlled vapor environment.

The occurrence of this anisotropic phase at the solution surface can result in a change in refractive index at or near the surface and a development of considerable density fluctuations due to heterogeneities in molecular density. This can easily account for the development of surface turbidity as observed in the " pool " of solution on an exposed substrate as discussed earlier.

Flory's theory does not appear to place exact geometric requirements which the growing phase will follow. Apparent, however, is that the texture could be of a fibrillar or rodlike character. The enthalpic effects and geometrical shape of the asymmetric particles will likely determine the " shape " and packing arrangements of the anisotropic phase. The " shape " of the anisotropic phase may in fact change as the concentration of the solute increases due to volatilization of solvent. This speculation is proposed based on the observations of Robinson(2) and Frenkel *et al.*(4) for PBLG solutions where liquid spherulites were observed initially but their collapse occurred as the solute concentration approached 100%. The kinetic considerations are presently lacking but it is suspected that the nucleation of these anisotropic phases (or " swarms ") and their orientation behavior are highly dependent on various external variables such as time, temperature, solvent-solute compatibility, etc.

The occurrence of optical activity, if present, can alter the scattering patterns in two ways: (a) the effect of an optical active medium will influence the polarization directions for both the incident and scattered rays and (b) the scattering will be enhanced due to the fluctuation in magnitudes and direction of optical rotation. One might describe these optically rotating effects respectively as external and internal. Both of these may complicate the interpretation of the scattering data.† The development of an optically active mesophase has been discussed by Robinson, Ward and Beevers(30) in light of the PBLG *solutions* where the solvent favors the formation of the α helix (e.g. chloroform). The occurrence of optical activity also depends on the type of mesophase. Specifically it seems to be noted in the cholesteric phase. Its origin is discussed in some detail by Robinson and will not be repeated here as it does not appear to be directly pertinent to the *film* morphology.

Picot and Stein,(31) in discussing effects on the scattering arising from spherulitic origin, found that " external " optical rotation affects the H_v scattering patterns considerably while the V_v pattern is affected less extensively. The H_v pattern found in undeformed spherulitic material is transformed from a distinctly four-fold symmetric pattern (as Fig. 2a) to a diffuse circular pattern as the optical rotation increases. Their same treatment cannot be directly applied to the case of rod scattering, but it is anticipated that similar results would be found. We are now considering the extension of this theory to anisotropic rod-like media in order to confirm our speculations. The effects of optical rotation, however, are not a highly significant factor in our data since it appears that the cholesteric mesophase does not dominate the texture in any of the films. For example, identical H_v " rod " patterns were obtained on films of varying thickness; this would not be expected if considerable optical activity existed. The film thickness maximum is, however, limited by the optical transmission of the film itself. For minimization of secondary scattering and higher order scattering the transmission should be of the order of 70% or higher. From our experience this requires a relatively thin film for the peptides systems e.g. $<$three mil.

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† It is worthwhile to mention that to this author's awareness the case of " internal " optical rotation effects has not been theoretically treated.

Our initial data spurred interest in the effects of temperature on the stability of existing film morphology and in PBLG in particular since Koleski and Lundberg(32) noted a mechanical damping peak at ~ 90 °C in compression molded films of PBLG (and 113 °C for PBDG) measured by torsion pendulum methods. They suggested that this damping peak might be due to the transformation to a mesophase, probably that of a nematic or smectic phase. To see if this effect could be noted by the scattering techniques and to see if any thermotropic response could be induced in the cast films of PBLG, we measured thermal dependence of H_v scattering patterns with a Mettler FP2-Hot Stage system. Figure 11 shows the typical results for PBLG films cast from chloroform while Fig. 12 shows the patterns taken on a thin film prepared by compression molding at 60 °C. Two points of significance arise from the data. First, there seems to be no apparent dependence of the H_v patterns on temperature for either the chloroform or compression molded films. Second, there is no azimuthal dependence of the scattering for the compression molded film. This implies strictly random orientation correlations rather than nonrandom orientation correlations as exist with the chloroform cast films. Furthermore, it seems that no further thermally induced mesophase transformations occur in these peptide materials. Similar observations were made on PMG film. This lack of transformation, even if thermodynamically favored, may be due to the fact that the viscosity is too high to allow flow to occur in the time scale of the experiments performed (anywhere from minutes to two hours). These experiments do not rule out that mesophase formations cannot occur over extended time periods, but it seems doubtful that such transitions exist for these materials.

