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Precholesteric liquid crystalline states of DNA

F. Livolant

Centre de Biologie Cellulaire (CNRS), 67, rue Maurice Günzburg, 94200 Ivry-sur-Seine, France

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Résumé. — L’ADN donne en solution aqueuse concentrée des phases cristallines liquides cholestériques dont les textures ont été analysées précédemment. La transition entre la phase isotrope et la phase cholestérique se fait généralement avec formation de sphérulites cholestériques qui naissent au sein de la phase isotrope et fusionnent ensuite pour donner une phase cholestérique homogène (transition du premier ordre). Nous décrivons ici avec le même polymère, l’existence d’une transition d’ordre supérieur entre la phase isotrope et la phase cholestérique. Cette évolution a été arbitrairement divisée en cinq stades que nous appelons précholestériques. Nous proposons une interprétation géométrique de cette évolution selon laquelle sont introduites successivement trois organisations hélicoïdales selon les trois directions de l’espace. Le modèle proposé ne présente aucune discontinuité des orientations moléculaires, les seules discontinuités possibles étant des lignes de disinclinaisons +1 et −1. La structure de ces édifices constitués de polymères présente de nombreuses analogies avec les phases bleues constituées de petites molécules, en particulier le double twist et la défrustration.

Abstract. — In aqueous concentrated solution, DNA forms cholesteric liquid crystalline phases whose textures were previously analysed. The transition between the isotropic and the cholesteric phase occurs generally with formation of cholesteric spherulites which grew inside of the isotropic phase and coalesce to give a homogeneous cholesteric phase (first order transition). Here is described with the same polymer, a higher order transition between the isotropic and the cholesteric phase. This evolution was arbitrarily divided into five steps that we called the precholesteric stages. We propose a geometrical interpretation of this evolution: three helical structures are introduced in the three directions of space. The proposed model does not present any discontinuity in the molecular orientations, the only possible singularities being +1 and −1 disclination lines. The organization of these polymer structures present numerous similarities with the blue phases made of small liquid crystal molecules, namely double twist and defrustration.

1. Introduction.

When molecules form spontaneously cholesteric liquid crystalline phases in concentrated solutions, cholesteric spherulites are usually found at the transition between the isotropic and the cholesteric phase. This occurs whatever the nature of the molecules: small liquid crystal molecules or polymers. Indeed, cholesteric spherulites were obtained as well with long molecules such as nucleic acids (DNA and RNA) [1-4], polypeptides (PBLG) [5, 6, 4] and polysaccharides (xanthan) [7, 4] as with small synthetic molecules such as MBBA twisted by addition of cholesterol benzoate and PAA [4]. Besides, a comparative analysis of these numerous kinds of spherulites was presented previously [4].

We are particularly interested in the study of liquid crystalline polymers and namely those of biological interest and we observed in DNA the occurrence of other ways to reach the cholesteric organization. These evolutions occur without formation of a definite interface between the isotropic and the cholesteric phase. Two kinds of structure will be considered as precholesteric structures. The first one, observed in DNA and encountered with numerous polymers is referred as the banded structures. The transformation of such structures into cholesteric ones was never followed but we show that nothing prevents this evolution to occur, at least in theory. The second evolution was followed in function of time and divided into several steps that we called precholesteric stages. The molecular organization is analysed in detail for each of them. This work completes the study of the liquid crystalline textures
exhibited by biopolymers (DNA, PBLG and xanthan) in concentrated solution [8, 9].

The transition described here in DNA is surely not specific to this molecule but more probably another way for long polymers to reach the cholesteric organization. The reasons which determine the occurrence of this higher order transition instead of the first order one (with formation of spherulites) will be discussed as well as the conditions required to make this evolution possible.

Moreover, the structure of these precholesteric states will be compared to those described in the blue phases. Indeed, these two systems present numerous similarities namely double twist and defrustration. However, blue phases were obtained with small liquid crystal molecules whereas precholesteric stages concern long polymers.

2. Material and methods.

Calf thymus DNA (Merck) was solubilized in 15 mM Tris-Cl- buffer pH 8 and sonicated to obtain short fragments. This method was described previously [3, 8]. The length of the DNA molecules was estimated by electrophoresis on agarose gels by comparison with DNA of clearly defined length (Lambda DNA cut by Hind III). DNA fractions with molecular lengths ranging from 150 to 5 750 base pairs (0.05 to 1.9 μm) were used in this study.

