EFFECTS OF HYDROSTATIC PRESSURE ON LIPID BILAYER MEMBRANES

I. Influence on Membrane Thickness and Activation Volumes of Lipophilic Ion Transport

ROLAND BENZ* AND FRANCO CONTI‡
*Lehrstuhl für Biotechnologie der Universität Würzburg, Röntgenring 11, D-8700 Würzburg, Federal Republic of Germany; and ‡Istituto di Cibernetica e Biofisica, c/o Dipartimento di Fisica, Universita di Genova, I-16146 Genova, Italy

SUMMARY Measurements of membrane capacitance, C_m, were performed on lipid bilayers of different lipidic composition (diphytanoyl phosphatidylcholine PPhPC, dioleoyl phosphatidylcholine DOPE, glycerlymonooleate GMO) and containing *n*-decane as solvent. In the same membranes, the absorption of the lipophilic ions dipicrylamine (DPA^-) and tetraphenylborate ($TPhB^-$), and the kinetics of their translocation between the two membrane faces have been studied. The data were obtained from charge pulse relaxation measurements. Upon increasing pressure the specific capacity C_m increased in a fully reversible and reproducible way reflecting a thinning of the membrane that is attributed to extrusion of *n*-decane from the black membrane area. High pressure decreased the rate constant, k_i, for lipophilic ion translocation. After correcting for changes in the height of the energy barrier for translocation due to membrane thinning the pressure dependence of k_i yields an apparent activation volume for translocation of ~ 14 cm^3/mol both for DPA^- and $TPhB^-$. Changes in lipophilic ion absorption following a step of pressure developed with a rather slow time course due to diffusion limitations in solution. The stationary concentration of membrane absorbed lipophilic ions increased with pressure according to an apparent volume of absorption of about -10 cm^3/mol. The relevance of the results for the interpretation of the effects of pressure on nerve membrane physiology is discussed.

INTRODUCTION

Pressure effects on biological membranes have attracted the interest of several investigators in recent years with the hope that they provide information about membrane structure and thermodynamic properties of functional membrane proteins (Heremans, 1982; MacDonald, 1984). Pressure promises to be a valuable test parameter, particularly in the study of excitable membranes, where it has long been known to have marked physiological effects (for a review see Wann and MacDonald, 1980), one of the most interesting ones being the reversal of the action of several anesthetic drugs (Spyropoulos, 1957; Kendig and Cohen, 1977; Franks and Lieb, 1982). In the membrane of the squid giant axon the pressure dependence of ionic currents (Conti et al., 1982*a*, *b*) has been described in terms of activation and reaction volumes involved in the conformational changes of ionic channels, an interpretation that is also supported by evidence that the structure of the lipid matrix of the membrane does not undergo phase transitions or drastic fluidity changes up to pressures of 80 MPa (Benz et al., 1984).

Studies of the influence of pressure on the transport properties of model membranes have first been reported by Johnson et al. (1973) and Johnson and Miller (1975). These experiments, dealing with the permeability properties of lipid vesicles, could not discriminate between the pressure effects on binding and those on transmembrane mobility of the transport systems. More recently Bruner and Hall (1983) reported measurements of the pressure dependence of the conductance and kinetics of the voltage-gated alamethicin pores in planar bilayers, and Moronne and Macey (1985) have described lipid bilayer measurements of apparent activation volumes involved in the translocation of ion carriers and lipophilic ions.

The experiments reported in this work address questions similar to those asked by Moronne and Macey (1985). They yield additional information concerning the absorption of lipophilic ions to lipid bilayers, which appears to be markedly affected by pressure. Our experiments also show that at high pressure *n*-decane is extruded from the hydrocarbon core of the lipid bilayers in the torus and microlenses which causes membrane thinning. After correcting for this effect we obtain estimates of the activation volumes for lipophilic ion translocation that are significantly higher than those reported by Moronne and Macey (1985).

MATERIALS AND METHODS

The lipid bilayer membranes were obtained in the usual way (Benz et al., 1973) from solutions of various lipids in *n*-decane (Fluka, Buchs, Switzerland, purum). The cell used for bilayer formations was made of Teflon and its design is described below. Dioleoyl phosphatidylcholine (DOPC), diphytanoyl phosphatidylcholine (DPhPC) and dioleoyl phosphatidylethanolamine (DOPE) were purchased from Avanti Biochemicals (Birmingham, AL). Glycerolomonooleate (monoolein, GMO) was obtained from NuCheck Prep (Elysian, MN). Stock solutions in ethanol of dipicrylamine (DPA^- Fluka, puriss.) and tetraphenylborate ($TPhB^-$ Merck, Darmstadt, German Federal Republic) were prepared at concentrations of 10^{-3} M. Small amounts of these solutions were added to the aqueous phases bathing the membranes to get final concentrations between 10^{-7} M and 10^{-8} M. In this concentration range the partition coefficient of the probe molecules between the aqueous phase and the membrane is expected to be constant (Wulf et al., 1977) and the absorbed lipophilic ions should not produce large boundary potentials (McLaughlin, 1977). The unbuffered aqueous solutions (pH ~6) contained either 0.1 M or 1 M NaCl (Merck, Darmstadt, German Federal Republic).

