

# LIPID EXCHANGE BETWEEN MEMBRANES

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**ABSTRACT** The exchange of lipid molecules between vesicle bilayers in water and a monolayer forming at the water surface was investigated theoretically within the framework of thermodynamics. The total number of exchanged molecules was found to depend on the bilayer curvature as expressed by the vesicle radius and on the boundary condition for exchange, i.e., whether during exchange the radius or the packing density of the vesicles remains constant. The boundary condition is determined by the rate of flip-flop within the bilayer relative to the rate of exchange between bilayer and monolayer. If flip-flop is fast, exchange is independent of the vesicle radius; if flip-flop is slow, exchange increases with the vesicle radius. Available experimental results agree with the detailed form of this dependence. When the theory was extended to exchange between two bilayers of different curvature, the direction of exchange was also determined by the curvatures and the boundary conditions for exchange. Due to the dependence of the boundary conditions on flip-flop and, consequently, on membrane fluidity, exchange between membranes may partially be regulated by membrane fluidity.

## INTRODUCTION

Exchange of molecules between membranes serves to transfer material, energy, or information between cells or parts of cells. Despite its widespread occurrence, this exchange is barely understood. Here I attempt to clarify some of the basic principles of exchange between membranes by investigating a simple example, the exchange of lipid molecules between two pure lipid membranes. The conclusions drawn hold analogously for other molecules such as proteins.

Any exchange process has two aspects, static and kinetic. The static aspect refers only to the equilibrium state reached after exchange has taken place and therefore describes the total number of exchanged molecules. The kinetic aspect includes the intermediate states and thus the detailed mechanism of exchange, e.g., whether the molecules are exchanged through the water phase or upon direct contact between the two membranes. Here I elaborate on only the static aspect, which can be studied within the framework of thermodynamics. The starting point is the free energy of a lipid membrane as a function of the lipid packing density. From the free energy, the chemical potentials of the two exchanging membranes are derived taking into account the prevailing boundary conditions for exchange in the two membranes. These boundary conditions specify whether, during exchange, the lipid packing density, i.e., the area per molecule, or the total membrane area remains constant. The final equilibrium state is reached when the chemical potentials of the two membranes have become equal.

Such an approach bears many analogies to the treatment of the stability of lipid aggregates as derived in detail by Israelachvili et al. (1). In the present study, however, different boundary conditions for exchange are considered.

This is of central importance, since the boundary conditions are decisive for the direction and the extent of exchange.

Recently, it has been recognized that lipid exchange can be investigated experimentally in a simple manner as bilayer-monolayer exchange (2, 3). Bilayers in the form of vesicles are injected into the aqueous subphase of a film balance and the formation of the monolayer at the water surface is observed (Fig. 1). The advantage of this approach as compared with bilayer-bilayer exchange is the ease with which the exchange process can be followed by measuring the monolayer surface pressure, and the ease with which one can vary the boundary condition for exchange in the monolayer. To set up the theory in close connection to experiment, we shall start with the treatment of bilayer-monolayer exchange and then turn to the case of bilayer-bilayer exchange. A kinetic model for bilayer-monolayer exchange has already been worked out by Schindler (3).

For clarity, planar bilayers in exchange with a monolayer are treated first. In a second step, the dependence of exchange on the bilayer curvature, i.e., on the vesicle radius, is included. This predicts whether small or large vesicles exhibit better monolayer spreading. Such knowledge is required for the preparation of a synthetic surfactant to treat the respiratory distress syndrome (5, for a review see reference 6), and it was in the course of this project that the present study emerged.

## PLANAR BILAYER-MONOLAYER EXCHANGE

### Free Energy of a Planar Bilayer

The free energy of a bilayer in water is determined by the interaction of the lipid molecules among themselves and

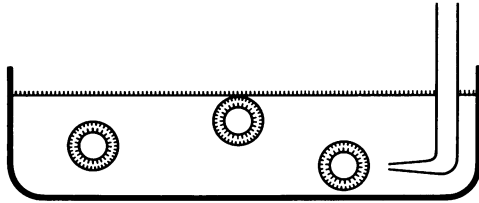


FIGURE 1 Schematic illustration of the equilibrium between vesicles and a monolayer.

