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Freeze-Fractures in Cholesteric Mesophases of Polymers

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Freeze-fracture techniques were used to study the cholesteric liquid crystalline phases of three polymer solutions (PBLG, Xanthan, HPC).

All fracture surfaces showed a regular stratification corresponding to the half-helical pitch, P/2. Electron microscopy permits very small helical pitches (P/2 < 300 Å) to be observed and defects and textures previously analyzed in the polarizing microscope can be easily recognized: edge dislocations, $+\pi$ and $-\pi$ disclinations etc. Fractures oblique with respect to the cholesteric stratification present a regular distribution of parallel series of nested arcs whose nature is directly related to the cholesteric organization but the shape of the arcs can be modified by the presence of steps in the fracture surface. The position of these steps is related to the fracture direction with respect to the cholesteric stratification.

Fractures through polygonal fields are very different from the classical patterns observed in the polarizing microscope since we are observing the distribution of molecular orientations in a plane instead of a projection of the whole structure. In such fields, cholesteric layers are distorted to form domes and basins and fractures normal to the axes of these deformations produce spiral and saddle-shaped patterns.

Keywords: freeze-fracture, cholesteric mesophase, polymers, electron microscopy, spirals, liquid crystals

INTRODUCTION

Numerous polymers of biological interest form liquid crystalline mesophases in concentrated solution. Among these are nucleic acids: DNA and RNA,[1] polysaccharides: xanthan, hydroxypropylcellulose (HPC)[2,3] and polypeptides: PBLG.[1] Their textures have been mainly studied by light microscopy.[1–6] We decided to analyse by freeze fracture methods the orientation of molecules in cholesteric mesophases. This technique is an appropriate method, classically applied to biological structures, but has been rarely used for the study of cholesteric liquid crystalline mesophases.

The freeze-fracturing method is applied here to three polymers: PBLG, xanthan and HPC which form cholesteric phases in concentrated solutions.

This work was presented at the 11th International Liquid Crystal Conference (Berkeley, USA).

MATERIAL AND METHODS

Polymers

1. The synthetic polypeptide, PBLG (M.W. = 60,000) type III, Sigma, was used in solution in dioxan.
2. Xanthan (M.W. = 150,000) is a polysaccharide secreted by the bacteria *Xanthomonas campestris*. A pure fraction of xanthan was kindly provided by Drs. Rinaudo and Milas (CERMAV, CNRS Grenoble, France). This polymer was dissolved either in a 30% glycerol solution or in the same solution with NaCl 0.1 M added. Different concentrations were used, ranging from 150 to 300 mg/ml.
3. HPC (M.W. = 60,000) was provided by Dr. Navard (Sophia-Antipolis, France) and used at a concentration of 55% in water.

Solutions of polymers were prepared a few days before the experiments. Small drops were deposited onto gold discs to stabilize for a few minutes (PBLG) or a few days (xanthan and HPC). It was not possible to wait longer with PBLG because of the high volatility of the dioxan solvent.

Freeze-fracture

Samples mounted on gold discs were rapidly frozen by immersion in liquid Freon 22 (-160°C) cooled by liquid nitrogen and stored in liquid nitrogen (-196°C). Freeze-fracturing was carried out in a Balzers BAF 400 T apparatus. During fracturing, the knife was maintained at -150°C and the specimen at -110°C under 2.10^{-7} Torr vacuum. After fracturing, specimens were immediately shadowed with platinum-carbon and coated with a layer of carbon. The shadowing was unidirectional (at an angle of 45°). Samples and replicas were reheated to room temperature and the replicas were separated from the samples by immersion in the appropriate solvent. For xanthan and HPC, replicas were washed twice in distilled water (2 × 1 hour), a third time with filtered sodium hypochlorite solution and finally with distilled water. For PBLG, replicas were immersed in absolute ethanol to remove condensing water, washed three times in dioxan and finally in absolute ethanol. Replicas were picked up on 300 mesh grids and observed in a Philips 201 electron microscope, with an accelerating voltage of 80 kV. Directions of fracture and shadowing are indicated by lines and arrows respectively on each micrograph.

