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The permeability and the effect of acyl-chain length for phospholipid bilayers containing cholesterol: theory and experiment

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The model of Cruzeiro-Hansson et al. (Biochim. Biophys. Acta (1989) 979, 166–1176) for lipid-cholesterol bilayers at low cholesterol concentrations is used to predict the thermodynamic properties and the passive ion permeability of lipid bilayers as a function of acyl-chain length and cholesterol concentration. Numerical simulations based on the Monte Carlo method are used to determine the equilibrium state of the system near the main gel-fluid phase transition. The permeability is calculated using an ansatz which relates the passive permeability to the amount of interfaces formed in the bilayer when cholesterol is present. The model predicts at low cholesterol contents an increase in the membrane permeability in the transition region both for increasing cholesterol concentration and for decreasing chain length at a given value of the reduced temperature. This is in contrast to the case of lipid bilayers containing high cholesterol concentrations where the cholesterol strongly surpresses the permeability. Experimental results for the Na^+ permeability of $C_{15}PC$ and DPPC ($C_{16}PC$) bilayers containing cholesterol are presented which confirm the theoretical predictions at low cholesterol concentrations.

I. Introduction

Ion transport in biological membranes is principally mediated by channels or pores formed by proteins which are able to discriminate between different types of substances and which can be open or closed to the flux of matter. A second means of the transmembrane ionic transport is via diffusion across the membrane lipids. This is known as passive transport and will be studied in this paper in the context of simple phospholipid bilayers.

Pure lipid bilayers are characterized by an extremely low permeability to the transverse diffusion of ions. However, an enhancement of the passive ion permeability has been experimentally observed on both sides of the main gel to fluid phase transition [1,2]. It has been proposed theoretically [1,3,4] that this enhancement can be related to the amount of interface formed between the gel and fluid domains in the bilayer in the neighborhood of the main phase transition. The model of Cruzeiro-Hansson et al. [3], which assumes that the interfacial region can be characterised by defects due to bad packing making the membrane leaky and allowing the ions to permeate it, succesfully describes the enhancement of the passive permeability of DPPC bilayers in the transition region.

Experimental observations, which are reported below, show that for lipid bilayers containing small concentrations of cholesterol, the passive permeability in the transition region increases for increasing cholesterol concentration. In this work we examine this effect theoretically using a version of the model of Cruzeiro-Hansson et al. [3]. Furthermore, we apply this model to dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) bilayers and we show that the effects of cholesterol are qualitatively the same for bilayers formed by lipids of different chain length. We also investigate the effect of the chain length on the permeability of lipid bilayers with a given cholesterol content.

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The experimental phase diagram and thermodynamic quantities such as the specific heat of lipid/cholesterol bilayers have been successfully described by the mean-field solution of a lattice model proposed by Ipsen et al. [5,6]. The model gives a description of lipid bilayers in terms of two degrees of freedom, one for the chain conformations and the other for chain positional order. It is based on the ten-state Pink model [7] combined with a modified multi-state Potts model which is used to treat the positional degrees of freedom (cystallinity) in an approximate manner. The theoretical phase diagram obtained form this model is in good agreement with the experimental phase diagram. There is a narrow coexistence region between the solid-ordered (gel) and the liquid-disordered (fluid) phases at low cholesterol concentrations, and a liquid-ordered phase at high cholesterol concentrations. Cholesterol dissolves easily in a phase with no crystalline structure but prefers neighboring acyl chains with high conformational order. The result is that at low cholesterol concentrations cholesterol prefers both lipid phases equally, but at high cholesterol concentrations, it induces conformational order in the fluid state and destroys the crystalline structure in the low temperature phase. This results in the liquid-ordered phase in which the conformational and the positional degrees of freedom are decoupled. Since this is the only phase at high cholesterol concentrations, there are consequently no interfaces and a very low permeability is expected relative to the permeability of the pure lipid bilayer. The model of Cruzeiro-Hansson et al. [3], which relates the permeability to the amount of interface, can be applied to the model of Ipsen et al. [5,6] which treats both the conformational and crystalline degrees of freedom. However, since our main interest here is to study the low cholesterol concentration regime we use the simpler model of Cruzeiro-Hansson et al. [8] which only is concerned with conformational variables but which nevertheless describes correctly the system in this regime.

