Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926090

Invite Article: The Liquid-Crystalline Phases of Double-Stranded Nucleic Acids in Vitro and in Vivo

Yu M. Yevdokimov[a]; S. G. Skuridin[a]; V. I. Salyanov[a]
[a] Institute of Molecular Biology, USSR Academy of Sciences, Moscow, USSR

To cite this Article Yevdokimov, Yu M. , Skuridin, S. G. and Salyanov, V. I.(1988) 'Invite Article: The Liquid-Crystalline Phases of Double-Stranded Nucleic Acids in Vitro and in Vivo', Liquid Crystals, 3: 11, 1443 — 1459
To link to this Article: DOI: 10.1080/02678298808086687
URL: http://dx.doi.org/10.1080/02678298808086687

LIQUID CRYSTALS, 1988, VOL. 3, NO. 11, 1443–1459

Invited Article

The liquid-crystalline phases of double-stranded nucleic acids *in vitro* and *in vivo*

by YU. M. YEVDOKIMOV, S. G. SKURIDIN and V. I. SALYANOV
Institute of Molecular Biology, USSR Academy of Sciences, Ul. Vavilova 32, Moscow 117334, U.S.S.R.

(*Received 6 April 1988*)

This article describes the state of and progress in experimental studies of liquid crystals of naturally occurring nucleic acids and synthetic polynucleotides. The areas considered in this review include: (i) the liquid-crystalline phase of nucleic acids in aqueous salt solutions, (ii) the liquid-crystalline phase of nucleic acids in aqueous polymer solutions, (iii) the liquid-crystalline phase of nucleic acids in living systems. Some unsolved problems which are of interest from both a physico-chemical and a biological point of view are discussed.

1. Introduction

The study of the physical state of aggregation of nucleic acid molecules in a living cell directly is a difficult problem. Two hypotheses have been suggested concerning the mechanism of packing for the genetic material. According to Kellenberger [1], in each cell there is a specific condensation factor which causes the aggregation of the genetic material in the cell nucleus. The alternative view of Laemmli and his co-workers [2], is that at some stage of the maturation of the phage DNA physico-chemical conditions are induced to provide a spontaneous ordered arrangement of the DNA molecules. Proceeding from the latter hypothesis, it is reasonable to design an *in vitro* model for the organisation of DNA molecules which would reflect the major features of the spatial ordering of nucleic acid molecules *in vivo*. Over the last few years, this problem has attracted the attention not only of molecular biologists but also of specialists in other branches of science. In particular, the state of DNA molecules in biological systems (for example, viruses and chromosomes) is of interest to these scientists who investigate liquid-crystalline phases formed from biopolymers.

The *in vitro* studies have been performed on two model systems that represent, in essence, lyotropic liquid crystals of nucleic acids. The first system I is a liquid-crystalline phase of nucleic acids arising spontaneously in a aqueous salt solution of a moderate or high ionic strength at some critical concentration of nucleic acid. The second model II is the liquid-crystalline phase of nucleic acids formed as a result of phase separation in aqueous polymer solutions.

In this review attention is focused on the properties of lyotropic liquid crystals of double-stranded nucleic acids and synthetic polynucleotides, an emerging but important area in the field of lyotropic liquid crystals. We discuss here experimental results which characterize the properties of lyotropic liquid crystals of nucleic acids. We do not consider theories for the formation of lyotropic liquid crystals of polymers,

instead we concentrate on the basic properties of the pure model; i.e. those of the liquid-crystalline phase prepared from nucleic acids only. Initially the properties of the phases formed under model conditions from [nucleic acids + poly cation (such as polyamines and polypeptides) complexes] are not considered either.

2. Lyotropic liquid crystals of nucleic acids in aqueous salt solutions

The formation of liquid crystals of natural double-stranded deoxyribonucleic acids has been discussed only in several publications prior to 1987. It should be noted that the basic information on the liquid-crystalline state of DNA was obtained (as in the case of liquid crystals of low molar mass) by means of X-ray analysis of gels, or DNA fibres prepared from them, and also from the analysis of optical textures.

The hypothesis that double-stranded DNA molecules are able to exist in the liquid-crystalline phase in concentrated solutions was first put forward in 1961 by Luzzati and his coworkers [3]. When taking the X-ray diffraction patterns of fibres pulled from concentrated solutions of calf thymus DNA they observed that on moistening the fibres (water content about 80 per cent) a birefringent phase of DNA occurred which gave, only one reflection in its small angle X-ray diffraction pattern. Further moistening of the fibres resulted in a transition of the birefringent DNA phase to the isotropic phase. The existence of birefringence combined with the small angle reflection in the X-ray patterns suggested that the packing of the DNA molecules in fibres at a certain level of hydration corresponds to a nematic ordering.

At the same time Robinson having obtained unequivocal evidence for the formation of cholesteric phases by polypeptides solutions reported the results of a single experiment on a concentrated calf thymus DNA solution [4]. A 6 per cent solution was placed in a capillary and stored for several days. Subsequent study of this solution with a polarizing microscope showed that it developed a texture with periodic alternating light and dark bands. Robinson expected the presence of protein admixtures in the DNA preparation, and so he tentatively suggested that in concentrated solution the DNA forms a mesophase structurally analogous to that of a cholesteric phase. Thus in 1961 the situation concerning the spontaneous formation of liquid crystals of DNA in concentrated solution was confused.

Later, several authors, Iizuka *et al.* [5, 6] and Potaman *et al.* [7] attempted to reproduce the observations of Robinson. In spite of the fact that the textures found by these investigators did not show features typical of classical nematic or cholesteric phases, their results were interpreted in terms of the formation of a cholesteric phase.

In 1983 a significant advance was made when unequivocal proof for the formation of a cholesteric phase in concentrated solutions of DNA was obtained. It was then that the investigators happened to prepare liquid crystals from short DNA molecules; the molecular weight was not greater than 1×10^6 [8–10]. Livolant *et al.* [9] showed that the storage of a concentrated KCl solution with an initial concentration of 10 mg/ml DNA for several days at 0°C caused an increase in the nucleic acid concentration which lead to the formation of an anisotropic phase. The optical texture of this phase showed a characteristic cholesteric finger-print pattern. The pitch (P) of the cholesteric helix of the DNA solution was determined from the texture to be 1·2 μm.

The properties of the phase formed from DNA (with a molecular weight of about 1×10^5) in 2M NaCl are described by Rill [10]. It should be noted that, in order to obtain the anisotropic phase, the concentrated solutions of DNA were heated and

cooled sequentially. This mesophase scattered light and formed the characteristic finger-print texture. An approximate phase diagram for the phases of DNA molecules in aqueous salt solutions was determined [10]. According to their results, at room temperature and concentration close to 200 mg/ml, DNA spontaneously form a liquid-crystalline phase.

The data available by 1987 demonstrate therefore that for biologically occurring DNA the formation of a cholesteric phase takes place spontaneously in concentrated solutions.

For synthetic polyribonucleic acids it should be stressed that the evidence relating the types and properties of their liquid-crystalline phases in concentrated solutions is not so unequivocal as for biologically occurring of DNA. The first attempt to prepare liquid-crystalline phases from polyribonucleotide was made in 1977 by Iizuka and his coworkers [11]. It was shown that double-stranded poly(A) × poly(U), poly(G) × poly(C), poly(C) × poly(I) and also three-stranded polyribonucleotides (poly(A) × 2 poly(U) and poly(A) × 2 poly(I)) form mesophases in concentrated solutions. Although these phases were birefringent their optical textures did not show the features expected for nematic and cholesteric phases. However the authors state that under the conditions used poly(A) × poly(U) and poly(A) × 2 poly(U) form cholesteric phases, the pitch of the helical structure being between 1 to 10 μm and proportional to $C_V^{-1\cdot1}$, where C_V is the volume concentration of the polyribonucleotides.

