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THE EFFECT OF DENSITY FLUCTUATIONS ON THE PASSIVE ION PERMEABILITY OF LIPID BILAYERS

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The influence of lateral density fluctuations in lipid membranes on passive ion permeability is studied. In an approximate model of the lipid bilayer, the bilayer permeability is accounted for by the existence of defects in the interfacial areas of clusters. The number of these defects depends on the state of neighbouring acyl chains. The ten-state Pink model is used. The analysis of snapshots of characteristic microscopic configurations obtained from Monte Carlo simulations of this model reveals that permeability properties of interfacial areas of cluster are heterogeneous and depend on the individual clusters. The results of these studies are compared with those from the Cruzeiro-Hansson model.

INTRODUCTION

The permeability of biomembranes is principally mediated by specific protein gates and pores which selectively direct the flux of matter across the membranes (Eisenman & Dani, 1987). However, the pure lipid bilayer is also not impermeable to ions.

Passive transbilayer permeability for alkali metal ions Na^+ (Papahadjopoulos, Jacobson, Nir & Isac, 1973), K^+, Rb^+, Ca^+ (Georgallas, MacArthur, Ma, Nguyen, Palmer, Singer & Tse, 1987) and such different molecules as TEMPO (2,2,6,6,-tetra- methylpiperidinyl-1-oxy)choline (Marsh, Watts & Knowles, 1976), ANS (8-anilino-1-napthalinesulfonate)(Kanehisa & Tsong, 1978) and water (Carruthers & Melchior, 1983; Blok, van Deenen & de Gier, 1977) exhibits a rapid increase in the phase-transition region. Investigations of this anomaly provide insight into the structure and function of the lipid components of biological membranes. In this connection, some attempts to explain this phenomenon based on many theoretical models of lipid membranes were made.

According to Nagle and Scott (Nagle & Scott, 1978), the anomalous peak is due to the enhanced lateral density fluctuations associated with high lateral compressibility which take place as the lipid bilayer is close to a critical point. These fluctuations open cavities which small ions can enter. Doniach (Doniach, 1978) also estimated the permeability using a simple Eyring form based on the concept of the critical fluctuations.

One of the first attempts to explain the anomalous behaviour of the lipid bilayer during the gel-to-fluid phase transition was given by Papahadjopoulos et al. (Papahadjopoulos, Jacobson, Nir & Isac, 1973). They suggested that the enhancement in the permeability could be attributed to the formation of domains in bilayers, with the enhanced diffusion occurring at the domain boundaries. Still Nagle and Scott (Nagle & Scott,1978) argued from the Gibbs phase rule that such an explanation could not be correct because the width of the transition is much smaller than the width of the sodium permeability peak.

The concept of domains is maintained at present in very successfully, extensively developed models of lipid membrane systems (Cruzeiro-Hansson, Ipsen & Mouritsen, 1989; Jřrgensen, Ipsen, Mouritsen, Bennett & Zuckermann, 1991a; Jorgensen, Ipsen, Mouritsen, Bennett & Zuckermann 1991b) which are based on the ten-state Pink model. This concept plays a very important part in considerations on permeability in pure and modified lipid bilayers. In this case, domain formation is a dynamic process and domains fluctuate in size and position in relation to lateral density fluctuations and lateral bilayer compressibility. Below the phase transition temperature, clusters of fluid phase are surrounded by acyl chains in a gel state, and above the phase transition temperature clusters of a gel phase in the fluid matrix are formed. It is believed that the heterogeneous microscopic picture of the pure lipid bilayer does not correspond to two-phase coexistence in a thermodynamic sense, and clusters are not macroscopic entities (Cruzeiro -Hansson & Mouritsen, 1988).

The interfacial regions between the clusters and the background matrix play a particularly important role. The total area of this interface reaches maximum at the phase transition temperature. According to Cruzeiro-Hansson & Mouritsen (1989) these areas are responsible for the transbilayer permeability.

