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# Pressure effects on mechanisms of charge transport across bilayer membranes

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**Ion transport across diphytanoylphosphatidylcholine/decane bilayer membranes was measured as a function of hydrostatic pressure over the range 0.1–100 MPa (1–1000 atm). Carrier-mediated $K^+$ conductance decreased with increasing pressure, yielding positive activation volumes of 45 $Å^3$ per complex for valinomycin mediated transport, and 74 $Å^3$ per complex in the case of nonactin. Comparison with the known pressure dependence of the viscosity of bulk alkane liquids supports the view that the rate limiting step for carrier-mediated transport is the translocation of the carrier-cation complex across an essentially fluid hydrocarbon membrane core. The parameters characterizing transient conductance by the hydrophobic anions, dipicrylaminate and tetraphenylborate, by contrast, were found to be insensitive to pressure over the range available. This was also the case for the steady-state conductance observed at elevated concentrations of both tetraphenylborate and the hydrophobic cation, tetraphenylarsonium. The quasi-stationary conductance observed at elevated concentrations of dipicrylaminate did, however, decrease significantly with increasing pressure, indicating a positive activation volume of 20 $Å^3$ per ion. Alternative explanations of this more complex response of hydrophobic ions to pressure are considered. Ancillary measurements of specific membrane capacitance revealed an increase of about 10% with an increase of pressure to 100 MPa, yielding an estimated membrane compressibility on the order of $10^{-9}$ $m^2 \cdot N^{-1}$, comparable to that of bulk liquid hydrocarbons.**

## Introduction

Hydrostatic pressure has been employed in a wide range of biophysical investigations [1–4]. Pertinent theory has been outlined, and additional references of general interest have been cited, in a recent paper describing pressure effects on bilayer membrane conductance introduced by the pore-forming antibiotic, alamethicin [5]. Any step of a membrane transport process may be viewed as a chemical reaction, the molecular rearrangements accompanying which produce a transient positive or negative volume change. Both the sign and magnitude of this 'activation volume' can be determined by measuring the pressure dependence of the rate constant characterizing the transport step of interest. A difference in volume of reactant and product states, the 'reaction volume', may also be inferred by measurement of the pressure dependence of the chemical equilibrium constant.

In this paper we report observed pressure effects on representative examples of two major carrier systems for charge transport across bilayer membranes. In the first system neutral macrocyclic antibiotics form ion-selective complexes with al-

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Abbreviations: $DPA^-$, dipicrylaminate anion; $BPh_4^-$, tetraphenylborate anion; $AsPh_4^+$, tetraphenyl arsonium cation; DPhPC, 1,2-diphytanoyl-3-*sn*-phosphatidylcholine.

kali cations. Charge transport is accomplished by electrodiffusion of these complexes across the membrane. Extensive reviews describing the experimental and theoretical basis of our understanding of these systems are available [6,7]. Our study utilized valinomycin and nonactin, both of which complex readily with potassium ions. The rate-limiting step for steady-state conductance in both cases is the electric field driven migration of the charged complex across the membrane, as evidenced by the observation of superlinear current-voltage characteristics. Measurement of the pressure dependence of steady-state conductance in these cases should provide information on the interaction of the carrier-ion complex with the membrane core.

This point is made evident by consideration of the Stokes-Einstein relation, which relates the mobility, $U$, (drift velocity/unit electric field) of a charged spherical particle of radius, $R$, moving through an insulating solvent medium, to the bulk viscosity, $\eta$, of that medium. In its simplest form, this relation is

$$U = \frac{q}{6\pi\eta R} \tag{1}$$

where $q$ is the charge on the particle. More refined treatments [8,9] modify Eqn. 1 to account for nonspherical particle shape, variable boundary friction between the migrating particle and the solvent, etc. Our interest, however, is in the reciprocal relationship between carrier mobility and solvent viscosity, which is preserved upon refinement. Eqn. 1 implies that, since conductance is proportional to carrier mobility, its pressure dependence should parallel that of the reciprocal viscosity of the membrane medium. Thus, comparison of the pressure dependence of membrane conductance with the known pressure variation of *n*-alkane viscosity [10] should provide an index of the extent to which the membrane core approximates the fluid properties of bulk hydrocarbons.

The second transport system to be dealt with here is that of hydrophobic ions, which owe their membrane permeability to charge delocalization and to the presence of hydrophobic groups. Transient conductance is of particular interest for anions of dipicrylamine ($DPA^-$) and of sodium tetraphenylborate ($BPh_4^-$). These ions were studied initially by Ketterer, Neumcke and Läuger [11], who related the relaxation time of the current transient, accompanying application of a transmembrane voltage jump, to the height of a central potential barrier which these ions had to surmount in moving from one interfacial potential minimum, across the membrane, to a corresponding minimum on the other side. Steady-state conductance is also observed with these and other related ions. Reviews of models and available experimental data may be consulted for details [7,12]. Studies of the pressure dependence of both transient and steady-state hydrophobic ion conductance would again be expected to provide information on the molecular rearrangements accompanying transports of these species across bilayer membranes.