Good preparations were obtained by addition to a drop of 10 to 50 mg/ml sonicated DNA (in buffer) of an equal volume of KCl solution (0.2 to 0.4 M) or pure water. The mixture is immediately deposited between slide and coverslip and stored either at 4 °C or at room temperature.

All preparations were observed with a Leitz (Orthoplan Pol) or a Nikon (Optiphot X Pol) polarizing microscope, between crossed polarizers. Molecular orientations were determined by the use of a quartz first order retardation plate inserted at 45° between crossed polarizers. The DNA molecule presents a positive form birefringence due to the polymer alignment which is small in absolute value relative to the negative intrinsic birefringence produced by the stacking of base pairs normally to the long axis of the molecule. The birefringence is then negatively uniaxial. This property was used to determine the molecular orientations.

3. Results.

3.1 BANDED STRUCTURES. — As will be seen later, precholesteric stages are highly organized structures. We obtained with long DNA molecules much more simple organizations whose patterns recall the « banded structures » described by many authors with various polymers. Usually, these patterns are described as relaxation processes which occur immediately after the end of a shear which was applied to the preparation [10-17]. In our case, these textures appear in a few hours or days by concentration of the polymer in solution and they are stable. Their analysis may be considered as an introduction to the study of the precholesteric stages.

These textures are presented in plate I. For particular orientations of the preparation, between crossed polarizers, large illuminated regions are divided into elongated domains by thin dark lines, more or less parallel to one polarizer direction (Pl. Ia). When the microscope stage is rotated, the dark lines move and their thickness increases progressively. After a rotation of 45°, this pattern is changed into an alternation of wide light and dark bands. The addition of a quartz first order retardation plate (λ), inserted at 45° between the two polarizers directions transforms the pattern of plate Ia into an alternation of blue and orange bands. This indicates that molecules lie obliquely in each domain and that their orientation changes from one band to the next one. These patterns correspond probably to long range undulations of the director orientation and this is illustrated in figure 1. When undulations are oriented parallel to one polarizer direction, the dark lines are very thin. They enlarge to reach a maximum size after a 45° rotation in both directions, one pattern being then the complement of the other.

Iizuka [18] also observed, in solutions of deoxyribonucleic acids, large domains showing parallel lines different from those observed in the cholesteric phase. They were supposed to be produced by shearing stresses and interpreted as a series of rod like assemblies (molecular clusters) of the polymer molecules. These structures were observed for very low concentrations (less than 1 %) and supposed to correspond to a poor ordering. There is no evidence that these observations correspond to the textures described here.

Resembling patterns were observed in hexagonal liquid crystalline phases of polymers [9]. Molecular distributions are the same but the polymer concentration is different in the two cases. In the banded structures, molecules align progressively from the isotropic phase to give a nematic structure. Besides, the illumination observed between crossed polarizers is less intense in the banded patterns regions than in the hexagonal textures.

Numerous defects are observed in these domains. They always correspond to addition or removal of two illuminated bands (and of two dark lines). However, two situations may be distinguished: one dark line divides into three (A in Pl. Ia) or two dark lines appear simultaneously inside an illuminated domain (B in Pl. Ia). Defects occurring in long range undulations textures correspond always to the addition of one period in the structure. This is the situation encountered here since the addition of one
Plate I. — "Banded structures" observed in concentrated aqueous DNA solution. a) The illuminated regions are divided into elongated domains by thin dark lines which are moving when the microscope stage is rotated. Defects observed in these regions always correspond to the simultaneous addition of two dark lines with formation of either triple points (A) or forks (B). Spindle-shaped domains are also encountered (arrows) (x 1,760). b), c), d) The area of the spindle-shaped domains reduces progressively when the microscope stage is rotated counterclockwise. See figure 3 for interpretation (x 1,600).