The purpose of this paper is to investigate an effect of lateral density fluctuations on the passive ion permeability in the interfacial areas of lipid

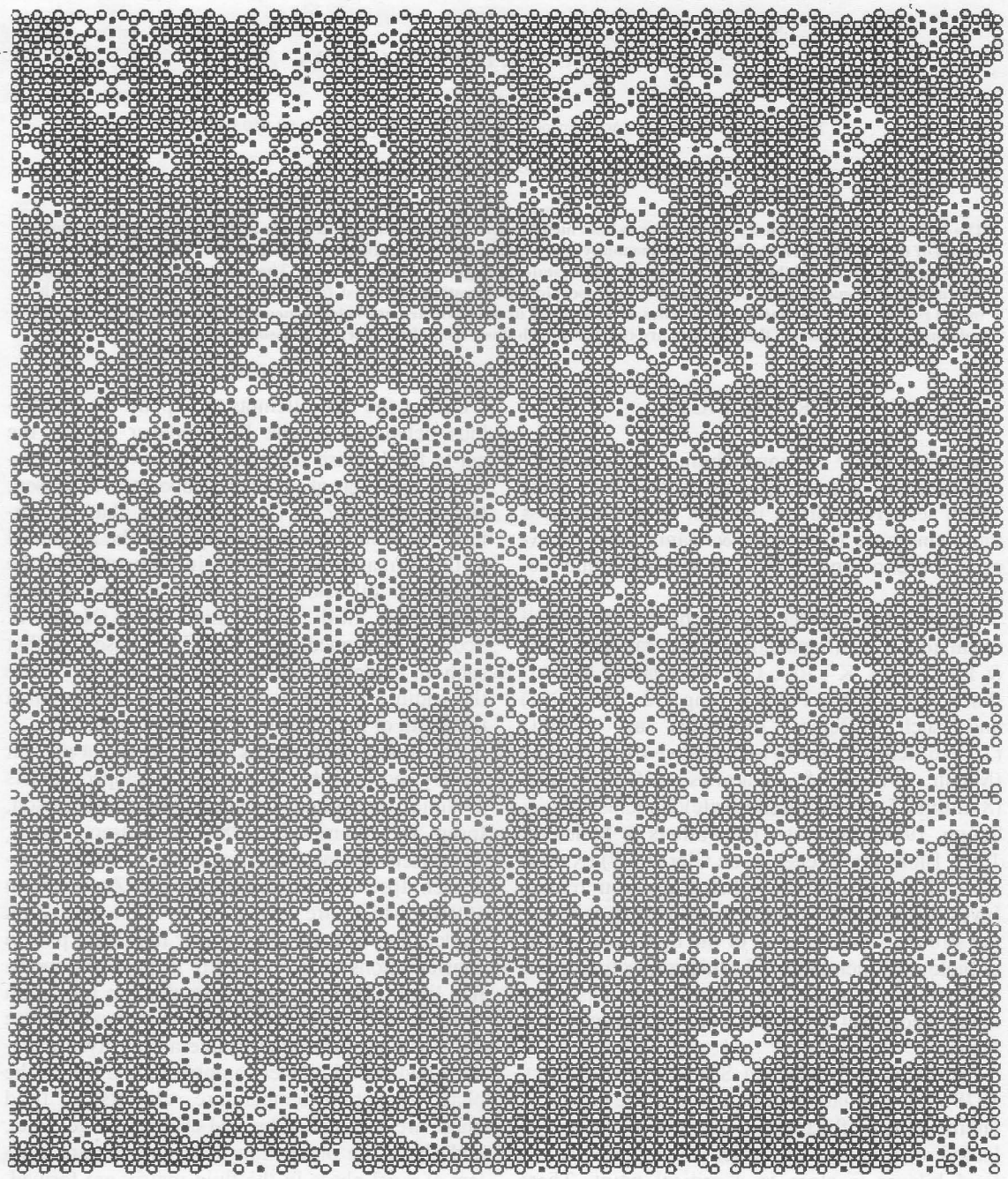


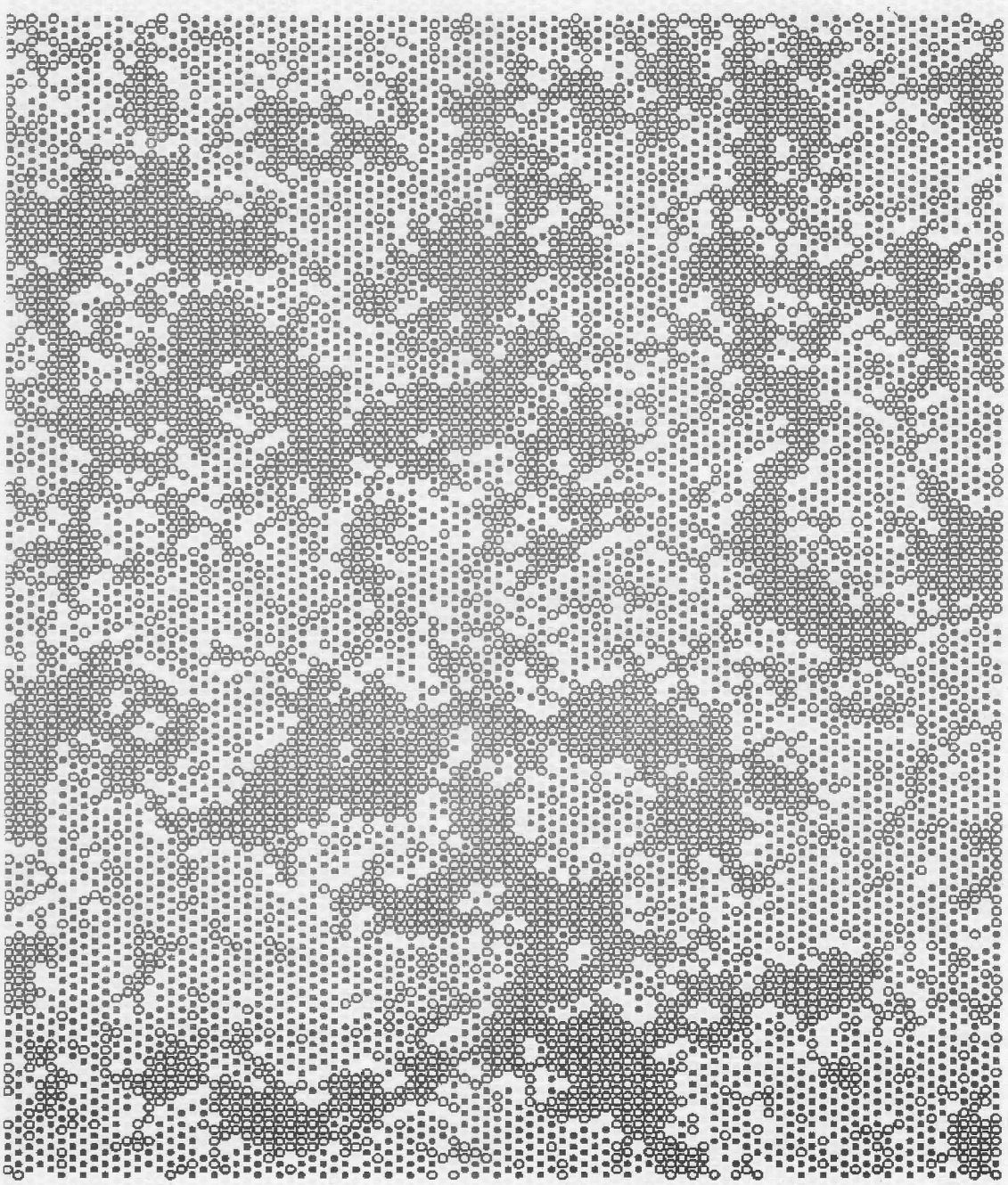
Fig. 1 Snapshots of microconfigurations of the lipid membrane. (a): 309 K (below the phase transition temperature), (b): 314 K (at the phase transition temperature), (c): 319 K (above the phase transition temperature). The system consists of 10000 chains on a triangular lattice. The single-chain states are represented as follows: ○: all-trans and intermediate states, ■: fluid state; holes are denoted by an empty lattice site. Figs 1b and 1c on next pages.