Since the bilayer constitutes a barrier to transport having a well-defined thickness we have also undertaken measurements of specific membrane capacitance as a function of hydrostatic pressure. Such measurements would be expected to permit a distinction between pressure-dependent conductance changes arising from purely geometric effects, such as membrane thinning, and changes resulting from modification by pressure of the interaction between permeant species and the membrane.

## Materials and Methods

### *Materials*

Membranes were formed by the brush technique using solutions of the synthetic lipid, 1,2-diphytanoyl-3-*sn*-phosphatidylcholine (DPhPC) in decane at 20–25 mg/ml. The lipid was purchased from Avanti Polar Lipids, Inc., Birmingham, AL, and decane was from Aldrich Chemical Co., Milwaukee, WI. This fully saturated lipid forms membranes of exceptional stability [13] essential for high pressure work, while at the same time the four methyl side groups on each phytol residue confer a high degree of membrane fluidity, as evidenced by the absence of observable phase transitions over the temperature range, $-120$°C to $+120$°C [14]. Clapeyron slopes for the variation with pressure of the transition temperature in unbranched, saturated phosphatidylcholines which do show a melting transition [15] are typically,

$dP/dT \simeq 20$–$30$ deg. C/kbar. These observations lead us to conclude that no pressure-induced phase transitions are to be expected with DPhPC membranes at room temperature and at pressures to approx. 1 kbar (100 MPa).

Valinomycin was purchased from Calbiochem, Los Angeles, CA, while nonactin was a gift from Dr. J.E. Hall. Dipicrylamine, sodium tetraphenylborate, and tetraphenylarsonium chloride were obtained from Aldrich. The antibiotics were dissolved in ethanolic stock solutions and added to the aqueous phases adjacent to the membrane at the time of use. The compounds yielding hydrophobic ions were similarly prepared and used as stock solutions in dimethyl sulfoxide. In no case did the final concentration of stock solvent in the aqueous phases bathing the membrane exceed 1% by volume. All salts used were reagent grade; water was glass distilled.

*High-pressure apparatus*

Generation of high pressure was accomplished in a manner essentially identical to that described previously [5], although a different apparatus was used. A motor driven oil pump delivered a rising column of oil to a high-pressure reservoir. The rising oil compressed the gas above it, typically He. The gas transmitted the pressure through a 2 m length of high pressure capillary tubing to the test cell containing the membrane under study. The system was precharged to cylinder pressure (10–15 MPa) with He, then isolated for further pressurization using the oil pump.

The test cell containing the teflon (E.I. du Pont de Nemours, Inc., Wilmington, DE) chamber in which membranes were formed is illustrated in Fig. 1. Pressure was contained by a heavy walled stainless steel vessel fitted with a bolted, O-ring sealed stainless steel lid. The vessel was fitted with an oriented single-crystal sapphire window (Adolf Meller Co., Providence, RI) and miniature lamps (Model No. 718, Miniature Lamp Works, Chicago, IL) mounted internally to permit viewing of the membrane. Membranes were formed by inserting a brush laden with membrane forming solution through the open front port shown in Fig. 1. Both front and rear ports were sealed prior to pressurization. Pressure was measured using a bonded silicon strain gauge (Model 8511-10K, Endevco,