Fig. 1. — Between crossed polarizers, long range undulations of director orientation (dotted lines) produce different patterns when the microscope stage is rotated. At 0°, all of the domain appears bright, except the thin lines where the molecules are aligned parallel to one of the polarizer directions. The dark lines extend and move to produce at 45° a regular alternation of light and dark bands. The occurrence of two different patterns in the core of the defect corresponds to the existence of two frequent insertion points of the added period: at an apex of the sine curve (A) or at an inflexion point (B). The two situations are illustrated in figure 2 and encountered in equal proportion in our preparations. Geometrically, the addition of a period can occur everywhere and the two favoured situations correspond probably to optimal situations from an energetic point of view. Spindle-shaped domains are also observed (Pl. Ia, arrow). They are more or less aligned parallel to one polar direction and their size changes when the microscope stage is rotated (Pl. Ib, c, d). The two extreme situations correspond to small dark flames in an illuminated field or to small illuminated flames in a dark field. These patterns correspond to the addition and immediate removal of one helicoidal period (Fig. 3).
Defects occurring in helical systems. Two layers (or one period $P$) are added simultaneously either at a point of maximum curvature (a) or at an inflexion point (b), giving either branched patterns (a) or flamed patterns (b).

We never follow a transition from these textures to cholesteric ones. However, nothing prevents this evolution to occur, at least in theory, as will be seen later.

3.2 PRECHOLESTERIC STAGES. — When a drop of highly concentrated DNA solution is deposited between slide and coverslip, the preparation dehydrates very quickly at the edge of the coverslip and KCl crystals form. This region creates a barrier inside of which the polymer concentrates progressively. A series of textures is observed between the centre and the periphery of the preparation. They correspond to steps leading to the cholesteric phase.

This evolution is continuous but it was convenient to divide it into five steps. The first ones are observed in a few minutes (from 5 to 20 min) at the periphery of the preparation just along the inner side of the crystallized region. The following stages appear a few days after in the central part of the preparation. Their succession in time and localization in space are schematically presented in figure 4.

A narrow illuminated border (state 0) appears first parallel to the limit of the coverslip. The addition of a quartz first order retardation plate indicates that the section of the ellipsoid of indices lies parallel to this border axis; molecules are thus normal to this direction.

Homogeneous birefringent ribbons appear later inside of the isotropic phase in regions where the illuminated border is no more visible (state 1). They are about 1.5 μm wide and the slow axis of the ellipsoid of indices is nearly normal to their elongation axis. They are regularly arranged parallel to the limit of the coverslip or to the air interface (Pl. IIa).
Plate II. — First steps of the precholesteric evolution. a) state 1: Birefringent threads appear in the isotropic phase (I) in the peripheral part of the preparation. The observed defects (arrows) always correspond to addition or removing of two bands (× 600; +). b) state 2A: Each cord presents an alternation of light and dark segments (× 800; +). c) state 2A: Cords with helical patterns which are joined together (× 1,760; +). d) state 3B: The first cholesteric layers (ch.) appear between the cords which are not helicoidal here but homogeneous or striated (× 1,600; +). e), f) state 3B: The same region is focused either in the plane of slide or in the plane of coverslip. The cholesteric domains which are well observed at each level are indicated by arrows. A circuit around the defect shows that a single band is added in each of the two planes (× 624; +).
When they are oriented at 45° between crossed polarizers, the intensity of the transmitted light is slightly different in two adjacent ribbons but we do not discern any colour difference between them when a quartz first order retardation plate is inserted parallel to their axis. This observation rules out the hypothesis of a significant difference in the molecular orientations between two contiguous elements. These patterns recall the « banded structures » but we do not follow any translation of the bands when the microscope stage is rotated. The cords simply extinguish for positions at 0 and 90° of the stage. Defects occur in this alignment and they always correspond to the simultaneous addition (or removal) of two cords (Pl. IIa). As already mentioned two situations are possible in a helicoidal structure according as the addition of the supplementary period occurs either at a point of maximum bend (Fig. 2a) or at an inflexion point (Fig. 2b). The two situations were equally observed in the « banded structures » whereas the first one is much more frequent in this case.

These cords undergo two parallel evolutions either simultaneously or successively : (1) an organization, often helicoidal, appears inside the cords and (2) the first cholesteric layers differentiate between them. These two steps are described separately.