with the surrounding water. The lipid-water interaction contains two contributions: (a) the hydrophobic interaction between the lipid hydrocarbon chains and water and (b) the hydrophilic interaction between the lipid polar heads and water (1). The hydrophobic interaction wants to decrease the lipid-water interfacial area, i.e., acts effectively as an attraction between the lipid molecules, whereas the hydrophilic interaction due to its demand for hydration wants to increase the interfacial area and acts in effect repulsively. The internal lipid-lipid interaction is determined mainly by steric hindrance of the lipid molecules, hence is repulsive. This contribution implicitly depends also on the orientational interaction of the lipids, since the orientational order and the packing density of the lipids are coupled (7). The free energy  $F_b$  of a bilayer can then be expressed in terms of the lipid packing density or the area  $a$ , per molecule as

$$F_b = N_b \phi(a) = N_b [\phi_{\text{phob}}(a) + \phi_{\text{phil}}(a) + \phi_{\text{int}}(a)], \quad (1)$$

$N_b$  denotes the number of lipid molecules in the bilayer of surface area  $A_b = N_b a$ . The qualitative behavior of the total repulsive contribution,  $\phi_{\text{phil}} + \phi_{\text{int}}$ , and the attractive contribution,  $\phi_{\text{phob}}$ , is shown in Fig. 2. The sum  $\phi$  exhibits a minimum at a certain area  $a^*$  per molecule. In equilibrium, the bilayer adopts this state of minimal free energy determined by  $(\partial F_b / \partial a)_{N_b} = 0$  (the subscript  $N_b$  indicates that  $N_b$  remains constant). With the definition of the

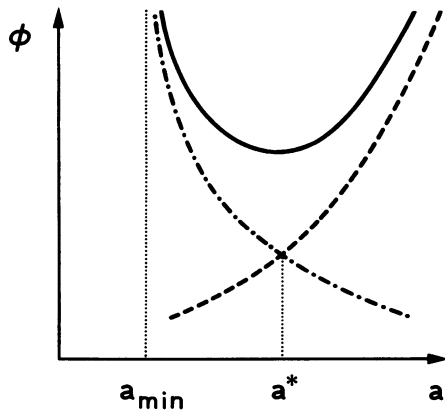


FIGURE 2 Variation of the total free energy per lipid molecule in a bilayer,  $\phi$  (—), and its effectively attractive and repulsive components,  $\phi_{\text{phob}}$  (---), and  $\phi_{\text{phil}} + \phi_{\text{int}}$  (-·-·-), respectively, with the area  $a$  per molecule.

surface tension  $\gamma_b = (\partial F_b / \partial A_b)_{N_b} = \partial \phi / \partial a$  and the surface pressures  $\pi_{\text{phob}} = \partial \phi_{\text{phob}} / \partial a$ ,  $\pi_{\text{phil, int}} = -\partial \phi_{\text{phil, int}} / \partial a$  (the signs are chosen such that  $\pi > 0$  in all cases), the equilibrium condition becomes

$$\gamma_b(a^*) = \pi_{\text{phob}}(a^*) - \pi_{\text{phil}}(a^*) - \pi_{\text{int}}(a^*) = 0. \quad (2)$$

Thus, equilibrium is characterized by a vanishing surface tension  $\gamma_b$ , the individual surface pressures compensating each other. This state was therefore called the saturated state by De Gennes and Taupin (8).

Near equilibrium the free energy may be expanded as

$$\phi(a) = \phi(a^*) + \frac{1}{2} k_c (a - a^*)^2 / a^*, \quad (3)$$

with  $k_c = a^* \partial^2 \phi / \partial a^2|_{a=a^*}$  representing an elastic constant equal to the inverse of the lateral compressibility. A relation of this kind has been used by Israelachvili et al. (9) to study the stability of lipid aggregates.

Derivation of the chemical potential of lipid molecules in a bilayer requires specification of the boundary condition for exchange. For a planar bilayer, the obvious assumption is that upon alteration of the number of lipid molecules, the area  $a$  per molecule remains constant. Then the chemical potential is given by  $\mu_b = (\partial F_b / \partial N_b)_a$ , and from Eq. 1

$$\mu_b(a) = \phi(a). \quad (4)$$

Hence, the chemical potential  $\mu_b(a)$  varies with  $a$  as the free energy per particle (Fig. 3).

### Free Energy of a Monolayer

Monolayers differ from bilayers in evincing a hydrocarbon-air interface. The free energy of this interface may be described simply by a surface tension term  $\gamma_{\text{ha}} A_m$ , where  $A_m$  is the monolayer area. A more difficult problem arises from the altered lipid-water interaction. In the case of low packing densities, the hydrocarbon chains of a monolayer may still avoid contact with water molecules by remaining in air, whereas for bilayers, if such low packing densities

