The Effect of a Transmembrane Osmotic Flux on the Ion Concentration Distribution in the Immediate Membrane Vicinity Measured by Microelectrodes

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ABSTRACT The osmotically induced transmembrane water flow is accompanied by solute concentration changes within the unstirred layer adjacent to membranes. Experimental concentration profiles, measured by means of microelectrodes in the immediate vicinity of a planar lipid bilayer, are compared with theoretical ones predicted from the standard physiological model in which the osmotic advection is countered by back-diffusion of the solute only. An increase of the apparent osmotic flow rate is induced by an increase of the osmotic gradient and by rigorous stirring. The polarization effect decreases in the latter case due to an increase of the transfer rate of solutes between the bulk solutions and the membrane surfaces, whereas it increases in the former case. The observations show that the concentration profile is not well described by the standard approximation. The discrepancy becomes increasingly large with increased volume flow. Based on a modified theoretical description of the interaction between water flux and diffusion, the hydraulic conductivity of the bilayer is calculated from the measured uniexponential concentration profiles. The common approximation that there is a discrete boundary between the stirred and unstirred regions adjacent to the membrane is substituted by the model of a stagnant point flow that takes into account a gradual change of the stirring velocity in the immediate membrane vicinity. Supported by experimental observations, this approach predicts a shortening of the unstirred layer if the transmembrane osmotic gradient is increased under gentle stirring conditions.

INTRODUCTION

Water movement across cell membranes is of central importance in many functions of any organism. In view of this fact, transmembrane water flux has been studied in the last years with great intensity (for reviews, see Haines, 1994; van Os et al., 1994; Verkman et al., 1996). Water crosses cell membranes by passive transport and by secondary active co-transport along with ions (Zeuthen, 1995). Two kinds of passive permeability properties of biomembranes are described: those that consist of aqueous pores traversing the membrane (Verkman et al., 1996) and those that do not. At least two independent nonspecific pathways for water diffusion across a lipid bilayer are suggested, the permeation according to a simple solubility diffusion mechanism (Hanai and Haydon, 1966) and through fluctuating defects (Deamer and Bramhall, 1986). The water diffusion across the hydrophobic barrier is related to membrane fluidity (Lande et al., 1995) and membrane structure (Subczynski et al., 1994). The hypothesis of a "hopping" mechanism, which proposes that small solutes fit into holes available in the acyl chain region of the bilayer, is supported by molecular dynamic simulation of solute diffusion carried out at an atomic level (Bassolino-Klimas et al., 1993).

The ratio of osmotic to diffusional water permeabilities (P_f/P_d), may provide useful information about the presence of a facilitated water-transporting pathway (Schafer and Andreoli, 1987). It may be overestimated due to the presence of an unstirred layer (USL) that acts as an additional diffusional barrier (Finkelstein, 1987; Barry and Diamond, 1984). Within the USL there is no convective mixing, and movement takes place solely by diffusion. It is well known that even in vigorously stirred systems there is usually a stagnant layer adjacent to a membrane that leads to concentration differences; i.e., water that passes through the membrane dilutes the solution it enters and concentrates the solution it leaves (Fettiplace and Haydon, 1980). The size and importance of the solute concentration gradients depends on their rate of dissipation through back-diffusion of the solute and on the various stirring effects that may be present. Large concentration differences would create density gradients (Hanai and Haydon, 1966). These, in turn, may cause streaming of the solution near the membrane (Eckert and Drake, 1959). It is evident that an assessment of the mode of water transport across membranes requires not only the traditional measurement of P_f and P_d, but also an explicit evaluation of the effects of USLs (Schafer et al., 1974; Andreoli and Troutman, 1971). Despite the large number of works devoted to this problem (for review, see Barry and Diamond, 1984; Fettiplace and Haydon, 1980; Schafer and Andreoli, 1987), an experimental verification of the theory in terms of concentration profile measurements has not yet been undertaken. The exact knowledge of the near-membrane solute concentration, however, is im-

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portant for the proper description of various transport phenomena. For example, the conductivity of a membrane, that is permeable for at least one ion may be reduced by diffusion polarization (Neumcke and Bamberg, 1975). Another example is the calculation of ion-binding constants that is possible when water flux and conductance measurements across a pore-containing membrane are carried out simultaneously—provided that the solute concentration in the immediate membrane vicinity is known (Dany and Levitt, 1981).