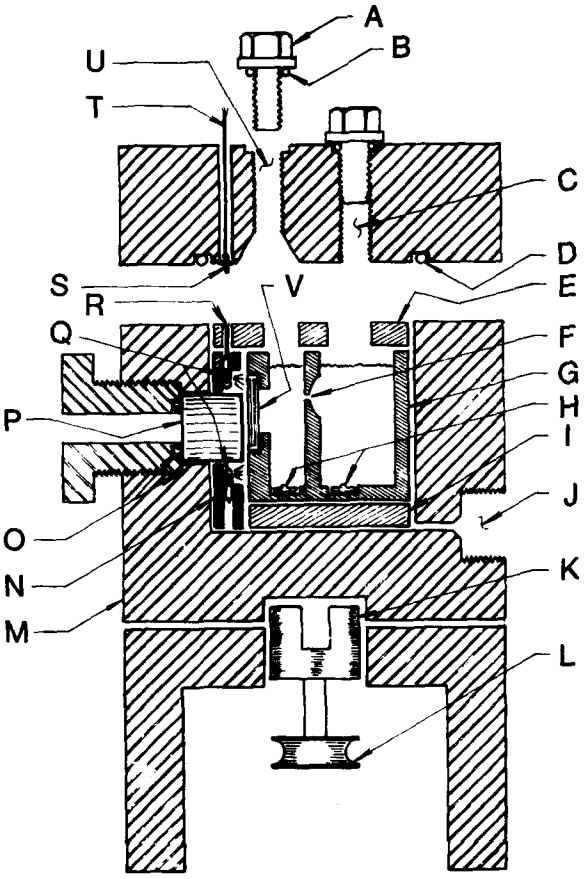


Fig. 1. A cross-sectional illustration of the high-pressure cell used for membrane conductance measurements is presented. A, Port closure bolt; B, O-ring seal; C, rear compartment access; D, O-ring seal for pressure vessel lid; E, nylon filler; F, membrane aperture; G, Teflon cup; H, stirring magnets; I, nylon filler; J, high-pressure port for gas entry; K, drive magnet; L, belt-driven shaft; M, stainless steel pressure vessel; N, light holder; O, O-ring seals; P, sapphire window; Q, miniature lamps; R, spring contact; S, electrical feedthrough for lamps; T, lamp input lead; U, front chamber access for membrane formation; V, fused quartz window. The Ag/AgCl electrodes and pressure transducer are not illustrated.

Inc., San Juan Capistrano, CA) threaded into a side port of the cell. Teflon-coated stirring bars, driven by a rotating magnet mounted externally, could be used to agitate the solutions bathing the membrane.

*Electrical measurements*

Electrical contact with the membrane was provided by Ag/AgCl electrodes of 1 $cm^2$ area (not shown in Fig. 1) immersed in the adjacent aqueous

phases. Hermetic feedthroughs mounted in the lid of the pressure vessel (Type 24916-19501; Astro-Seal, Inc., South El Monte, CA) provided external connections to the electrodes and to the internally located miniature lamps.

Voltage pulses for membrane conductance measurements were produced by a microprocessor-based programmable pulse generator built in our laboratory, using a system design kit (SDK-85) available from Intel Corp., Santa Clara, CA. It could provide up to eight output pulses per cycle, each having independently programmable duration and amplitude. When the pulse generator was used, a current sensing resistor was placed in series with the membrane, so that the voltage drop across it provided a measure of the transmembrane current. This resistor was always chosen to be small in comparison to the membrane resistance.

Capacitance measurements were made by applying a triangular waveform (50 Hz; 50 mV, p-p) to a membrane placed in the input circuit of an operational amplifier (Model 43-K; Analog Devices Inc., Cambridge, MA) connected as a current-to-voltage converter. The differentiated output produced by this circuit configuration was a square wave having an amplitude proportional to the membrane capacitance. Some membrane conductance measurements were also made using the same circuit configuration, but with a much lower frequency (0.2 Hz) triangular waveform. This permitted direct display of membrane current-voltage characteristics on an $X$-$Y$ recorder.

A much more detailed account of all aspects of the experimental methods employed in this work has been published elsewhere [16].

## Results

A preliminary account of our observations on the pressure dependence of carrier-mediated transport and of hydrophobic anion conductance has appeared in abstract form [17]. A detailed presentation of these results is given below, together with additional data on the pressure dependence of facilitated transport and of membrane capacitance.

*Carrier-mediated transport*

In measuring the pressure dependence of carrier-mediated transport of potassium ions by valinomycin and nonactin, particular care was taken to wait long enough (2–3 h) after membrane formation to minimize the effects of time-dependent loss of carrier to the membrane torus [18].

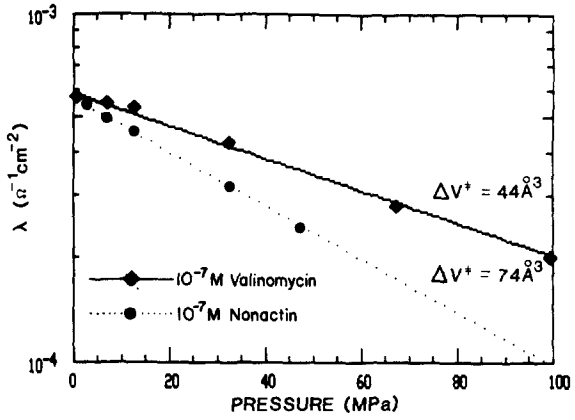


Fig. 2. Semi-logarithmic plots of ohmic conductance versus pressure are illustrated for carrier-mediated transport by valinomycin and nonactin. Activation volumes shown are those determined for the particular runs depicted. In the case of valinomycin twelve pressure runs were made, yielding $\Delta V^{\ddagger} = 45 \pm 6$ Å$^3$. For the single nonactin run made, the error estimate was $\pm 9$ Å$^3$. The aqueous phases were unbuffered and contained 0.1 M KCl. The membrane lipid was DPhPC.

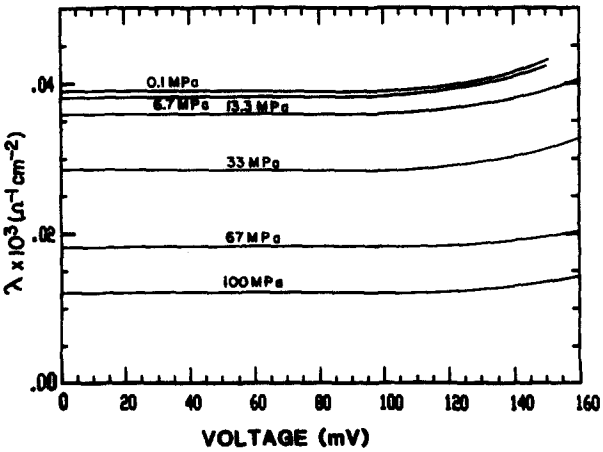


Fig. 3. Chord conductance is plotted versus transmembrane applied voltage for the case of valinomycin mediated $K^+$ transport. Results at various fixed pressures between ambient and 100 MPa are shown. Current-voltage characteristics are seen to be superlinear at all pressures reached. Unbuffered aqueous phases contained $10^{-6}$ M valinomycin and 0.1 M KCl.