bilayers. We will use the ten-state Pink model (Pink, Green & Chapman, 1980; Mouritsen, Boothroyd, Harris, Jan, Lookman, MacDonald, Pink & Zuckermann,1983) and the Monte Carlo simulation techniques (Metropolis, Rosenbluth, Rosenbluth, Teller & Teller, 1953).

MODEL OF PHASE TRANSITION

The two dimensional ten-state Pink model describes the main transition of the lipid bilayer. Each acyl chain is assigned to a site in a triangular lattice. The translational degrees of freedom and interactions between monolayers are ignored. The acyl-chain conformations are represented by ten single states. There are a nondegenerate all-trans conformation, eight intermediate excited states, and a highly excited fluid state. These states are characterized by the internal energy E_α, area A_α and internal degeneracy D_α.

The model Hamiltonian of the pure lipid bilayer is written as follows:



$$H = \sum_i \sum_\alpha \left(E_\alpha + \Pi A_\alpha\right)\alpha_{i\alpha} - \frac{J_o}{2}\sum_{i,j}\sum_{\alpha,\beta} I_\alpha I_\beta \alpha_{i\alpha}\beta_{j\beta} \quad (1)$$

Π is the internal lateral pressure, $\alpha_{i\alpha}$ is the projection operator for the conformational states α of the chains on the site i.