The charge pulse relaxation measurements were performed as previously described (Benz et al., 1976; Benz and Conti, 1981). Electrical connection to the solutions on both sides of the membrane was obtained via two Ag-AgCl/platinum-black electrodes simply prepared by electrolytic deposition of AgCl and Pt on clean Ag wires. The membrane capacitance was charged up to a voltage of ~10 mV by a brief current pulse (20–50 ns duration) delivered by a pulse generator (Philips PM5712) via a diode with a reverse resistance $>10^{12}$ Ohm. The voltage transient following a charge pulse was amplified 20 times using a Burr-Brown 3551J operational amplifier (Burr-Brown Corp., Tucson, AZ), and stored in a digital oscilloscope (Nicolet Explorer III; Nicolet Scientific Corp., Northvale, NJ). The time resolution of the relaxation measurements was better than 500 ns. The analysis of the records was carried out with a Hewlett-Packard computer (model 9825; Hewlett-Packard Co., Palo Alto, CA). To measure the membrane capacitance a relatively fast discharge of the membrane potential after a charge pulse was artificially produced by adding to the circuit a resistor of known value in parallel with the membrane. The specific membrane capacitance, C_m, was obtained from the time constant of the discharge and from an estimate of the black membrane area.

The pressurizing system (Nova Swiss Werk, Zürich, Switzerland) used vaseline oil as pressure transmitting medium and has already been described previously (Conti et al., 1984; Benz et al., 1984). It consisted of a pressure bomb (internal cylindrical volumed of 100 cm^3, 25 mm cross-sectional diameter) and capable of withstanding pressures up to 150 MPa (~1,500 atm), a hand driven hydraulic pump, and a pressure gauge with 0.1 MPa sensitivity. During the experiments >90% of the inner volume of the bomb was occupied by the cylindrical Teflon cell (see Fig. 1).

First a standard cell with two identical compartments was used. However, we could not pressurize such a system above 5 MPa because of pressure gradients across the membranes, which resulted in their rupture. A solution to this problem was to reduce as much as possible the volume to surface ratio in one of the two compartments, and keep it tightly closed. In the cell that was finally used the volume of the large compartment was ~1.5 ml, that of the small compartment was ~1 μl, and the membrane hole (diameter 1 mm) was horizontal. The small compartment was not accessible from outside after formation of the membrane because it was closed on the other side by the electrode. A schematic drawing and a photograph of one of the membrane cells used is shown in Fig. 1. With this cell a hydrostatic pressure of 150 MPa could be reached without membrane disrupture. The horizontal bilayer had also the advantage that the membrane formation and the magnitude of the torus could be easily inspected from above.

After having followed the formation of an optically black membrane the chamber was closed with a thin Teflon cover to avoid contact of the aqueous phase with the oil and the chamber was transferred into the pressure bomb. At the end of every experiment the membrane cell was cleaned with organic solvents to remove completely the vaseline oil, and dried under vacuum. Before starting a new experiment the membrane hole was pretreated with a droplet of lipid solution in chloroform and the chloroform was evaporated. This procedure was needed to obtain membranes which were stable for hours. After the cell was filled with the aqueous solution it was kept under vacuum for 5 min to remove any trapped air in the small compartment which could cause bulging of the membranes at high pressure. The experiments were performed at temperatures between 20 and 22°C, usually 15 to 20 min after the membranes had become black and stationary conditions were reached.

