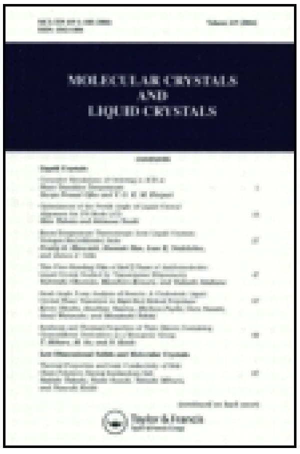

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DETERMINATION OF THE LIQUID CRYSTALLINE PROPERTIES OF ERYTHROCYTE MEMBRANES

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Abstract A study has been made on the mesomorphic properties of membrane preparations from normal and abnormal human red blood cells using a variety of physical techniques such as Fourier Transform Infrared Spectroscopy, Optical Microscopy and Differential Scanning Calorimetry. FTIR spectra of normal and abnormal ghosts confirmed that both have the same composition. Textures of the lyotropic neat phase were observed in normal ghosts. In addition, it was found that amphiphilic systems (such as membranes) may exhibit a nematic phase similar to that observed in thermotropics. DSC thermograms of normal ghosts showed that they go through several mesophases such as smectic and nematic. At the physiological temperatures, the membranes are in a lamellar smectic phase further lending support to the fluid mosaic model of cell membranes. Similar tests performed on membranes of thalassemic (abnormal) erythrocytes yielded no results that are similar to the liquid crystalline behavior of normal membranes.

INTRODUCTION

Lyotropic liquid crystals are mixtures of amphiphilic compounds and a polar solvent, most often water. An example of such a mixture found in living systems is the cell membrane. With the use of physical techniques, we hope to determine the liquid crystalline properties of membranes of normal and abnormal red blood cells. The analysis of differences in the structure, function, and dynamics of normal and abnormal cell membranes is of value in the study of disease processes, and could prove useful in the search for preventive and curative medicine.

THE EXPERIMENT

To compare the differences in the mesomorphic properties of membranes of normal and abnormal RBCs, RBCs afflicted with thalassemia were obtained. In thalassemias, there is a decrease or complete lack of α or β globin chain synthesis. Since there is an unbalanced chain synthesis, there is a relative excess of one of the globin chains which form inclusions that reduce cell deformibility, act as an oxidant stress causing irreversible membrane damage and subsequently lead to decreased survival of the cell.

Erythrocyte ghosts were prepared using an extraction protocol by Antony et. al. They were then tested for mesomorphic properties using Fourier Transform Infrared (FTIR) spectroscopy, optical microscopy, and differential scanning calorimetry (DSC).

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy

The IR profiles of the normal and abnormal erythrocyte ghosts were very similar indicating that they are of the same composition. Comparing the obtained peaks (such as the C=O, NH, CH_2, and CH_3 functional groups) with the molecular structure of the phospholipids found in membranes, we saw that the peaks correspond to the molecules present in lipids.

Optical Microscopy

Table I lists the textures observed as water was allowed to evaporate from the normal and abnormal ghosts and as the ghosts were heated up.

TABLE I Textures observed for the two ghosts under a polarizing microscope.

	Normal Ghost	Abnormal Ghost
Letting water evaporate:		
initial	positive units (Figure 1a)	none (Figure 1b)
after 25 hours	fine mosaic, stippled, and schlieren textures (Figures 2-3)	none
after 14 days	crystalline	none
Upon heating:		
at room temperature	unit texture	none
40°C - 60°C	more units	none
60°C - 80°C	schlieren texture	none
above 80°C	isotropic	none

When water was allowed to evaporate from the ghosts, the normal RBC ghost exhibitied characteristic textures of the lyotropic neat phase (Figures 1a-2). This indicates that in an aqueous environment, the normal membranes are in a lamellar (or bilayer) configuration, further validating the fluid mosaic model of cell membranes.

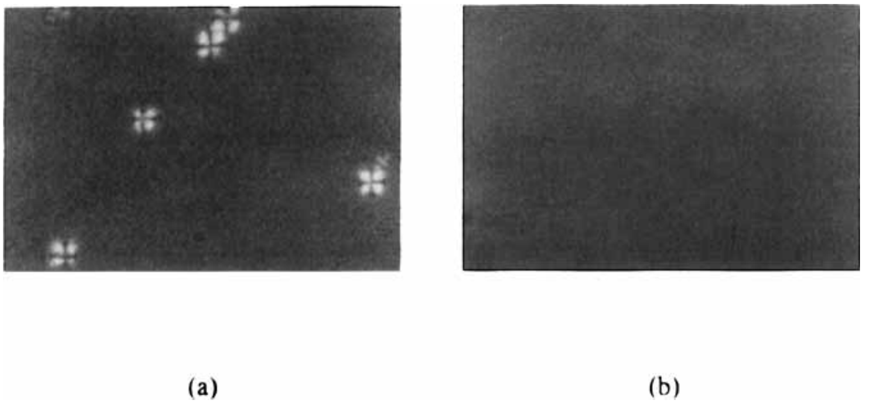

FIGURE 1 Initial appearance of (a) **normal** and (b) **abnormal** ghosts at room temperature. 400x.