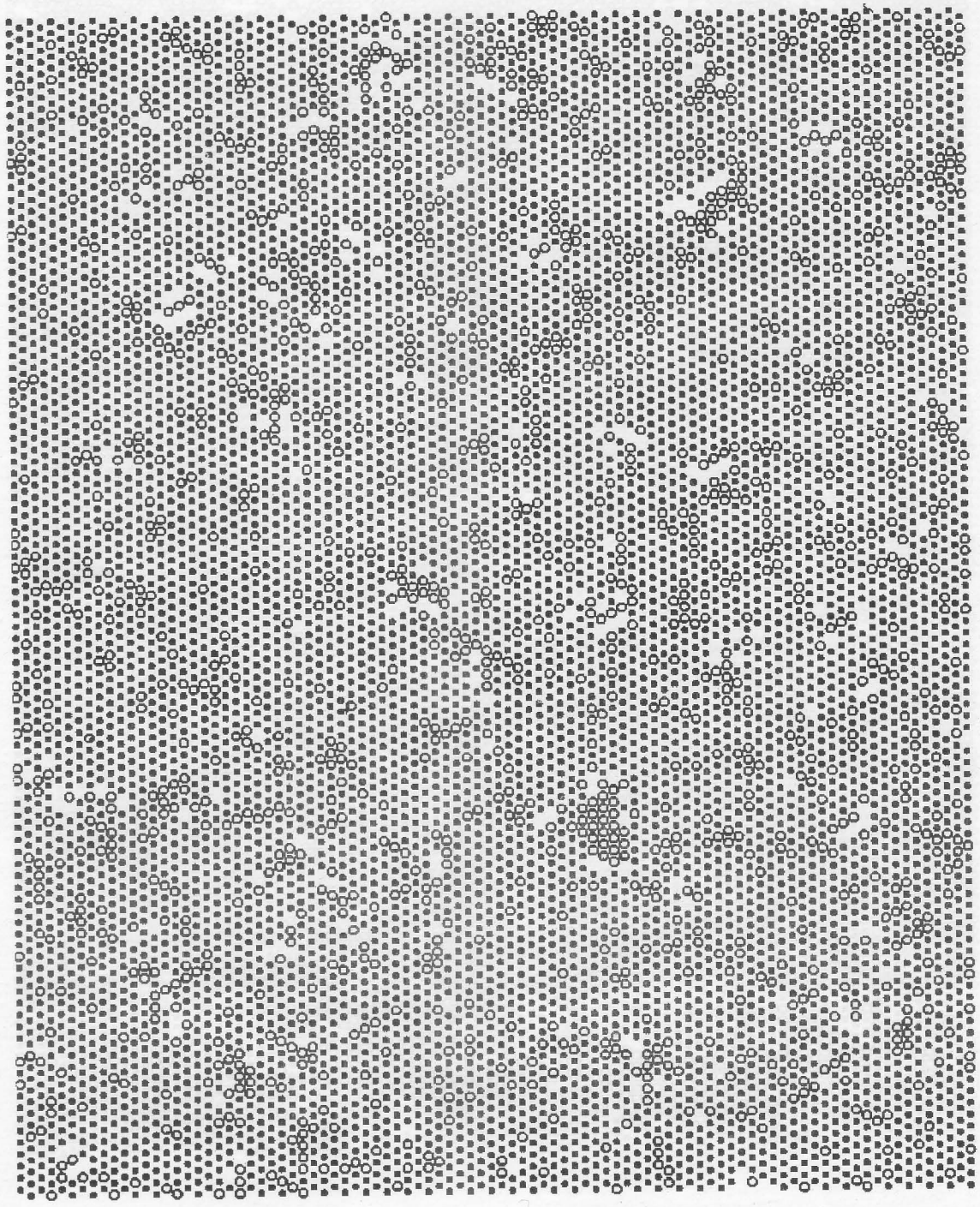
The second term of the Hamiltonian represents interaction between chains with the nematic factors I_α and the coupling constant J_o. The summation in this term is over all lattice sites i and over only the nearest neighbours j of the acyl chain i.

The interaction parameter values of this model for dipalmitoyl phosphatidylcholine (DPPC), internal energies, areas and degeneracies are taken from Cailé et al. (Cailé, Pink, de Verteuil & Zuckermann, 1980).

As usual, the all-trans state and intermediate states form together the gel phase.

We have used the Monte Carlo method. Computer simulations were performed on the lattices with 10000 lattice sites and periodic boundary conditions.

In an attempt to connect directly the passive ion



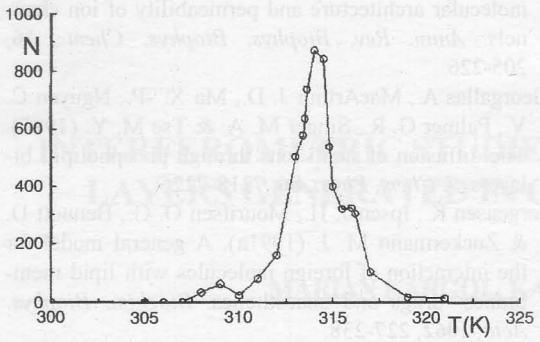


Fig. 2. The temperature dependence of a total number of holes (N) localized in the interfacial areas.

permeability with microscopic lateral fluctuations we would like to find chains which undergo strong lateral fluctuations. For such an analysis we consider acyl chains in intermediate states.

There are strong fluctuations if on one side of such an acyl chain, acyl chains (at least one) occur in the all-trans state, and on the other side in the fluid state. In such cases there arise defects (for convenience hereafter called holes) which are a microscopic effect of mismatch in a molecular packing between ordered and fluid lipid.

In our analysis we assume that the lipid bilayer with 10000 acyl chains after 1000 Monte Carlo steps per site achieves a state of thermodynamic equilibrium in a common way and then following the above described procedure we are looking for the holes.