The goal of the present work is to measure the concentration distribution of ions in the immediate vicinity of a planar bilayer lipid membrane under the conditions of an osmotic volume flow. Now the experimental data can be obtained with great accuracy with the help of the microelectrode technique (Lucas et al., 1975) adapted to concentration measurements near planar bilayer membranes (Antonenko and Bulychev, 1991). The experimentally measured concentration profiles are compared with theoretical ones, and it is shown that satisfactory agreement between theory and experiment is achieved only if stirring motions of the liquid near the membrane are taken into account.

THEORY

Within the USL, the concentration of solute is a function of the distance to the membrane, and it is not equal to that in the bulk solution. The thickness of the layer δ is defined in terms of the concentration gradient at the membrane water interface (e.g., see Dainty and House, 1966):

$$\frac{|C_s - C_b|}{\delta} = \left.\frac{\partial C}{\partial x}\right|_{x=0} \tag{1}$$

where x is the distance from the membrane. C_b and C_s denote the solute concentrations in the bulk and at the interface, respectively.

The effect of the USLs is assessed based on the assumptions that the only motion in the USL is the osmotic flux itself, and that C depends only on the distance x from the membrane (Dainty, 1963). In the steady state, the flux, J, of an impermeable solute in the USL is zero:

$$J = D(dC/dx) + vC = 0 \tag{2}$$

where v is the linear velocity of osmotic volume flow. Integrating between the edge of the USL ($x = \pm\delta$) and the membrane surface ($x = 0$), one obtains under the assumption that $C(\pm\delta) = C_b$:

$$C(x) = C_b e^{(\delta - x)v/D} \tag{3}$$

on the hypotonic side of the membrane. The volume flow is directed toward the other side of the membrane, where the concentration distribution of the solute is given by

$$C(x) = C_b e^{(x - \delta)v/D} \tag{4}$$

after replacing v by $-v$ in Eq. 2 (Barry and Diamond, 1984). It has long been recognized that the last assumption is not to be taken literally, because the concentration at the distance δ is never equal to the bulk concentration (Pedley, 1983). δ determined in terms of the concentration gradient at the wall (Eq. 1) is commonly regarded as the effective USL thickness that, if inserted into Eqs. 3 and 4, allows calculation of the near-membrane concentration shift (Finkelstein, 1987).

According to Eqs. 3 and 4 the greatest gradient of solute concentration is not located at the membrane-solution interface, but it occurs at the outer edge of the USL at the hypertonic side of the membrane (Pedley and Fischbarg, 1978). This conclusion may be a consequence of the inconsistency of the commonly accepted model of the USL, assuming the existence of a completely unstirred region that yields abruptly to a perfectly stirred region.

For the diffusion of weak acids it has been shown that the concentration distribution of the acid within the USL is describable by an empirical function (Pohl et al., 1993):

$$C(x) = |C_s - C_b|e^{-x/\delta} + C_b \tag{5}$$

As we are going to show below, Eq. 5 fits well the experimental profiles obtained for an impermeable solute under the conditions of a transmembrane volume flow. The uniexponential Eq. 5 conflicts with the theoretical Eqs. 3 and 4, because according to this equation the maximum concentration gradient is located at the membrane surface.

If a gradual change of the stirring conditions along the USL is considered, the agreement between theory and experiment should be much better. In the most simple case the stirring motion is represented as a two-dimensional stagnation point flow against the infinite plane occupied by the bilayer lipid membrane (Pedley, 1983). The convective flow caused by the stirrer arrives from the direction (x axis) perpendicular to this plane and impinges on the membrane placed at $x = 0$. There it divides into two streams on the membrane and leaves in both directions ($-y$ and $+y$) parallel to the membrane. The viscous flow must adhere to the wall, whereas the frictionless potential flow slides along it (Schlichting, 1979). The velocity distribution in frictionless potential flow in the neighborhood of the stagnation point at $x = y = 0$ is given by:

$$U = -\alpha x; \quad W = \alpha y \tag{6}$$

where α is the stirring parameter measured in units of s^{-1}. Near the membrane a viscous boundary layer (VBL) is defined, where the flow velocity u along the x axis differs from that in the bulk (U). In the absence of osmosis the velocity u of the viscous flow may be approximated by a quadratic function of the distance x to the membrane (Schlichting, 1979; Pedley 1983):

$$u = -0.6165\,(\alpha^{3/2}/\nu_k^{1/2})x^2 = -ax^2 \tag{7}$$

where ν_k is the kinematic viscosity of the fluid. u in Eq. 7 has to be evaluated very close to the membrane, where $u \lll U$. Within the USL ($-\delta \le x \le \delta$), it is assumed that