RESULTS

Cholesteric stratification and arced patterns

All freeze-fracture replicas of the cholesteric mesophases show a regular stratification which corresponds to the cholesteric periodicity. Most fractures also show parallel series of nested arcs within these stratifications. This is illustrated in Figure 1 with HPC (a), xanthan (b) and PBLG (c).

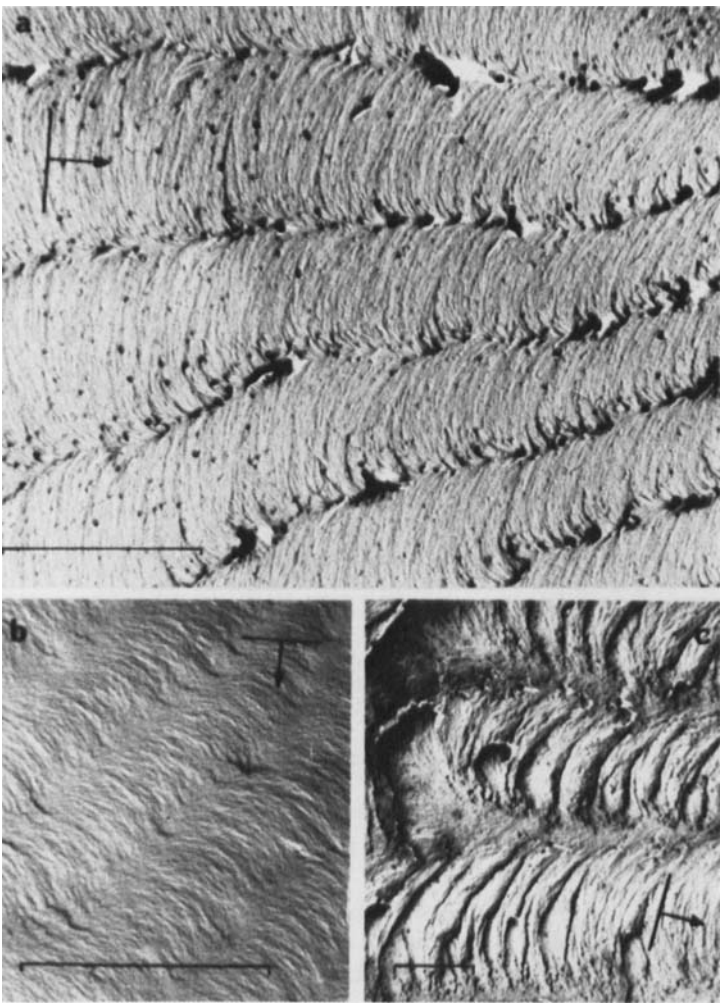

FIGURE 1 Series of nested arcs observed in cholesteric samples of HPC (a), xanthan (b) and PBLG (c). Such patterns correspond to fracture planes oblique with respect to the cholesteric stratification. The replicas show reliefs which follow two main directions either parallel to the series of arcs or parallel to the arcs themselves (clearly visible in c). Bars correspond to 1 μm. Bar and arrow indicate the knife and the shadow direction.

The existence of the series of arcs is directly related to the cholesteric geometry of the structure. Their nature was extensively studied in sections of various polymerized materials.[7-9] The arced patterns were shown to correspond to fracture planes, oblique with respect to the cholestric stratification, the arcs being drawn by the regular succession of molecular orientations in projection onto the oblique plane (Figure 2).

Although the patterns seen in freeze-fracture replicas are reminiscent of those observed in thin sections[7] they show additional important features due to the complexity of the fracturing process.