A further study of polyribonucleotide liquid-crystalline phases was made [12] and it was shown that in the C.D. spectrum of the anisotropic phase of poly(A) × poly(U) a negative band ($\lambda_{max} \sim 500$ nm) appeared outside the absorption region of the nitrogen bases. It was suggested [12] that the band was a selective reflection resulting from the helical structure of the poly(A) × poly(U) mesophase. The pitch was determined to be about 0·5 μm. The study of the alignment of poly(A) × poly(U) and poly(A) × 2 poly(U) liquid crystals in a magnetic field led to a somewhat unexpected result. The value of P for the helical structure of the poly(A) × poly(U) cholesteric phase determined in the absence of the magnetic field was 3·4 μm, in contrast to the estimates based on the C.D. spectrum. The origin of this discrepancy is not clear.

Senechal *et al.* found that there is a critical concentration of poly(A) × poly(U) above which the anisotropic phase is formed [13]. Although textures typical of cholesteric phases were not obtained in this work, the angular dependence of the intensity of laser scattering for the anisotropic phase allowed the periodicity to be estimated. If it is assumed that this periodicity is the pitch of the cholesteric helix, then P was found to be proportional to $C_V^{-0\cdot5}$, which is at variance with the earlier data [11].

In 1983 Iizuka again studied the properties of the liquid-crystalline phases of single-stranded polyribonucleotides to try to determine the sign of the cholesteric twist [6]. Using an external chromophore [14] he analysed the anomalous optical properties of acridine orange dissolved in the liquid-crystalline phase. On the basis of his results he concluded that the cholesteric phase formed from poly(A) was left-handed, and that from poly(I), poly(C), and poly(U) right-handed.

So, above a critical concentration both biologically occurring nucleic acids and synthetic polyribonucleotides are able to form liquid-crystalline phases spontaneously in aqueous salt solutions. Two questions which are of interest not only physico-chemically but also to biology were left unanswered in these studies. First, what is the relationship between the physico-chemical properties of the nucleic acids and the structure of the mesophase? Secondly what is the role of the solvent in the formation of liquid crystals from nucleic acid? For an *in vivo* modelling of the spatial ordering

of nucleic acids, another path might be followed, namely, to study the liquid-crystalline phases of nucleic acids formed in aqueous salt-polymer solutions because the molecular structure and properties of these can be varied over a wider range.

3. Lyotropic liquid crystals of nucleic acids in aqueous-polymer solutions

Here, we shall consider the properties of nucleic acid liquid crystals prepared in aqueous salt solutions containing poly (ethyleneglycol), PEG. This system was used initially for the formation of ordered phases of double-stranded DNA in two laboratories, almost simultaneously—in those of Lerman [15] and Varshavsky [16].

A scheme has been described [17, 18] (see figure 1) for the preparation of liquid-crystalline phases from double-stranded nucleic acid and synthetic polynucleotide molecules in aqeous salt-PEG solutions under definite critical conditions [19]. In the first stage, as a result of mixing equal volumes of nucleic acid aqueous salt solution ($C_{NA} \leqslant 50\,\mu g/ml$) and that of PEG ($C_{PEG} \leqslant 600\,mg/ml$) a dispersed phase appears; this phase consists of microscopic droplets of nucleic acid (microphases [20]). According to estimates based on measurements of sedimentation and diffusion coefficients of the DNA microphases, 10^4 molecules of nucleic acid form one droplet (as $C_{DNA} \to 0$). In the second stage, the dispersed phase is centrifuged (at 5000 rev/min for 40 min) and a microscopic liquid-crystalline phase of nucleic acid is obtained. This nucleic acid mesophase was studied by X-ray diffraction and polarization microscopy.

Water-salt solution of nucleic acid (NA)
(ionic content, pH and t^0 are fixed; molecular mass of NA $\leqslant 1 \times 10^6$; C_{NA} – 5–50 μg/ml; volume V_1)

Stage 1 This is mixed with

Water–salt solution of poly(ethyleneglycol) (PEG)
(ionic content, pH and t^0 are fixed; molar mass of PEG = $1 - 40 \times 10^3$; C_{PEG} = 0–600 mg/ml; volume V_1)

A dispersed liquid-crystalline phase of nucleic acid in PEG-containing solution is formed. Size of particles of dispersed phase $\approx 10^3$ Å; each particle contains 10^4 DNA molecules (as $C_{DNA} \to 0$)

Stage 2 The dispersed phase of the nucleic acid is stored ($\sim$20 hr, at 4–20°C) and then centrifuged (5000 rev/min for $\sim$1 hr)

A liquid-crystalline phase of nucleic acid in PEG-containing solution is formed

Figure 1. A two-stage scheme for the preparation of liquid-crystalline phases of nucleic acids in PEG-containing solutions.

3.1. *X-ray analysis of nucleic acid mesophases*

X-ray scattering patterns of liquid-crystalline phases prepared from double-stranded nucleic acids (DNA [21–23] and RNA [24, 25]) and also from synthetic double-stranded polynucleotides [25–27] contain only one maximum of significant intensity (see figure 2, curves 1–5). Phases prepared in PEG-containing solutions from single-stranded ribosomal RNA [25] or heat-denatured DNA [6] the rigidity of which is significantly less than that of double-stranded nucleic acid molecules do not contain a distinct small angle reflection in their X-ray diffraction patterns (see curve 6 in

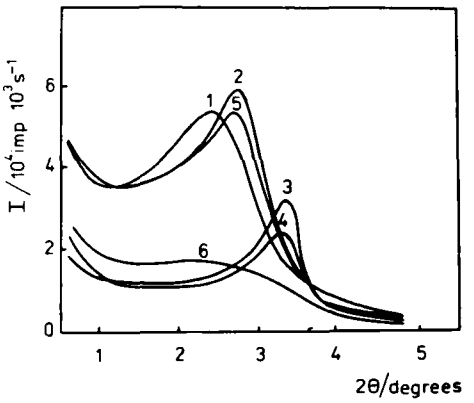

Figure 2. X-ray diffraction curves of the liquid-crystalline phases prepared from DNA in PEG-containing solutions. Curves 1–4, native DNA (molar mass = 0·6 × 10^6); Curve 6, heat-denatured DNA; Molar mass of PEG 4000; 0·3M NaCl; C_{PEG} (mg/ml): 1, 140; 2, 180; 3, 260; 4, 300; 5, 180 (molar mass of DNA = 1·5 × 10^6); 6, 180.

figure 2). The fact that higher order small angle reflections are not observed in the X-ray patterns indicates that there is no regular three dimensional order and that there can only be short range translational order typical of a mesophase.