In light of the work of Samulski we also tried to orient films of PBLG by solvent casting in the presence of a magnetic field. We wished to observe whether this electropic effect would alter the superstructure in any notable manner. Samulski's X-ray data clearly showed that distinct layer lines could be noted when X-ray diagrams were taken with the beam normal to the plane of the film—see Fig. 13a. The strength of the magnetic field in his studies was of the order of 10 K gauss. Using a Fieldial electromagnetic system we were able to reach field strengths as high as 25 K gauss during film casting. X-ray patterns are shown in Fig. 13 b and c taken on

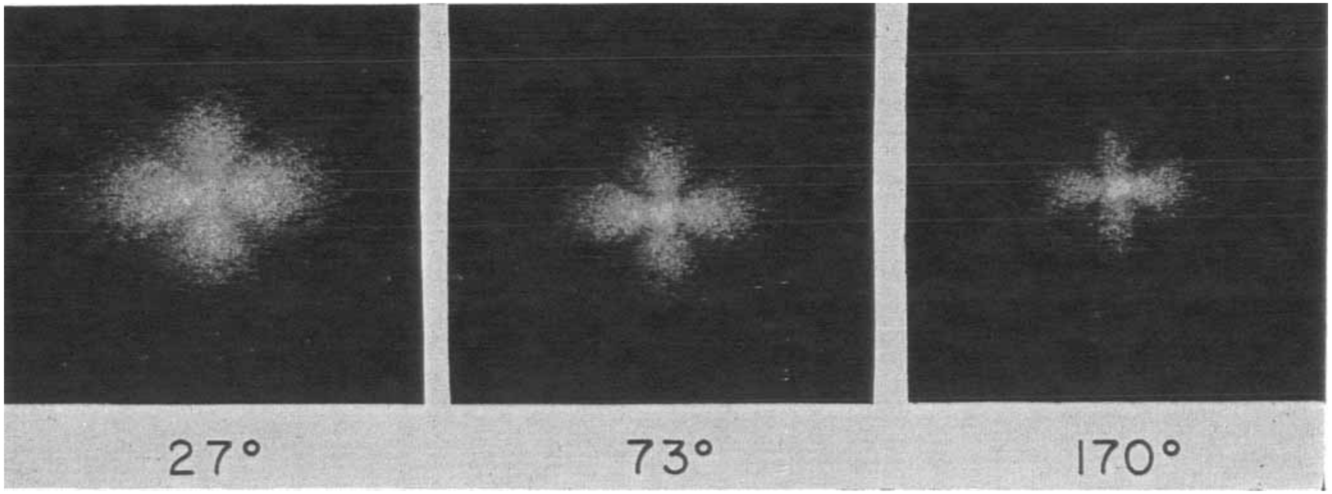

Figure 11. H_v patterns taken as a function of temperature from a film of chloroform cast PBLG.