— Different patterns may appear inside of the cords, either a fine striation normal to the ribbon axis (Pl. IId) or a regular alternation of bright or dark oblique bands (Pl. IIb) or also a more or less evident helicoidal organization (Pl. IIc). Cords with oblique bands present different orientations of molecules in the thickness of the preparation since the bands are moving when the focus plane is slightly changed. In addition, the patterns (bands or helices) are shifted from one cord to the next one which produces long-range oblique striations.

— Besides, these ribbons join side by side and the first cholesteric layers appear between them, in contact with the glass surfaces. At this stage, the cholesteric domains are very elongated and they separate groups of two cords, both in planes of slide and coverslip but they are in phase opposition in the two levels. This is schematically represented in figure 5. We observe nearly all the possible orientations of the cholesteric axis in these regions, either parallel, oblique or normal to the preparation plane. However, in most cases, the cholesteric layers lie parallel to the ribbons as in plates IId and III. At this stage of the evolution, defects do not present the same nature than previously. This is illustrated in plates IIe, f : the same region is focused either in the coverslip plane or in the slide plane. In each case, only one band is added. However, a focus in the middle plane (which corresponds to the superposition of the two levels) shows the addition of two bands in the same defect, each one being half the size of the first ones. This is schematically represented in figure 6. Defects showing the addition of one band only are possible in cholesteric structures (when molecules can align parallel or antiparallel). Defects showing the addition of two bands simultaneously are encountered in helicoidal systems, as shown previously.

The cholesteric domains which are first very narrow (often one or two layers) enlarge later in contact with the glass surfaces and sometimes progress also in the thickness of the preparation.

Plate IIIa corresponds to a region of high polymer concentration gradient, between the middle part of the preparation which is less concentrated (bottom) and the peripheral region near the crystallized zone (top). We can therefore observe in the same view the different steps of this evolution : the isotropic phase, the homogeneous birefringent ribbons, the joined ribbons with or without oblique striation and the regions with helicoidal ribbons separated by cholesteric layers.

In these latter regions, the parameters of the helices may be measured on micrographs. Their average values are as follows :

— Helix diameter \((D)\) : 2.4 \(\mu m\)
— Helicoidal pitch \((P)\) : 2.6 \(\mu m\)
— Thickness of the helicoidal strand \((e)\) : 1 \(\mu m\)
— Distance between two helices \((d)\) : 0.8 \(\mu m\)

Fig. 6. — Schematic drawing of the defect of Plate IIc, f. One layer is added in planes of slide and coverslip (a, b) whereas two layers are added in the preparation thickness (c). The star indicates the same position in the three cases.
Plate III. — a) The precholesteric stages appear in a zone limited by the dilute isotropic phase (I) (bottom) and the edge of the coverslip (top). This region presents a strong variation of the polymer concentration. Different steps of the evolution may be recognized from bottom to top of the micrograph: states 1, 3B, 3A (× 928: +). b), c), d) state 3A: The preparation was brought into focus at three levels in the same region: in the plane of the coverslip (b), in the intermediate plane (c) and in the slide plane (d). Each cord presents a helicoidal organization and this helix is slightly shifted from one cord to the next one. The cholesteric layers (CH) follow the cords direction and they are located alternatively near the coverslip and near the slide. The reference marks (*) are useful to compare the three patterns (× 1,480: +).

and the mutual arrangement of the helices is schematically represented in figure 7. Each helix is slightly shifted with respect to the next one which produces oblique lines easily followed when micrographs are seen obliquely.

These patterns correspond probably to true helices since slight changes in the focus plane transform the helix into a series of oblique segments. These helices appear black in our micrographs when they are oriented at 45° with respect to the polarizers directions. They are illuminated for other orientations and they present an alternation of blue and yellow segments when a quartz first order retardation plate is added. Therefore, molecular orientations probably follow this helix inside of the cord. Besides, in the illuminated regions separating the black helices, molecular orientations are aligned parallel to their helices axes (Fig. 8).
The last steps of this evolution are observed in the central region of the preparation, a few days later. The transition between the previous stages and these ones was not followed under the microscope because this evolution is too slow. However, they probably correspond to a direct transformation of the preceding stages since the cords are still visible and well individualized (Pl. IVa). However, the cholesteric organization is no more observed between the ribbons but inside of them. Each cord appears then as a « string of cholesteric beads » (state 4). These strings gather and concentrate into small regions.
limited by air whose area decreases progressively whereas DNA concentration increases. The cords disappear progressively and the « cholesteric cells » join to give homogeneous domains where the cholesteric stratification is much more easily observed (state 5) (Pl. IVb).