Eq. 7 works, because hydrodynamic studies have demonstrated that δ is usually much smaller than the VBL, defined by the velocity gradient at the interface (Dainty and House, 1966). Stagnation point flow is convenient to study, because the boundary layer thickness does not vary along the membrane (Schlichting, 1979). Furthermore, the velocity u of the flow toward the membrane does not depend on the space coordinates parallel to the membrane, and neither does the solute concentration (Pedley, 1983). Although the flow parallel to the membrane affects the system by convecting away part of the solute, the steady-state concentration is a function of the distance to the membrane only. If v in Eq. 2 is substituted by the scalar sum of both fluid velocities perpendicular to the membrane (the stirring velocity u and the velocity v of the osmotic volume flow) a one-dimensional differential equation is obtained ($u < v$):

$$J = D(dC/dx) + (v - ax^2)C = 0 \tag{8}$$

The boundary condition on C is

$$C(x) \rightarrow C_s \quad \text{as} \quad x \rightarrow 0 \tag{9}$$

From Eqs. 8 and 9 the concentration course within the USL is found as:

$$C(x) = C_s e^{(-vx/D)+(ax^3/3D)} \tag{10}$$

for $-\delta \leq x \leq \delta$. Eqs. 8–10 hold for the hypertonic and hypotonic sides of the membrane. At both sides of the membrane the concentration gradient induced by the osmotic flow is decreased due to fluid motions caused by the stirrer. From concentration profile measurements it is possible to find the unknown parameters v and a. With the knowledge of v the transmembrane water permeability, P_f, can be calculated:

$$P_f = \frac{v}{C_{osm} V_w} \tag{11}$$

where V_w is the partial molar volume of water, and C_{osm} the near-membrane concentration of the solute used to establish the transmembrane osmotic pressure difference.

Our purpose is to calculate the hydraulic membrane permeability, P_f, of planar bilayer lipid membranes from experimental concentration profiles. We do this by using two different models for the estimation of v. In the first model v is determined from Eqs. 3 and 4 and in the second from Eq. 10. Whereas the first model is based on the assumption of a strict boundary between the regions of complete stirring and diffusion, the second model considers a gradual change of convection in agreement with the hydrodynamic theory of a VBL. This way the validity of the simplification made in the first model can be checked, and the resulting error can be assessed. The conclusions to be drawn are of importance for the correction of measured osmotic membrane permeability (Fischbarg et al., 1993), reflection coefficient (Hamada and Imai, 1995), and hydraulic conductivity of channels and pores (Wang et al., 1995).

MATERIALS AND METHODS

The planar black lipid membranes (BLM) were formed by a conventional method (Mueller et al., 1963) of 20 mg diphytanoyl phosphatidylcholine (DPhPC; Avanti Polar Lipids, Alabaster, AL) or 40 mg Asolectin (Fluka, Buchs, Switzerland) per ml *n*-decane (Merck, Darmstadt, Germany). The membranes (0.4 mm in diameter) were spread across a circular hole in a diaphragm separating two aqueous phases of a polytetrafluorethylene chamber. The bathing solution consisted of 10 mM Tris (Fluka), 10 mM 2-(*N*-morpholino)ethanesulfonic acid (Boehringer Mannheim, Mannheim, Germany), and 100 mM NaCl (Merck). It was agitated by magnetic stirrer bars.

An osmotic gradient was induced by urea (Laborchemie Apolda, Apolda, Germany) added to the *trans* side of the membrane only. Concentration changes of sodium ions in the immediate membrane vicinity due to the water flow across the membrane were monitored with the help of microelectrodes. The sodium-sensitive electrodes were made of glass capillaries containing mixture A of sodium ionophore II (Fluka) according to the procedure described by Ammann (1986). Their tips had a diameter of about 1–2 μm. Electrodes with a 90% rise time of less than 0.6 s were selected. Artifacts due to a very slow electrode movement are therefore unlikely. Nevertheless, possible effects of time resolution or distortion of the USL were tested by making measurements while moving the microelectrode toward and away from the bilayer. Because no hysteresis was found, it can be assumed that an electrode of appropriate time resolution was driven without any effect on the USL.