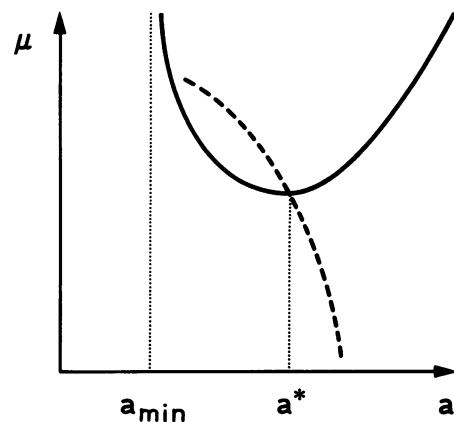


FIGURE 3 Variation of the chemical potential  $\mu$  of a lipid molecule in a bilayer (—) or in a monolayer (---) with the area  $a$  per molecule.

could be reached, water molecules would penetrate between the hydrocarbon chains. Thus, in general, the hydrophobic pressure is larger for a bilayer than for a monolayer. Because we are interested in monolayer-bilayer exchange equilibria, we can, however, restrict our description of monolayers to packing densities that are comparable to those of bilayers. In this case, monolayers and bilayers behave analogously, because at such packing densities penetration of the water molecules between the hydrocarbon chains does not occur to any appreciable extent. It is this restriction to monolayer packing densities comparable to those of bilayers that permits us to approximately equate the lipid-water interaction in mono- and bilayers. The lipid-lipid interaction between the two halves of a bilayer is negligibly weak compared with the interaction within one layer, so that also the lipid-lipid interaction is approximately the same in mono- and bilayers. Thus, for our purposes, the free energy of a monolayer may be described as

$$F_m = N_m \phi(a) + \gamma_{ha} A_m. \quad (5)$$

The surface tension of this monolayer-covered surface,  $\gamma_m = (\partial F_m / \partial A_m)_{N_m}$ , follows as

$$\gamma_m(a) = \gamma_b(a) + \gamma_{ha}. \quad (6)$$

The so-called Langmuir surface pressure  $\pi_L$ , measured against a free water surface of surface tension  $\gamma_{wa}$ , results as

$$\pi_L(a) = \gamma_{wa} - \gamma_{ha} - \gamma_b(a). \quad (7)$$

To calculate the chemical potential of lipid molecules in a monolayer, one must consider that upon changing the particle number the monolayer area  $A_m$  remains constant so that the area  $a$  per molecule changes. Eq. 5 then yields for  $\mu_m = (\partial F_m / \partial N_m)_{A_m}$  the result

$$\mu_m(a) = \phi(a) - a \frac{\partial \phi(a)}{\partial a}. \quad (8)$$

The first term on the rhs of Eq. 8 is the same as for a bilayer, Eq. 4, and the second term accounts for the change of  $a$  upon changing the particle number. For  $a$  near  $a^*$ , the harmonic approximation Eq. 3 can be used, which leads to

$$\mu_m(a) = \phi(a^*) + \frac{1}{2} k_c (a^{*2} - a^2) / a^*. \quad (9)$$

The variation of  $\mu_m$  with  $a$  is shown in Fig. 3. It implies that to add a molecule for example at  $a > a^*$  requires a free energy  $\mu_m$  that is smaller than  $\phi(a^*)$ . This is because upon addition of a molecule,  $a$  approaches  $a^*$  so that the packing density becomes more favorable.

### Equilibrium Condition

If bilayers are injected into the aqueous subphase of a monolayer trough, lipid molecules will undergo exchange to form a monolayer on the water surface. Equilibrium is

reached when the chemical potentials of mono- and bilayer, regarded as two phases, have become equal. Assuming that many more lipid molecules are in the bilayer phase (a bulk dispersion) than in the monolayer phase, the bilayers can be regarded as a reservoir with a constant chemical potential  $\mu_b(a^*)$ . The equilibrium condition then reduces to a condition for the area  $a_m$  per molecule in the monolayer

$$\phi(a_m) - a_m \phi'(a_m) = \phi(a^*). \quad (10)$$

Remembering that  $\phi'(a^*) = 0$ , Eq. 2, the solution of this equation is  $a_m = a^*$ . In equilibrium with a saturated bilayer, a monolayer also adopts the saturated state. The number of exchanged molecules is  $N_m = A_m / a^*$ . The corresponding Langmuir surface pressure  $\pi_L(a^*)$ , called equilibrium surface pressure, follows from Eq. 7 as

$$\pi_L(a^*) = \gamma_{wa} - \gamma_{ha}, \quad (11)$$

and is completely independent of the internal pressure  $\pi_{int}$  and the pressures  $\pi_{phob}$  and  $\pi_{phil}$ . The lipid monolayer simply reduces the surface tension of the trough surface to that of a hydrocarbon-air interface as pointed out already by Nagle (10). Typical values are  $\gamma_{wa} \approx 70$  dyn/cm and  $\gamma_{ha} \approx 30$  dyn/cm (11),<sup>1</sup> so that  $\pi_L(a^*) \approx 40$  dyn/cm. This value fits well with experimental results (12) as discussed later.<sup>2</sup> Hence, equivalence between mono- and bilayer is ensured for a monolayer surface pressure of  $\pi_L \approx 40$  dyn/cm.