The succession of these steps follows a progressive increase of the DNA concentration. Conversely, the addition of distilled water on the edge of the coverslip produces a reversion of this process (from the highly organized patterns of plate III to the homogeneous cords and the isotropic phase). This evolution is schematically represented in figure 9.

We must note that a large polymorphism exists as well in the succession of the different steps than in the precise geometry of each of them. In our interpretation we will then consider only the main and constant lines of this process.

Fig. 9. — Table summarizing the precholesteric evolution.

4. Interpretation.

4.1 BANDED PATTERNS. — The banded patterns are mainly observed with very long DNA molecules. These patterns and their defects may be interpreted as helicoidal organizations of molecules. However, true helices, flattened helices or sinusoids lying in the preparation plane would produce very similar patterns between crossed polarizers since in the three cases, molecular orientations would project in the same way onto the observation plane. Therefore, it is difficult to ascertain whether there are true helices or not in these textures. However, parallel sinusoids will not produce any twist between them whereas we strongly suppose that this twist exists between DNA molecules. Therefore, we retain the helicoidal hypothesis, i.e. a chiral smectic C organization (without the layers) which can be described as follow:

\[
\begin{align*}
    n_x &= \cos \frac{2 \pi z}{p} \\
    n_y &= \sin \frac{2 \pi z}{p} \\
    n_z &= k/\sqrt{1+k^2}.
\end{align*}
\]

In this situation, twist and bend are constant.

In theory, this organization can transform continuously into a cholesteric one by a progressive decrease of the component \( n_x \). For the cholesteric structure, \( k \) equals zero and the \( n \) components read:

\[
\begin{align*}
    n_x &= \cos \frac{2 \pi z}{p} \\
    n_y &= \sin \frac{2 \pi z}{p} \\
    n_z &= 0.
\end{align*}
\]

In this case, the cholesteric axis lies parallel to the helicoidal axis of the chiral smectic C structure but we never observed this transition. On the other hand, we follow the reverse evolution from cholesteric to « chiral smectic C » in certain biological structures made of DNA. Indeed, in the stallion sperm nucleus, DNA is highly condensed into a quite dehydrated structure [19]. Biochemical treatments permit to decondense this structure into a helicoidal one which was shown to be analogous to a chiral smectic C organization (without layers) [19].

Patterns resembling those described here were first reported in kevlar (an aramid fibre) [10, 11] and also in thermotropic and lyotropic liquid crystalline polymers [12-17]. They are produced by shearing processes and they form when the solution is relaxed. Molecular orientations are also described as sinusoidal or helicoidal in these domains and these textures remain under discussion at the present time.

In our preparations of DNA, these patterns are probably not produced by shearing forces; they appear in a few days from the isotropic phase and they can remain stable a long time.

4.2 PRECHOLESTERIC EVOLUTION. — We propose a geometrical interpretation of the phenomena which occur during the precholesteric transition. We do not claim to interpret in details all the observed patterns but to give the general rules which permit to understand this evolution in its major lines.

This evolution would correspond to successive introductions of helical structures in three directions of space. They are named \( h_1 \), \( h_2 \) and \( h_3 \) and their orientation is given with respect to the edge of the
coverslip which is considered here as the reference direction ($D$).

Molecules lie first normally to the direction $D$ in the birefringent border. They rotate by about 90° to form the birefringent cords since they are then align parallel to the cords direction. We propose that this rotation occurs by a helicoidal condensation of molecules according to a direction $h_1$ which lies in the preparation plane, normally to $D$. Molecular orientations produced by this way are presented in figure 10a. This corresponds to a chiral smectic C organization without layers; the structure superimposes to itself by any translation parallel to $D$. Between crossed polars, this organization would produce the pattern of figure 10b: a series of light bands parallel to $D$ separated by dark lines. This organization is coherent with the observed defects in which two cords are always added simultaneously. However, we must suppose that molecules are nearly parallel to the direction $D$ since there is no difference between two consecutive cords in the colours produced by the addition of a quartz first order retardation plate. This representation was used to facilitate the understanding and could correspond to an intermediate state. However, this situation is not observed and the only explanation would be that molecules are not condensed enough to present a visible illumination since the total birefringence of DNA is very weak.