The experimental arrangement was similar to the one described previously (Antonenko et al., 1993; Pohl et al., 1993). Voltage sampling was performed by an electrometer (model 617; Keithley Instruments Inc., Cleveland, OH) connected via an IEEE interface to a personal computer. The microelectrode was moved perpendicular to the surface of the BLM by a hydraulic microdrive manipulator (Narishige, Tokyo, Japan). The touching of the membrane was indicated by a steep potential change (Antonenko and Bulychev, 1991). Because the velocity of the electrode motion was known (2 μm s^{-1}), the position of the microsensor relatively to the membrane could be determined at any instant of the experiment. The accuracy of the distance measurements was estimated to be ± 8 μm.

RESULTS

Concentration profiles of sodium ions in the immediate membrane vicinity under the conditions of a transmembrane volume flux were monitored. It is assumed that the observed effects are qualitatively similar for any other solute. Sodium-selective microelectrodes have the advantage that they are easy to prepare; furthermore, interactions between sodium ions and other components of the experimental system, i.e., the lipid and the buffer mixture, may be neglected.

In the absence of an osmotic gradient no concentration shift in the immediate membrane vicinity was detected (Fig. 1 *A, 1*). After the addition of urea to the *trans* side of the membrane a dilution of sodium ions was observed at the hypertonic side within the USL (Fig. 1 *A, 2*). An increase of the ion concentration occurred at the opposite side of the membrane (Fig. 1 *A, 2*). The absolute value of the difference between the near-membrane and the bulk concentrations at the *cis* side was equal to that at the *trans* side. As demonstrated in Fig. 1 *B*, the course of the experimental profiles was certainly uniexponential. If the sodium concentration shift is plotted against the distance to the membrane on a semilogarithmic scale, a straight line is obtained.

The thickness of the USLs at the *cis* and *trans* sides was calculated with the help of Eq. 5. The parametric equation

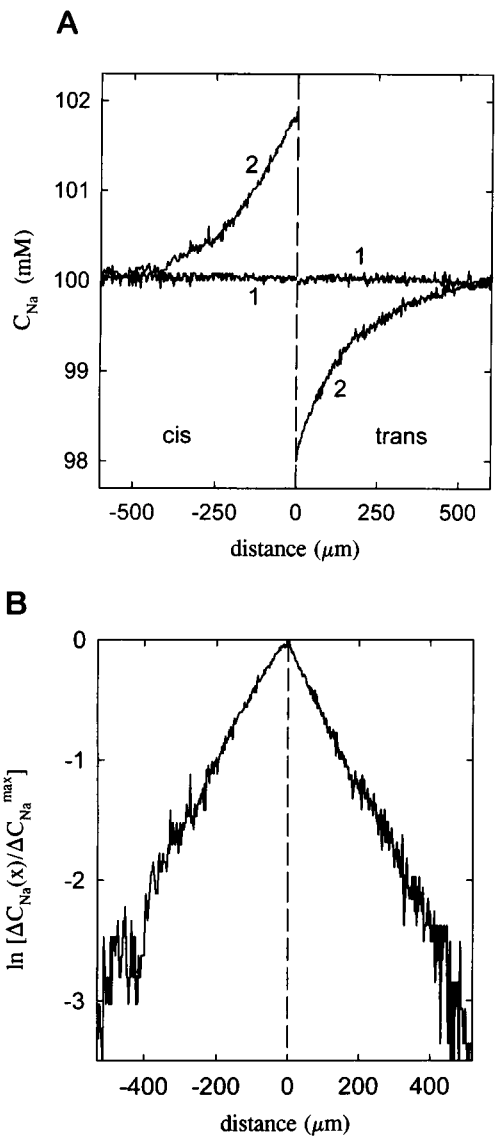

FIGURE 1 (*A*) Sodium ion concentration profiles in the immediate vicinity of a planar bilayer lipid membrane (made from DPhPC) in the absence of an osmotic gradient (*1*) and in the presence of 400 mM urea at the *trans* side (*2*). (*B*) The concentration distribution obtained under the conditions of an osmotic volume flow is drawn in a semilogarithmic scale. $\Delta C_{Na}(x)$ denotes the difference between the actual concentration at the distance x from the membrane and the bulk concentration. It is equal to ΔC_{Na}^{max} at the membrane surface ($x = 0$).

was fitted to the experimental data set. For the minimization of the least square residuals the program SigmaPlot (Jandel Corporation, San Rafael, CA) was used. This approach is preferred over the simple differentiation of the concentration profile as required by Eq. 1, because the SD is kept small, and the noise caused by the high impedance of the microelectrode is averaged.