According to the definition of equilibrium, the monolayer adopts the saturated state independently of its initial state. This implies that in the usual type of monolayer experiments where lipid molecules are spread onto the water surface, the monolayer also tries to reach the saturated state. Hence, except for the case  $a = a^*$ , the monolayer is not in an equilibrium state. For  $a < a^*$ , lipid molecules try to enter the subphase by forming vesicles (large vesicles to approach the saturated state). The spontaneous formation of vesicles, however, is a rare event, so that densely packed monolayers are relatively stable, although metastable in a strict sense. This point has been stressed by Horn and Gershfeld (14) on the basis of experimental results. For  $a > a^*$ , not enough lipid molecules are present, hence equilibrium can never be reached. The monolayer may try, however, to reduce its free energy

<sup>1</sup>Reported values for the surface tension of hexane and octane at 20°C are 18.4 and 21.8 dyn/cm, respectively. A value of  $\gamma_{ha} = 20$  dyn/cm has often been quoted in the literature (10), but obviously the surface tension increases with increasing chain length so that by extrapolation to the chain length of typical lipids one would expect  $\gamma_{ha} \approx 30$  dyn/cm. More experimental data would be required to decide upon this point.

<sup>2</sup>For spreading of a solid lipid an equilibrium surface pressure of 50 dyn/cm has been measured (13). This case, however, differs from bilayer-monolayer exchange, since solid lipids are not in the saturated state due to the lack of water.

by forming saturated domains and leaving part of the water surface as a free surface. Such behavior seems to have been observed by von Tscharner and McConnell (15).

## CURVED BILAYER-MONOLAYER EXCHANGE

### Curvature Elasticity

If a bilayer is curved, e.g., in the form of a spherical vesicle, the packing is no longer optimal. In the simplest case, one may assume that the number of lipid molecules is the same in the two halves of the bilayer. Then the outer layer is more loosely packed than the inner one and the areas per molecule may be expressed as  $a_{\text{outer}} = \bar{a} + \Delta a_b$  and  $a_{\text{inner}} = \bar{a} - \Delta a_b$ ,  $\bar{a}$  denoting the average value. If  $\bar{a} = a^*$ , the free energy of the curved bilayer follows from Eq. 3 as

$$F_b = N_b [\phi(a^*) + 1/2 k_c (\Delta a_b)^2 / a^*]. \quad (12)$$

$a_{\text{outer}}$  and  $a_{\text{inner}}$  may be specified further as the molecular areas at the lipid-water interface, where the hydrophobic effect is most effective, so that with  $R$ , which denotes the outer vesicle radius, and  $d$ , which denotes the bilayer thickness, one obtains

$$\Delta a_b = a^* \left[ \frac{1}{\left(1 - \frac{d}{2R}\right)^2} - 1 \right]$$

or assuming  $d \ll R$

$$\Delta a_b = a^* \frac{d}{R}. \quad (13)$$

Insertion into Eq. 12 yields

$$F_b = N_b \phi(a^*) + 1/2 A k_c \left(\frac{d}{R}\right)^2. \quad (14)$$

The essential point is that the curvature term is proportional to  $1/R^2$ . This dependence is of much more general validity than suggested by the above derivation under the assumption of equal numbers of lipid molecules in the two halves of the bilayer. Under the opposite assumption of equal packing densities  $a = a^*$  in the two halves, the free energy formally would turn out to be  $N_b \phi(a^*)$ , i.e., the same as for a planar bilayer with  $a = a^*$ . This, however, is an oversimplification. Actually, for a curved bilayer with  $a = a^*$  at the two surfaces, the packing of the lipid chains in the interior of the bilayer is not optimal. In the inner layer, the area per molecule is larger than  $a^*$  on the average; in the outer layer it is smaller. This implies that the steric and van der Waals interactions between the lipid chains are different from the planar case leading to a higher free energy of the curved bilayer. To describe this effect one may treat each segment along the lipid chains individually. Attributing an area per molecule  $a_n$  and an elastic constant  $k_n$  to the  $n$ th segment, the segmental free energy per

molecule is given, in analogy to Eq. 12, by

$$\phi(a_n) = \phi(a^*) + 1/2 k_n (a_n - a^*)^2 / a^*, \quad (12a)$$

and the total free energy per molecule by  $\phi = \sum_{n=0}^N \phi(a_n)$ . Here the optimal area per molecule,  $a^*$ , has been assumed to be the same for all segments, thus avoiding a tendency for spontaneous curvature of a layer. For a curved bilayer with  $a_0 = a^*$  at both lipid-water interfaces, the area per molecule in the inner layer increases linearly with  $n$  and reaches  $a_N = a^*(1 + d/2R)$  at the chain ends, so that