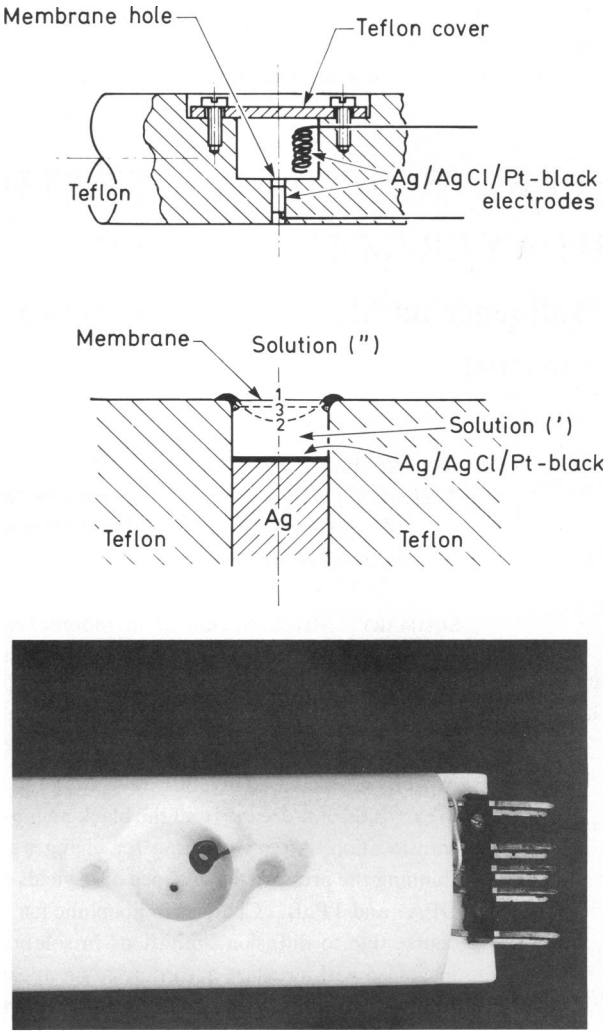

FIGURE 1 Schematic diagram of the membrane cell. (*A*) *upper* part: section of the chamber; *lower* part: detailed section of the chamber near the membrane hole. Three possible configurations of the lipid bilayer are shown schematically. (*1*) Normal configuration at atmospheric pressure; (*2*) possible bulged configuration at high pressure caused by the compression of solution ("); (*3*) displaced configuration, by which the compression of solution (') is likely compensated at high pressure. (*B*) Photograph of the cell.

THEORY

The theory of the movement of the lipophilic ions across lipid bilayer membranes has been given in full detail in previous publications (Ketterer et al., 1971; Benz et al., 1976; Benz and Conti, 1981). Here we will only summarize the basic assumptions and list the simplified equations that

allow the calculation of the transport parameters from our present experimental data. It is assumed that the lipophilic ions are absorbed to relatively deep free energy minima on both sides of the membrane with a total concentration per unit surface, N_t. For symmetry reasons (equal solutions and symmetric bilayer) it is assumed that at zero membrane potential the lipophilic ions are equally distributed between the two membrane-solution interfaces and cross the intermediate free energy barrier with the same rate constant, k_i, in either direction. Under these conditions the shape of the barrier has no influence on the characteristics of the voltage relaxations measured in a charge pulse experiment as long as the initial membrane potential perturbation is much <25 mV (Benz and Zimmermann, 1983). Finally, we neglect the exchange of lipophilic ions between the membrane and the aqueous phase, during a single relaxation measurement, because it is rate limited by slow aqueous diffusion (Benz et al., 1976) and it has a characteristic time much longer than the time constant of the lipid bilayer membranes.

In a charge pulse experiment the system is in equilibrium at times $t < 0$, and the membrane capacitance is charged instantaneously at $t = 0$ to an initial voltage V_m^o The following decay of the membrane voltage $V_m(t)$, is given by two exponential relaxations:

$$V_m(t) = V_m^o[a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)], \quad (1)$$

where a_1, $a_2 = 1 - a_1$, τ_1 and τ_2 are known functions of k_i, N_t, and the membrane time constant, τ_m (Benz and Conti, 1981; Benz and Zimmermann, 1983). The inverse relations are given by

$$k_i = (a_1/\tau_2 + a_2/\tau_1)/2 \quad (2)$$

$$N_t = 2RTC_m(1/\tau_1 + 1/\tau_2 - 2k_i - 1/\tau_1\tau_2k_i)/k_iF^2, \quad (3)$$

where R is the gas constant, T is the absolute temperature, and F is the Faraday constant.

RESULTS

Influence of Hydrostatic Pressure on Membrane Capacitance

Fig. 2 shows the influence of hydrostatic pressure, P, on the discharge process of a DOPE membrane across a 104 KΩ resistor, following brief charge pulses of 50 ns duration. The relaxation curves shown in the figure were recorded at atmospheric pressure (0.1 MPa) (trace 1), at 70 MPa (trace 2), at 150 MPa (trace 3), and again at 0.1 MPa (trace 4). The exact superposition of records 1 and 4 demonstrates the excellent reversibility of this type of measurement. The data were very accurately fitted by single exponentials with the following time constants: 362 μs at 0.1 MPa (curve 1); 387 μs at 70 MPa; 403 μs at 150 MPa, and 365 μs after return to 0.1 MPa (curve 4). Thus, increasing pressure increases the membrane capacitance up to ~12% at 150 MPa. The specific capacitance at 0.1

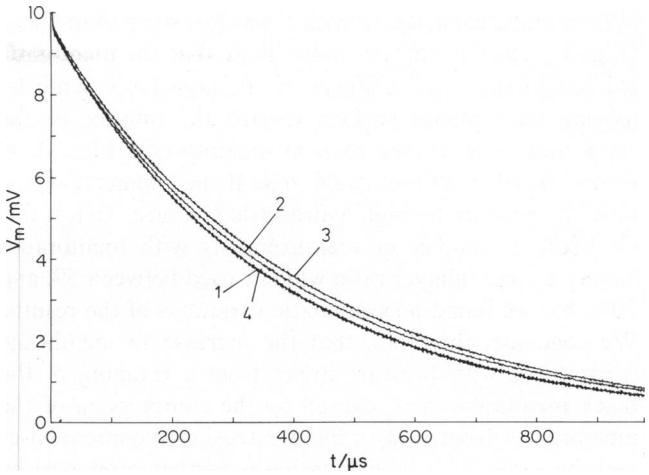

FIGURE 2 Effect of pressure in membrane capacitance. Membrane potential relaxations following charge pulses in a DOPE/*n*-decane membrane with a resistor of 104 KΩ placed in parallel to the membrane electrodes at the hydrostatic pressures indicated; 0.1 M NaCl.