Studies of X-ray diffraction from liquid-crystalline phases at large angles [21, 27] (which were undertaken to obtain information about the secondary structures of DNA and poly(I) × poly(C) relating to the B- and A- families, respectively) show that the transition from the isotropic to the liquid-crystalline phase is not accompanied by any change in the structural parameters of individual molecules. Heating liquid-crystalline phases which were prepared at various PEG concentrations from DNA [28] or poly(I) × poly(C) [27] causes a progressive displacement of the position of the maximum of the scattering curves into the small angle region (see figure 3). That is

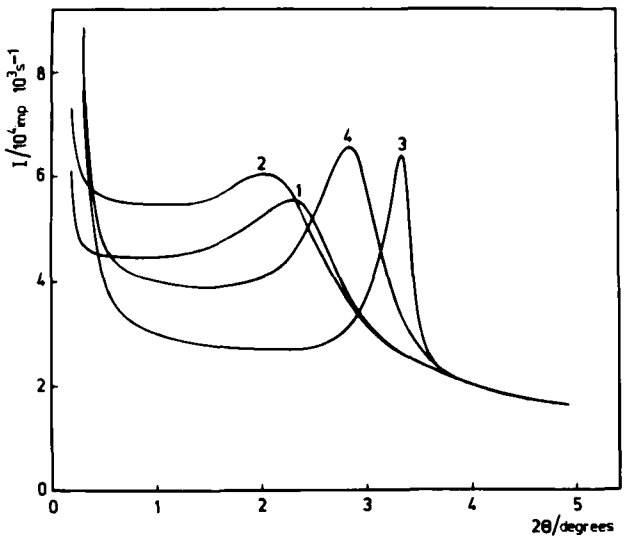

Figure 3. X-ray diffraction curves of the liquid-crystalline phases prepared from DNA molecules at different temperatures. Molar mass of DNA = 0·5–0·7 × 10^6; molar mass of PEG = 4000; 0·3 M NaCl C_{PEG} (mg/ml): 1, 120, 20°C; 2, 120, 80°C; 3, 300, 20°C; 4, 300, 80°C.

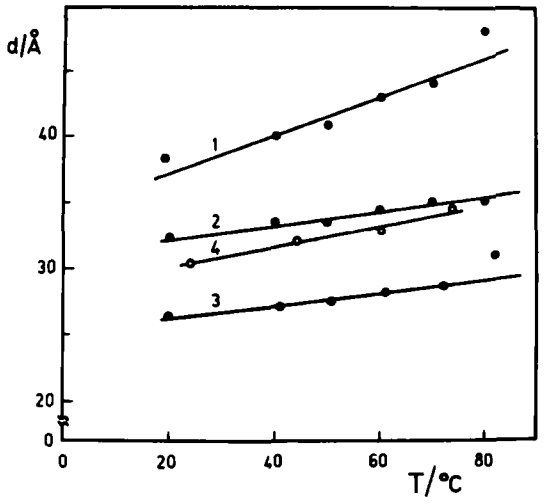

Figure 4. The temperature dependence of the periodicity d. Curves 1–3, DNA; C_{PEG}: 120, 170, 300 mg/ml, respectively; Curve 4, poly(I) × poly(C); C_{PEG} = 190 mg/ml.

by an increase in the average distance (d) between neighbouring molecules. This change in d with increasing temperature (see figure 4) continues until the nucleic acid is denatured. When this denaturation temperature is reached, the double strands separate, the small angle reflection in the X-ray patterns disappears, and a transition from the liquid-crystalline to the isotropic phase is observed.

The decrease of d with increase in the PEG concentration (see figure 5) indicates an increase of the packing density of the nucleic acid molecules. The maximum density appears to occur for PEG concentrations greater than 250 mg/ml. Using the dependence of d on the PEG concentration we can estimate the critical volume concentration (V_{crit}) of the nucleic acid (for DNA, in particular) starting from which an ordered

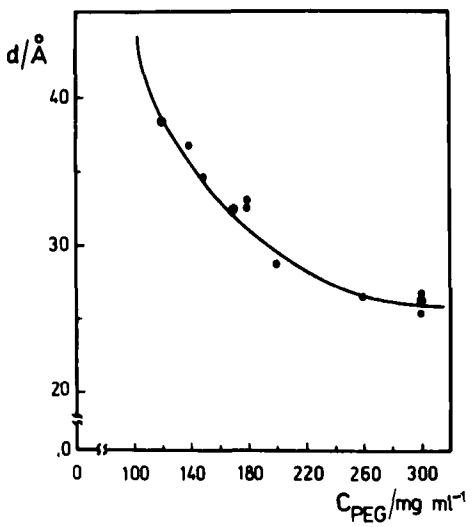

Figure 5. The dependence of the periodicity d on the concentration of PEG.

phase is formed. According to our calculations [20], the critical volume concentration of DNA for an aqueous salt (0·3M NaCl) solution of PEG (C_{PEG} = 110 mg/ml; molar mass of PEG = 4000) is about 0·2; this values seems to be close to that obtained by other authors [10, 29] for the formation of the DNA liquid crystalline phase in a concentrated solution. This equivalence shows that the conditions to induce the ordering of rigid double-stranded nucleic acid molecules in aqueous salt solutions containing PEG are not very different from those necessary for the formation of the nucleic acid liquid-crystalline phase in concentrated solutions.

The decrease of d with increasing PEG concentration indicates that the packing density of the nucleic acid molecules in liquid-crystalline phases prepared in aqueous salt-PEG containing solutions may depend on the properties of the solvent. It is to be expected that the dielectric constant of the PEG solution (E_m) is an important factor [21]. This supposition can be tested since the dielectric constant of PEG solutions can be lowered not only by increasing the PEG concentration [30], but also by adding low molecular weight alcohols to the PEG solution of a fixed concentration. The study of the X-ray scattering of liquid-crystalline phases of DNA prepared in PEG solutions which contain methanol, ethanol, isopropanol etc. shows that the decrease of the dielectric constant of these solutions is indeed accompanied by a reduction in d [31] similar to the decrease observed on increasing the PEG concentration (cf. figures 5 and 6). The estimate of the dielectric constant of a PEG solution containing isopropanol and corresponding to the conditions when d is close to 25 Å shows that E_m equals 57 (C_{PEG} = 150 mg/ml + 20 vol.% of isopropanol). This result is in accord with the known dielectric constants of aqueous PEG solutions (at $C_{PEG} \approx 300$ mg/ml; $E_m \approx 60$ [30]). Therefore, by changing the properties of the solvent it is possible to affect the packing density of nucleic acid molecules in the liquid-crystalline phases.

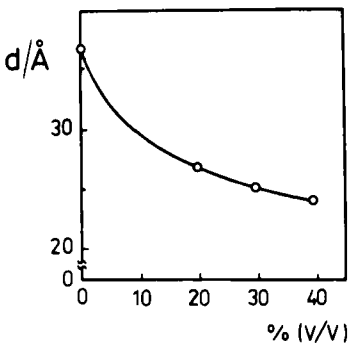

Figure 6. The dependence of the periodicity d on the volume concentration of isopropanol in PEG-containing solutions used for the preparation of liquid crystals of DNA. C_{PEG} = 150 mg/ml; 0·3 M NaCl.

It is more difficult to explain the changes in the X-ray patterns of the liquid-crystalline phases prepared in aqueous salt-PEG containing solutions from nucleic acids after their modification either chemically or by complex formation with different biologically active compounds. For example, the liquid-crystalline phases prepared from DNA complexed with the antibiotic daunomycin have been studied by X-ray diffraction [32]. The daunomycin molecules intercalate between the nitrogen bases of the nucleic acids. Over the entire composition range of the DNA—antibiotic mixture

the one dimensional ordering of DNA molecules is preserved. The density of their packing is somewhat changed, with d decreasing from 34 Å at $r = 0$ to 30·7 Å at $r = 0{\cdot}16$; where r is the ratio of the molar concentration of daunomycin bound in a complex with DNA to that of the nucleotides or DNA.