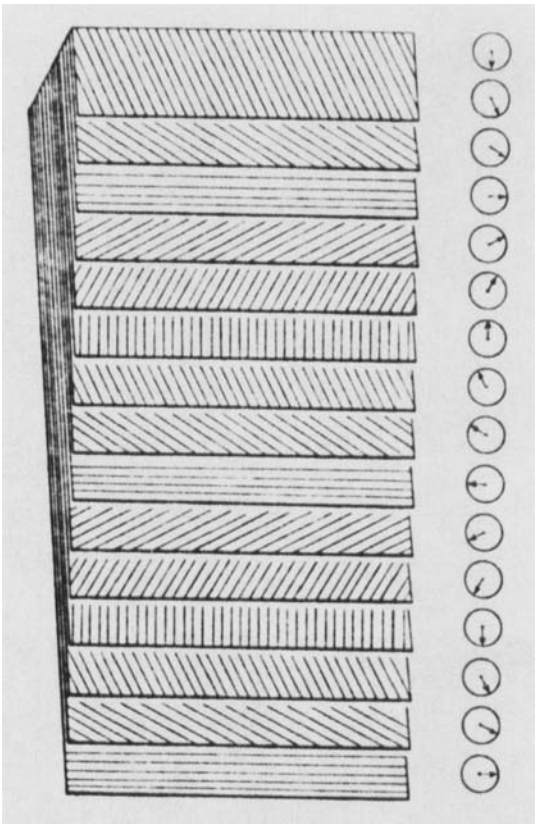

FIGURE 2 Origin of nested arcs in oblique fractures. Polymer orientation in each layer is indicated in the corresponding dial in the right part of the figure.

Regular steps follow two main directions: (i) the cholesteric layering and (ii) the arced patterns. These features significantly modify the shape of the arcs as shown in Figure 3. Steps parallel to the series of nested arcs may be located at different places and producing series of arcs which are either sharp (Figure 3a), asymmetric (Figures 3b, 1a) or flattened (Figures 3c, 1b,c).

Defects and textures

Fracture planes normal to the cholesteric stratification present a regular periodicity corresponding to the half-helical pitch and this spacing can be measured directly if the fracture plane is exactly parallel to the cholesteric axis. Most patterns are reminiscent of the fingerprint textures observed in polarizing microscopy. Defects are easily recognized: $+\pi$ and $-\pi$ disclinations (Figure 4a, b), and edge dislocations (Figure 4c). The latter often have a Burgers vector corresponding to a large number of half cholesteric pitches. Other regular associations of defects (not shown here) are also observed. The freeze fracture method facilitates the analysis of defects and textures in mesophases whose helical pitch is too small to be resolved in the optical microscope. It should also be a great help in the determination of molecular orientations in the core of the defects.