II. Model and Theory

Our model study is based on the microscopic interaction model used by Cruzeiro-Hansson et al. [8]. This model is basically the multi-state lattice model of Pink [7] extended to include the presence of cholesterol and which gives the correct phase behavior at low cholesterol concentrations. The extended model can only account for the chain-melting transition but not for the change in the lateral crystalline structure. The interaction between lipid molecules and cholesterol is modelled by assuming that cholesterol is a bulky, stiff molecule with no internal degrees of freedom. The interaction between two cholesterol molecules and the interaction between a cholesterol molecule and a lipid chain are assumed to have the same form as the interaction between two lipid chains and described by a strength parameter which is determined [8] by the requirement that the mean-field phase diagram resembles that of PC/cholesterol mixtures for cholesterol concentrations up to 10%, i.e., with a narrow coexistence region and only a small decrease in the melting temperature.

The model for the passive permeability of lipid/cholesterol bilayers [8] is a direct extension of the model for pure lipid bilayers [3]. The probability of an ion crossing a membrane is within this model given by

$$P(T, x_c) = a_b(T, x_C)p_b + a_c(T, x_C)p_c + a_{il}(T, x_C)p_i + a_{iC}(T, x_C)p_{iC} \qquad (1)$$

where b stands for bulk, c for clusters, and i for interface. p_b, p_c and p_i denote the corresponding transfer probabilities, and a_b, a_c and a_i are the fractional areas occupied by the bulk, the clusters and the interface in the transition region. The terms bulk, cluster and interface are formally defined in Section III. For now it is sufficient to use the word cluster to refer to an aggregation of lipids in the minority phase immersed in a background of lipids in the majority phase which we will refer to as the bulk. The interfaces separate the clusters from the bulk. The interfacial contribution has in Eqn. 1 been separated into two parts, $a_{il}(T, x_C)$ which is the part of the interface formed by lipids, and $a_{iC}(T, x_C)$ which is the part formed by cholesterol.

This model of passive ion permeability requires the knowledge of the fractional areas occupied by the bulk, the clusters and the interface, as well as the values of the transfer probabilities. The transfer probabilities are assumed to be independent of temperature. A constraint is imposed on the transfer probabilities by assuming that the interfacial area formed by the lipids is associated with a very high transfer probability $p_i \gg p_b$, p_c, whereas those formed by cholesterol are assumed to have a low probability of transfer. The principal hypothesis underlying this simple model of passive permeability is that, at the interfacial regions, packing faults make the membrane leaky and allow the ions to permeate it. The model does not assume a specific mechanism for the ionic transport.

We have used the same values of the transfer probabilities for the lipid regions as in Ref. 3. The choice of these values is based on the assignment of a higher probability of transfer to fluid regions than to gel regions. The model for the permeability of lipid/cholesterol bilayers also requires the knowledge of the transfer probability, p_{iC}, of the interfacial region containing cholesterol. In order to avoid the introduction of new parameters, the interfacial probability of trans-

fer at sites occupied by cholesterol molecules is assumed to be equal to the bulk probability of transfer below the transition [8], i.e., the smallest value for a probability of transfer used in the model for pure lipid bilayers. We have calculated the reduced permeability

$$R(T, x_C) = A(T, x_C)^{-1/2} T^{1/2} P(T, x_C) \qquad (2)$$

which is proportional to the logarithm of the fraction of ions retained in a liposome.

III. Numerical results

Monte Carlo simulations are performed on a 100 × 100 triangular lattice in which each site of the lattice is occupied by either a lipid chain in one of the ten different conformational states or a cholesterol molecule. Periodic boundary conditions are imposed on the system in order to minimize the finite-size effects. To implement the Monte Carlo Metropolis algorithm [9] for the lipid membrane containing cholesterol, both single lipid chain excitations as well as cholesterol–lipid chain exchange are employed.

Thermodynamic properties

In this subsection numerical results of Monte Carlo simulations are presented for systems with three different chain lengths, corresponding to DMPC, DPPC and DSPC lipid bilayers. The interaction constant describing the lipid–lipid interactions depends on the chain length and is fitted for the three systems in order to reproduce the experimental transition temperatures for the pure lipid bilayers.