Representative semi-log plots of ohmic membrane conductance versus pressure are illustrated in Fig. 2 for both valinomycin and nonactin as carriers of $K^+$ ions.

While the measurement of steady-state conductance alone does not suffice to determine uniquely all the rate constants characterizing carrier-mediated transport, the observation of superlinear current-voltage characteristics does establish that an electric field driven step, namely, translocation of the carrier-ion complex across the membrane, is rate limiting [19]. In Fig. 3 we present, for the valinomycin-$K^+$ complex, plots of chord conductance versus transmembrane applied voltage, at various pressures between 0.1 and 100 MPa. The plot clearly indicates superlinearity at all pressures.

*Hydrophobic ion conductance*

The hydrophobic anions produced by dissociation of dipicrylamine ($DPA^-$) and of sodium tetraphenylborate ($BPh_4^-$) show a large field-independent initial conductance, $\lambda_{00}$, at low transmembrane voltage, which decays with a characteristic relaxation time, $\tau_0$, after application of a voltage step [11]. We have measured the pressure dependence of these transient conductance parameters, and found the associated activation volumes to be zero within an estimated error of $\pm 5$ Å$^3$. Fig. 4(a, b) depicts, for two different membranes in solutions containing $DPA^-$, the dependence of both $\tau_0$ and $\lambda_{00}$ upon pressure. Similar data for two different membranes bathed in aqueous solutions containing $BPh_4^-$ ions are presented in Fig. 5(a, b).

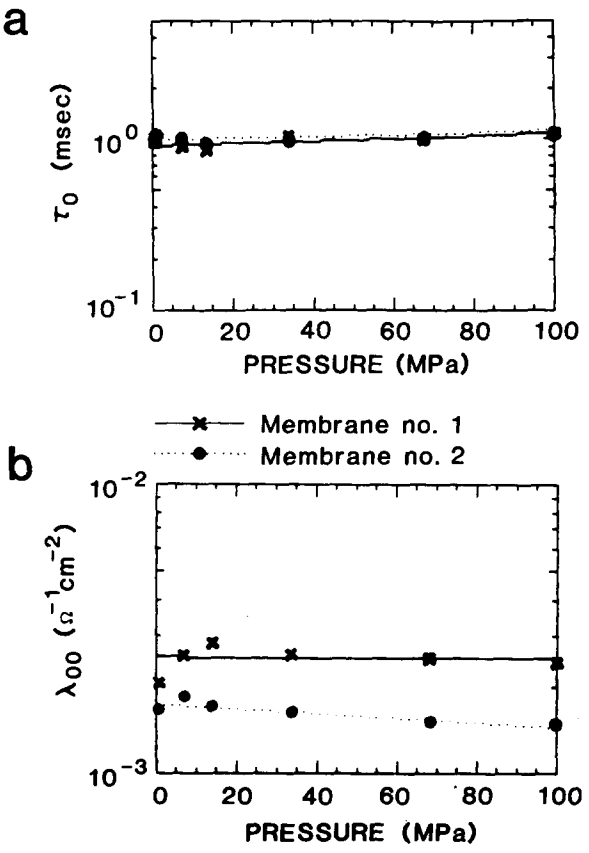


Fig. 4. The pressure dependence of the parameters characterizing transient membrane conductance due to $DPA^-$ is illustrated for two different DPhPC membranes. The unbuffered aqueous solutions contained $5 \cdot 10^{-8}$ M $DPA^-$ and 0.1 M NaCl. (a) The relaxation time, $\tau_0$, is plotted versus pressure. (b) The initial conductance, $\lambda_{00}$, is plotted versus pressure.