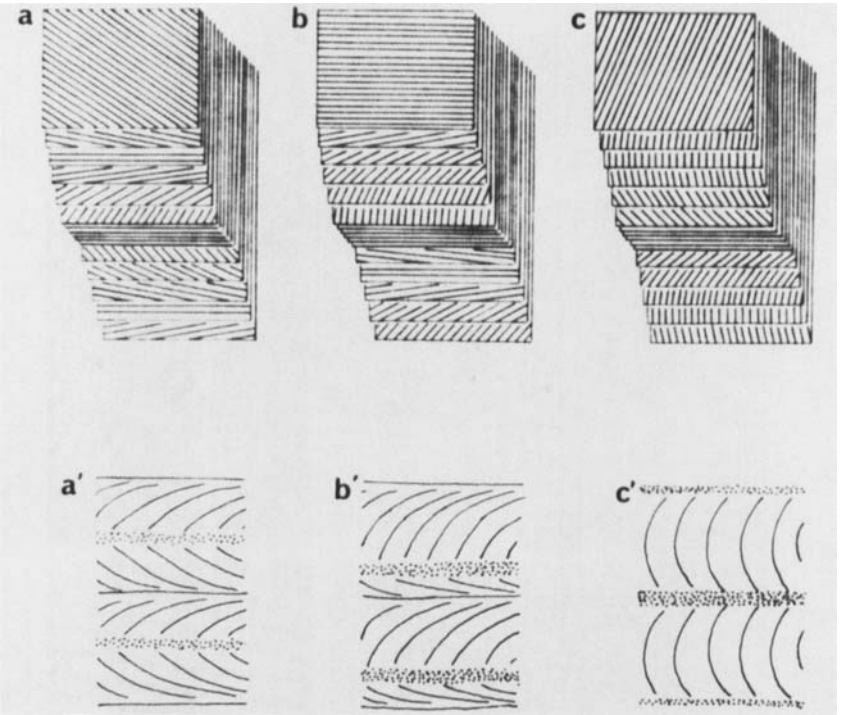

FIGURE 3 Modulations of arc shapes for three different step profiles. a, b, c: different positions of steps relative to cholesteric structure; a', b', c': corresponding shapes of the arcs.

Single and double spirals

Polygonal textures observed in freeze-fracture replicas show very beautiful fields of spiral patterns as shown in Figures 5 and 6. A single spiral is directly visible at a low magnification (Figure 5). This feature is formed by surface steps parallel to the cholesteric layers. At higher magnification, this single spiral superimposes to a double spiral of nested arcs as observed in Figure 6.

In polygonal fields, the cholesteric layers are distorted to form domes and basins. Such features are very numerous in xanthan and appear as a field of quadilaterals in the polarizing microscope. The origin of the double spiral of nested arcs is shown in Figure 7. In polygonal fields, the cholesteric layers are wrapped around focal lines and are therefore more or less conical. Many fracture planes cut obliquely or nearly perpendicularly the corresponding focal line. Filaments projecting along a given direction in the fracture plane belong to nested conic surfaces as shown in Figure 7a. When viewed from above, one sees a double spiral of arcs which, at lower resolution appears as a single spiral corresponding to the surface step produced by the fracture. Similar steps have been observed on both faces of thin sections of twisted fibrous materials (the biological analogues of cholesteric liquid crystals). Detailed discussions of this topic are given in refs. 8, 11–13.

Cholesteric layers often assume a saddle shape, for example in regions lying between two neighbouring domes or basins in a polygonal field. A fracture tan-