The second helicoidal organization occurs along a direction $h_2$, parallel to $D$, inside each cord. The molecular orientations still superimpose by translations oblique with respect to the cord axis. Indeed, we start each helix parallel to $h_2$ from a line parallel to $h_1$ and molecular orientations were not identical all along this line (Fig. 11a). This organization produces cords with an alternation of oblique bright and dark bands whose orientation changes from one cord to the next one (Fig. 11b). This pattern was observed sometimes in our preparations.

![Fig. 10.](image)

- a) Top view of molecular orientations produced by a helicoidal condensation along a direction $h_1$, normal to the reference direction $D$. We used the classical nail convention and the obliquity of molecules was significantly increased to clarify the drawing. In reality, molecules are nearly parallel to $D$. b) Corresponding pattern observed between crossed polarizers: alternation of light and dark bands parallel to $D$. Loci of directors parallel to the section plane are indicated by double lines.

![Fig. 11.](image)

- A second helicoidal organization occurring along $h_2$ produces the molecular distribution presented in (a) and the pattern (b) between crossed polarizers: each cord appears as an alternation of oblique light and dark bands.
If we consider now the dark regions between the threads in which molecules are more or less normal to the preparation plane, we see that they can organize to follow the orientations presented in figure 12a, i.e. a cholesteric organization. This is in agreement with our observations since the first cholesteric layers appeared between the cords and we saw that in these regions the cholesteric axis was oriented either parallel, oblique or normal to the cord direction. According to this hypothesis, molecular orientations may be underlined by another representation which is given in figure 12b. The loci where molecules lie parallel to the preparation plane are indicated by double lines and they draw herringbone patterns. The arrowed lines follow the molecular orientations. There is a regular distribution of +1 and -1 singular lines along directions parallel to the long axis of the cords. These singular lines do not present any discontinuity in the molecular orientations. This organization can transform to give a succession of cholesteric concentric structures aligned parallel to the direction D. We think therefore that this organization is not far from those presented in plate IVa. This pattern can be transformed continuously to give homogeneous cholesteric textures such as in plate IVb.

Topologically, there is possibly a continuous passage from figures 10 to 12 as indicated in the footnote (1), but there is no clear evidence about it. The resolving power of light microscopes is not sufficient to follow the complete evolution of the system.

The distribution of directors, in the plane of figure 12 presents symmetries (four binary axes per unit cell, Fig. 12b), which corresponds to the two-dimensional space group p211. (The unit cell is generally represented by a parallelogram in textbooks but it can be verified than transforming continuously this parallelogram into a rectangle does not add any new symmetry.)

It remains to explain the organization which occurs in the thickness of the preparation and which is revealed by changes in the molecular orientation observed when the focus plane is changed. We propose the existence of a third helicoidal axis h3 normal to the preparation plane. The molecular orientations obtained by this way must be analysed in the whole preparation volume. They are given in figure 13 in certain section planes normal to the cord direction. We note the formation of cylindrical cholesteric rods and the three-dimensional structure is more easily perceived if we follow the axes of these bundles. They form two families of intertwined sinusoids which are included into two families of orthogonal planes. These sinusoids have the same period (a) and amplitude (3/8a). They are more clearly observed in section planes parallel to the preparation one, at levels 0 and ±a/2 (Fig. 14a, b).

Section planes parallel to the cord axes are presented in figure 15a, b. The underlined sinusoids do not correspond to the structures previously described in sections normal to the cord axes. Indeed, each section plane parallel to the cord axis will present the same pattern (with a translation) and this sinusoid is not therefore the axis of a cholesteric bundle.

The true sinusoids are therefore associated by pairs and their distribution is given in figure 16 in a perspective view. The elementary cell of the lattice is a cube of side (a). However, sinusoids do not correspond to the patterns observed in our preparations since we described helicoidal cords but two intertwined sinusoids may be considered as two particular lines running at the surface of a helicoidal cord (Fig. 17). The twist is always left-handed between the sinusoids of one pair (and also between with

\[ D = [1 + \beta^2 \sin^2 (2 \pi y/q)]^{-1/2}. \]

D is a factor introduced to have |n| = 1; β and \( \mu \) are two parameters; p and q are the two periods of the structure along y and z. This corresponds to a rectangular unit cell of edges p and q.

