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ON PHASE TRANSITIONS IN ERYTHROCYTE MEMBRANES AND EXTRACTED MEMBRANE LIPIDS

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Summary

X-ray diffraction patterns show that extracted erythrocyte membrane lipids from which almost all cholesterol has been removed undergo a rigid crystalline to liquid crystalline transition over the range 2 to 20° C. Increasing the cholesterol content reduces the temperature at which rigid crystalline phase appears; no rigid crystalline material is detectable above −10° C with cholesterol contents of 7.3% and greater. Also no rigid crystalline state was detected, down to −20° C, in unmodified erythrocyte membranes and in membranes depleted of up to 1/3 of their cholesterol. These results do not substantiate the indications of a phase transition at 18° to 20° C in erythrocyte membranes and extracted membrane lipids by viscosity measurements (Zimmer, G. and Shirmer, H. (1974) Biochim. Biophys. Acta 345, 314—320).

Zimmer and Schirmer [1] have recently reported viscosities of suspensions of sonicated human erythrocyte membranes, of extracted membrane lipids adjusted to varying cholesterol contents, and also of $CHCl_3$—CH_3OH (1/1, v/v) solutions of the extracted membrane lipids. In some cases the viscosity—temperature curves showed a discontinuity in the range 18° C—20° C. This was interpreted as a phase transition. Zimmer and Schirmer pointed out that the rates of glucose transport across erythrocyte membranes also showed discontinuities in the 18—20° C temperature range, and accordingly suggested a role for lipid—protein interactions in glucose transport.

The above mentioned paper prompted us to report on X-ray diffraction studies made on human erythrocyte membranes and on mixtures of water with extracted erythrocyte membrane lipids of varying cholesterol contents.

The X-ray diffraction results only partially support the indications of a phase transition by the viscosity measurements.

It is well known that phospholipids, which constitute the major membrane lipids, can exist in two state [2]. In the state stable at lower temperatures, termed the rigid-crystalline state, the hydrocarbon chains of the lipids rotate freely about their axes, but are otherwise rigidly aligned. At higher temperatures a liquid-crystalline state is formed in which the hydrocarbon chains have a liquid-like flexibility. The presence of each state can be readily detected by X-ray diffraction methods, the rigid-crystalline state giving a sharp line at 4.2 Å, and the liquid-crystalline state giving a diffuse line at about 4.5 Å [3,4]. Transitions between these states occur in bacterial membranes as well as in isolated lipids [5—7].

TABLE I

CRYSTALLINE STATES PRESENT IN 50/50 WT % MIXTURES OF WATER WITH EXTRACTED ERYTHROCYTE MEMBRANE LIPIDS OF VARIOUS CHOLESTEROL CONTENTS

Cholesterol content (% of total lipids)	T (°C)	Crystalline state
3	25	Liquid crystalline
	20	Liquid crystalline + trace rigid crystalline
	15	Liquid crystalline + rigid crystalline
	2	Rigid crystalline + trace liquid crystalline
7.3	0	Liquid crystalline
	−10	Liquid Crystalline + trace rigid crystalline
	−20	Liquid crystalline + rigid crystalline
13.7	2	Liquid crystalline
	−20	Liquid crystalline
31.5	2	Liquid crystalline
	−20	Liquid crystalline

X-ray diffraction results on extracted membrane lipids of various cholesterol contents are given in Table I. The sample with 31.5% cholesterol is the complete lipid extract of erythrocyte membranes; the 3% cholesterol material is lipid extract from which cholesterol was removed by column chromatography. The two intermediary cholesterol contents were attained by adding pure cholesterol to this latter cholesterol-depleted lipid. The relative amounts indicated for the two crystalline states were estimated assuming a correspondence between the relative amounts and the relative intensities of the respective X-ray lines. Since the rigid-crystalline X-ray line is much sharper than the liquid-crystalline line, it can be safely concluded that only the liquid-crystalline state is present when an X-ray line corresponding to that state occurs and there is none corresponding to the rigid-

crystalline state. It is clear from Table I that the transition takes place over a wide temperature range and that cholesterol markedly reduces the temperature at which the rigid-crystalline state forms. This latter result is in accord with many other measurements of the effects of cholesterol on phospholipids [8,9]. The absence of a transition, in the temperature range covered, in the case of whole membrane lipids is in accord with the differential thermal analysis data of Ladbrooke and Chapman [10].

The viscosity measurements of Zimmer and Schirmer show a discontinuity at 18—20° C with suspensions of extracted membrane lipids from which all cholesterol has been removed. This seemingly correlates well with the appearance of rigid-crystalline phase at 20° C in the X-ray pattern of extracted lipids containing 3% cholesterol (Table I). However, the viscosity measurements also show a discontinuity at 18—20° C with extracted lipids containing 10% cholesterol while, as shown in Table I, X-ray diffraction gives no indications of rigid-crystalline state at temperatures above —10° C with samples containing 7.3% and 13.7% cholesterol. The viscosity measurements on material with 50% cholesterol showed no discontinuities over the range 15° C to 23° C, which is in accord with the data of Table I.

In the case of suspensions of sonicated erythrocyte membranes, the viscosity measurements of Zimmer and Schirmer also show a discontinuity at 18° C to 20° C. Our X-ray diffraction measurements on suspensions of intact erythrocyte membranes show no evidence of rigid-crystalline state down to —20° C; indeed we have also found no evidence for a rigid-crystalline state down to —20° C with membranes depleted of up to 1/3 of their cholesterol.

One possible source of differences between the X-ray diffraction and the viscosity results may lie in the physical form of the materials. In the case of the whole membranes, the viscosity measurements concern dispersions of the fragments produced by sonication of the membranes, while the X-ray measurements were made on the essentially intact membrane structures produced by osmotic hemolysis [11].

Different physical forms are also involved in the case of the extracted lipids. The X-ray measurements were made on a bulk material which is multilamellar in structure [12], while at least a portion of lipids in sonicated dispersions of the type used in the viscosity measurements are present as single bilayer liposomes [13]. However, the phase transition behavior of sonicated dispersions [14] and bulk lipids [15] are similar, as indicated by calorimetry, both for the cases of pure phospholipid and phoslipid—cholesterol mixtures.

While viscosity measurements on sonicated suspensions of extracted membrane lipids and of whole membranes may eventually prove to be a valuable tool for identifying phase transitions, we suggest that caution be used in drawing conclusions from such measurements until they are better understood.

Erythrocytes were obtained from fresh human blood collected with ACD anticoagulant. The blood was separated from plasma by centrifuging at 500 × *g* for 20 min and washed 3 times with a balanced salt solution.

Membranes were prepared by pH 7.4 osmotic hemolysis as described by Dodge et al. [11]. The dilute membrane suspensions obtained from this procedure were concentrated, by slow evaporation of water, to solids contents of 30%—50% for the X-ray measurements.

Lipids were extracted from the membranes following the procedure of Rand and Luzzatti [12]. Cholesterol was removed from the extracted lipids by elution from a Unisil column with 10 volumes of $CHCl_3$. The remaining lipids were eluted with 10 volumes of CH_3OH. Eluates were evaporated to near dryness on a rotary evaporator and to complete dryness under N_2 at room temperature. Lipids were stored in $CHCl_3$ at $-20°C$. Measurements were made within 2 weeks of preparation of the lipids.

The membranes were depleted of cholesterol by incubation in preincubated plasma, as described by Murphy [16].

Cholesterol was determined by the σ-phthalaldehyde method of Zlatkis and Zak [17].

X-ray diffraction measurements were made by standard procedures, as described [4].

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