RESULTS

Figs 1a, 1b and 1c show the microscopic membrane pictures of DPPC at 309 K, 314 K (the phase transition temperature of DPPC) and 319 K, respectively. It can be seen (Fig. 1a) that the lipid bilayer undergoes strong lateral density fluctuations which is manifested in cluster formation when approaching the phase transition temperature.

At every temperature studied the holes are localized very often within the interfacial areas which are not homogeneous entities.

It is well known (Cruzeiro-Hansson & Mouritsen, 1988) that in the interfacial areas acyl chains are distributed in different states. In our approximate model we study another kind of heterogeneity of these areas related to different permeability properties which are the result of the state of neighbouring acyl chains.

We carried out computer simulations for the temperatures in a range from 298 K to 321 K.

The greatest fluctuations was expected, take place in the vicinity of the phase transition temperature.

It is worth noting that the holes are most preferably distributed in the interfacial areas at each temperature studied.

Fig.2 shows the curve of the temperature dependence of a total number of holes localized in the interfacial areas. The shape of the total number of holes vs. the temperature curve reveals a maximum at the phase transition temperature.

DISCUSSION

In the structure of the lipid bilayer, the domain interfacial area is believed to be responsible for passive ion permeability (Mouritsen & Jørgensen 1992; Cruzeiro-Hansson & Mouritsen, 1988; Marsh, Watts & Knowles, 1976). According to Cruzeiro-Hansson & Mouritsen (1988) an increase in the total interfacial area in the phase transition region is correlated with an increase in the passive ion permeability of the lipid bilayer. On the contrary, the bulk and cluster regions of the membrane are associated with very low regional permeabilities.

Direct experimental observations of clusters in a lipid membrane are very difficult because of very short relaxation time of the fluctuations. However, such observations have been carried out latelly by fluorescence lifetime heterogeneity measurements (Ruggiero & Hudson, 1989).

In an approximate model of the lipid bilayer which we adopt here, we account for the bilayer permeability by the existence of defects (holes) in the interfacial areas of clusters. The number of these holes depends on the state of neighbouring acyl chains.

These defects are an effect of mismatch in molecular packing between the ordered and fluid lipids. We can see that the most preferable site for holes are the interfacial areas in Fig. 1. These observations support the idea of a great significance of these regions for passive ion permeability. However, our results show that the interfacial areas are not homogenous entities and their different regions have various probabilities of the ions transfer.

Comparison of the temperature dependence of the total number of holes distributed in the interfacial areas obtained in this work (Fig. 2) with the experimental results of diffusion rates of Na^+ through DPPC vesicles (Papahadjopoulos, Jacobson, Nir & Isac, 1973) also supports the idea that holes in the interfacial areas play an important part in permeability properties.

It should be noted here that the influence of the interfacial areas in the Cruzeiro-Hansson model (Cruzeiro-Hansson & Mouritsen, 1988) on passive ion permeability depends only on their total size. The results described above suggest that in more precise considerations, microscopic contributions to the passive ion permeability of individual lattice sites from the inside of the interfacial areas should be analyzed. These contributions depend on the state of order of neighbouring acyl chains.

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REFERENCES

Blok M. C., Van Deenen L. L. M. & De Gier J. (1977). The effect of cholesterol incorporation on the temperature dependence of water permeation through liposomal membranes prepared from phosphatidylcholines. *Biochim. Biophys. Acta*, **464**, 509-518.

Cailé A., Pink D. A., De Verteuil F. & Zuckermann M. J. (1980). Theoretical models for quasi-two- dimensional mesomorphic monolayers and membrane bilayers. *Can. J. Phys.*, **58**, 581-611.

Carruthers A. & Melchior D. L. (1983). Studies of the relationship between bilayer water permeability and bilayer physical state. *Biochemistry*, **22**, 5759-5807.