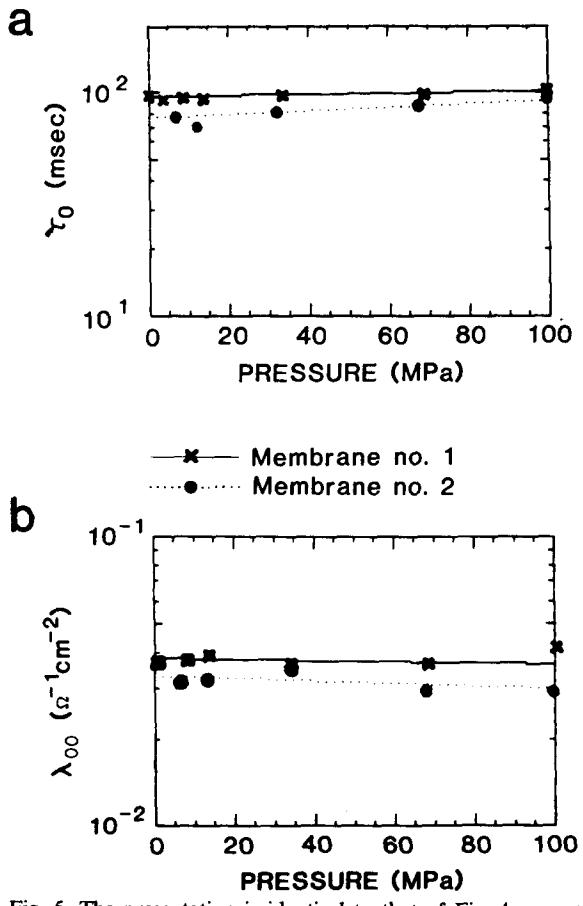


Fig. 5. The presentation is identical to that of Fig. 4, except that the hydrophobic anion in this case is $BPh_4^-$ at a concentration of $10^{-7}$ M.

When the anion, $DPA^-$, is present at higher concentrations ($\geq 10^{-5}$ M), a 'quasi-stationary' conductance, similar to that described by Ginsburg and Stark [20] for dinitrophenolate and for picrate ions, is observed. While this conductance (current) decays slowly over tens of seconds after application of a voltage step, it does not appear to be diffusion limited by aqueous unstirred layers adjacent to the membrane. Arguments supporting this assertion will be presented below. A measurement of the pressure dependence of this conductance was made by noting, at each pressure, the current flowing at a fixed time, 120 ms, after application of a voltage step. This time was sufficient to permit the decay of 'early' transients, described above, but short enough so that negligible decay of the quasi-stationary component of conductance could occur. The logarithm of this conductance has been plotted versus pressure in Fig. 6. A positive activation volume of 20 $Å^3$ is inferred from the data.

When the concentration of the anion, $BPh_4^-$, is elevated to $10^{-4}$ M or higher, a steady current is observed upon application of a voltage step. This current does not decay slowly as in the case of $DPA^-$ conductance. Efforts to measure the pressure dependence of this steady-state $BPh_4^-$ conductance were complicated by the fact that the pressure effect was small and was superposed on a time-dependent effect of undetermined origin. The results are presented in Fig. 7, where the consecutively numbered points were taken at approximately 3-min intervals as the pressure was first increased from 0.1 to 100 MPa, then cycled between 33 and 100 MPa. Points taken on the final pressure cycle, together with the starting point at 0.1 MPa, yielded the straight line illustrated on the semi-log plot. The corresponding activation volume is positive and equal to 7 $Å^3$. While this result is considered definitive, it is close to the limit of resolution of our experiment. Assuming an uncertainty of $\pm 10\%$ in the measurement of conductance, this limit would be $\pm 4$ $Å^3$. Since the conductance of $BPh_4^-$ ions at elevated concentration has been reported to be diffusion limited by aqueous unstirred layers [21], we note that all conductances reported here are ohmic, and as such reflect properties of the interaction between permeant anions and the membrane.

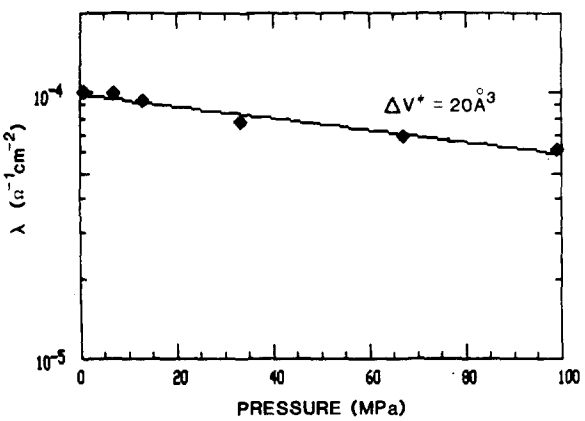


Fig. 6. The pressure dependence of the quasi-stationary conductance of $DPA^-$, described in the text, is shown. The concentration of $DPA^-$ is $10^{-5}$ M in 0.1 M NaCl.

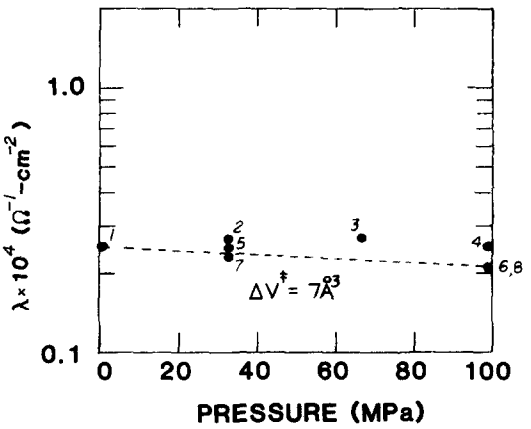


Fig. 7. Steady state conductance due to $10^{-4}$ M $BPh_4^-$ is plotted versus pressure. The numbers indicate the temporal order in which the data points were taken, as explained in the text. The dashed line represents an estimate of the results expected in the absence of time-dependent effects.

