parameter (oxygen diffusion-concentration product) were obtained from saturation-recovery curves. All of these profiles provide unique information about the depth-dependent physical properties and the three-dimensional dynamic organization of the membrane. Additionally, saturation-recovery measurements allow discrimination of membrane domains because the collision rate of molecular oxygen with the nitroxide spin label may differ in these domains. All membranes saturated (but not oversaturated) with cholesterol are homogenous on the EPR timescale. When properties of the phospholipid-cholesterol membrane are monitored with phospholipid analogue spin labels (by measuring the alkyl chain order parameter), the membrane shows high rigidity that decreases gradually toward the membrane center. However, when membrane properties are measured by monitoring movement and/or concentration of small molecules like molecular oxygen or water, the monitored properties change abruptly between the C9 and C10 positions (depth to which the rigid cholesterol ring-structure is immersed), showing low membrane fluidity and hydrophobicity to the depth of the ninth carbon and high membrane fluidity and hydrophobicity in the membrane center. Based on these observations, it can be concluded that the bulk physical properties of membranes saturated with cholesterol (with a cholesterol-to-phospholipid mole ratio close to one) are mainly determined by the presence of the saturating amount of cholesterol and are practically independent on membrane phospholipid composition.

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Comparative Characterization of Lateral Organization and Packing Properties of Lipids in Pulmonary Surfactant Membranes and Interfacial Films

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The main function of the lipid-protein complex pulmonary surfactant is to stabilize the respiratory surface by formation of a surface active interfacial film on top of the thin aqueous layer that covers the alveoli. From synthesis in the pulmonary epithelium, until formation of the functional film, surfactant complexes adopt different lamellar structures during its storage and secretion. The functional structure of surfactant makes Langmuir monolayers especially useful for the analysis of structure-function correlations in the pulmonary surfactant system, although they are also widely used as a model to obtain structural information about biological membranes. However, the exact correspondence between lateral organization and molecular packing in bilayers and monolayers is still a matter of controversy.

The fluorescent probe laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) is a lipophilic dye used to analyze the structure of membranes due to its spectral characteristics. Once inserted in membranes, the fluorescence emission of laurdan is very sensitive to the level of hydration of the phospholipid headgroups. Changes in packing or lateral organization of the membrane produce a shift of the emission maximum of the label from 440 nm in ordered membranes to 490 nm in disordered membrane phases. Taking advantage of the properties of this probe, films from pulmonary surfactant lipids containing laurdan were prepared and compressed to obtain surface pressure-area isotherms. Surface pressure and area occupied per molecule along compression were obtained in parallel with the generalized polarization function (GPF) of laurdan -as calculated from its interfacial fluorescence emission spectra- and compared with the fluorescence of laurdan in multilamellar suspensions of the same surfactant lipids at different temperatures.

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Nystatin Action On POPC/sterol Membranes Along Its Phase Diagram

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Nystatin is a membrane active polyene antibiotic that has been studied for a long time due to its permeabilization activity. Its clinical use is based on the different potency in membranes containing ergosterol (fungi) vs membranes containing cholesterol (mammal). It has been proposed that the mechanism of permeabilization is through the formation of transmembrane pores constituted of several monomers of the drug in a barrel stave configuration. The greater selectivity in ergosterol containing membranes as compared with those containing cholesterol has been proposed to be due to greater pore stability produced by ergosterol. This hypothesis has been questioned over the time and alternative models have been suggested. Experimental evidence has shown that sterol is not a requirement for membrane permeabilization, then the proposal of an indirect role of sterol through the effect on membrane structure. This idea has been supported by experimental evidence showing a different action in different phases of the membrane. In order to evaluate the effect of phase changes of the membrane on the pores formed by Nystatin, single channel experiments were performed along the phase diagram of 1-palmitoyl-2-oleoyl-sn-glycero-phophocholine (POPC) membranes containing ergosterol (erg) or cholesterol (chol). The results show that for POPC-erg membranes there is a region of maximum permeabilization consistent with the liquid-ordered (lo) + liquid disordered (ld) mixed region. For the POPC-chol membranes the maximum permeabilization occurs also in the mixed region but at lower temperatures. These results are taken as strong evidence supporting the idea that the phase of the membrane is determinant for the activity of polyene antibiotics.

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Changes in Membrane Fluidity of Blood Platelets in Myeloid Neoplasm

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In this study we evaluate the alterations in membrane fluidity of blood platelets in patients with various entities of myeloid malignancies.

The clinical history of myeloproliferative and myelodysplastic disorders is often complicated by thromboembolic or hemorrhagic events.The mechanism of these major complications remains unclear. Since there is a weak correlation between the risk of these life threatening complications and the number of blood platelets, our research is focusing on qualitative defects of platelets.

Membrane fluidity is an important parameter which influences many of the unique cellular functions and which is strongly correlated (among other factors) to the membrane lipid composition. We try to correlate changes in cell membrane fluidity with the clinical status of the patient disease.

32 patients with various entities of myeloid neoplasm were selected (Department of Hematology, Emergency University Hospital of Bucharest). They were diagnosed according to the WHO criteria. 11 normal healthy volunteers, non smokers, drug-free, were used as controls.

Membrane fluidity was assessed by fluorescence anisotropy measurements. The platelet membrane shows to be more rigid compared with controls/normal regardless of the clinical type of myeloproliferative disorder. However patients with severe clinical status due to acute myeloid leukemia have a more fluid membrane compared to the same patients found previously in a better state. Thus, the activity (or severity) of the disease correlates with the increase in membrane fluidity, as other studies revealed on lymphocytes. We consider that detection of these modifications may be useful for a better insight in cell abnormalities occurring in this pathology.

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NSAIDs Alter Lipid Bilayer Mechanical Properties

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Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are a class of widely prescribed medications that have analgesic, antipyretic, anti-thrombotic and anti-inflammatory properties. Their primary mechanism of action is through non-selective inhibition of the Cyclo-oxygenase enzymes (COX-1 and COX-2), which mediates many of the clinical - and side - effects of NSAIDs. Other effects are mediated through COX-independent mechanisms, however. Given that NSAIDs are amphiphiles, that they modulate the function of many different, structurally unrelated membrane proteins, and that the lipid bilayer serves as a gate-keeper/regulator for many different cell functions, we tested whether NSAIDs could alter lipid bilayer material properties. To measure such changes in bilayer material properties, we used gramicidin A (gA) channels as molecular force transducers. We found that salicylate, ibuprofen, diclofenac, sulindac sulfide and flurbiprofen are potent modifiers of bilayer properties. At pH 7, NSAIDs were found to increase both the lifetime and appearance rate of channels formed by both short (13-residue) and long 15-residue gramicidin analogues, with the larger effects on the shorter channels - the channels with the larger hydrophobic mismatch, which shows that NSAIDs decrease lipid bilayer stiffness by increasing the bilayer elasticity. These effects were achieved at the high end of clinically relevant concentrations. This suggests that in both the clinical and research setting, NSAIDs may have effects that arise from modulation of lipid bilayer mechanical properties.