MPa was estimated to be ~0.35 μF/cm² (black-membrane area ~1.0 mm²) in good agreement with earlier published results (Benz and Janko, 1976). Similar results were also obtained with membranes composed of other lipids. At 150 MPa the capacitance of DOPC membranes increased by ~11% and a similar effect was found for GMO/*n*-decane membranes. Attempts to study GMO/hexadecane membranes were not successful because these bilayers usually broke at pressure ~60 MPa. The overall data of capacitance measurements with different lipids using *n*-decane as a solvent are given in Fig. 3.

We could think of only two possible artifacts that would produce a capacitance increase at high pressure. First, the compression of the aqueous volume of the closed compartment could force the membrane to bulge. However, considering the isothermal compressibility of water (~6% decrease in volume at 150 MPa [McGowan, 1976]) and the geometry of the chamber, we expect from this effect an increase in membrane surface at 150 MPa of 5% at most. Furthermore, it can be shown that such an effect would be roughly proportional to P^2 (the compressibility of water decreases only slightly with P) while the observed increase

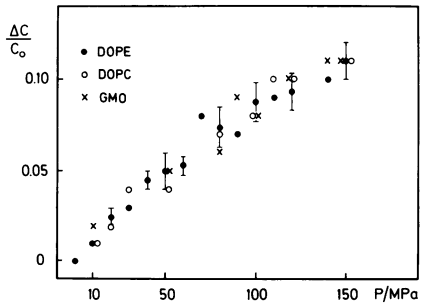

FIGURE 3 Relative change of the membrane capacitance of DOPE/*n*-decane, DPhPC/*n*-decane and GMO/*n*-decane membranes at various hydrostatic pressures.

of membrane capacitance with P was less steep than linear (Fig. 3). Thus, it appears more likely that the membrane reacted to the small changes of the aqueous volume by moving as a planar surface toward the interior of the supporting hole, rather than by bulging (see Fig. 1). A second possible artifact could arise if the geometry of the torus changed under high hydrostatic pressure. To test this we made a number of measurements with membranes having a torus/bilayer ratio which varied between 5% and 20%, but we found no systematic variations of the results. We conclude, therefore, that the increase in membrane capacitance with pressure arises from a thinning of the black membrane area, caused by the compression of the membrane solvent, and/or by its extrusion into microlenses and the torus. This interpretation is further supported by an indirect argument given later.

Pressure Dependence of DPA^- Absorption and Translocation

Fig. 4 shows records of the voltage relaxation following brief charge pulses applied to a DOPE membrane separating two solutions containing 1 M NaCl and 10^{-7} M DPA^-. These records were obtained in a series of experiments in which within ~10 min the pressure was raised from 0.1 MPa (trace 1) to 150 MPa (trace 2), and lowered again to 0.1 MPa (trace 3). It is obvious that increasing P strongly influences the relaxation processes. Furthermore, the influence of pressure is only partially reversible within a time scale of a few minutes.

The analysis of the measurements in terms of changes in k_i and N_t (compare the legend of Fig. 4) shows that the pressure effect on k_i was in fact fairly reversible from a 40% decrease at 150 MPa. On the other hand, N_t increased with pressure but this process was so slow that we observed some kind of hysterisis. In another set of experimental conditions we studied this hysterisis in more detail. One of these experiments is illustrated in Fig. 5. P was rapidly taken to 100 MPa (in <1 min) and the kinetics of the lipophilic ions was repetitively tested at constant P for ~20 min. The pressure was then released within 10 s and we took again charge pulse experiments at certain time intervals. The whole procedure was then repeated for 150 MPa. Fig. 5 *A* shows the time course of the ratio between N_t and its initial value at 0.1 MPa and Fig. 5 *B* shows similar data for k_i. It is obvious that N_t changed slowly with time after a sudden pressure change while k_i showed a smaller time dependence and reached a stationary value much more quickly. The pressure dependence of the stationary values of N_t and k_i appears fully reversible in these experiments and it implies a negative volume of DPA^- absorption by the membrane and a positive activation volume for DPA^- translocation.