Hydrophobic cations such as tetraphenylarsonium ($AsPh_4^+$) and tetraphenylphosphonium show little or none of the transient response under voltage clamp which is exhibited by hydrophobic anions [22]. Cationic $AsPh_4^+$ does, however, display a low level of steady-state conductance at elevated concentration. We observed an ohmic conductance of $2 \cdot 10^{-7}$ $\Omega^{-1} \cdot cm^{-2}$ with $5 \cdot 10^{-2}$ M $AsPh_4Cl$ added to unbuffered aqueous solutions containing 0.1 M NaCl. No measurable change in this conductance was observed upon pressurization to 100 MPa (data not shown).

*Facilitated transport*

The experiments reported by Ginsburg and Stark [20] in which the transport of picrate and of dinitrophenolate ions was facilitated by addition of valinomycin led us to conduct similar experiments using $DPA^-$. In both their experiments and ours, potassium ions were excluded, so that conductance by carrier-cation complexes was negligible. We first examined the effect of valinomycin on the parameters characterizing transient conductance by $DPA^-$, obtaining the results illustrated in Fig. 8. In this experiment the relaxation time, $\tau_0$, of the transient current decreased by a factor of four upon addition of valinomycin, while the initial conductance, $\lambda_{00}$, increased by the same factor. The constancy of the $\lambda_{00}\tau_0$ product implies that the surface density of $DPA^-$ is unaffected by added valinomycin [23], while the reduction of $\tau_0$ implies that addition of the antibiotic introduces a dipole potential making the membrane interior more positive relative to the interfaces with the aqueous phases [24,25].

While the addition of valinomycin markedly alters the parameters characterizing transient $DPA^-$ conductance, pressurization to 100 MPa produces no further measurable change in either $\tau_0$ or $\lambda_{00}$ (data not shown). Thus neither the adsorption equilibrium of $DPA^-$ nor the charge transfer mechanism governing its transient flow across the membrane is appreciably modified by pressure, regardless of the presence of valinomycin.

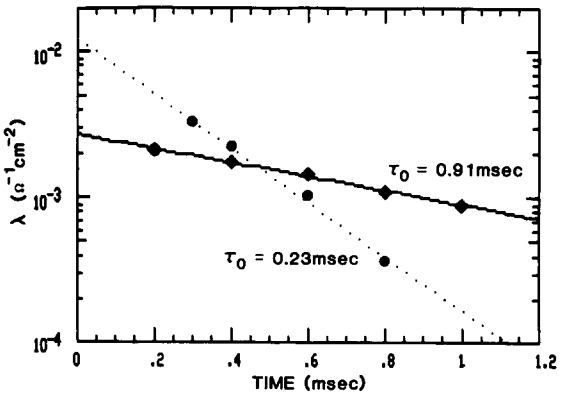


Fig. 8. Semi-logarithmic plots of DPhPC membrane conductance versus time illustrate the effects of valinomycin on the transient conductance of $DPA^-$. The line ◆———◆ indicates the conductance decay in the presence of $2\cdot10^{-8}$ M $DPA^-$. The effect of adding $10^{-7}$ M valinomycin is indicated by ●·····●. No further change of response occurs, however, upon pressurization to 100 MPa.

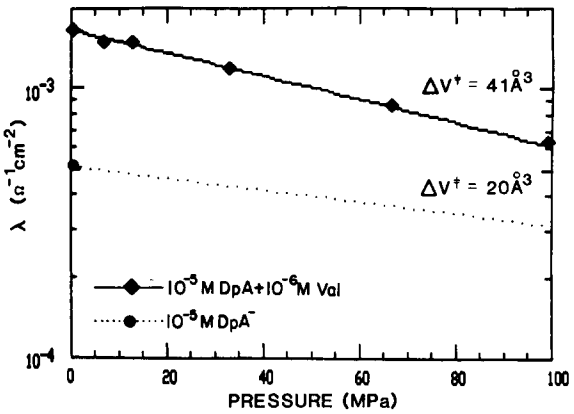


Fig. 9. The upper plot shows the effect of pressure on the quasi-stationary conductance of $DPA^-$, after the latter has been facilitated by addition of valinomycin. The lower plot, with a single data point, illustrates the response to pressure which would have been expected had valinomycin not been added. The single data point indicates the actual conductance of the membrane prior to valinomycin addition.

The quasi-stationary conductance of $DPA^-$ ions at $10^{-5}$ M concentration is also modified by valinomycin at ambient pressure, being increased by a factor of three in the presence of $10^{-6}$ M of the antibiotic. This is shown in Fig. 9. The pressure sensitivity of this facilitated quasi-stationary conductance is also greater than for the non-facilitated case, with a positive activation volume of 41 Å$^3$ being measured.

