PHASE TRANSITION IN LIPID MULTILAYERS INDUCED BY BENZENE. A RAMAN SPECTROSCOPIC STUDY

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<u>Summary</u>: The interaction of increasing amount of benzene with dipalmitoyl lecithin multilayers has been investigated by Raman spectroscopy. It is shown that benzene is able to promote a gel—liquid crystalline phase transition.

Experimental:  $L-\beta$ , $\gamma$ -dipalmitoyl- $\alpha$ -lecithin (DPL) was checked for purity by thin layer cromatography and mixed in capillary tubes with 30% (w/w) water and a given amount of benzene (Carlo Erba, spectroscopic grade). The sealed capillary tubes were equilibrated at 80 °C in a water bath for 18 hours. All spectra were recorded at room temperature by a Cary 82 Raman spectrometer equipped with a Spectra Physics 164 argon ion laser at 488 nm and 400 mW.

Results and Discussion: The interaction of DPL with benzene can be analysed by investigating changes in two regions of vibrational spectra of lipids; the 1000-1200 cm<sup>-1</sup> region assigned earlier to C-C skeletal stretching modes (1,2) and the 2800-2900 cm<sup>-1</sup> region assigned to C-H stretching region (3-5). Both vibrational modes have proven to be useful in studying conformational changes of the hydrocarbon chains of lipids (6-8).

DPL with 30% (w/w) water forms a gel phase at room temperature (9). A typical spectrum is shown on Fig.1a and the ratio of the intensities of  $1090~\rm{cm}^{-1}$  band to  $1130~\rm{cm}^{-1}$  band is also given. Increasing amounts of benzene cause changes in the vibrational properties of the hydrocarbon chains

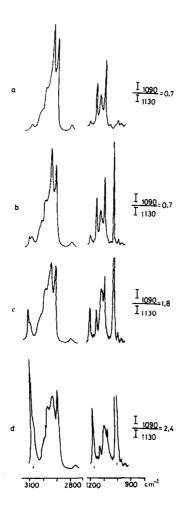


Fig.1.

The effect of benzene on vibrational spectrum of dipalmitoy1 lecithin in the presence of 30% (w/w) water and a given amount of benzene. a-pure lecithin with water, b-10%, c-20%, d-30% (w/w) benzene added. Instumental setting: 2700-3100 cm<sup>-1</sup> region - slit width 4 cm<sup>-1</sup>, scan speed 1.0 cm<sup>-1</sup>/s, pen period 2 s, sensitivity 50 000 counts/s; 900-1200 cm<sup>-1</sup> region - slit width 4 cm<sup>-1</sup>, scan speed 0.4 cm<sup>-1</sup>/s, pen period 5 s, sensitivity 5000 counts/s. Marks show benzene lines at 3046 cm<sup>-1</sup>, 1178 cm<sup>-1</sup>, and 991 cm<sup>-1</sup>.

which are responsible for the changing intensity ratio of Raman bands at 1090  $\rm cm^{-1}$  and 1130  $\rm cm^{-1}$  (1,6) and similarly in the case of 2850  $\rm cm^{-1}$  and 2880  $\rm cm^{-1}$  bands (6-8)(Fig.1b-d).

The intensity ratio of 1090 cm<sup>-1</sup> and 1130 cm<sup>-1</sup> bands has been interpreted as a measure of the order of the relative hydrocarbon chains within the bilayer (6).

The value of  $I_{1090}/I_{1130}$  given on Fig.1a is characteristic of DPL in the gel phase at room temperature. It has been shown in a previous paper (9) that the spectrum of DPL undergoes well-defined changes as the temperature is raised. These changes correspond to the gel-liquid crystalline phase transition. In this work the temperature has been kept constant below the phase transition temperature for DPL-water multilayers (10), but the concentration of benzene added to the multilayers was varied. It can be seen on Fig. 1b-d that adding benzene in concentration of 10%, 20%, 30% (w/w) causes successive changes in both regions of the Raman spectrum at room temperature. An estimated temperature 43-44  $^{\rm O}{\rm C}$  would be necessary to obtain the same value of relative intensities of  $I_{1090}/I_{1130}$  in the case of pure DPL as those obtained from DPL with 30% (w/w) benzene. That is 30% (w/w) benzene brings about a gel-liquid crystalline phase transition well below the transition temperature usually observed in water-DPL systems. Thus on the basis of this analogy one may conclude that benzene alters the ordered hydrocarbon structure of the gel producing chain liquid crystallinity at lower temperatures.

We interpret this effect as a solubilization of benzene in the hydrocarbon part of lipids which might be one explanation for the toxic effect of benzene in living systems e.g. It can destroy the ordered structure of lipid parts in biomembranes and by that way can cause a dangerous change in their barrier properties.

It is interesting in this connection to compare the recent work of Sanioto and Schreier (11), who found that aromatic hydrocarbons having carcinogenic activity showed greater effects on membrane disordering than non-carcinogenic molecules.

We have shown that benzene is able to cause a gel-liquid crystalline phase transition in lipid multilayers. However it might be added that the amount of benzene necessary to produce this phase transition is an order of magnitude larger than for naphtalene they added to bilayers. In contrast to recent spin label study we have found that even benzene may have a similar disordering effect as bigger hydrocarbons. Thus Raman spectroscopy seems to be a suitable method to investigate such problems.

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