Cholesterol is expected to decrease the hydraulic conductivity of membranes in which the acyl side chains of the phospholipids are unsaturated (Finkelstein, 1987). Indeed the difference, ΔC_{Na}, between the solute concentration in the membrane vicinity and in the bulk was dramatically decreased by the addition of cholesterol to the membrane-forming solution (Asolectin) if the osmotic concentration gradient was kept constant (Fig. 2).

On the contrary, an increase of ΔC_{Na} should be observed with an increase of volume flow. This situation was produced by an increasing osmotic gradient. The experimental result (Fig. 3) is qualitatively consistent with the theory. Within the osmotic pressure interval studied, however, ΔC_{Na} was found to be a linear function of the osmotic gradient only if the bulk solution was stirred very well (Fig. 5). The nonlinear dependence obtained at low stirring rates (Fig. 5) was due to a diminishing in size of the USL δ induced by the enhanced osmotic stress (Figs. 4 and 6). At a high stirring rate δ was found to be 140 $\pm$ 8 μm and did not depend on the osmotic gradient established (Fig. 3).

From the data shown in Fig. 5 it is not possible to make any conclusions about the effect of the stirring rate on ΔC_{Na}, because the lipid composition of the membranes investigated was different. Fig. 7 reports that the concentration changes at the surface of the membrane produced by osmotic flow actually decreases in size when the solution is stirred more vigorously. Stirring is known to increase the apparent osmotic flow rate, which implies smaller polarization effects due to an increase of the transfer rate of solutes between the bulk solutions and the membrane surfaces (Pedley, 1980).

DISCUSSION

Concentration profiles of solutes in the immediate vicinity of a bilayer lipid membrane generated by osmotic volume flow were measured for the first time. As predicted from theoretical considerations (Dainty, 1963; Pedley and Fischbarg, 1978), flux (Barry and Diamond, 1984), and streaming

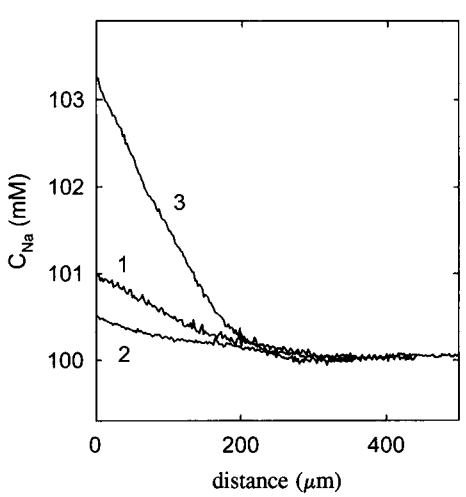

FIGURE 2 The difference between the solute concentration in the membrane vicinity (*cis* side) C_{Na} and in the bulk is dramatically decreased by the addition of cholesterol to the membrane-forming solution (*1*, cholesterol:Asolectin, 1:2; *2*, cholesterol:Asolectin, 1:1; *3*, pure Asolectin). The *trans* compartment contained 500 mM urea.

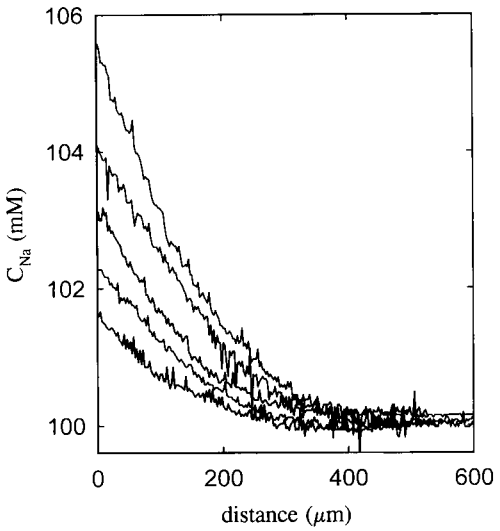

FIGURE 3 An increase of the urea concentration (300, 400, 500, 600, and 700 mM) at the *trans* side was accompanied by an increasing sodium concentration shift within the *cis*-USL. The bulk solution at both sides of the membrane made from Asolectin was stirred vigorously.