The results of the simulations for the pure lipid bilayer show an abrupt change in the energy, area and order parameter at the main transition located at T_m. With the addition of cholesterol the transition becomes broadened and the difference in the areas of the two phases becomes smaller than for the pure system. The relation between the area and the cholesterol concentration below the transition is quite complicated. In

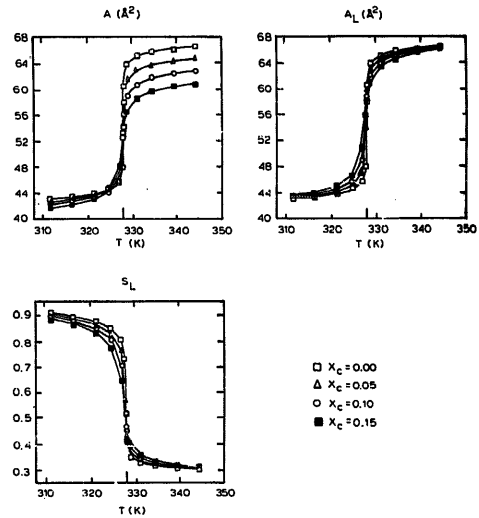

Fig. 1. Numerical results for the total area per molecule, A, the lipid area per lipid molecule, A_L, and the lipid order parameter, S_L, of DSPC/cholesterol bilayers.

order to observe the effect of cholesterol on the lipid chains, we calculated the lipid area per lipid molecule given by

$$A_L = \frac{A - x_C A_C}{1 - x_C} \tag{3}$$

where A_C is the area of a single cholesterol molecule [10], A is the total area per molecule and x_C is the cholesterol concentration of the system. Fig. 1 shows that cholesterol has an expansion effect in the gel phase and a condensation effect in the fluid phase and this behavior is observed for all three systems under consideration. This observation is related to the fact that cholesterol induces conformational disorder in lipid chains below the transition temperature and conformational order above the transition temperature. This effect is confirmed by the behavior of the average nematic order parameter, S_L, of the lipid chains shown in the same figure. The model therefore predicts that cholesterol makes the lipid chains of the membrane more disordered in the low-temperature phase and more rigid in the high-temperature phase.

The specific heat is calculated from the energy fluctuations

$$C_p = \frac{1}{k_B T^2}(\langle \mathcal{H}^2 \rangle - \langle \mathcal{H} \rangle^2) \tag{4}$$

and the lateral area compressibility is calculated from the fluctuations in the area

$$\kappa_T = \frac{1}{k_B T \langle A \rangle}(\langle A^2 \rangle - \langle A \rangle^2) \tag{5}$$

where A is the total area of the system. The results in Figs. 2 and 3 show that the transition is accompanied by strong fluctuations. The peak in both response functions decreases with the addition of cholesterol, but the addition of cholesterol increases the response functions at the 'wings' of the transition. This indicates that cholesterol causes the thermal fluctuations to decrease at the transition but to increase away from the transition. This is illustrated in Fig. 2 for the lateral compressibility of DPPC, but again we emphasize that the same effect is observed for all three systems studied.

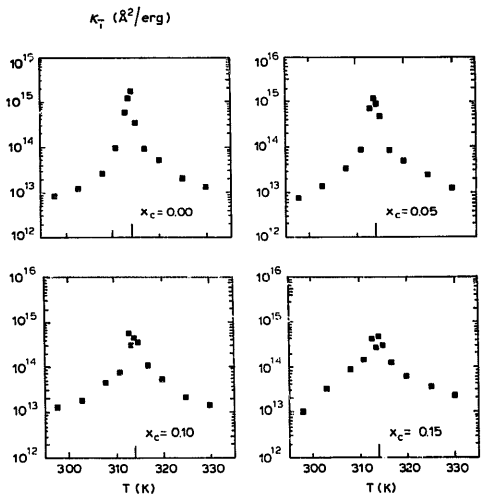

Fig. 2. Numerical results for the isothermal lateral compressibility, κ_T, of DPPC/cholesterol bilayers.