The observation of nematic textures (Figure 3) proves that the nematic phase, more commonly associated with thermotropic liquid crystals, can also occur in lyotropics.

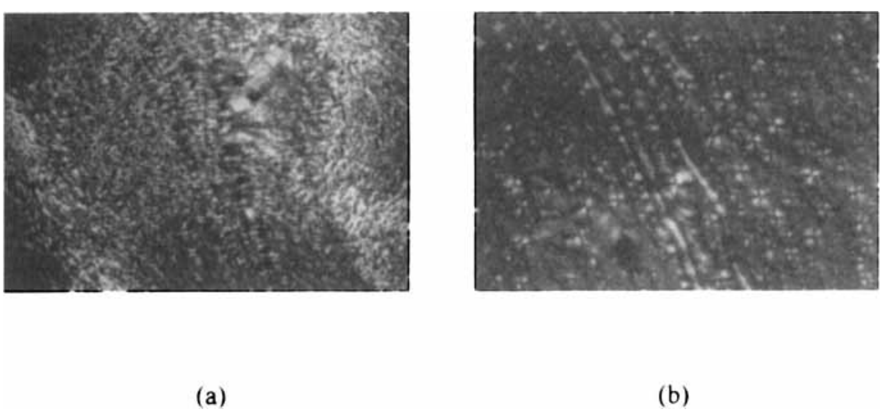

FIGURE 2 The neat phase (fine mosaic texture) of the **normal** ghost after 25 hours. (a) 400x. (b) 1000x.

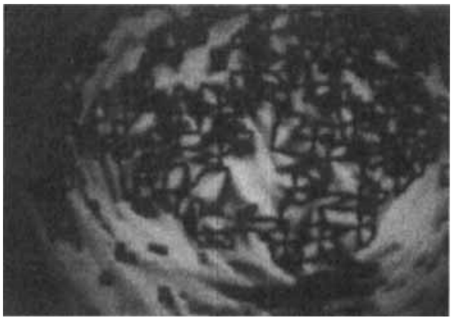

FIGURE 3 Nematic (schlieren) structure. Note the "untwisting" of the arms of individual droplets. 1000x..

Taking another set of samples, when the normal ghost was heated up from room temperature, the sample exhibited different textures characteristic of thermotropic liquid crystals, such as droplets and the schlieren texture.

Letting the water evaporate from it or heating it up yielded no liquid crystalline texture for the abnormal ghosts as can be seen in Figure 1b.

Differential Scanning Calorimetry

Table II is a summary of the thermodynamic data for the phase transitions of normal and abnormal ghosts.

TABLE II Thermodynamic data for the phase transitions of normal and abnormal ghosts.

	Normal Ghost	Abnormal Ghost
1st transition:		
peak temperature (°C)	27.66	106.72
ΔH (kJ/g)	1.00	1.57
2nd transition:		
peak temperature (°C)	58.16	-
ΔH (kJ/g)	0.0057	-
3rd transition:		
peak temperature (°C)	71.07	-
ΔH (kJ/g)	0.0096	-

DSC thermograms of normal ghosts yielded three peaks. The first peak, at 27.66°C, corresponds to a C - Sm transition. This transition to a smectic liquid crystal was verified by the positive units (similar to Figure 1a) observed when the sample was heated. The second peak, observed at 58.16°C, corresponds to a Sm - N transition and this was confirmed by the schlieren texture (similar to Figure 3) encountered when the sample was heated. The third peak, at 71.07°C, corresponds to a N - I transition.

The above data strongly suggest that at the physiological temperatures, as membranes are found in the body, normal membranes are liquid crystalline arranged in a lamellar (bilayer) pattern.

Meanwhile, DSC thermograms of ghosts of abnormal RBCs consistently yielded one melting peak. This peak, found at above 100°C, corresponds to a transition from crystalline gel to a homogeneous solution. This was confirmed by the isotropic appearance of abnormal ghosts upon heating.

Compared to the mesomorphic behavior of normal membranes, the apparent non-liquid crystallinity of the abnormal membranes suggests that for the membrane to function normally, the membranes must be in a liquid crystalline state.

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