$$\Delta a_n = a^* \frac{d}{2R} \frac{n}{N}, \quad (13a)$$

whereas in the outer layer the area per molecule decreases and  $\Delta a_n$  is given by Eq. 13a with a minus sign. Insertion into Eq. 12a and summation over  $n$  leads to

$$F_b = N_b \phi(a^*) + \frac{1}{2} A \left( \sum_{n=1}^N k_n n^2 \right) \left( \frac{d}{2R} \right)^2. \quad (14a)$$

Compared with Eq. 14, the elastic constant  $k_c$  is replaced by  $\sum_{n=1}^N k_n n^2$  and the thickness  $d$  by  $d/2$ , but the curvature free energy is still proportional to the square of the curvature  $1/R$ . Thus, in general the free energy may be described as in Eq. 14 with an effective bilayer thickness  $d_e$ .

A further contribution to the curvature energy arises from the nonparallel orientation of the preferred axes of lipid order. This effect has been investigated by Helfrich (4) and the energy results as in Eq. 14 with  $k_c d^2$  replaced by the orientational elastic constant  $k_0$  (1). Hence, using the total elastic constant  $k = k_c d_e^2 + k_0$  instead of  $k_c d^2$ , Eq. 14 can be considered as the general expression for the free energy of a curved bilayer. For an estimate of the order of magnitude, we use  $k_c = 100 \text{ erg/cm}^2$  derived from measurements of the lateral compressibility of monolayers (16) and  $d_e = d = 50 \text{ \AA}$  to obtain  $k_c d_e^2 = 2.5 \cdot 10^{-11} \text{ erg}$ . Helfrich's estimate for  $k_0$  based on the analogy to liquid crystals is  $k_0 = 10^{-12} \text{ erg}$ . Thus, the orientational contribution to the curvature energy is small and will therefore be neglected in the following.

Eq. 14 becomes extremely simple for a spherical vesicle for which  $A \approx 2 \cdot 4\pi R^2$ , so that

$$F_b = N_b \phi(a^*) + 4\pi k_c d_e^2. \quad (15)$$

The curvature elastic energy of a vesicle is independent of the vesicle radius  $R$ . For large  $R$  (and  $N_b$ ), however, the curvature term becomes negligible compared with the ordinary term  $N_b \phi(a^*)$ .

### Equilibrium Conditions

For a curved bilayer at least three different boundary conditions for exchange can be envisaged according to which quantity remains constant: the area  $a$  per molecule,

the mean area  $\bar{a}$ , or the total area  $A$ . These three cases will be discussed separately.

The first boundary condition of constant area  $a$  per molecule does not apply to vesicles, because upon release of lipid molecules a vesicle shrinks with a concomitant alteration of the packing density. This boundary condition may hold, however, for protuberances of cell membranes whose curvature is kept constant during exchange by external constraints and lipid molecules are diffusing into the protuberances to keep the packing density constant. To treat this case, the vesicle radius  $R$  has to be replaced by the radius of curvature of the protuberances. The chemical potential  $\mu_b = (\partial F_b / \partial N_b)_a$  follows from Eq. 14 as

$$\mu_b = \phi(a^*) + \frac{1}{2} a^* k_c \left( \frac{d_c}{R} \right)^2. \quad (16)$$

For the chemical potential of the monolayer lipids, Eq. 9 still holds and inserting  $a = a^* + \Delta a_m$  yields, neglecting terms of order  $(\Delta a_m)^2$ ,

$$\mu_m = \phi(a^*) - k_c \Delta a_m. \quad (17)$$

Regarding the bilayers again as a reservoir of constant chemical potential, the equilibrium condition  $\mu_m = \mu_b$  leads to

$$\Delta a_m = -\frac{1}{2} a^* \left( \frac{d_c}{R} \right)^2. \quad (18)$$

This result states that the monolayer reaches a packing density higher than that of the saturated state which implies that the number of exchanged molecules,  $N_m = A_m / \{a^* [1 - 1/2 (d_c/R)^2]\}$ , is larger than for exchange with a planar bilayer. To understand this behavior, one may consider the situation where the monolayer has just reached the saturated state. This state is energetically more favorable for lipid molecules than the state in the curved bilayer, hence they will continue to populate the monolayer until the equilibrium state, Eq. 18, is reached.