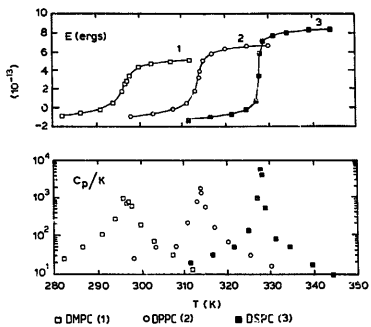

Fig. 3. Numerical results for the internal energy, E, and specific heat per molecule, C_p, of bilayers with a cholesterol concentration of $x_C = 0.05$.

It is of interest to examine the effect of the chain length on the different thermodynamic functions. From the results for the membrane area and the order parameter it is clear that for all cholesterol concentrations studied, the shorter the chain length the smoother is the transition. This is illustrated in Figs. 3 and 4. For all cholesterol concentrations studied, the peak in the specific heat and lateral area compressibility decreases for decreasing chain length. This indicates that the longer the chain length the larger the fluctuations at the transition temperature. However, in the 'wings' of the transition an enhancement is observed in both response functions for decreasing chain length. This is illustrated in Figs. 3 and 5 and indicates that the thermal fluctuations are larger in the 'wings' of the transition for shorter chains. This behavior is observed for all cholesterol concentrations studied.

Interfaces and passive permeability

A cluster analysis of the configurations obtained from the Monte Carlo simulations is required for the calculation of the fractional areas occupied by the clusters, the bulk and the interface. In the case of the pure lipid bilayer where there are only chains in gel or in fluid conformational states, a cluster is defined as a set of chains in the minority phase connected by nearest-neighbor bonds. In the case of lipid/cholesterol bilayers there is no unique way to define the clusters. We have therefore decided to classify the cholesterol molecules as follows. Only three or more connected fluid (gel) chains are considered to form a fluid (gel) cluster. If a cholesterol molecule is a nearest neighbor of a lipid chain belonging to a cluster, it is considered as part of the cluster. If a cholesterol molecule in a cluster is a nearest neighbor of a site that is occupied by another cholesterol molecule, the second cholesterol molecule is not considered as part of the cluster. The interface is defined as the set of sites that are nearest neighbors to the cluster sites. The bulk is defined as the set of points that are neither clusters nor interface. Following Cruzeiro-Hansson et al. [3], a cut-off of 14 lattice sites was chosen implying that clusters smaller than 14 lattice sites were considered as part of the bulk, and *clusters in clusters* (small domains of bulk within the clusters) with less than 14 lattice sites were considered as part of the cluster.

The temperature dependence of the fractional areas show clear anomalies at the transition temperature. As the transition temperature is approached from either side, the area of the bulk decreases and the area occupied by the clusters increases and has a peak at T_m. As a consequence of the increase in the number of clusters, the lipid fractional interfacial area increases as the transition is approached. The higher the cholesterol concentration the higher the cluster and interfacial fractional areas and the smaller the bulk fractional area. This indicates that cholesterol induces the formation of clusters above and below the transition temper-

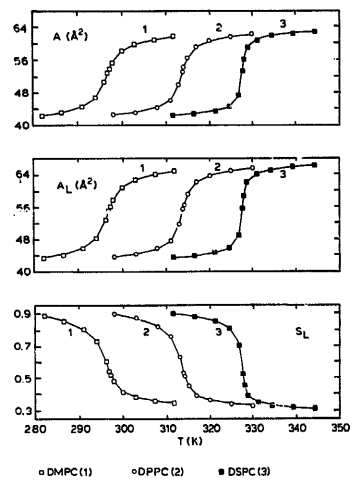

Fig. 4. Numerical results for the total area per molecule, A, the lipid area per lipid molecule, A_L, and the lipid order parameter, S_L, of bilayers for a cholesterol concentration of $x_C = 0.10$.