The fact that the quasi-stationary conductance attributed to $DPA^-$ is facilitated by valinomycin provides the clearest evidence that this conductance is not diffusion limited. There is no plausible mechanism by which valinomycin could facilitate diffusion of $DPA^-$ ions in unstirred aqueous layers adjacent to the membrane. Any complexation which might occur would produce a larger and hence more slowly diffusing entity.

*Membrane capacitance*

Before presenting results we note that the capacitance per unit area (specific capacitance) of a membrane is given by

$$C' = \frac{\varepsilon\varepsilon_0}{d} \qquad (2)$$

where $\varepsilon_0$ is the permittivity of free space, $\epsilon$ is the relative permittivity of the dielectric medium comprising the membrane, and $d$ is its thickness. Differentiation yields

$$\frac{\delta C'}{C'} = \frac{\delta \epsilon}{\epsilon} - \frac{\delta d}{d} \tag{3}$$

which indicates that pressure-dependent variation of $C'$ can arise both from thickness change and from change of relative permittivity with pressure. Since guidance from theory or experiment is lacking, we will assume that the membrane responds isotropically to hydrostatic pressure, so that

$$\frac{\delta d}{d} = \frac{1}{3}\left(\frac{\delta V}{V}\right) \tag{4}$$

where $V$ is the molar volume. To deal with pressure effects on $\varepsilon$, we will use the Clausius-Mosotti relation [26], for non-polar media, namely

$$\frac{\varepsilon - 1}{\varepsilon + 2} = \frac{4}{3}\pi\left(\frac{\gamma}{V}\right) \tag{5}$$

where $\gamma$ is a molar polarizability dependent upon the species only, and not upon temperature or pressure. Differentiation of Eqn. 5 yields

$$\frac{\delta \varepsilon}{\varepsilon} = -\frac{(\epsilon - 1)(\epsilon + 2)}{3\varepsilon}\left(\frac{\delta V}{V}\right)$$

$$\cong -\frac{2}{3}\left(\frac{\delta V}{V}\right) \tag{6}$$

where the approximation assumes $\epsilon \approx 2$ and that changes in $\varepsilon$ with pressure are small. This assumption is in good accord with experimental data for $n$-alkanes [27]. The isothermal compressibility of a medium is defined as

$$\kappa = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T = -\frac{1}{V}\left(\frac{\delta V}{\delta P}\right) \tag{7}$$

assuming $\kappa$ to be independent of pressure over the range spanned experimentally. Combining Eqns. 3, 4, 6, and 7 yields

$$\frac{\delta C'}{C'} \cong \kappa \delta P \tag{8}$$

from which the compressibility of the membrane medium may be calculated using data on the pressure dependence of specific membrane capacitance.

TABLE I

VARIATION WITH PRESSURE OF SPECIFIC MEMBRANE CAPACITANCE

Diphytanoylphosphatidylcholine/decane membrane: $T = 20°C$
Compressibility calculated from Eqn. 8, $\kappa = 2.1 \cdot 10^{-9}$ $m^2 \cdot N^{-1}$.

| Pressure (MPa) | Specific capacitance ($\mu F/cm^2$) |
|---|---|
| 0.1 | 0.271 |
| 3.4 | 0.277 |
| 6.8 | 0.280 |
| 13.5 | 0.297 |
| 33.9 | 0.304 |
| 67.8 | 0.312 |

Representative data of this type are presented in Table I, together with a compressibility calculated from Eqn. 8. This calculated value, though larger, is of the same order as compressibilities determined for $n$-alkanes from the data of Snyder [28]; e.g., $\kappa = 0.76 \cdot 10^{-9}$ $m^2 \cdot N^{-1}$ is an average value for $n$-decane at 25°C over the pressure range 0.1–100 MPa.

## Discussion

In the light of the results obtained we now consider the idea developed in the Introduction, namely, that we should compare pressure dependences of the bulk viscosities of appropriate media with the pressure dependence of membrane conductance due to various transport mechanisms. Such a comparison should provide a measure of the usefulness of the Stokes-Einstein relation in linking membrane conductance with membrane 'fluidity'. In the upper portion of Table II we present, for several hydrocarbons and for water, data on the variation of viscosity with pressure [10]. It appears for the hydrocarbons that both branching and the presence of *cis* double bonds, which would interfere with close packing and promote a more open and fluid structure, produce a greater variation of viscosity with pressure. The viscosity of water changes only slightly over the pressure range covered in this work. For those transport systems which showed a measurable sensitivity to pressure we have listed, in the lower