Quite a different variation in d is observed for the packing of nucleic acids in the liquid-crystalline phases prepared from DNA whose structure is modified by chemical reaction with an anti-tumour compound of the platinum(II) group, for example cis-dichlorodiammineplatinum(II) (cis-Pt(II) [33]. Increase in the extent of modification of DNA with cis-Pt(II) as well as heating the liquid-crystalline phases of nucleic acids is accompanied (see figure 7) by a progressive shift in the position of the maximum of the scattering curve to the small angle region (cf. figures 3 and 7). This implies that at higher levels of modification of DNA with cis-Pt(II) the probability of forming an ordered phase from such molecules in PEG-containing solutions is strongly reduced. So, unlike thermotropic polymeric liquid crystals, the packing density of the molecules in lyotropic liquid crystals depends on both the properties of the solvent and the properties of the polymeric molecules themselves.

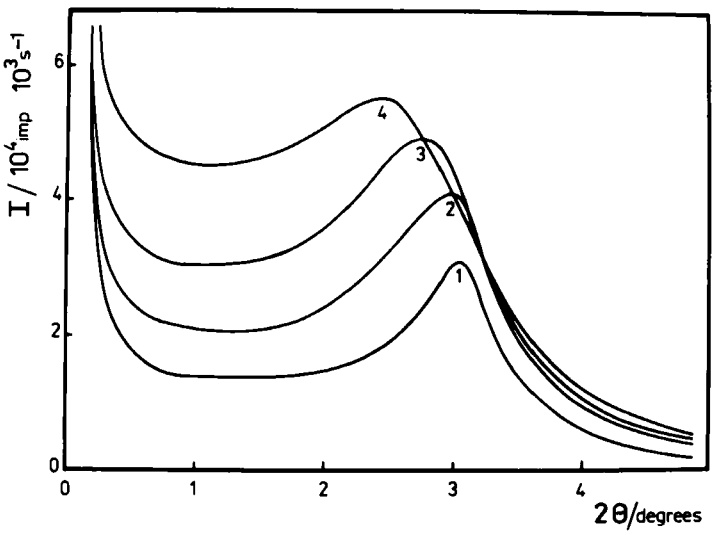

Figure 7. X-ray diffraction curves for the liquid-crystalline phases prepared from DNA modified by cis-Pt(II). Molar mass of PEG 4000; C_{PEG} = 170 mg/ml; 0·3 M $NaClO_4$ 1, $r = 0$; 2, $r = 0{\cdot}01$; 3, $r = 0{\cdot}03$; 4, $r = 0{\cdot}1$ (r is the ratio of the molar concentration of cis-Pt(II) in solution to that of the DNA nucleotides).

3.2. *Textures of liquid-crystalline phases of nucleic acids*

In parallel with these X-ray diffraction investigations of nucleic acid liquid-crystalline phases the optical textures of the phases prepared from the original and modified DNA were studied in order to identify the types which form liquid crystals [33–35]. Such studies had to overcome the experimental difficulties of preparing thin layers of liquid-crystalline phases of nucleic acids and synthetic polynucleotides in concentrated solutions. Depending on the PEG concentration, the optically active double-stranded DNA molecules form either cholesteric (see figure 8 (*a*)) or nematic (see figure 8 (*b*)) phases [34]. The pitch of the helical structure of the cholesteric phase of DNA in PEG-containing solutions varies very slowly with the PEG concentration

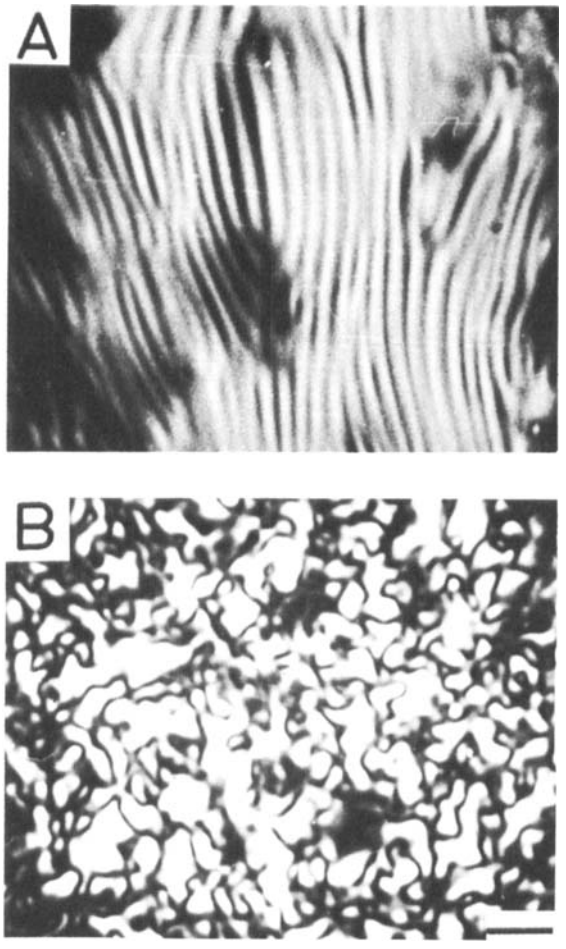

Figure 8. Optical textures of DNA liquid-crystalline phases in PEG-containing solutions. (*A*) the finger-print texture in polarized light (crossed nicols). (C_{PEG} = 130 mg/ml; 1M NaCl.) The length of the bar is 10 μm. (*B*) the black bent lines texture in polarized light (crossed nicols). (C_{PEG} = 300 mg/ml; 1M NaCl.)

in the original solutions and with temperature; it is equal to 2–4 μm (within the temperature range from 25 to 85°C). This result agrees with the data obtained for cholesteric phases prepared from synthetic double-stranded polynucleotides poly(*A*) × poly(*U*) in concentrated solutions [11, 13]. The pitch obtained in [34] correlates with the estimate of that for the cholesteric helix of liquid crystals formed by DNA in concentrated solutions [9, 10]. At the denaturation temperature of DNA a transition from the liquid-crystalline to the isotropic phase is observed. In this case the small angle reflection and the birefringence disappear.

The texture of the cholesteric phase of DNA shown in figure 8 (*a*) has a number of features which distinguish it from the textures of thermotropic cholesteric liquid crystals of low molecular weight compounds. These features may reflect the specificity of molecular ordering of lyotropic liquid crystals formed by DNA and relate mainly to the structure of linear defects, that is dislocations associated with translational disordering of the quasi-nematic layers of the cholesteric phase. All dislocations form four types of pairs: $(\tau^+ \lambda^-)$, $(\tau^- \lambda^+)$, $(\tau^- \tau^+)$ and $(\lambda^- \lambda^+)$. It has been shown [35] that

the number of $(\tau^- \lambda^+)$ pairs is considerably greater than that of the $(\tau^+ \lambda^-)$ pairs, for pair formation with the Burgers vector equal to $P/2$. The latter observation apparently contradicts the data obtained for low molecular weight thermotropic cholesteric phases [36], however it is in good agreement with the results of the analysis of textures of lyotropic cholesteric liquid crystals formed by DNA or the polysaccharide, xanthane, in concentrated solutions and also by the synthetic polypeptide poly-γ-benzyl-L-glutamate in organic solvents [37].

It should be added that the molecules of nucleic acids contain chromophores, which nitrogen bases, absorb in the U.V. region ($\lambda_{max} \approx 260$ nm). Therefore the cholesteric phases of nucleic acids are, in fact, dyed cholesterics. In accordance with the theory [14, 38] for dyes (that is external chromophores) which are dissolved in thermotropic cholesteric phases, an abnormally intense band is induced in the U.V. region (see figure 9) of the C.D. spectrum of lyotropic liquid crystals of nucleic acids [39]. The sign of this band depends not only on the sense of the cholesteric helix but also on the orientation of the bases with respect to the long axis of the nucleic acid molecule. For cholesteric phases the intense band in the C.D. spectrum can be used as an additional criterion with which to study the different factors that influence the properties of the original nucleic acids as well as the properties of the liquid crystals formed from them.