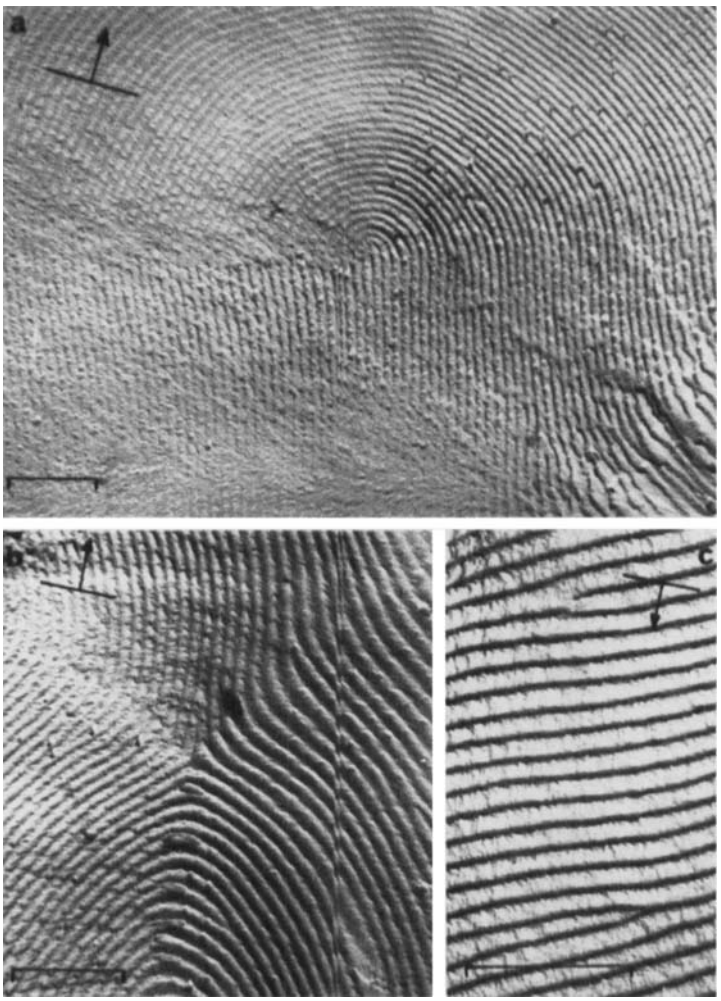

FIGURE 4 Defects observed in cholesteric mesophases of xanthan. a: $+\pi$ disclination; b: $-\pi$ disclination; c: edge dislocations with P/2 Burgers vectors. Bars correspond to 1 μm. Bar and arrow as in Figure 1.

gential to such a saddle layer shows series of nested arcs which form a system of concentric hyperbolae.[10] There are also steps which form a slightly different system of lines and this will be described in a further report.

Diversity of fracture patterns

Individual polymer molecules can not be resolved by the freeze-fracture technique. The polymers studied here have diameters which are far below the resolution of the freeze-fracture method (50 Å or more). Micrographs show a large series of fracture patterns which depend on the local distribution of polymers and also on

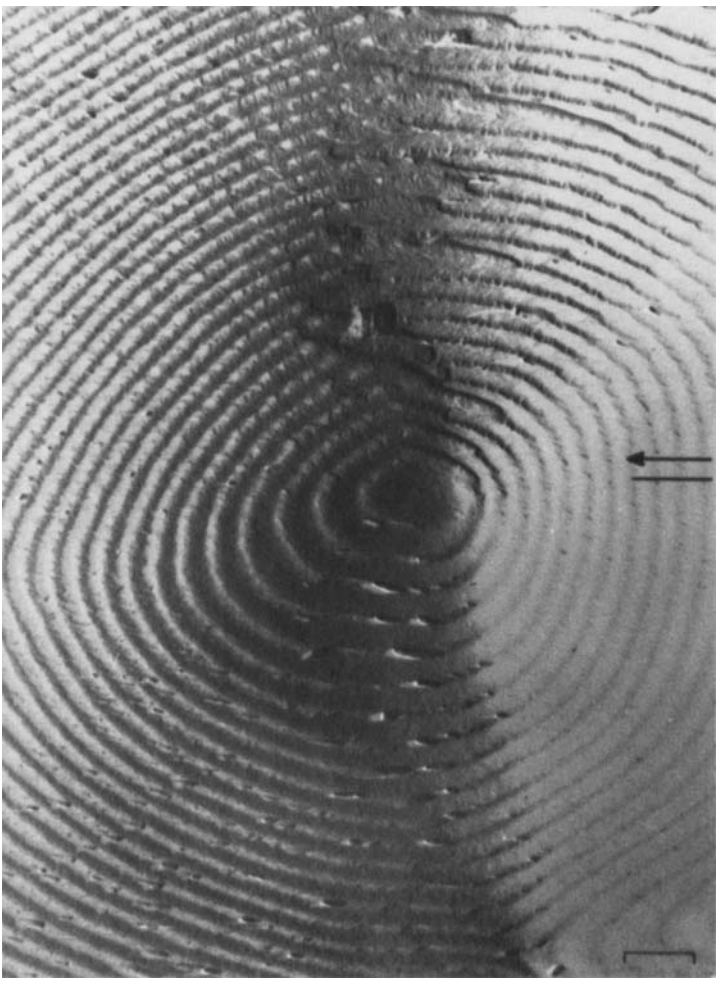

FIGURE 5 Spiral patterns are observed when polygonal textures are fractured. Here a dome of cholesteric xanthan shows a single spiral within the fracture plane. Bar: 1 μm. Bar and arrow as in Figure 1. However the shadow orientation lies in projection parallel to the knife pass.