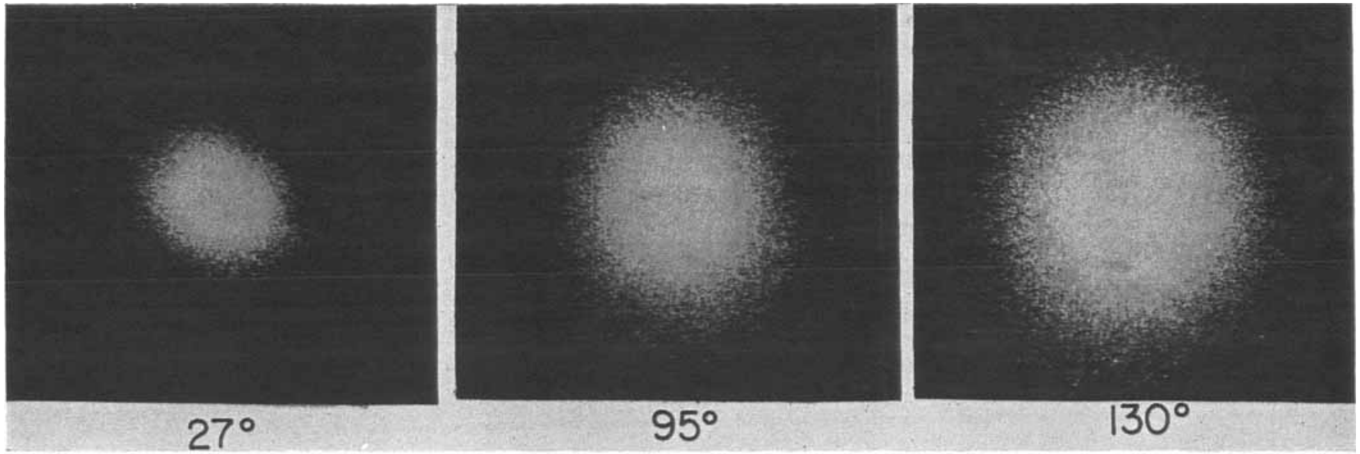

Figure 12. H_v patterns taken as a function of temperature on a compression molded film of PBLG.

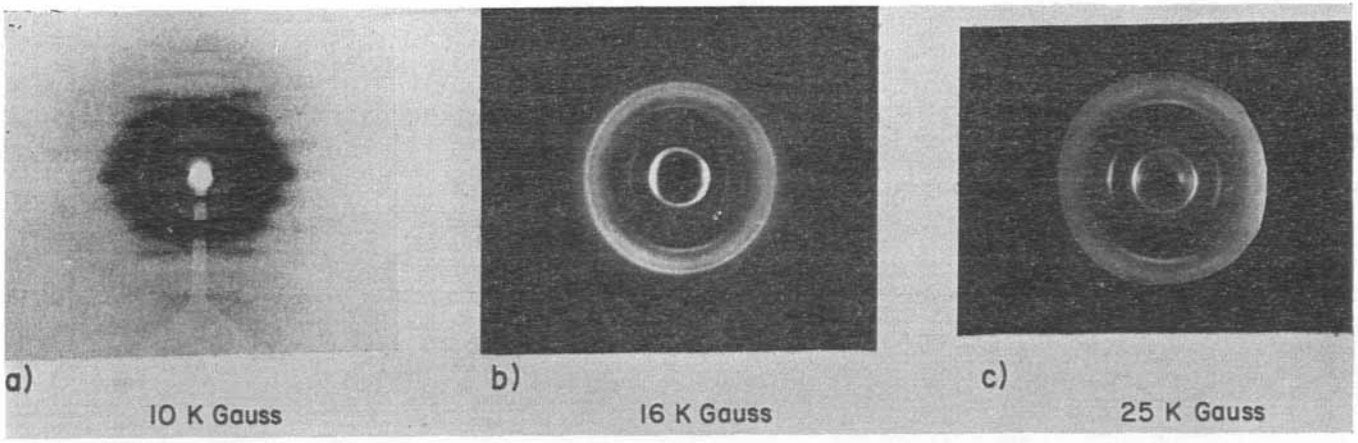
a) 10 K Gauss b) 16 K Gauss c) 25 K Gauss

Figure 13. Wide angle X-ray diffraction patterns taken on chloroform cast PBLG films. Films were cast in a magnetic field with the field parallel to the film plane. In the figure the field direction is vertical. (Fig. 10a courtesy of E. T. Samulski.)

thin films cast at 16.5 K gauss and 25 K gauss. Before presenting the corresponding light scattering data it is worth commenting on these X-ray patterns. A significant point is that at these higher field strengths we did not see the formation of layer lines but found only well-defined arcs as have been noted in drawn fibers. A typical H_v scattering pattern taken from these films is shown in Fig. 14.