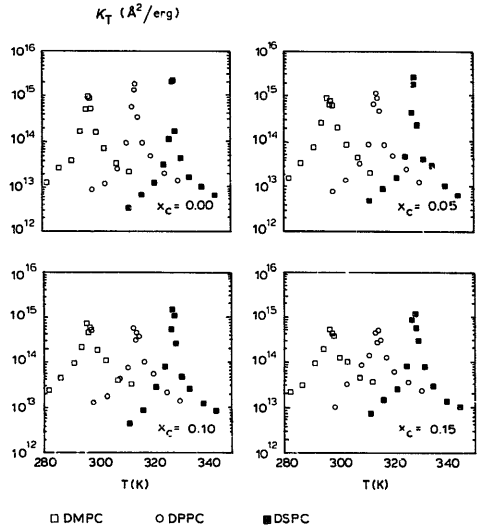

Fig. 5. Numerical results for the isothermal compressibility, κ_T, of bilayers containing cholesterol.

ature and consequently causes the amount of interface to increase. This is illustrated in Fig. 6 where snapshots of configurations of DSPC are shown for several cholesterol concentrations. For this figure the temperature was fixed at a value above the transition temperature. From the figure it is clear that there are more clusters and there are more interfaces for the bilayer containing cholesterol than for the pure lipid bilayer. Furthermore, the number of lattice sites forming the clusters and the amount of interface increase with increasing cholesterol concentration. This was observed for all three systems studied.

The behavior of the passive ion permeability is closely related to the behavior of the interfaces. Fig. 7 shows the relative permeability as a function of temperature for all three systems and for all cholesterol concentrations studied. An enhancement of the relative permeability as the transition temperature is approached from both sides is obtained for all cholesterol concentrations studied.

As a consequence of both the assumption of a high probability of transfer in the lipid interfacial regions and the increase found in the amount of interface for increasing cholesterol concentration, the model predicts an increase in the membrane permeability for increasing cholesterol concentration for all temperatures in the transition region, the effect being more pronounced in the 'wings' of the transition. This is one of the main predictions of the model and it holds for lipid bilayers formed by lipids of different chain lengths.

As stated in the previous subsection, the thermal fluctuations increase in the 'wings' of the transition with decreasing chain length for all the cholesterol concentrations studied. Consequently, there are more clusters and there is more interface at a given value of T/T_m for short chains than for long ones. The cluster and interfacial fractional areas are larger for shorter chains at a given value of T/T_m in the 'wings' of the transition. A concomitant decrease in the bulk fractional area is observed for short chains at all cholesterol concentrations studied. The increase in the amount of clusters and interface is illustrated in Fig. 8 where snapshots of configurations for three chain lengths are shown. In the figure, the reduced temperature T/T_m is fixed to be above the transition temperature and the cholesterol concentration is also fixed. The figure clearly shows that there are more clusters and more interface for bilayers formed of short chains

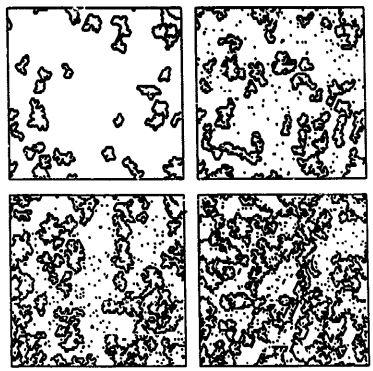

Fig. 6. Snapshots of configurations for several cholesterol concentrations in DSPC at $T = 228.88$ K. Only the interfacial regions and the cholesterol sites are shown. The upper left figure corresponds to $x_C = 0.00$, the upper right figure corresponds to $x_C = 0.05$, the lower left figure corresponds to $x_C = 0.10$ and the lower right figure corresponds to $x_C = 0.15$.

than for the ones formed by long chains at the same value of the reduced temperature. As a consequence of the increase in the amount of interface, the model predicts that the relative permeability increases with decreasing chain length at a given value of T/T_m. This is another important prediction of the model and it was observed for all the cholesterol concentrations studied. This result is presented in Fig. 9. From this figure it can be seen that for a given cholesterol concentration, and for a given value of the reduced temperature, the relative permeability is larger for shorter chain lengths.

IV. Experimental results

Multilamellar liposomes were formed by well established techniques [11,12]. Briefly, phospholipid/cholesterol mixtures were evaporated and dried from chloroform and then dispersed in excess water containing 50 mM NaCl, 5 mM Tris-HCl, ^{22}NaCl (3 μCi/ml), pH 7.5 at a temperature above that of the main transition temperature, T_m, of the lipid system. The liposomes were dialysed overnight at 278 K against buffer lacking the isotope to remove untrapped tracer, and then incubated at different temperatures in stoppered glass tubes to measure the efflux rates [11]. C_{15}PC and D_{16}PC lipids were chosen for several experimental reasons. Shorter-chain phosphatidylcholines such as DMPC have such a high permeability in the region of T_m that further enhancement with low cholesterol concentrations is not easily detectable. Conversely, longer-chain phosphatidylcholines such as DSPC have a very small permeability peak in the region of T_m such that a decrease in that permeability with high cholesterol concentrations is difficult to measure.