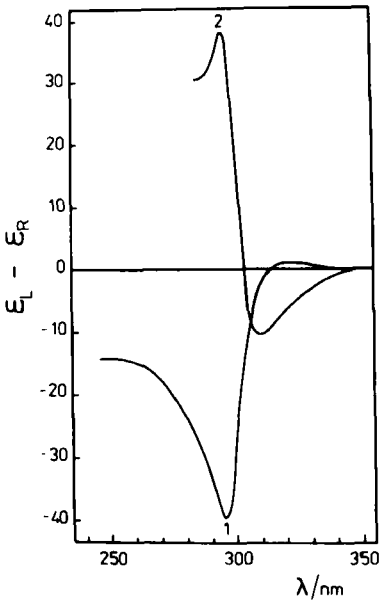

Figure 9. C.D. spectra of thin layers of liquid-crystalline phases prepared from DNA (curve 1) and poly(I) × poly(C) (curve 2). Molar mass of PEG 4000; 0·3 M NaCl; C_{PEG} = 150 mg/ml. The thickness of the layer is 30 μm.

Abnormally intense bands in the C.D. spectra of films prepared from DNA and synthetic polynucleotide poly(dA–dT) × poly(dA–dT) have been described by Brunner and Maestre [40]. Although the textures of these films were not investigated by means of polarization microscopy it was noted that the intense band appears in C.D. spectra of these films only at a definite hydration, i.e. at a definite mode of spatial packing

of the nucleic acid molecules. At PEG concentrations greater than 250 mg/ml, i.e. in conditions when the packing density of DNA in the liquid-crystalline phase increases, according to the X-ray analysis, the optical textures of these phases exhibit a system of black bent lines (see figure 8 *b*). According to Wendorf [41] such textures are typical of nematic polymeric liquid crystals. Since the increase of the PEG concentration up to 250 mg/ml is accompanied by a reduction of the dielectric constant from 80 to 60, then the formation of a nematic phase from optically active DNA molecules indicates that the solvent influences not only the packing density but also the mode of spatial organisation of the nucleic acid molecules.

The formation of nematic liquid crystals of DNA is not accompanied by the appearance of abnormal optical activity. Therefore, unlike aqueous solutions, a directed change of the properties of the aqueous polymeric solvent allows the formation of either cholesteric or nematic phases from optically active DNA molecules. These results correlate well with the theoretical concepts developed by Samulski and Samulski [42] as well as by Osipov [43]. According to these ideas optically active polymeric molecules can form different types of lyotropic liquid crystal.

The study of the optical textures of liquid-crystalline phases prepared in an aqueous-salt solution with a fixed concentration of PEG (C_{PEG} = 170 mg/ml) from DNA-daunomycin complexes showed [35] that a regular change of physico-chemical properties of the original linear DNA molecules is also accompanied by the formation of either cholesteric (see figures 10 (*a*) and (*c*)) or nematic (see figure 10 (*b*)) phases. Cholesteric phases of DNA-daunomycin complexes (cf. the textures in figures 10 (*a*) and (*c*)) have abnormal optical activities of different signs. This means that it is possible to prepare cholesteric phases with different senses of the helical twist depending on the extent of the bonding of daunomycin to DNA.

Modification of DNA by reaction with cis-Pt(II) is accompanied by a decrease in the pitch of the cholesteric phase formed [33]. In particular at r = 0·01, P does not equal 3–4 μm as for the original unmodified DNA, but is about 2 μm. Greater modification of DNA with cis-Pt(II) (r = 0·1) leads to the formation of the isotropic DNA phase which gives a texture called an isotropic drop-shaped phase (see figure 11). The existence of this texture observed only in natural light, together with the one dimensional ordering of the DNA molecules shown by the X-ray analysis (see figure 7) proves that DNA forms a phase whose properties differ from those of classical liquid-crystalline phases of nucleic acids. We infer that the change of energy of interaction between adjacent DNA molecules whose structure has been modified by reaction with cis-Pt(II), for instance, reduces the positive charge which appears on the molecules and results in the formation of an optically isotropic phase. This isotropic phase has the unusual texture of isotropic football shaped tactoids; a similar texture has been observed by Krigbaum for optically active molecules of another polymer, poly-γ-benzyl-L-glutamate [44]. Theoretical calculations by Khokhlov *et al.* [45] show that charged polyelectrolytes can, in principle, form a wider range of liquid-crystalline phases than neutral polymeric molecules also; this support the feasability of the formation of an ordered, optically isotropic DNA phase.

So, the change of the physico-chemical properties of the original DNA molecules which occurs following interaction with certain compounds is a factor with which to control the spatial organisation of their mesophases.

These results demonstrate that the change of properties of either the solvent or of the nucleic acid is accompanied not only by a change in the mode of spatial organisation of the nucleic acid molecules in the liquid-crystalline phases but also that of