the knife pass. Let us call "fracture domains" regions of the replica, where the fracture seems to be planar or slightly curved, the steps being considered as microstructures of these domains. The boundaries of domains are often well defined and correspond to sharp dihedrons, but obviously this is not always the case. The presence of clear-cut dihedrons in replicas shows that the successive domains of fracture separate by large angles from the plane defined by the knife motion. Our micrographs therefore show very different local orientations of fractures and this generates extremely different angles of shadowing. Moreover, cholesteric axes present all the possible orientations. This leads to a considerable variety of situations.

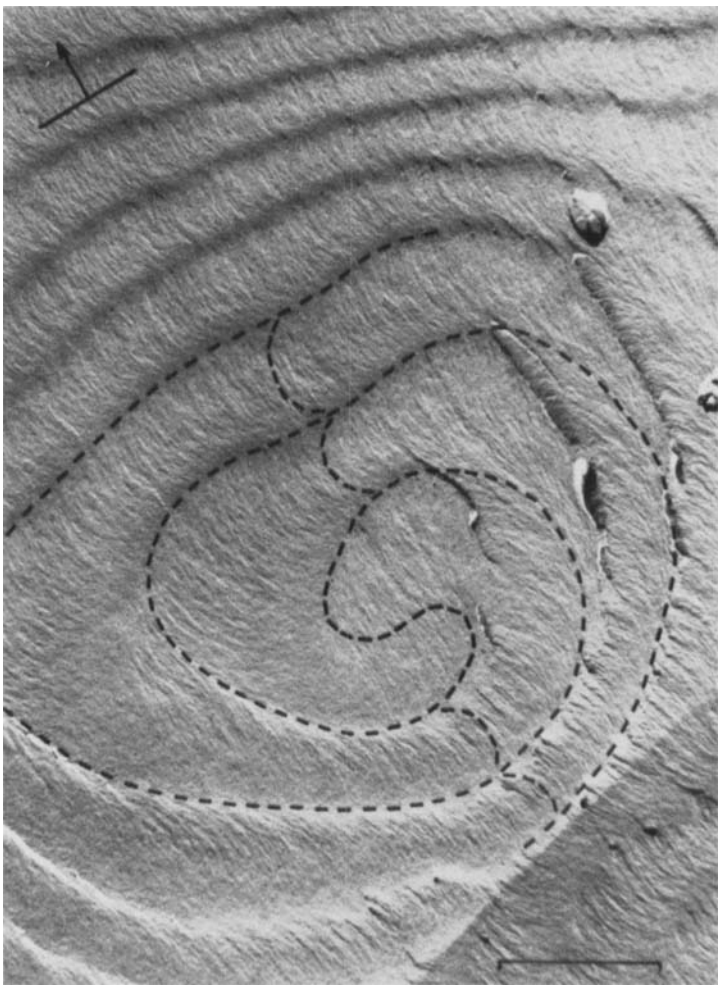

FIGURE 6 The two spiral patterns are superimposed: a double spiral of nested arcs (dotted lines) and a single spiral due to the fracture. Bar: 1 μm. Bar and arrow as in Figure 1.

In spite of this complexity there are constant patterns: *nested arcs* and their modulations due to *steps*. Arced patterns are pictured as following the polymer orientation in projection onto the fracture plane. These arced fractures are often gathered into small fan-shaped groups (Figure 1c). Arcs can be also replaced by more or less parallel sinusoids which cross the cholesteric layers. The steps themselves can be more or less sharply defined: a small angle between cholesteric layers and the fracture plane gives no steps or very smooth steps; on the contrary steps are more important, when this angle is greater and when the series of arcs lie in the direction of fracture. There are also a set of well defined patterns observed along the edges of dihedrons at the limit of fracture domains.