Initially, \( \beta = \mu = 0 \) and therefore \( D = 1 \). This corresponds to the cholesteric structure expressed by equations (2). Passing from \( \beta = 0 \) to \( \beta = 1 \), one gets a rectangular array of disclinations +1 and -1 with continuous cores. Now, changing \( \mu \) from 0 to 1 transforms continuously the director into that of figure 12. However, there is little doubt that these simple equations are solutions minimizing the energy of the system, which is highly complex. These equations correspond to patterns similar to those of figure 12, with their symmetries. The parameters β and \( \mu \) express the possibility of continuous changes of a director distribution within a single homotopy class.

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(1) Consider three perpendicular axes Oxyz, Oy being parallel to h1 and Oz parallel to D. To pass continuously from figures 10 to 12, the following steps are proposed:

1. Align all directors of figure 10 along direction h1.
2. Rotate continuously directors in the plane of figure 10, so that they align parallel to D:

\[ n_x = n_y = 0; \quad n_z = 1. \]

3. Equations (1) show the continuous passage to a cholesteric when k varies from infinity to zero.
4. The following equations of a director field |n| = 1 indicate the passage to a picture very close to that of figures 12a, b.

\[ n_x = D \cos \left\{ 2 \pi \left[ z + \mu \sin (2 \pi y/q) \right]/p \right\} \]
\[ n_y = D \sin \left\{ 2 \pi \left[ z + \mu \sin (2 \pi y/q) \right]/p \right\} \]
\[ n_z = D \beta \sin (2 \pi y/q) \quad (3) \]
Fig. 12. — The regions separating the threads can organize in such a way that molecules follow a cholesteric organization. The global structure is given with two different representations: the nail convention (a) and the arrowhead convention (b). The understanding is easier in the second situation: the directors parallel to the observation plane follow zig-zag lines; the arrowed lines follow force lines and reveal an alternation of +1 and −1 singular lines. This structure is continuous everywhere. The unit cell with its two-fold axes (⊗) has been superimposed to the drawing in (b). Points lettered A, B, C, D, E, F, G, H are reference marks which will be useful in the following drawings.

Fig. 13. — Section planes normal to the preparation plane along the lines AB and CD (see Fig. 12), after the introduction of the three helicoidal organizations h₁, h₂ and h₃. The axes of concentric cholesteric rods are underlined. They form two families of intertwined sinusoids: one is lying in the section plane and the other one is normal to this plane and therefore alternatively in front of (thick lines) and behind the plane (dotted lines). The pairs of sinusoids are localized at levels 0 and −a along CD, and at level −a/2 along AB.
Fig. 14. — Top views of molecular orientations at levels 0 and $-a/2$. Families of intertwined sinusoids are indicated at the two levels.

Fig. 15. — Section planes normal to the preparation plane along the lines EF and GH (see Fig. 12). The sinusoids underlined by dotted lines do not correspond to the axes of cholesteric rods since patterns obtained in all sections parallel to $D$ will be the same excepting a slight lateral displacement.

Fig. 16. — Perspective view of the distribution of sinusoids in a cube whose side equals $a$.

(2) DNA is a right-handed helix which is known to form left-handed cholesteric phases, at least in vivo [19].
Fig. 17. — Deux sinusoides identiques séparés par $\pi/2$ sont dessinés sur la surface d'une corde hélicoidale. Ils sont situés dans le plan de l'observation et dans le plan perpendiculaire à ce dernier. Les sinusoides présentent un twist hélicoidal gauche et la corde hélicoidale est donc gauche aussi.

Fig. 18. — Formation d'une organisation cholesterique (ch.) entre deux cordes hélicoidales concentriques, en vue latérale (a) et en vue de dessus (b).