Corvera E., Mouritsen O. G., Singer M. A. & Zuckermann M. J. (1992). The permeability and the effect of acyl-chain length for phospholipid bilayers containing cholesterol: theory and experiment. *Biochim. Biophys. Acta*, **1107**, 261-270.

Cruzeiro-Hansson L. & Mouritsen O. G. (1988). Passive ion permeability of lipid membranes modelled via lipid-domain interfacial area. *Biochim. Biophys. Acta*, **944**, 63-72.

Cruzeiro-Hansson L., Ipsen J. H. & Mouritsen O. G. (1989). Intrinsic molecules in lipid membranes change the lipid-domain interfacial area: cholesterol at domain boundaries. *Biochim. Biophys. Acta*, **979**, 166-176.

Doniach S. (1978). Thermodynamic fluctuations in phospholipid bilayers. *J. Chem. Phys.*, **68**, 4912-4916.

Eisenman G. & Dani J. A. (1987). An introduction to molecular architecture and permeability of ion channels. *Annu. Rev. Biophys. Biophys. Chem.*, **16**, 205-226.

Georgallas A., MacArthur J. D., Ma X. -P., Nguyen C. V., Palmer G. R., Singer M. A. & Tse M. Y. (1987). The diffusion of small ions through phospholipid bilayers. *J. Chem. Phys.*, **86**, 7218-7226.

Jørgensen K., Ipsen J. H., Mouritsen O. G., Bennett D. & Zuckermann M. J. (1991a). A general model for the interaction of foreign molecules with lipid membranes: drugs and anaesthetics. *Biochim. Biophys. Acta*, **1062**, 227-238.

Jørgensen K., Ipsen J. H., Mouritsen O. G., Bennett D. & Zuckermann M. J. (1991b). The effects of density fluctuations on the partitioning of foreign molecules into lipid bilayers: application to anaesthetics and insecticides. *Biochim. Biophys. Acta*, **1067**, 241-253.

Jørgensen M. I. & Tsong T. Y. (1978). Cluster model of lipid phase transition with application to passive permeation of molecules and structure relaxations in lipid bilayers. *J. Amer. Chem. Soc.*, **100**, 424-432.

Marsh D., Watts A. & Knowles P. F. (1976). Evidence for phase boundary lipid. Permeability of Tempo-choline into dimyristoylphosphatidylcholine vesicles at the phase transition. *Biochemistry*, **15**, 3570-3578.

Metropolis N., Rosenbluth A. W., Rosenbluth M. N., Teller A. H. & Teller E. (1953). Equation of state calculations by fast computing machines. *J. Chem. Phys.*, **21**, 1087-1092.

Mouritsen O. G., Boothroyd A., Harris R., Jan N., Lookman L., MacDonald L., Pink D. A. & Zuckermann, M. J. (1983). Computer simulation of the main gel-fluid phase transition of lipid bilayers. *J. Chem. Phys.*, **79**, 2027-2041.

Mouritsen O. G. & Jørgensen K. (1992). Dynamic lipid-bilayer heterogeneity: a mesoscopic vehicle for membrane function? *BioEssays*, **14**, 129-136.

Nagle J. F. & Scott H. L. (1978). Lateral compressibility of lipid mono- and bilayers theory of membrane permeability. *Biochim. Biophys. Acta*, **513**, 236-243.

Papahadjopoulos D., Jacobson K., Nir S. & Isac T. (1973). Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol. *Biochim. Biophys. Acta*, **311**, 330-348.

Pink D. A., Green T. & Chapman D. (1980). Raman scattering in bilayers of saturated phosphatidylcholines. Experiment and theory. *Biochemistry*, **19**, 349-356.

Ruggiero A. & Hudson B. (1989). Critical density fluctuations in lipid bilayers detected by fluorescence lifetime heterogeneity. *Biophys. J.*, **55**, 1111-1124.