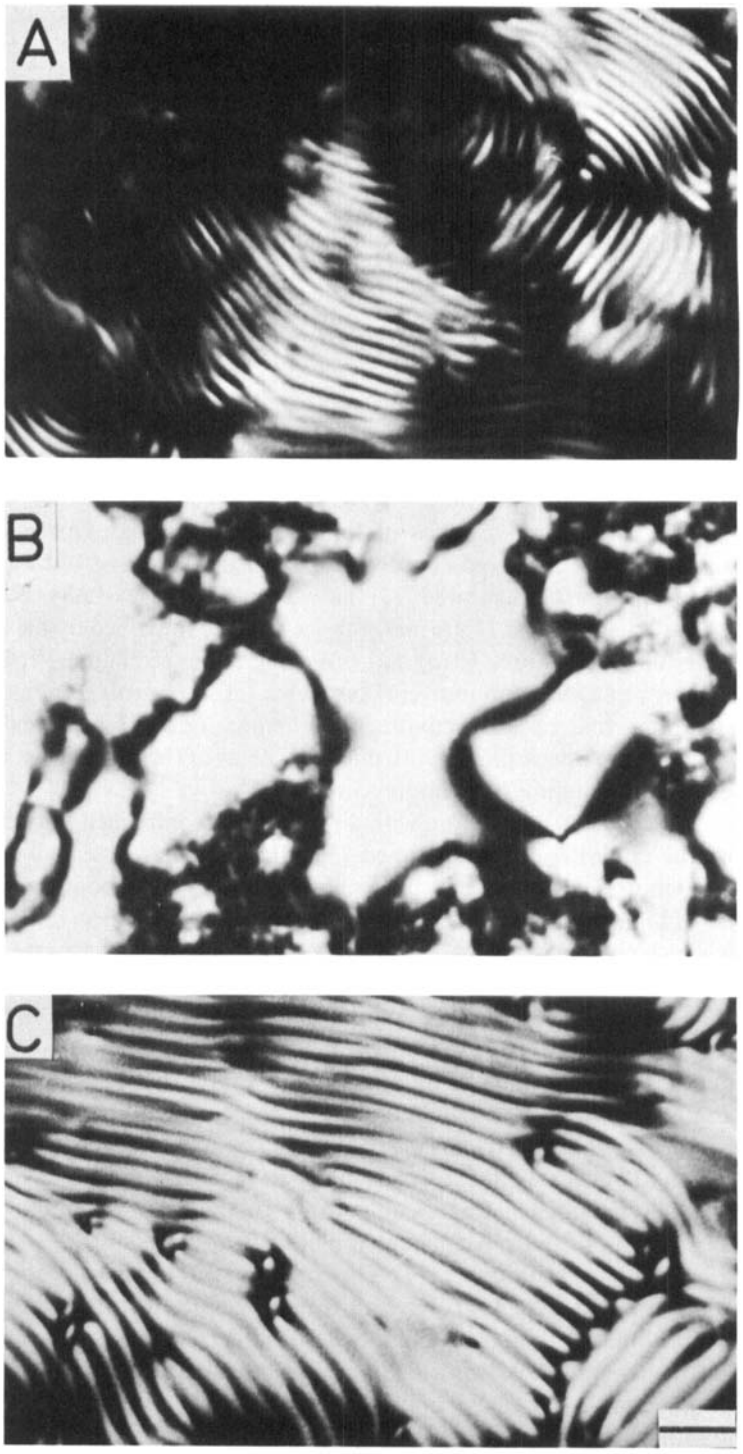

Figure 10. Textures of liquid-crystalline phases prepared from complexes of DNA with daunomycin. Molar mass of PEG 4000; 0·3 M NaCl; C_{PEG} = 170 mg/ml. (*A*) r = 0·05; (*B*) r = 0·165; (*C*) r = 0·675. (r is the ratio of molar concentration of daunomycin to that of the DNA nucleotides.) The lenglth of the bar is 10 μm.

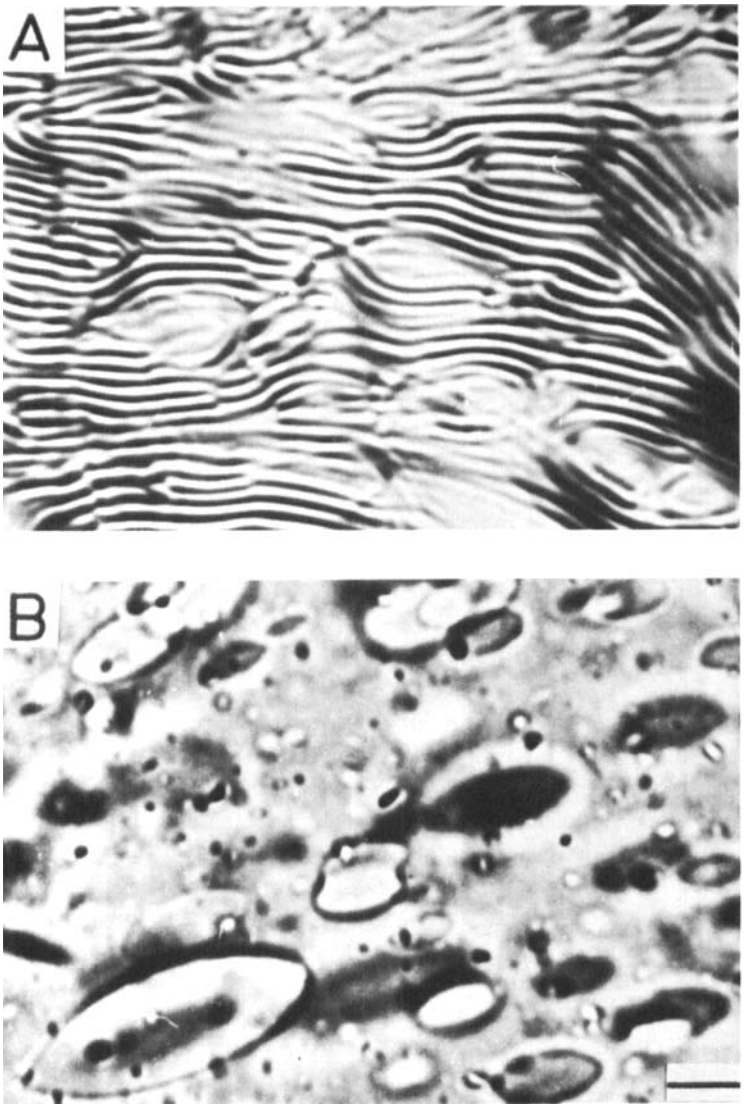

Figure 11. Textures of liquid-crystalline phases prepared from DNA modified by cis-Pt(II). (*A*) the finger-print texture in natural light. Molar mass of PEG 4000; C_{PEG} = 170 mg/ml; 0·3 M $NaClO_4$; r = 0 (*B*) the drop-shaped texture in natural light. Molar mass of PEG 4000; C_{PEG} = 170 mg/ml; 0·3 M $NaClO_4$; r = 0·1. The length of the bar is 10 μm.

the abnormal optical activity in the absorption region of the nitrogen bases of the nucleic acids.

4. The liquid-crystalline phase of nucleic acids in biological systems

4.1. *A PEG-like situation in biological systems*

When studying the assembling of bacteriophage T4 in *E. coli* cells Laemmli and his coworkers [2] focused their attention on the process of the organisation of phage DNA under cell conditions. At a definite stage of the cell cycle which is of interest physico-chemically, splitting of protein P22 takes place. This is cleft into two low

molecular weight proteins, II and VII. These proteins contain glutamine and asparagine amino acid residues at a level of 80 and 40 per cent, respectively. Within the cell these proteins are negatively charged and do not form stoichiometric complexes with DNA. The concentration of proteins II and VII in a cell at the packing site of the phage particles is estimated to reach about 500 mg/ml, which, in combination with their physico-chemical properties, induces the formation of the DNA phase due to phase separation. This implies that in a cell the water soluble biopolymers create conditions which correspond to those when nucleic acid molecules are excluded from aqueous salt solutions containing PEG; such a situation is termed a PEG-like situation. Taking into account the high concentration of glutamine and asparagine amino acids residues in proteins II and VII, Laemmli *et al.* [2] showed that addition of polyglutamine and polyasparagine acids (as well as the addition of PEG) to the aqueous salt DNA solutions is accompanied by a change in the hydrodynamic properties of this macromolecule, which suggests the formation of the DNA phase. Therefore, the process of packing phage particles *in vivo* appears to occur in a similar fashion to that of the liquid-crystalline phases of nucleic acids formed *in vitro*. This means that using the data describing the formation of liquid-crystalline phases of nucleic acids, it is possible not only to model and conserve the phase of the nucleic acid in biological systems *in vitro* but also to elucidate the methods and factors for direct control of the packing process for the genetic material *in vivo*.

4.2. *Pecularities of the organization of nucleic acid molecules in biological systems*

Before we discuss the possible phases of nucleic acids in biological systems (for example, viruses and chromosomes), it is necessary to determine the general features of the organisation of nucleic acid molecules. Such an analysis allows us to distinguish the following pecularities.

(i) High concentration of nucleic acids. The local concentration of DNA in biological systems appears to be rather high. For instance, the concentration of DNA in bacteriophage heads is approximately 800 mg/ml [1]. In certain regions of interphase nuclei of human somatic cells the concentration of DNA reaches 200 mg/ml [46]. While in isolated chromosomes of *E. coli* the concentration of DNA reaches 600 mg/ml [47]. The data quoted indicate that the volume concentration of DNA in biological systems corresponds to the range of concentration at which spontaneous ordering of DNA is possible, i.e. a liquid-crystalline phase is formed.