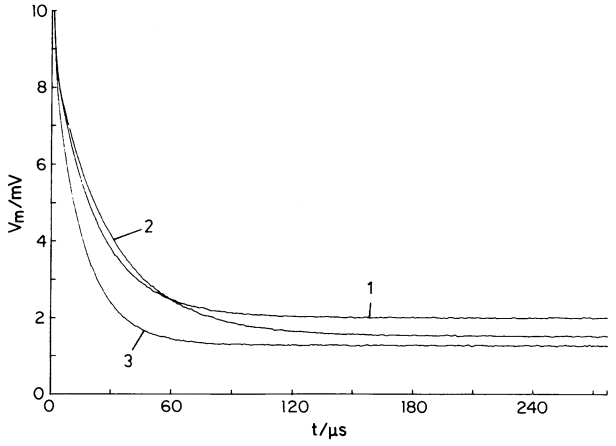

FIGURE 4 Records of voltage relaxations following charge pulses applied to a DOPE/*n*-decane membrane separating two solutions containing 0.1 M-NaCl and 10^{-7} M-DPA^-. Traces 1 to 3 represent measurements at 0.1 MPa, 150 MPa and return to 0.1 MPa. The measurements are part of a longer series lasting ~10 min in which P was raised stepwise up to 150 MPa and decreased again to 0.1 MPa. The analysis of the records in terms of Eqs. 1–3 gave the following results: (*1*) 0.1 MPa, τ_1 = 22 μs, τ_2 = 73 ms, a_1 = 0.78, k_i = 5,000 s^{-1}, N_t = 1.4 pmol/cm^2; (*2*) 150 MPa, τ_1 = 29 μs, τ_2 = 74 ms, a_1 = 0.83, k_i = 3,000 s^{-1}, N_t = 1.9 pmol/cm^2; (*3*) 0.1 MPa, τ_1 = 16 μs, τ_2 = 100 ms, a_1 = 0.85, k_i = 4,600 s^{-1}, N_t = 2.3 pmol/cm^2. The temperature was 20°C. C_m = 370 nF/cm^2.

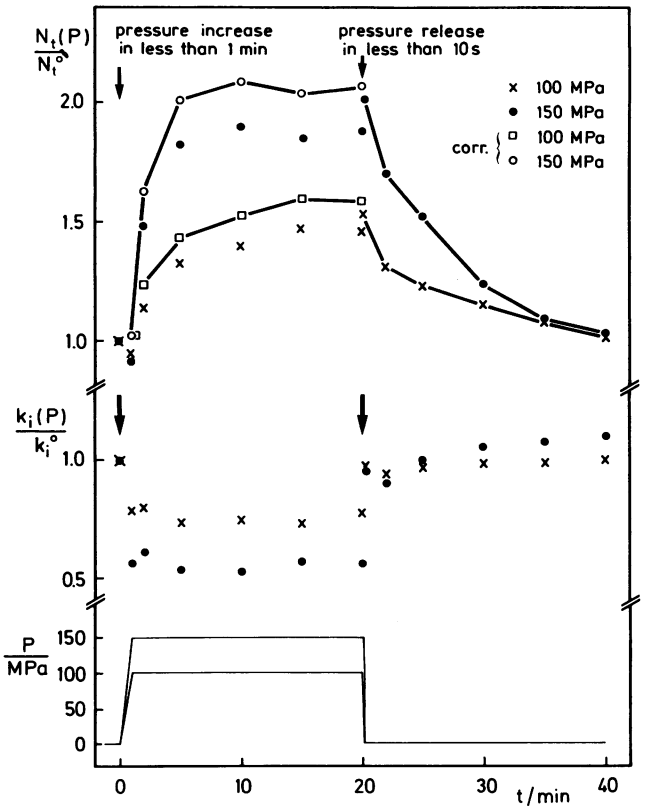

FIGURE 5 Time course of the translocation rate constant, k_i, and the surface concentration, N_t, of DPA^- absorbed on a DOPE/*n*-decane membrane, in response to step changes in pressure, P. k_i° and N_t° are estimates of k_i and N_t prior to the application of the step pressure increase diagrammed in *C*. The N_t data in *A* were corrected, yielding the points connected by lines, according to the change in specific membrane capacity estimated from the smooth line of Fig. 3. Bathing solutions: 0.1 M NaCl; 10^{-7} M DPA.

The systematic weavy time course of k_i immediately after a step in P can be easily explained on the basis of small temperature changes. This was shown in control experiments in which a small thermistor was placed at the membrane location. After a sudden rise of pressure from 0.1 to 150 MPa we observed 3 phases: (*a*) a small rapid increase of T by ~1.5°C; (*b*) a further increase of T by 1°C within 1 min because of the heat convection from the oil bath (which warmed up more than water); and (*c*) a final reequilibration due to heat absorption by the pressure vessel walls and the Teflon chamber, which was complete in <5 min. The translocation rate constant k_i followed rather faithfully these temperature changes because of its positive activation energy (Benz et al., 1976).

The slow time course of N_t is expected because changes in the concentration of membrane adsorbed molecules are rate limited by diffusion in the aqueous phase (Conti et al., 1974). The characteristic time constant for such processes, τ_D, is given by β^2/D, where β is the membrane to water partition coefficient of DPA^- and D is its diffusion constant in water. In our case, $\beta \sim 10^{-2}$ cm (Benz and Läuger, 1977, and this paper), and assuming D of the order of 10^{-6} cm^2/s, τ_D is expected of the order of 100 s.

An interesting feature of the N_t changes shown in Fig. 5 is the apparent sudden jump following every step in P. This discontinuity in the time course of N_t is completely removed if we account for the change of the specific capacitance with pressure when we calculate N_t using Eq. 3. This is shown by the open symbols in Fig. 5 representing N_t values corrected for the average change of C_m at 100 MPa and 150 MPa according to the data of Fig. 3. This result provided further independent evidence that the specific capacitance, rather than the membrane area is pressure dependent.