Fig. 10 shows ^{22}Na effluxes for dipentadecanoylphosphatidylcholine (C_{15}PC)/cholesterol and DPPC (C_{16}PC)/cholesterol mixtures. Several observations are evident from this figure. Both lipids (in the absence of cholesterol) show a peak in permeability in

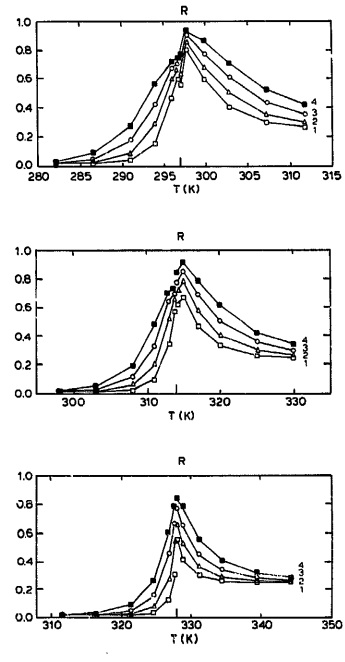

Fig. 7. Numerical results for the relative permeability, R, of DMPC/cholesterol, DPPC/cholesterol and DSPC/cholesterol bilayers. The units are arbitrary.

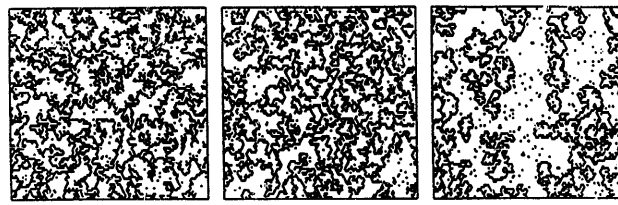

Fig. 8. Snapshots of configurations for several chain lengths at $T/T_m = 1.003$ for $x_C = 0.10$. Only the interfacial regions and the cholesterol sites are shown. The figure to the left corresponds to DMPC, the central figure corresponds to DPPC and the figure to the right corresponds to DSPC.

the region of the main transition temperature (305 K for $C_{15}PC$, 314 K for DPPC). The height of this peak was less for DPPC than for $C_{15}PC$. In the case of $C_{15}PC$, all cholesterol concentrations examined increased ^{22}Na efflux at temperatures below the T_m. Cholesterol mole fractions of 0.10 and 0.20 increased ^{22}Na efflux in the region of T_m.

Higher cholesterol mole fractions decreased ^{22}Na efflux in the temperature region of T_m and in fact a cholesterol mole fraction of 0.33 eliminated the peak associated with T_m and produced a flat permeability profile independent of temperature. Similar results were obtained with DPPC/cholesterol mixtures but only two cholesterol mole fractions were examined for this lipid.

V. Discussion and Conclusion

We have presented both theoretical predictions based on numerical simulations of a lattice model and new experimental results for lipid/cholesterol bilayers. The theoretical results are valid for low cholesterol concentration and can be summarized as follows:

(i) The effect of cholesterol on DMPC, DPPC and DSPC bilayers is to broaden the main phase transition.

(ii) For all three systems it was shown, by calculating the response functions, that cholesterol causes the thermal fluctuations to decrease at the main phase transition but to increase in the wings of the transition. In addition we found that, for all cholesterol concentrations studied, the longer the chain length the larger the fluctuations at the transition temperature. In the wings of the transition, however, an enhancement is observed in the response functions for decreasing chain length. This corresponds to the behavior found by Ipsen et al. [13] for the pure system.

(iii) It was shown that the permeability of systems with low cholesterol contents increased substantially as a function of cholesterol concentration for all temperatures in the transition region. This is in contrast to the case of lipid bilayers containing high cholesterol concentrations where cholesterol strongly suppresses the permeability. Furthermore, the model predicts that the relative permeability increases with decreasing chain length at a given value of the reduced temperature for all cholesterol concentrations studied.