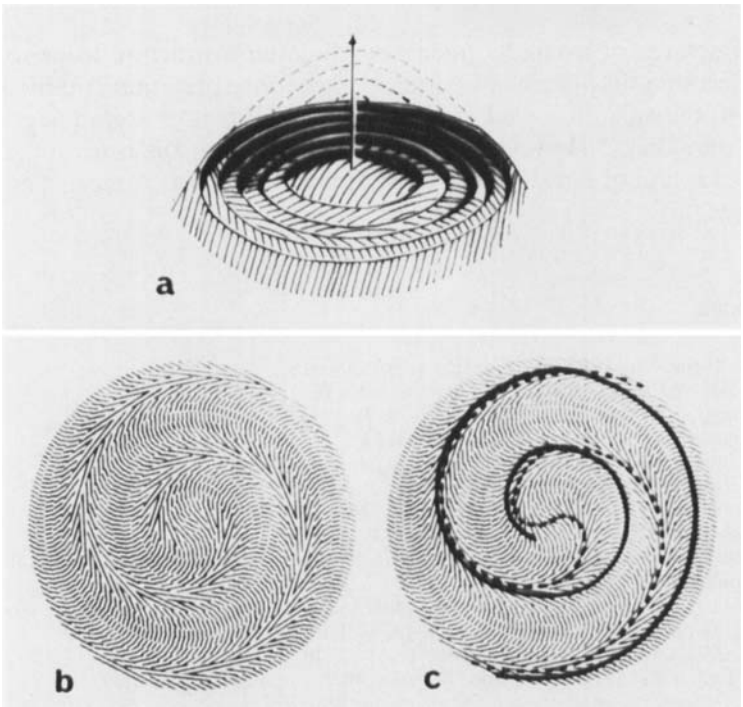

FIGURE 7 Origin of the spiral patterns observed in freeze-fracture. (a) a mound of cholesteric layers fractured along a horizontal plane. The concentric conical surfaces are intended as a conceptual aid to represent the cholesteric structure. (b) Figure a viewed from above. (c) the double spiral corresponding to the edges of the nested arcs (indicated by dotted lines) and the single spiral corresponding to the surface step shown as a solid line now superimposed to Figure b. Redrawn from reference (8).

DISCUSSION AND CONCLUSION

The freeze-fracture technique has been frequently applied to lyotropic mesophases (lamellar and hexagonal) but there are few papers dealing with cholesteric liquid crystals. There is however a study due to Lydon and Robinson,[14] (1972) on the structure of various cholesteryl esters rapidly frozen in their cholesteric (and smectic) phases. A more recent study due to Costello *et al.*[15] (1984) compares a cholesteric phase with its blue phase. In these two works, there are no clear signs of the presence of arcs corresponding to the twisted distribution of molecules in the studied cholesteric phases. However, the micrographs show series of parallel stripes which arise from the cholesteric periodicity (i.e. the half helicoidal pitch).

The three distinct polymers give very similar fracture patterns and there are only slight differences. Arcs, steps, double spirals and saddle patterns are recognizable in these different materials. Cholesteric phases of DNA were prepared *in vitro* and were compared with the cholesteric chromosomes of certain microorganisms[4] (Livolant, 1984). We intend to do a similar comparison at the ultrastructural level between these cholesteric phases of DNA and the cholesteric chromosomes which show remarkably distinct patterns of arcs in freeze-fracture preparations.[16]

The fractures of a rapidly quenched cholesteric structure leads to mechanical events resulting in a series of patterns whose interpretation is difficult. Arcs are related to the twist observed in oblique view and regular steps underline the cholesteric periodicity. However, problems remain about the origin of surface steps, of sinusoids and of fan-shaped groups of arced patterns. A more detailed study is necessary.

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