The equilibrium surface pressure follows from the expansion  $\pi_L(a) = \pi_L(a^*) - k_c \Delta a_m / a^*$  as

$$\pi_L = \pi_L(a^*) + \frac{1}{2} k_c \left( \frac{d_c}{R} \right)^2. \quad (19)$$

With decreasing  $R$  or increasing curvature the surface pressure increases, as shown in Fig. 4.

The second type of boundary condition for exchange, constant mean area  $\bar{a}$ , applied to vesicles. Upon release of molecules, the vesicles shrink and their curvature increases. Hence, the packing density in the outer and inner vesicle layer varies while the packing density in the midplane may remain constant,  $\bar{a} = a^*$ . Then Eq. 15 for the free energy applies and the chemical potential  $\mu_b = (\partial F_b / \partial N_b)_{\bar{a}}$  results as

$$\mu_b = \phi(a^*). \quad (20)$$

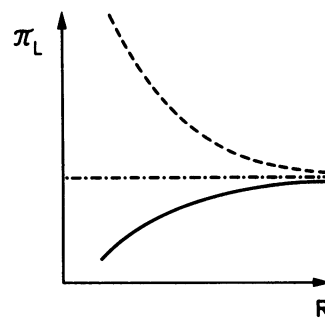


FIGURE 4 Dependence of the surface pressure  $\pi_L$  on the vesicle radius for the three different boundary conditions: Constant area  $a$  per molecule (---), constant mean area  $\bar{a}_0$  per molecule (-·-), and constant vesicle area or radius (—).

The equilibrium condition  $\mu_b = \mu_m$  leads to  $\Delta a_m = 0$  or  $a_m = a^*$ , i.e., the monolayer adopts the saturated state. Compared with the above case, Eq. 18, less lipid molecules are exchanged. The reason for this lies in the increase of vesicle curvature during exchange, which is energetically unfavorable and restricts the exchange to the monolayer. The equilibrium surface pressure in this case is

$$\pi_L = \pi_L(a^*), \quad (21)$$

independent of the vesicle radius (Fig. 4).

In the third type of boundary condition the area  $A$  of the bilayer is kept constant, which for vesicles implies that the radius  $R$  remains constant. Such a boundary condition may hold if only lipid molecules from the vesicle outer layer participate in exchange, while the vesicle inner layer remains unaltered and keeps the radius constant. Obviously, this requires that the time for flip-flop of the lipid molecules from the inner to the outer vesicle layer is large compared with the time for bilayer-monolayer exchange. Therefore, the state reached under this boundary condition is a preequilibrium, as recognized already by Schindler (3). The overall equilibrium is attained via flip-flop of the lipids and shrinkage of the vesicles, corresponding to the second type of boundary condition discussed above. Under the boundary condition for preequilibrium, constant area  $A$ , the expression for the chemical potential of the bilayer lipids is the same as for the monolayer lipids, Eq. 17, and the preequilibrium condition becomes  $a_m = a_b$ . Assuming again the bilayer lipids to act as a reservoir, i.e., high vesicle concentration, preequilibrium is reached when the packing density in the monolayer is the same as in the vesicle outer layer

$$a_m = a^* \left( 1 + \frac{d_c}{R} \right). \quad (22)$$

This monolayer packing density is lower than in the saturated state, fewer lipid molecules than in the other two cases of boundary conditions are exchanged. The preequi-

brium surface pressure results as

$$\pi_L = \pi_L(a^*) - k_c \frac{d_c}{R} \quad (23)$$

With increasing curvature  $\pi_L$  decreases, as shown in Fig. 4. Such behavior has been observed experimentally by Schindler (12) providing evidence for the existence of the preequilibrium state. A fit of Eq. 23 to the experimental data, shown in Fig. 5, yields  $k_c d_c = 5.2 \cdot 10^{-5}$  dyn and  $\pi_L(a^*) = 40.4$  dyn/cm. Insertion of  $k_c = 100$  dyn/cm from monolayer experiments (16) leads to the value  $d_c = 52$  Å, which agrees with the bilayer thickness  $d$ . This result indicates that for vesicles in the preequilibrium state, the number of lipid molecules in the outer and inner layer is the same and their packing densities differ correspondingly. The result for  $\pi_L(a^*)$  coincides with the previous estimate based on Eq. 11. Thus, the evaluation of experimental data lends support to the theory of exchange as developed above.

The appearance of preequilibrium depends on whether the time  $\tau_{\text{flip}}$  for flip-flop within the bilayer is larger than the time  $\tau_{\text{exch}}$  for exchange between mono- and bilayer. These times are of comparable order of magnitude, namely hours. Their exact values depend on parameters such as bilayer fluidity (for  $\tau_{\text{flip}}$ ) or vesicle concentration (for  $\tau_{\text{exch}}$ ). Hence, by variation of these parameters, one may induce or suppress the appearance of preequilibrium. This point deserves further experimental investigation.