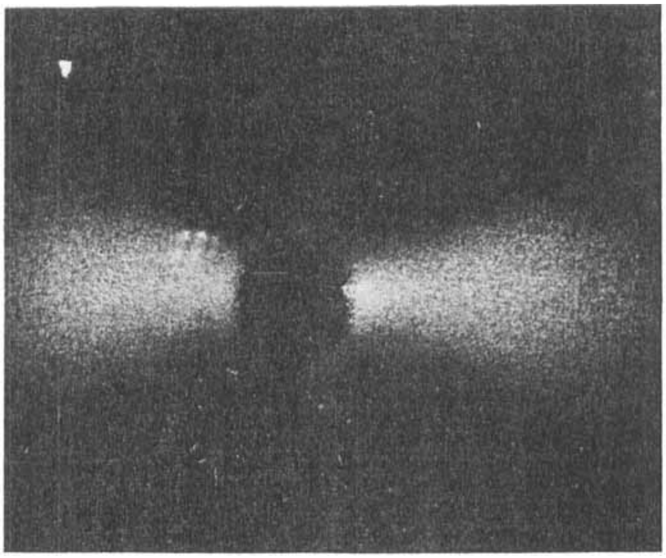

Figure 14. H_v scattering pattern taken on PBLG film cast in a magnetic field of 25 K gauss. The field direction is horizontal.

Similar results were obtained from their film. It is clearly apparent that the magnetic orientation has affected the texture of the superstructure as well. One's first thought might be to compare the observed H_v pattern (Fig. 14) with a theoretical contour plot where the orientation parameter, ϵ, is greater than unity. Doing so (see Fig. 1 $\epsilon = 4$) clearly reveals that the experimental pattern is rotated 90° from that given theoretically. This simply implies that the " rods " are oriented perpendicular to the magnetic field directions rather than being parallel. We, in fact, generated a theoretical scattering pattern in agreement with the experimental by using an ϵ value of 0.25 which nearly corresponds to a perpendicular arrangement. A possible model for this texture might be as shown in Fig. 10c where it is noted that the molecular axis lies along the direction of the magnetic field, the optic axis is at 45° to the rod axis and the rod axis is perpendicular to the field. This model is strictly tentative and is only presented to help interpret the observed X-ray and light scattering patterns. It should also be pointed out that in view of these two types of information one is forced to postulate that the principal optic axis of the rod and the principal axis of the assumed α helix of PBLG do not coincide as might be initially expected. This discrepancy may well be due to the orientation of the highly polarizable side chain (benzyl ester). Further efforts involving both X-ray and light scattering studies will be helpful in better understanding this point.

It was of interest to see if similar superstructure could be found in peptides of natural origin. We chose to investigate collagen since we were carrying out some mechanical studies on the system at that time. It should be realized that the preparation techniques for purification of collagen from tendon are numerous and vary widely in chemical treatment, pH etc. Investigation was, therefore, carried out on two common forms of collagen film—one that was prepared by acidic means, CA film,(25) and a second by an alkaline method, CB film.(26) Figure 15a shows the H_v pattern for the CA film while Figure 15b shows the H_v pattern for the CB materials. Both films were identical in appearance and were optically clear.

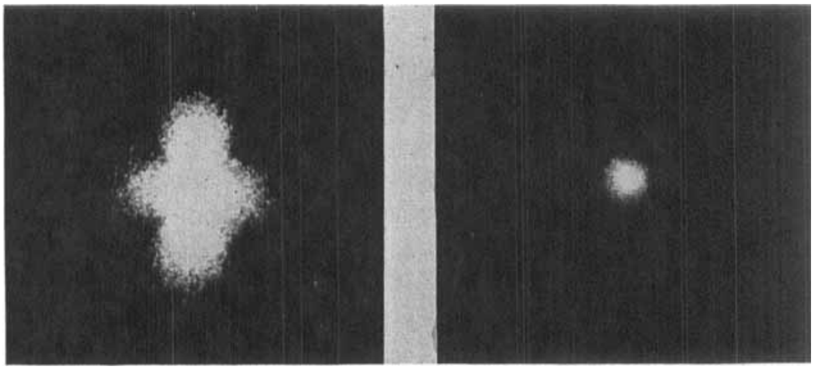

Figure 15. H_v patterns from reconstituted collagen films: (left) acidic preparation (CA); (right) alkaline preparation (CB). STF = 12 in.

The data demonstrates conclusively that the similar " rod-like " patterns can be obtained for the CA film but not the CB film. To obtain the H_v pattern for the CA film, it was necessary to use a sample to film distance that was greater than for the synthetic peptide films. Yet it is clear that the pattern is " smaller " indicating a larger " rod " structure than noted in the other peptide films.