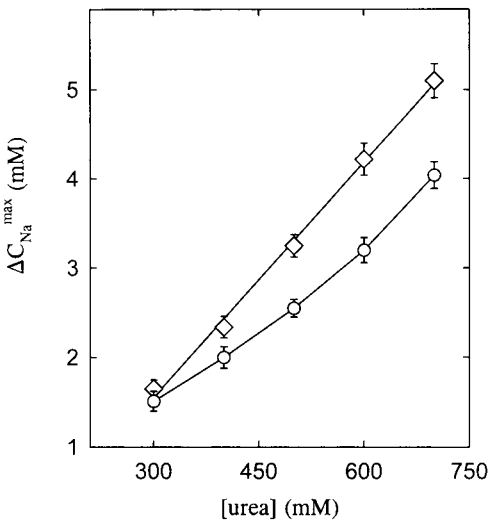

FIGURE 5 Absolute value of the difference between the sodium concentration adjacent to the membrane and in the bulk as a function of the urea bulk concentration at one side of the membrane (the urea concentration at the opposite side of the membrane was kept at zero) obtained for Asolectin and DPhPC bilayers at, respectively, small (*lower plot*) and high (*upper plot*) stirring rates of the bulk solution.

potential measurements (Levitt et al., 1978; Rosenberg and Finkelstein, 1978; Tu at al, 1994), the water flow alters the solute concentration at both sides of the membrane. The concentration profiles obtained by a microelectrode technique are in qualitative agreement with interferometrical measurements performed on synthetic membranes (Lerche, 1976; Kargol, 1994)

The standard physiological model of the USL (Eqs. 2–4) assumes that the only motion in the layer is the osmotic flux itself. Limitations caused by diffusion give rise to a concentration shift in the immediate membrane vicinity. The solute concentration in the near-membrane layers at the hypertonic side of the membrane increases, whereas it is decreased at the opposite side. C_s is picked directly from the experimental profiles, whereas δ is obtained after fitting the parametric Eq. 5 to the experimental data set. The hydraulic membrane permeability can be calculated according to Eqs. 4 and 11:

$$P_f = -\frac{D}{\delta C_{osm} V_w} \ln \frac{C_s}{C_b} \approx \frac{D(C_b - C_s)}{\delta C_{osm} C_b V_w} \qquad (12)$$

for small concentration differences ($C_b - C_s$). At the hypotonic side Eqs. 3 and 11 give an analogous result, where the concentration shift, $C_b - C_s$, is replaced by $C_s - C_b$. C_{osm} has to be corrected for urea dilution at the hypertonic side. Because the diffusion coefficients of urea (D_{urea}) and sodium (D_{Na}) are very close ($D_{urea} = 1.38\times10^{-5}$ cm^2/s; $D_{Na} = 1.33 \times 10^{-5}$ cm^2/s), Eq. 4 may be used to calculate the near-membrane concentration of urea, $C_{s,urea}$ from the sodium concentration at the interface C_s and the bulk concentrations of urea, $C_{b,urea}$, and sodium, C_b:

$$\frac{C_{s,urea}}{C_{b,urea}} = \frac{C_s}{C_b} \qquad (13)$$

With respect to the concentration gradient of NaCl that is induced by the volume flow, the transmembrane osmotic gradient is diminished further:

$$C_{osm} = C_{s,urea} - 4\Delta C \qquad (14)$$

For high flow velocities a nonsymmetrical concentration distribution is predicted by the standard physiological model of the USL; i.e., the solute concentration at the hypotonic side is believed to increase more rapidly than to decrease at the hypertonic side. Under our experimental

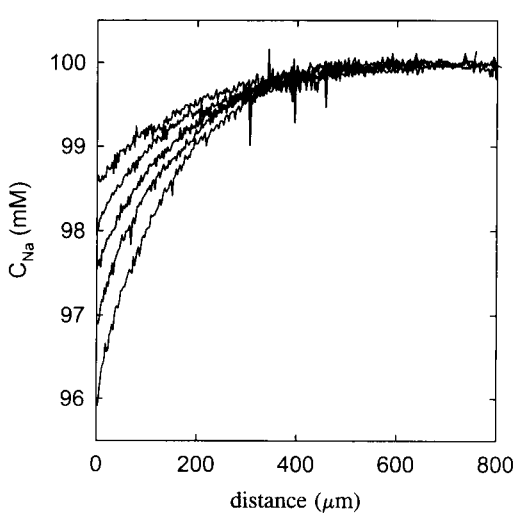

FIGURE 4 An increase of the urea concentration (300, 400, 500, 600, and 700 mM) at the *trans* side was accompanied by an increasing sodium concentration shift within the *trans*-USL. The stirring rate of the bulk solution was low. The planar membrane consisted of DPhPC.

conditions the predicted difference between the concentration shifts does not exceed the experimental error. It is therefore not surprising that for a given volume flow velocity the same absolute value of the concentration shift was measured at both sides of the membrane.