The results obtained allow us to answer the question of whether small or large vesicles exhibit better spreading. For long periods of equilibration, when true equilibrium is reached, spreading is independent of the vesicle radius. However, for short periods of equilibration and  $\tau_{\text{flip}} > \tau_{\text{exch}}$ , i.e., when preequilibrium is reached, large vesicles are

advantageous in order to obtain high spreading. This behavior has been exploited in the preparation of a synthetic lung surfactant (5).

#### BILAYER-BILAYER EXCHANGE

The above treatment of exchange between a mono- and a bilayer can easily be extended to exchange between two bilayers of different curvature, e.g., a vesicle and a planar bilayer (as the limiting case of a large vesicle). The planar bilayer is described by a constant area per molecule,  $a_p = a^*$ , and a chemical potential,  $\mu_p = \phi(a^*)$ , Eq. 4. This behavior may be simulated with a monolayer if instead of the total film area the surface pressure is kept constant at  $\pi_L(a^*)$ . Evidently, the equilibrium state of lowest free energy of a vesicle and a planar bilayer in exchange is a planar bilayer solely, i.e., all lipid molecules in the vesicles have moved to the planar bilayer. However, analogous to the vesicle-monolayer exchange, preequilibrium states may occur depending on the prevailing boundary condition for exchange in the vesicles.

Formally, the equilibrium state consisting of a planar bilayer solely may be derived from the boundary condition of constant area per molecule in the vesicles,  $a_c = a^*(1 \pm d/R)$ , so that the chemical potential is  $\mu_c = \phi(a_c)$ . The chemical potentials  $\mu_c$  and  $\mu_p$  (subscripts c and p indicating curved and planar) cannot become equal, hence, the equilibrium state is given solely by a planar bilayer (Fig. 6 a).

The boundary condition of constant area per molecule, however, is unrealistic for a vesicle, as discussed, since upon exchange the vesicle radius varies and only the mean area,  $\bar{a}$ , may be considered constant. Then the chemical potential of the vesicle lipids is  $\mu_c = \phi(a^*)$ , according to Eq. 20. This expression agrees with the chemical potential of

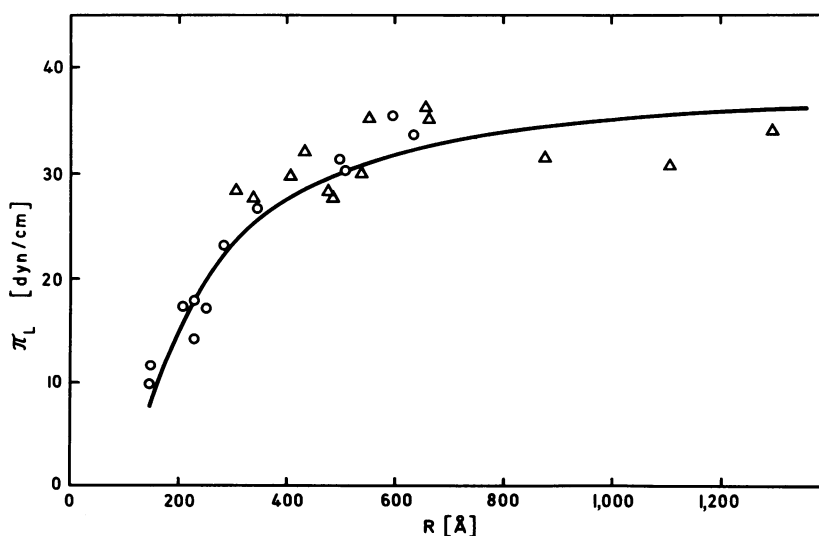


FIGURE 5 Comparison between the theoretical result for the preequilibrium surface pressure, Eq. 23 with  $k_c d_c = 5.2 \cdot 10^{-5}$  dyn and  $\pi_L(a^*) = 40.4$  dyn/cm (—), and the experimental results of Schindler (12) for vesicles of dioleoylphosphatidylcholine (o) and soybean phosphatidylcholine ( $\Delta$ ).