(ii) The local ordering of DNA molecules (or DNA segments). The first studies of bacteriophages T2 and T4 showed the presence of a reflection in the small angle region of the X-ray pattern. This reflection corresponds to d equal to 24 Å [48]. This implies that the packing density of adjacent DNA segments in the bacteriophage heads corresponds to that of DNA molecules in a liquid-crystalline phase. The results of an X-ray analysis of chromatin gels, while indicating a dense packing of DNA, are not sufficient to develop an exact picture of their spatial organisation. In consequence models for the packing of DNA in chromatin, specifically at levels higher than nucleosomal, remain under discussions [49, 50].

(iii) Specific character of the organization of nucleic acids. The electron microscope pictures obtained for the mild conditions of destroying the bacteriophage heads show that the double-stranded molecules of DNA are torus shaped. The diameter of the particles is about 1000 Å [51]. The data obtained from electron microscope visualization of thin sections of bacteriophages indicates the existence of crystalline

domains. The distance between the neighbouring DNA molecules in these domains is about 25 Å [52].

The study of thin sections of the protozoa chromosome, *Protocentrum micans*, shows striations [53, 54]. These striations indicate the packing of DNA to be more exact than that of the nucleo-protein complex. The character of the striations depends on the angle of the chromosome section. The reconstruction of the model of DNA packing based on the analysis of the sections obtained led to the conclusion that the DNA molecules in the protozoa chromosomes (which are typical of a low content or complete absence of proteins) are packed similarly to polymer cholesterics [54].

4.3. *Optical properties of nucleic acids with the composition of the biological systems*

The optical rotatory dispersion spectra of 16 bacteriophages obtained in [55] prove that the *T*-even bacteriophages have negative optical rotation whereas *T*-odd have a positive sign in the part of the spectrum where the nitrogen bases do not absorb ($\lambda > 300$ nm). In 1980 an unusual C.D. spectrum of bacteriophage ϕKZ [56] was found containing two negative bands. These are located in the absorption region of the nitrogen bases. It was supposed that the spatial organisation of DNA in this bacteriophage differs from that of DNA in bacteriophage T2 which is similar in size to phage ϕKZ.

The data on the state of DNA molecules in sperm heads are of interest; these are also typical of the dense packing of DNA [57–60]. In the C.D. spectrum of chromatin isolated from equine sperm [57] an intense band is present, the sign and the shape of this band are similar to those of bacteriophage ϕKZ. However, the amplitude of the band corresponds to the amplitude of the negative band found in the C.D. spectra of nucleic acid liquid crystals. The C.D. spectrum of octopus sperm heads also has a band whose sign is positive and not negative [60].

Comparison of these observations for DNA in biological systems with those for the nucleic acid liquid-crystalline phases prepared in PEG solutions allows a number of conclusions to be made. (1) The properties of DNA with the composition of some bacteriophages are similar to the properties of nematic liquid crystals of DNA. This is shown by the presence of small angle reflections in the X-ray scattering patterns, the electron microscopy data and the absence of abnormal optical activity. In addition there are examples which show that the properties of DNA in bacteriophages are similar to those of cholesteric liquid crystals. The different signs for the optical activity found for *T*-even and *T*-odd bacteriophages can also be interpreted in terms of the cholesteric packing of DNA molecules. The change of the nucleotide content of DNA, the alteration of the character of interaction of DNA molecules with phage proteins, different sense of helical twist of DNA molecules may all be associated with the different signs of the abnormal optical activity observed for bacteriophages. (2) The properties of DNA packed in sperm heads of some organisms are probably closely analogous to those of DNA arranged in cholesteric phases, i.e. a high packing density and the presence of abnormal optical activity observed in the absorption region of the DNA bases. (3). The nature of the organisation of DNA in chromatin is more complex and less studied than the packing of DNA in bacteriophages. To solve this problem experimental work is necessary to fix chromatin (or the chromosomes) and to vary the interaction of DNA with the protein. In this case, based on the notion of the packing of DNA in liquid-crystalline phases, we would expect the appearance of abnormal optical activity for the chromosomes.

The study of nucleic acid liquid crystals opens up therefore a perspective for verifying the validity of the concept that DNA or segments of DNA in complex biological objects from liquid-crystalline phases.

In concluding this review, we should say that in the field of lyotropic liquid crystals of nucleic acids there exist a number of unanswered questions:

(1) Is it possible, in practice, to prepare liquid-crystalline phases from nucleic acids with a molecular weight exceeding 25–50 $\times$ 10^6? Is a DNA molecule with such a molecular weight able to form an intramolecular liquid crystal and what properties would it possess?

(2) Does the type of structural organization of DNA in biological systems affect its biological activity? Do the intracellular enzymes distinguish between the right-handed and the left-handed twist in the cholesteric phases of DNA?

(3) Is it possible to study the dependence of the spatial organization of DNA in chromosomes which have been fixed in some way on the properties of the solvent? Does the abnormal optical activity depend on definite properties of the solvent? Do proteins bound to DNA in chromosomes create a dielectric medium in which the potential mesogenic properties of DNA can be realized?

While questions 1 and 2 appear to be amenable to theoretical and experimental approaches, questions 3 and 4 are more elusive. Studies in the field of lyotropic liquid crystals of nucleic acids may then lead to valuable novel data interesting from both physico-chemical and biological points of view.

References

[1] KELLENBERGER, E., 1961, *Adv. Virus Res.*, **8**, 2.

[2] LAEMMLI, U. K., PAULSON, J. R., and HITCHINS, V., 1974, *J. supramolec. Structure*, **2**, 276.

[3] LUZZATI, V., NICOLAIEFF, A., and MASSON, F., 1961, *J. molec. Biol.*, **3**, 185.

[4] ROBINSON, C., 1961, *Tetrahedron*, **13**, 219.

[5] IIZUKA, E., 1977, *Polym. J.*, **9**, 173.

[6] IIZUKA, E., 1983, *Polym. J.*, **15**, 525.

[7] POTAMAN, V. N., ALEXEEV, D. C., SKURATOVSKY, I. YA., RABINOVICH, A. Z., and SHLYAKHTENKO, L. S., 1981, *Nucl. Acids Res.*, **9**, 55.

[8] RILL, R. L., HILLARD, P. R., and LEVY, G. C., 1983, *J. biol. Chem.*, **258**, 250.

[9] LIVOLANT, F., 1984, *Europ. J. cell. Biol.*, **33**, 300.

[10] RILL, R. L., 1986, *Proc. Natn. Acad. Sci. U.S.A.*, **83**, 342.

[11] IIZUKA, E., and YANG, J. T., 1977, *Liquid Crystals and Ordered Fluids*, Vol. 3, edited by J. F. Johnson and R. S. Porter (Plenum Press) p. 197.

[12] IIZUKA, E., 1978, *Polym. J.*, **10**, 293.

[13] SENECHAL, E., MARET, G., and DRANSFELD, K., 1980, *Int. J. biol. Macromolec.*, **2**, 256.

[14] SAEVA, F. D., 1979, *Liq Crystals*, edited by F. D. Saeva (Marcel Dekker Inc.), p. 249.

[15] LERMAN, L. S., 1971, *Proc. Natn. Acad. Sci. U.S.A.*, **68**, 1886.

[16] EVDOKIMOV, YU. M., PLATONOV, A. L., TIKHONENKO, A. S., and VARSHAVSKY, YA. M., 1972, *FEBS Lett.*, **23**, 180.

[17] YEVDOKIMOV, YU. M., PYATIGORSKAYA, T. L., POLYVTSEV, O. F., AKIMENKO, N. M., TSVANKIN, D. YA., and VARSHAVSKY, YA. M., 1976, *Molekul. Biol.* (Russ. Ed.), **10**, 1221.