Numerous variations occur around this basic scheme. In particular, we observed quite frequently the formation of the cholesteric layers and their disposition in phase opposition in planes of slide and coverslip prior to the helicoidal organization inside the cords (Pl. IId). Our model remains correct in this case if the helicity along the vertical direction is introduced before the helicity parallel to $D$. This does not modify the whole topology of the structure but the observed patterns (i.e. the projections onto the observation plane) will be different since the three series of sinusoids are not equivalent, as mentioned previously.

Meyer [21] indicated that splay is proportional to the difference between numbers of bottom and top ends of molecules in a volume element. The splay elastic constant is therefore very high for very long polymers and this prevents in general the splay to occur. However, in the studied DNA, the length/diameter ratio varies between 20 and 950 and this is not incompatible with a certain splay. Molecules folded back into hair-pins are not excluded and this could be another contribution to splay. Now, it must be noticed that splay is very weak in all proposed models from figures 10 to 18. The splay is zero in planar cholesterics and in chiral smectics C. It can be zero around continuous $+1$ disclinations with a cylindrically twisted core and very low in the core of continuous $-1$ disclinations. In our three-dimensional models of figures 13 to 17, the left-handed helicoidal cords are the most representative structures and are almost devoid of splay, since they correspond mainly to a cylindrical twist, also called « double twist ».

Sénéchal et al. [22] with high molecular weight polynucleotides (170 to 1 000 nm long) observed a transition from the isotropic phase to a phase which was not of a simple cholesteric type. They proposed the existence of cholesteric cylinders but the long...
range packing of these cylinders was ignored. Their three-dimensional tubular structure is probably related to the structure described here.

A few ideas will be retained here as a conclusion:

1. A helicoidal organization of molecules is a favourable situation to produce a cholesteric order and three possibilities may be considered:

   (i) Alignment of molecules form helices as in "chiral smectic C" the structure being however nematic, without any layering and this structure transforms directly into a cholesteric one. However, this evolution, which probably occurs with small liquid crystal molecules, is nearly impossible with polymers on account of their high viscosity.

   (ii) A three-dimensional helicoidal organization of molecules produces a cholesteric organization in the bulk with formation of concentric cholesteric cords.

   (iii) Small cholesteric domains are produced locally between two regions of different molecular orientation. This situation occurs probably in our preparations between the helicoidal cords, in contact with the glass surfaces. Molecules follow helicoidal paths at the surface of cylindric cholesteric bundles. Molecular orientations are different between two neighbouring cords and a cholesteric organization may appear between them (Fig. 18).

   The two last processes (ii and iii) probably work together to produce the cholesteric liquid crystalline phases of DNA in our preparations.

2. The analysis of textures presented in this work supports the hypothesis that molecular orientations are continuous in the bulk. The only possible defects are +1 and −1 disclinations lines. All these defects do not present any discontinuity of the molecular orientations in their core.

3. The occurrence of the precholesteric evolution described in this work seems to be related to different parameters, the more important one being the length of the molecules. Indeed, small DNA fragments (146 base pairs) give cholesteric spherulites in equilibrium with the isotropic phase for an appropriate range of concentration (23) whereas long DNA molecules (150 to 5,750 base pairs) follow preferentially the precholesteric evolution (cholesteric spherulites were difficult to obtain with these DNA fractions). This is also true for other molecules such as xanthan, a polysaccharide which is secreted by a bacteria. This polymer is known to form cholesteric liquid crystalline phases in concentrated solution [7, 24, 8] and very beautiful cholesteric droplets are observed at the transition between the isotropic and the cholesteric phase [4]. However, we recognized precholesteric states with xanthan of high molecular weight (about 980,000) (3). We think therefore that the precholesteric organization is a general way for long polymers to reach the cholesteric organization.

4. This organization presents numerous analogies with the blue phases described with small liquid crystal molecules [25]. They both correspond to precholesteric stages and they are defrustrated structures. However, all the models of the blue phases contain defects with singular distributions of molecules whereas this one does not. The differences observed between these phases are probably due to difference in molecular length between small liquid crystal molecules and long polymers. This problem of frustration in polymers was considered theoretically by Kléman (26, 27).

5. Such precholesteric evolutions are probably involved in many morphogenetic processes in biological structures which are mainly composed of long polymers. Besides, a helicoidal organization is frequently observed in chromosomes [28-31] and such analyses would probably be useful in the understanding of processes involved in the condensation of chromatins into chromosomes.

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