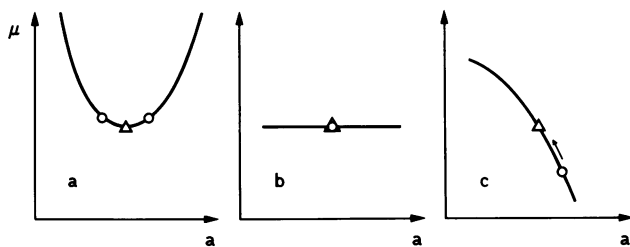


FIGURE 6 Equilibria between vesicles (o) and a planar bilayer ( $\Delta$ ) for three different boundary conditions in the vesicle: (a) constant area  $a$  per molecule, (b) constant mean area  $\bar{a}_0$  per molecule and (c) constant vesicle area or radius.

the planar bilayer lipids, which implies that the vesicle is already in equilibrium with the planar bilayer and no exchange takes place (Fig. 6 b). In a strict sense, however, this equilibrium is a preequilibrium state because true equilibrium corresponds solely to a planar bilayer (this notion differs from the one in the preceding chapter, where a vesicle was considered as an equilibrium state and the preequilibrium state was of different origin). The vesicle simply cannot release molecules and shrink due to the concomitant increase in curvature. One way to circumvent this barrier is to fuse the vesicle with the planar bilayer. Here no shrinkage of the vesicle takes place, instead the vesicle bilayer locally fuses with the planar bilayer and then flattens.

The third possible boundary condition in the vesicle is a constant radius  $R$  or total area  $A$ , if only lipid molecules from the vesicles outer layer participate in exchange. This case corresponds to the previous preequilibrium and requires  $\tau_{\text{flip}} > \tau_{\text{exch}}$ . The chemical potential of the vesicle lipids is given by Eq. 9,  $\mu_c = \phi(a^*) + 1/2 k_c (a^{*2} - a_c^2)/a^*$ . The condition for preequilibrium,  $\mu_c = \mu_p$ , leads to  $a_c = a^*$ . Thus, the area per molecule in the vesicle outer layer is decreased from  $a^*(1 + d/R)$  to  $a^*$ , to reach the saturated state (Fig. 6 c). In this case, exchange takes place from the planar bilayer to the vesicle.

Note that for bilayer-bilayer exchange even the direction of exchange depends on the curvature and the boundary conditions in the two bilayers. We have discussed different boundary conditions in the more curved bilayer, a vesicle, but different boundary conditions may also exist in the less curved bilayer represented by the planar bilayer. The type of boundary condition is determined by the time  $\tau_{\text{flip}}$  for flip-flop in relation to the time  $\tau_{\text{exch}}$  for exchange. Hence, by varying these times or the bilayer curvature, the direction of exchange between bilayers can be regulated.

## CONCLUSION

Bilayer-monolayer exchange of lipids was investigated to clarify some basic principles of the exchange of molecules between membranes. The equilibrium state, which is reached after exchange takes place, was found to depend on the curvature of the bilayer as expressed by the vesicle

radius and on the boundary conditions for exchange, i.e., whether the area of the bilayer or the area per molecule remains constant.

In the planar bilayer the lipid packing is optimal, this being called saturated state. If upon exchange this packing remains constant, the monolayer of constant area is also in the saturated state. If the bilayer is curved, then the lipid packing is not optimal and three types of exchange equilibria can be distinguished according to the boundary condition for exchange in the bilayer. First, the packing remains constant and unfavorable, and the lipids prefer the monolayer; exchange is high. This may hold for protrusions of bilayer membranes. Second, upon exchange the vesicles shrink. Due to shrinkage the bilayer packing becomes even more unfavorable, so that the monolayer is only populated up to the saturated state; exchange is moderate. Third, only the lipids of the vesicle's outer layer participate in exchange. Upon exchange their low packing density decreases further becoming extremely unfavorable so that the monolayer packing does not even reach the saturated state; exchange is weak. This latter state is a preequilibrium, because via flip-flop of lipid molecules between the inner and outer vesicle layer the true equilibrium state of shrunken vesicles is reached. Hence, the appearance of preequilibrium depends on the time for flip-flop in comparison with the time for exchange between membranes. If flip-flop is slow, preequilibrium establishes at short times. In this situation, exchange increases with increasing vesicle radius. For long times, when the true equilibrium is reached, exchange is independent of the vesicle radius.

The description of exchange was then extended to lipid exchange between two bilayers, two kinds of vesicles of different curvature. This led to the result that the direction of exchange between bilayers is determined by the curvature and the prevailing boundary conditions in the two bilayers. According to the above arguments, this implies that the direction of exchange can be regulated by the flip-flop time in the bilayers. Because the flip-flop time is known to depend on the fluidity of the bilayers, it is concluded that lipid exchange between membranes can be regulated by membrane fluidity.

In establishing the theory, exchange of lipid molecules has been treated, particularly between spherical vesicles. Obviously, the results obtained hold analogously for other molecules such as proteins or polypeptides. Furthermore, these results may be applied to cell membranes of arbitrary shape, if the vesicle radius is replaced by the local radius of curvature, e.g., of protuberances of cell membranes. In this way, the theory of exchange presented may be relevant to some complex exchange processes that occur between cell membranes.

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