According to Eq. 12 the membrane permeability, P_f, varies from 30 to 45 μm/s as the transmembrane urea concentration gradient increases from 300 to 700 mM, respectively (Fig. 3). In view of the small errors of the estimation of δ (<7%) and C_{osm} (<0.5%), the variation is significant. According to the standard equations of irreversible thermodynamics, however, P_f is a constant for an isothermal system, independent of the nature or the concentration of the impermeant solute (House, 1974). Moreover, the value measured at 700 mM is twice the one given by Hanai and Haydon (1966) (19 μm/s) and Finkelstein (1976) (22 μm/s).

At a slow stirring rate the discrepancy between experiment and standard physiological theory becomes even worse. It is not only P_f that varies from 18 to 33 μm/s if calculated according to Eq. 12, but also the nonlinear dependence of the near-membrane concentration shift on C_{osm} (Fig. 5). If Eq. 12 were true, a linear function should have been observed. It is obtained, however, only for well-stirred conditions (Fig. 5). Most probably the nonlinear dependence (Fig. 5) is originated by a decrease of the USL thickness (Fig. 6). δ is nearly doubled by the increase of the osmotic gradient under our conditions. P_f should not depend on the stirring rate also. Nevertheless, if P_f is calculated according to the standard physiological model from the profiles shown in Fig. 7, its value increases from 38 to 43 μm/s as far as stirring rate increases.

Inadequacies of the conventional USL model have been known for almost as long as the approximation has been used. Introducing the correction for USL effects (see Eqs. 2–4) Dainty (1963) has emphasized that it is approximate and probably an overcorrection. Hydrodynamic studies have demonstrated that the USL is related to the VBL (Dainty and House, 1966). A complete description of the transmembrane convective flow requires consequently that fluid motions are taken into account. Proper solutions of the equations for simultaneous convection and diffusion have only proved possible for a few special geometries. According to the theory of Levich (1962), δ is a function of the diffusion coefficient of the solute, the velocity and viscosity of the solution. Pedley (1980) has developed a model for the interaction between stirring and osmosis in which he proposed that the stirring motions in the bulk solution, which counter the osmotic advection, can be represented as a stagnation point flow. His examination of the hydrodynamic description of the two-dimensional flow reveals that it is possible for the solute concentration to be independent of the coordinate parallel to the membrane (see Theory). Indeed, because the diameter of the microelectrode is two orders of magnitude smaller than that of the membrane, the latter can be represented as an infinite plane. It is further considered that the convective flow is uniformly oriented so that changes of the concentration over the surface of the membrane may be neglected—at least in the vicinity of the electrode. Because the USL is much thinner than the VBL, it is only the velocity very near the membrane that is important (Pedley, 1983). Consequently, Eq. 10 can be applied for $-\delta \leq x \leq \delta$ only. In view of the restrictions mentioned above, it seems impossible to give a simple theoretical basis for empirical relation 5.

From Eq. 10 it is obvious that the USL thickness is a function of the osmotic gradient if the stirring parameter a is small. This prediction is in agreement with the experimental observations (Fig. 6). In this sense the current anal-

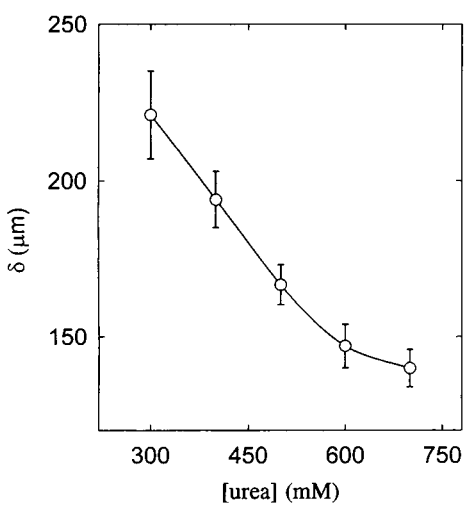

FIGURE 6 At a low stirring rate (conditions as in Fig. 4) the thickness of the USL δ was a function of the osmotic stress, induced by the addition of urea to the bulk solution at one side of the membrane. δ was found from uniexponential fittings of the experimental concentration profiles (compare with Fig. 4).