(iv) The model predicts that, for DMPC/cholesterol and DPPC/cholesterol bilayers, the peak in the relative permeability occurs slightly above T_m for all cholesterol concentrations studied as can be seen in Fig. 7. This is in agreement with the experimental results of Fig. 10. This effect is due to the aggregation of clusters very close to T_m which causes the fractional area of the interfaces to decrease.

Experimental results for $C_{15}PC$/cholesterol and $C_{16}PC$ (DPPC)/cholesterol bilayers shown in Fig. 10 demonstrate that there is an increase in the permeability between the pure bilayers and bilayers containing 10% cholesterol in accordance with the theoretical predictions. An enhancement in passive permeation of K^+ ions [14] and of membrane fluctuations [15] for low levels of cholesterol has previously been reported for DMPC liposomes. Furthermore, Fig. 10 shows that the peak in the permeability decreases with increasing

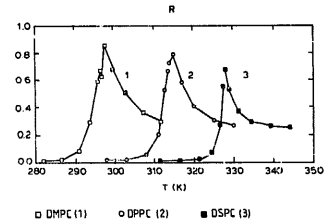

Fig. 9. Numerical results for the relative permeability, R, of bilayers with $x_C = 0.05$. The units are arbitrary.

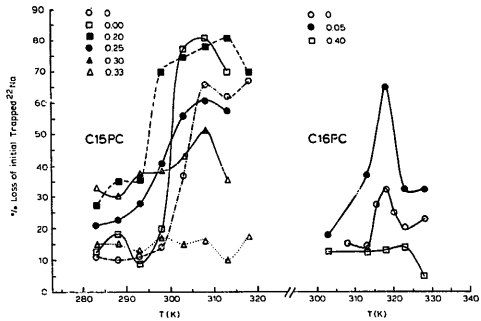

Fig. 10. Experimental results for ^{22}Na effluxes in liposomes composed of different C_{15}PC-cholesterol and DPPC (C_{16}PC)/cholesterol mixtures. The ordinate refers to the percentage of initial trapped ^{22}Na lost over 60 min. Experimental points for each temperature represent one, or in some cases two separate experiments, each performed in triplicate. The results illustrated are the mean values. To simplify the figure, error bars have not been given but the range of values did not exceed 15% for any of the mean values. The different cholesterol mole fractions are indicated in the figure.

chain length. This confirms the predictions given in (iii).

Fig. 10 also shows that, for values of the concentration greater than 20%, the permeability decreases with increasing cholesterol concentration. This behavior can be inferred from the experimental and the corresponding theoretical phase diagrams for DPPC/cholesterol mixtures [6,16]. As stated in the introduction, the theory of the full phase diagram is based on a combination of two models: the modified Pink model used in the present work, which describes the 'rigidifying' effect of cholesterol on neighboring lipid chains and the modified Potts model which describes the 'ice-breaker' effect of cholesterol. This leads to a phase separation between the low concentration cholesterol phase examined in the present work and a high concentration phase in which the chains are relatively rigid and the permeability is correspondingly low. This implies that the permeability increases until the phase boundary of the low concentration phase is reached. The permeability should then decrease with concentration until the phase boundary for the high concentration phase is reached. The decrease in the permeability should then continue with increasing cholesterol concentration. The theory presented in this paper is capable of describing the low value of the permeability of the high cholesterol phase [3] but cannot account for the phase separation region.

We wish finally to make a few remarks on some possible limitations of the comparison made in the present work between theoretical predictions and experimental measurements of passive permeability. The calculations are carried out for a well-defined unilamellar lipid/bilayer membrane whereas the lipid vesicles used in the experiments may have different degrees of multilamellarity. It is likely that the multilamellarity of the vesicles change as the vesicles are taken through the phase transition. Furthermore, it has been pointed out by E. Evans (private communication) that lipid vesicles often break in the transition region which may lead to part of the permeability anomaly observed right at the transition temperature. Recent micromechanic measurements of water permeability (Evans, E., private communication) of unilamellar SOPC vesicles indicate a suppression of the permeability above the transition in the presence of small amounts of cholesterol which is at variance with the model calculation presented here.

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