From experiments such as that just discussed a quantitative description could be obtained for the effect of pressure on the absorption of DPA^- on DOPE/*n*-decane membranes. The pressure dependence of N_t, corrected for C_m changes is shown in Fig. 6. Since it is known that the absorption of lipophilic ions to membrane-solution interfaces is fairly independent of membrane thickness (Benz and Läuger, 1977; Benz and Gisin, 1978) these data reflect the pressure dependence of the partition coefficient of DPA^-. The straight line is a least squares fit of the data according to

$$\ln(N_t(P)/N_t^\circ) = -\Delta V^*(P - P_o)/RT, \qquad (4)$$

where N_t° is the value of N_t at atmospheric pressure (0.1 MPa) and $\Delta V^* = -12$ cm^3/mol. According to classical arguments of thermodynamics ΔV^* represents the volume change per mol DPA^- absorbed to DOPE membranes.

A correct description of the effect of pressure on the translocation of lipophilic ions must also account for the fact that an important contribution arised from changes in membrane thickness. When the latter is decreased the Born electrostatic energy barrier is lowered and results in an increase of k_i. The dependence of the Born energy on membrane thickness is well characterized both by a detailed theory (Parsegian, 1969) and by measurements on membranes containing different solvents (Benz and Läuger, 1977; Benz and Gisin, 1978; Pickar and Benz, 1978; Dilger and Benz, 1985). For a change of $\Delta W \cdot kT$ in the height of the barrier, the translocation rate constant, k_i, is expected to change by a factor $\exp(-\Delta W)$. When a change in thickness from d^* to d is the only structural modification responsible for ΔW, it is given by (Benz and Läuger, 1977)

$$\Delta W = h(1/d^* - 1/d)$$
$$h \approx 17.8 \text{ nm}. \qquad (5)$$

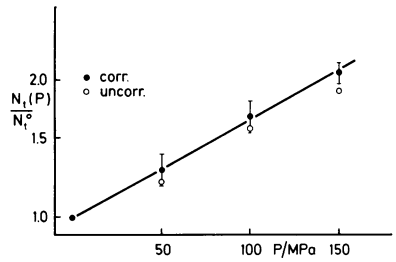

FIGURE 6 Pressure dependence of the equilibrium concentration, N_t, of absorbed DPA^- on DOPE/*n*-decane membranes. The data are plotted as ratios to the estimates obtained at atmospheric pressure, before pressurization. Each measurement was obtained after long equilibration time and was corrected for specific capacity changes as discussed in connection with Fig. 5. The straight line through the data obeys Eq. 8 with $\Delta V^* = -12$ cm^3/mol. The latter quantity is the estimated volume change associated with the absorption of a mole of DPA^- from water into the lipid bilayer.

The change in k_i can be estimated on the basis of capacitance measurements as was demonstrated by Pickar and Benz (1978) and Dilger and Benz (1985). The results for the pressure induced capacitance changes are given in Table I. The change of k_i was estimated to be ~1.45 at 150 MPa. After correcting the thickness effect the direct influence of pressure on k_i appears to be stronger. The data for DPA^- in DOPE/*n*-decane membranes are shown in Fig. 7, together with similar data for $TPhB^-$ which are given below. The semilogarithmic plot of the corrected k_i vs. pressure is fairly well fitted by a straight line. Both DPA^- and $TPhB^-$ data are fitted by approximately the same straight line with equation:

$$\ln(k_i(P)/k_i^\circ) = -\Delta V^\ddagger (P - P_o)/RT, \qquad (6)$$

where $P_o = 0.1$ MPa and $\Delta V^\ddagger$ is ~14 cm^3/mol. The latter quantity would represent the true activation volume for lipophilic ion translocation if we could assume that extrusion of *n*-decane does not affect significantly the free energy barrier of the process, apart from the above discussed modifications caused by the thickness changes. We would like to stress the point, however, that without correcting for the thickness change $\Delta V^\ddagger$ is already ~8

TABLE I
INFLUENCE OF HYDROSTATIC PRESSURE ON MEMBRANE CAPACITANCE AND CORRECTION FOR THE TRANSLOCATION RATE CONSTANTS OF LIPOPHILIC IONS

P/MPa	C_m/C_m^o	exp $(-\Delta W)$	DPA$^-$ $\frac{k_i(P)}{k_i^o}$	TØB$^-$ $\frac{k_i(P)}{k_i^o}$
30	1.033	1.125	0.84	0.83
40	1.042	1.166	0.78	0.75
50	1.05	1.195	0.74	—
60	1.06	1.23	0.71	0.71
70	1.068	1.274	0.59	0.61
80	1.074	1.301	0.62	0.60
90	1.08	1.33	0.60	0.59
100	1.085	1.354	0.55	0.58
120	1.095	1.403	0.50	0.52
150	1.105	1.454	0.41	0.45

Mean relative change with pressure of the rate constant, k_i, for DPA$^-$ and TPhB$^-$ translocation across DOPE/*n*-decane membranes, after correction for the effect of thickness changes. The corresponding factors (column 3) were obtained from mean capacity changes (column 2).

cm^3/mol and thus considerably larger than that found by Moronne and Macey (1985).