[18] YEVDOKIMOV, YU. M., PYATIGORSKAYA, T. L., POLYVTSEV, O. F., AKIMENKO, N. M., KADYKOV, V. A., TSVANKIN, D. TA., and VARSHAVSKY, YA. M., 1976, *Nucl. Acids Res.*, **3**, 2353.

[19] SKURIDIN, S. G., SCHASCHKOV, V. S., YEVDOKIMOV, YU. M., and VARSHAVSKY, YA. M., 1979, *Molekul. Biol.* (Russ. Ed.), 13, 804.

[20] YEVDOKIMOV, YU. M., SKURIDIN, S. G., AKIMENKO, N. M., 1984, *Vysokomolek. Soedin.* A, **26**, 2403.

[21] SKURUDIN, S. G., DAMASCHUN, H., DAMASCHUN, G., YEVDOKIMOV, YU. M., and MISSELWITZ, R., 1986, *Studia biophys.*, **112**, 139.

[22] YEVDOKIMOV, YU. M., SKURIDIN, S. G., DEMBO, A. T., SCHTYKOVA, E. V., KADYKOV, V. A., VARSHAVSKY, YA. M., 1979, *Molekul. Biol.* (Russ. Ed.), **13**, 1110.
[23] MANIATIS, T., VENABLE, J. M., and LERMAN, L. S., 1974, *J. molec. Biol.*, **84**, 37.
[24] YEVDOKIMOV, YU. M., PYATIGORSKAYA, T. L., KADYKOV, V. A., POLYVTSEV, O. F., DOSKOCIL, J., KAUDELKA, J., and VARSHAVSKY, YA. M., 1976, *Nucl. Acids Res.*, **3**, 1533.
[25] LORTKIPANIDZE, G. B., YEVDOKIMOV, YU. M., DEMBO, A. T., VARSHAVSKY, YA. M., 1984, *Molekul. Biol.* (Russ. Ed.), **18**, 446.
[26] DEMBO, A. T., STIKOVA, E. V., LOPTKIPANIDZE, G. B., and YEVDOKIMOV, YU. M., 1982, *Studia biophys.*, **87**, 275.
[27] SKURIDIN, S. G., BADAEV, N. S., DEMBO, A. T., LORTKIPANIDZE, G. B., and YEVDOKIMOV, YU. M., 1988, *Liq. Crystals*, **2**, 51.
[28] SKURIDIN, S. G., DEMBO, A. T., OSIPOV, M. A., DAMASCHUN, H., DAMASCHUN, G., and YEVDOKIMOV, YU. M., 1985, *Dokl. Akad. Nauk SSSR*, **285**, 713.
[29] BRIAN, A. A., FRISCH, H. L., and LERMAN, L. S., 1981, *Biopolymers*, **20**, 1305.
[30] ARNOLD, K., HERRMANN, A., PRATSCH, L., and GAWRISCH, K., 1985, *Biochim. biophys. Acta*, **815**, 515.
[31] YEVDOKIMOV, YU. M., SALYANOV, V. I., DEMBO, A. T., SCHRAGO, M. I., and KHANINA, L. A., 1986, *Kristallografiya*, **31**, 736.
[32] YEVDOKIMOV, YU. M., SALYANOV, V. I., DEMBO, A. T., and BERG, H., 1983, *Biomed. biochim. Acta*, **42**, 855.
[33] SKURIDIN, S. G., SCHTYKOVA, E. V., DEMBO, A. T., BADAEV, N. S., CHELTSOV, P. A., and YEVDOKIMOV, YU. M., 1988, *Biofisika*, **33**, 55.
[34] YEVDOKIMOV, YU. M., SKURIDIN, S. G., and BADAEV, N. S., 1986, *Dokl. Akad. Nauk SSSR*, **286**, 997.
[35] SKURIDIN, S. G., BADAEV, N. S., LAVRENTOVICH, O. D., and YEVDOKIMOV, YU. M., 1987, *Dokl. Akad. Nauk SSSR*, **295**, 1240.
[36] DEMUS, D., and RICHTER, L., 1978, *Textures of Liquid Crystals* (Deutscher Verlag für Grundstoffindustrie), p. 219.
[37] LIVOLANT, F., 1986, *J. Phys., Paris*, **47**, 1605.
[38] PROKHOROV, V. V., and KIZEL', V. A., 1985, *Kristallografiya*, **30**, 958.
[39] YEVDOKIMOV, YU. M., SALYANOV, V. I., and PALUMBO, M., 1985, *Molec. Crystals liq. Crystals*, **131**, 285.
[40] BRUNNER, W. C., and MAESTRE, M. F., 1974, *Biopolymers*, **13**, 345.
[41] WENDORFF, J. H., 1978, *Liquid Crystalline Order in Polymers*, edited by A. Blumstein (Academic Press), p. 15.
[42] SAMULSKI, T. V., and SAMULSKI, E. T., 1977, *J. chem. Phys.*, **67**, 824.
[43] OSIPOV, M. A., 1985, *Chem. Phys.*, **96**, 259.
[44] KRIGBAUM, W. R., 1982, *Polymer Liquid Crystals*, edited by A. Ciferri, W. R. Krigbaum, R. B. Meyer (Academic Press), p. 275.
[45] NIRKOVA, I. A., and KHOKHLOV, A. R., 1986, *Biofisika*, **31**, 771.
[46] GOLOMB, H. M., and BAHR, G. F., 1974, *Chromosoma (Berl.)*, **46**, 233.
[47] KAVENOFF, R., and BOWEN, B. C., 1977, *Stadler Symp. University of Missouri*, **9**, 159.
[48] NORTH, A. C. T., and RICH, A., 1963, *Nature, Lond.*, **191**, 1242.
[49] GEORGIEV, G. P., and BAKAEV, V. V., 1978, *Molekul. Biol.* (Russ. Ed.), **12**, 1205.
[50] WOODCOCK, C. C. F., FRADO, L. Y., and RATTNER, J. B., 1984, *J. cell. Biol.*, **99**, 42.
[51] KLIMENKO, S. M., TIKCHONENKO, T. I., and ANDREEV, V. M., 1967, *J. molec. Biol.*, **23**, 523.
[52] LEPAULT, J., DUBOCHET, J., BASCHONG, W., and KELLENBERGER, E., 1987, *EMBO J.*, **6**, 1507.
[53] BOULIGAND, Y., 1977, *Am. chem. Soc. Polym.*, Preprint, **18**, 33.
[54] BOULIGAND, Y., and LIVOLANT, F., 1984, *J. Phys., Paris*, **45**, 1899.
[55] MAESTRE, M. F., and TINOKO, I., 1967, *J. Molec. Biol.*, **23**, 323.
[56] TJAGLOV, B. V., KRILOV, V. N., PLOTNIKOVA, T. G., MANAEV, V. E., and PERMOGOROV, V. I., 1980, *Molekul. Biol.* (Russ. Ed.), **14**, 1019.
[57] WAGNER, T. E., MANN, D. R., and VINCENT, R. C., 1974, *J. exp. Zoology*, **189**, 387.
[58] PHILLIPS, D. M., 1976, *J. Ultrastruct. Res.*, **54**, 397.
[59] SIPSKI, M. L., and WAGNER, T. E., 1977, *Biopolymers*, **16**, 573.
[60] MAESTRE, M. F., BUSTAMANTE, C., HYAYES, T. L., SUBIRANA, J. A., and TINOCO, J., 1982, *Nature, Lond.*, **298**, 773.