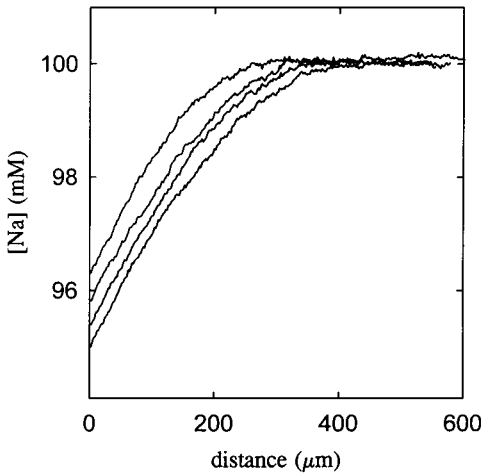

FIGURE 7 Sodium concentration profiles in the immediate membrane vicinity at different velocities of the magnetic stirrer bars. The near-membrane concentration shift decreases in parallel with an increase in the stirring rate (from bottom to top). Urea concentration at the hypertonic side of the membrane was 600 mM. Asolectin was used to form the membranes.

ysis is an improvement of the treatment carried out by Pedley (1980, 1983) in which the USL thickness was assumed to be a constant that can be derived from the diffusion coefficient, the stirring parameter, and the kinematic viscosity. Considering that the velocity of the transmembrane volume flow v is the only fluid velocity that is different from zero at the membrane water interface, it becomes obvious that the transmembrane flow must affect the concentration distribution in the immediate membrane vicinity.

Modifications of the USL governed by an increase of the osmotic pressure gradient are reported also for rabbit proximal convoluted tubules (Berry and Verkman, 1988). The underlying mechanism, however, is completely different from the one described herein. A physiological downregulation of the osmotic water permeability is explained by a decrease of the solute diffusion coefficient within a complex cytoplasmic USL (Berry and Verkman, 1988).

v is found by fitting Eq. 10 to the concentration profiles within the interval $-\delta < x < \delta$. The dependency of the parameter determined by the least squares approximation is always better than 96%. After inserting v into Eq. 10, the calculation of P_f gives 25 ± 2 and 20 ± 2 μm/s for membranes made from Asolectin and DPhPC, respectively. The result of the calculations does not depend on the stirring rate (Fig. 7) or the transmembrane osmotic gradient (Figs. 3 and 4). It is in reasonable agreement with literature data obtained for planar bilayer lipid membranes (Hanai and Haydon, 1966; Finkelstein, 1976).

From Eq. 10 it is not possible to predict the near-membrane concentration of the solute even if its bulk concentration and the hydraulic membrane permeability are known. This circumstance is easy to understand, because diffusion limitations are mainly determined by the geometry of the particular system under investigation. Indeed, the size of the USL is correlated to the dimensions of the object under study (Mierle, 1985). From the combination of Eqs. 1 and 10 it would be possible to obtain an expression that may be helpful for the estimation of the solute concentration in the immediate membrane vicinity if δ and P_f (and, therefore, v) are known. There is, however, no guarantee that the size of the USL obtained from time course measurements (compare Cotton and Reuss, 1989) is identical to that derived from combined Eqs. 1 and 10. The error introduced should increase when there is an interaction of osmosis with stirring as when there is none ($a = 0$ in Eq. 10). The same conclusion was drawn earlier for the standard physiological model (Barry and Diamond, 1984; Pedley, 1983).

Provided that the profile course is known, a comparison of the results obtained with both models shows clearly that the flow velocity may be overestimated by the standard physiological model. The discrepancy becomes increasingly large with increased volume flow and enhanced stirring rate. Under our conditions an overestimation by a factor of 2 was the worst case. At a low stirring rate δ is a function of the volume flow velocity. Consequently, an additional error is introduced if the estimation of the near-membrane concentration is carried out assuming a constant δ. At high stirring rates Eqs. 3 and 4 correctly predict a constant USL thickness, which does not vary with the osmotic gradient. The hydrodynamic theory gives the same result, because the large velocity gradient that develops in this case at the membrane-water interface is only slightly modified by the comparatively low velocity of the transmembrane water flow. Because the concentration gradient at the interface is assumed to be a function of the velocity gradient, it is not expected to be changed either.

Although the hydrodynamic description of the transmembrane volume flow given in Eq. 10 is derived due to an oversimplification of the system, the concentration distribution within the USL is described accurately. Microelectrode measurements of solute concentration profiles in the immediate membrane vicinity provide a potentially useful way of determining the hydraulic conductivity of the bilayer.

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