Scanning electronmicroscopy, to support our data, was used to look at the film surfaces of both CA and CB films as well as films of PBLG cast from chloroform. Limited data could be obtained on the collagen due to poor stability in the electron beam. Figure 16 shows an electronphotomicrograph of the surface of the CA film. Although the film shows electron damage (the presence of cracks)

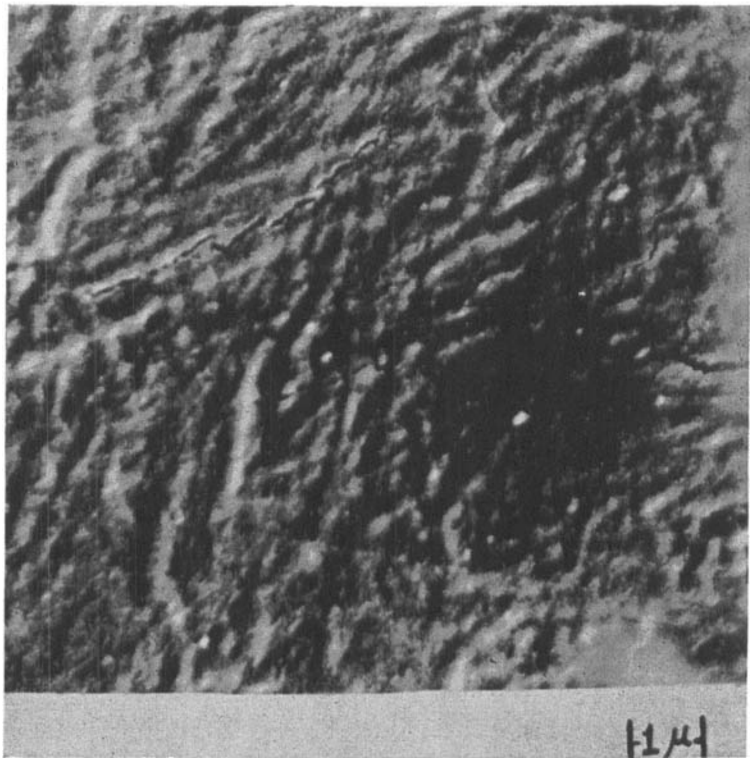

Figure 16. Scanning electronmicrograph showing the surface of the CA collagen film (10,000 ×).

it is clear that a fibrillar texture is visible. This texture was completely lacking in the CB film, in fact, the surface appeared to be homogeneous and smooth even at higher magnifications. This difference in collagen superstructure between the CA and CB films is somewhat surprising, as the difference appears to depend on the two types of chemical treatment of the collagen preparation. We are presently investigating this further. It is clear, however, that the scattering behavior of the collagen is in agreement with the microscopy data. As with the CB film, no fibrillar texture was apparent at the surface of the PBLG film, but in light of the scattering pattern characteristics this was expected. That is, since the PBLG scattering pattern occurred at larger scattering angles relative to CA it indicated that the " rods " were of a smaller size. Since it was difficult to observe the CA texture it is not surprising that it was lacking at the surface of the PBLG films. There was evidence of fibrillar texture in an edge view of PBLG film. Samulski(5) reported similar observations. We can reasonably conclude that the preliminary microscopy studies are in agreement with the light scattering data and its present interpretation. Further studies however are necessary to delineate the fine points of interest.

In conclusion it is postulated that the presence of rodlike or fibrillar superstructure, above the molecular level, is generally induced in other solid peptide systems of natural or synthetic origin. The inducement of this superstructure and its character will depend on the degree of helicity retained at the molecular level and certainly upon such factors as rate of solvent evaporation, type of solvent, and/or chemical treatment. We also found no major transformation of superstructure over a considerable temperature range for the cases of the synthetic peptide films investigated. There is, however, a definite and reproducible thermally induced transformation in the case of the CA collagen film. The nature of this transition, dependent on chemical environment, will be reported elsewhere since the structural features of collagen are not of direct importance to the work reported here. We have clearly shown that very high orientation can be induced in PBLG films solvent cast in the presence of extremely high magnetic fields. The ordering as noted by X-ray diffraction is also found by light scattering, and indicates that the superstructural elements are preferentially aligned perpendicular to the magnetic field but the " type " of elements remain the same. Further studies are now in progress to further elucidate manv of the points of interest presented in this paper.

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