The influence of the pressure on DPA$^-$ absorption and transport was also tested with other lipid bilayer systems, such as DOPC/*n*-decane and DPhPC/*n*-decane. The volume of absorption was of the same sign and had about the same magnitude for all membrane systems. For DOPC-membranes it was estimated -9 cm^3/mol and for DPhPC-membranes -10 cm^3/mol. Also the pressure dependence of the DPA$^-$ translocation rate constant was very similar in all membrane systems.

We tested also the influence of the aqueous salt concentration on our data. We could not detect any significant difference upon varying the NaCl-concentration from 0.1 to 1 M. Thus, we conclude that the ionic strength of the bathing solutions has no strong influence on the reaction and activation volume involved ion the absorption and mobility of DPA$^-$ in lipid bilayer membranes.

Pressure Dependence of TPhB$^-$ Absorption and Translocation

In the past it has been shown that the membrane structure has a similar effect on the kinetics of TPhB$^-$ and DPA$^-$, apart from the fact that the k_i for TPhB$^-$ is 20–40 times smaller than for DPA$^-$ (Benz et al., 1976; Benz et al., 1983). Thus, it is no surprise that pressure has more or less the same effect on TPhB$^-$ transport as on DPA$^-$ transport. Fig. 8 illustrates charge pulse experiments with a DOPE/*n*-decane membrane in the presence of 10^{-7} M TPhB$^-$ in the aqueous phase, performed at different hydrostatic pressures with a protocol similar to that of Fig. 4. Trace 1 was taken at atmospheric pressure, trace 2 at 150 MPa. Trace 3 shows the control after return to 0.1 MPa. As in

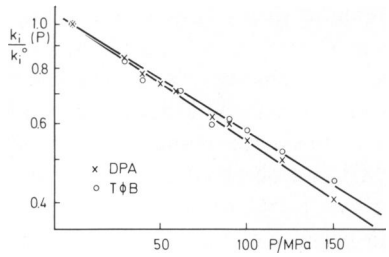

FIGURE 7 Pressure dependence of the rate of lipophilic ion translocation across DOPE membranes. The data obtained from voltage relaxation measurements were corrected for the effect expected from changes in membrane thickness. The straight line is a fit by hand of the data according to Eq. 6 and corresponds to an apparent activation volume of 14.7 cm^3/mol (DPA$^-$) and of 13.5 cm^3/mol (TPhB$^-$).

the case of DPA$^-$ the short term effects of pressure on TPhB$^-$ transport showed a marked hysterisis of change of N_t while variations of k_i were rapidly reversible. A pressure of 150 MPa caused an apparent decrease of k_i of ~30%. This would correspond to a $\Delta V^{\#}$ (Eq. 6) of ~7 cm^3/mol. Upon correcting for thickness changes this would correspond to a much larger effect on k_i. The average pressure dependence of the corrected k_i estimated from three different membranes is shown in Fig. 7. The apparent activation volume for TPhB$^-$ translocation obtained from the fit of the data according to Eq. 6 is ~14 cm^3/mol, practically indistinguishable from that of DPA$^-$. The concentration of absorbed TPhB$^-$ showed a pressure dependence similar to that described above for DPA$^-$. From experiments with membranes pressurized for long periods of time the reac-

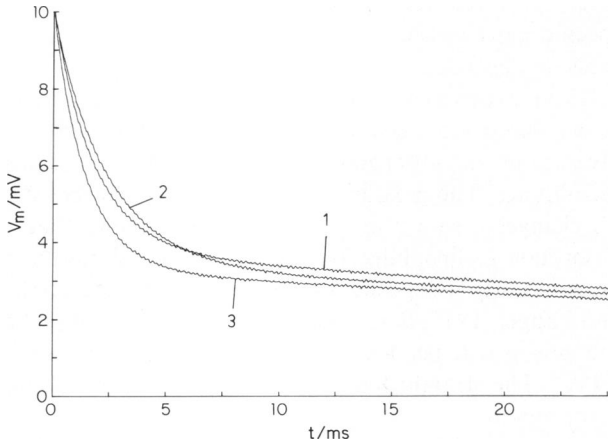

FIGURE 8 Charge pulse relaxation records from a DOPE/*n*-decane membrane separating identical solutions containing 0.1 M-NaCl and 10^{-7} M-TPhB$^-$. Records 1 to 3 were obtained in the same order in successive measurements at 0.1 MPa, 150 MPa, and 0.1 MPa. The measurements are part of a longer series lasting ~10 min between 1 and 3 in which P was raised stepwise up to 150 MPa and decreased again to 0.1 MPa. The analysis of the records in terms of Eqs. 1–3 gave the following results: (*1*) 0.1 MPa, τ_1 = 1.9 ms, τ_2 = 82 ms, a_1 = 0.62, k_i = 110 s^{-1}, N_t = 0.57 pmol/cm^2; (*2*) 150 MPA, τ_1 = 2.4 ms, τ_2 = 100 ms, a_1 = 0.66, k_i = 74 s^{-1}, N_t = 0.65 pmol/cm^2; (*3*) 0.1 MPa, τ_1 = 1.5 ms, τ_2 = 94 ms, a_1 = 0.67, k_i = 110 s^{-1}, N_t = 0.72 pmol/cm^2. The temperature was 20°C. C_m = 370 nF/cm^2.

tion volume for $TPhB^-$ absorption on DOPE membranes was estimated about -10 cm^3/mol very close to that found for DPA^-.

DISCUSSION

The arguments presented in Results should leave little doubt that the increase in membrane capacitance, produced by increasing pressure, is due to membrane thinning and not to an increase of the black membrane area. The sublinear P dependence of membrane thickness is qualitatively consistent with the isothermal compressibility of a light oil such as *n*-decane being about halved when P is increased from 0.1 to 100 MPa (McGovan, 1976). However, the thickness change expected for a membrane having the bulk compressibility of *n*-decane would be <4% at 150 MPa and solvent-free dipalmitoyl phosphatidylcholine (DPPC) bilayers are reported to be less compressible (Liu and Kay, 1977). Furthermore, the effect of 80 MPa on the specific capacitance of squid axon membrane was negligible (Benz et al., 1984). Thus, it seems likely that the major cause of the thinning observed in our experiments is the extrusion of some *n*-decane into microlenses and the torus, occurring because the partial molar volume of *n*-decane in the bilayer region is larger than in its bulk phase. Unfortunately, it is difficult to quantify this interpretation as in the case of the C_m temperature dependence studied by White (1976) because we are unable to account properly for the expected anisotropy of the elastic properties of lipid bilayer (Evans and Simon, 1975). Indeed, x-ray diffraction measurements (Stamatoff et al., 1978) indicate that the thickness of solvent-free DPPC bilayers is increased by pressure (5% at 80 MPa) at the expense of larger compressibility within the bilayer plane. Our present data lead at least to the qualitative conclusion that the solubility of *n*-decane into lipid bilayers decreases upon increasing pressure.

It has been demonstrated that one of the reasons of the anesthetic effect of short *n*-alkanes on nerve membranes comes from an increase in membrane thickness due to the absorption of these molecules into the bilayer (Haydon and Kimura, 1981; Haydon et al., 1984). Our findings suggest that such effects should be at least partly reversed by pressure via the simple squeezing away of the anesthetics from the lipid bilayer and from possible target sites (proteins) within it (Franks and Lieb, 1982).

The activation volumes of k_i obtained here for solvent containing membranes are about three times larger than those estimated from similar experiments on nerve membranes (Benz et al., 1984). This may imply some difference between the structure of model and natural membranes. However, it is also possible that the effects reported here are dominated by the phenomenon of solvent depletion. In fact, if we calculate the pressure effects in the absence of membrane thinning we come to activation volumes of k_i which are close to those of the (solvent free and incompressible) nerve membranes. In apparent contrast with both our present results and those of Benz et al. (1984) on squid axon membranes. Moronne and Macey (1985) reported trivially small effects of pressure on $TPhB^-$ and DPA^-. One major difference of the interpretation of the data arises certainly from the fact that we corrected our results for the measured thickness changes, while Moronne and Macey (1985) did not. Another factor that might explain why they observed smaller effects could be temperature variations that we believe were larger and longer-lasting in their system than in ours. In the case of Moronne and Macey (1985) 100 ml of the inner volume of the pressure bomb was occupied by liquids (water and tetradecane). Since their measurements were taken within few tenths of seconds after any pressure step, the increase of k_i due to the temperature increase following any pressurization must have partly masked the decrease of k_i due to pressure.

The estimated volumes of absorption, in the range of -9 to -12 cm^3/mol, have opposite sign to those expected from relaxation of electrostriction of water around the ions in solution, a phenomenon that plays a major role in many reactions involving the displacement of a charged group between environments of different polarity (Low and Somero, 1975). Major contributions from such a mechanism are also excluded by our finding that the ionic strength in solution has no influence upon our results. This is not surprising if one assumes that the lipophilic ions are positioned at the membrane solution interface with their polar group facing water, thus not modifying the packing of water around this group. On the other hand, there appears to be considerable gain in packing efficiency upon removing the nonpolar moiety of the lipophilic ions from water and placing it facing the hydrocarbon region of the bilayer. Indeed it seems plausible that a negative reaction volume characterizes the absorption on lipid bilayers of most amphipathic molecules. This suggests that the pressure reversal of the anesthesia produced by amphipathic molecules in nerve membranes is unlikely to occur by simple drug extrusion from the membrane lipids. Whenever such reversal occurs it is more likely that anesthesia arises from the binding of the drug to specific membrane protein sites (Franks and Lieb, 1982). Such binding must involve a volume increase large enough to overcome the negative volume associated with the uptake of the anesthetic by the membrane lipids.

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