TABLE II

THE RELATIONSHIP OF BULK VISCOSITY TO MEMBRANE CONDUCTANCE: A COMPARISON OF PRESSURE DEPENDENCES

| Substance | | Bulk viscosity * Relative viscosity $(\frac{\eta(100\ \text{MPa})}{\eta(0.1\ \text{MPa})})$ |
|---|---|---|
| *n*-Octane | | 2.12 |
| Isoamyl-decane | | 2.72 |
| Oleic acid | | 4.13 |
| Water | | 1.05 |
| **Transport system** | **Fig. No.** | **Membrane conductance ** Relative reciprocal conductance $(\frac{\lambda^{-1}(100\ \text{MPa})}{\lambda^{-1}(0.1\ \text{MPa})})$** |
| Valinomycin – $K^+$ | 2 | 2.97 |
| Nonactin – $K^+$ | 2 | 5.97 |
| $DPA^-$ ($10^{-5}$ M) | 6 | 1.67 |
| $BPh_4^-$ ($10^{-4}$ M) | 7 | 1.18 |
| $DPA^-$ ($10^{-5}$ M) (facilitated) | 9 | 2.69 |

* Data from Bridgman (1958) ($T = 30°C$).

** All membranes are DPhPC/decane ($T = 20°C$).

portion of Table II, the ratio of reciprocal conductance measured at 100 MPa and at ambient pressure.

In comparing table entries it appears quite feasible to relate macrocyclic carrier-mediated transport to the fluid properties of liquid hydrocarbons as suggested in the Introduction. To press the point closely, however, is not feasible because no data on viscosity versus pressure are available for a more directly comparable bulk hydrocarbon, such as tetramethylhexadecane. Furthermore, the membrane core is a mixture of both lipid and decane solvent. Virtually solvent free membranes at ambient pressure have specific capacitances more than two times larger than those listed in Table I [29].

A more direct question arises, however, when comparing the markedly different activation volumes measured for $K^+$ ion conductances mediated by valinomycin and by nonactin (45 and 74 $Å^3$, respectively: see Fig. 2). Since the membrane medium is the same in both cases, why should this large difference be observed? Note that differing radii of the two complexes would not be significant, since these radii are not expected to change appreciably with pressure. One could simply assert that the Stokes-Einstein relation, being based on macroscopic theory, does not adequately describe the interaction between complexes and the membrane. A more specific possibility is that transport of the complex may be 'self-facilitated' by the uncomplexed form to an extent dependent upon the particular carrier. This suggestion is made plausible by our observation of facilitated conductance of $DPA^-$ in the presence of valinomycin (Fig. 6). Still another complication could arise if partitioning of the carrier into the membrane were pressure dependent to an extent which varied with the carrier being used. An argument against this possibility is provided by our observation (Fig. 8) that the parameters characterizing transient $DPA^-$ conductance, though markedly altered by introduction of valinomycin, are not further altered by application of hydrostatic pressure. Any pressure-dependent partitioning of valinomycin in this system would be expected to produce some corresponding variation in the parameters of transient $DPA^-$ conductance. We conclude that, while a rigorous demonstration is not possible, carrier-mediated conductance can justifiably be modeled by regarding the complexes as charged particles moving subject to classical viscous forces through a fluid hydrocarbon membrane.

Johnson and Miller [30] have measured the pressure dependence of valinomycin-mediated $K^+$ permeability in solvent-free liposomes, reporting an activation volume of about 40 ml/mol (66 $Å^3$ per complex). They used egg phosphatidylcholine mixed with 2–6% of negatively charged phospholipids and, in one experiment, 50% cholesterol. The variation of observed activation volume with lipid composition was small and not considered to be significant. Water was used as the pressure transmitting medium. Morrone and Macey [31] have reported in abstract the same activation volume for this system, using planar bilayer membranes formed from egg phosphatidylcholine suspended in decane. They also reported an activation volume of 37 ml/mol (61 $Å^3$ per complex) for nonactin-mediated $K^+$ conductance. They used oil as a pressure transmitting medium. Discrepancies

between the above described results and our own (Fig. 2) show no systematic trend. We note in particular that our measured activation volume for nonactin-$K^+$ conductance is larger, while that which we obtained for valinomycin-$K^+$ conductance is smaller than the corresponding values reported by these other investigators. Thus we conclude that the discrepancies noted, which are not large in any case, cannot be ascribed to any specific differences in experimental method, such as our use of He gas as a pressure transmitting medium, or to our use of solvent-containing membranes. The long aging times (2–3 h) which we allowed prior to pressurization may be a factor in the case of valinomycin.

Our observations of the negligible pressure dependence of hydrophobic ion conductance (Figs. 4 and 5), by contrast, do not permit plausible interpretation in terms of charged particle electrodiffusion through a hydrocarbon membrane core. The theoretical picture of Ketterer et al. [11] accounted for transient conductance by $DPA^-$ and $BPh_4^-$ ions in terms of their motion over 'smooth' barriers resulting from the superposition of electrostatic and hydrophobic forces only. Their rate theoretical treatment took no explicit account of diffusive flow. More detailed analysis [12] shows, however, that diffusive flow of ions across a membrane can be described by the superposition of an additional low amplitude periodic potential. Either a Nernst-Planck or a rate theoretical analysis will then incorporate the effects of diffusive flow by including an appropriate multiplicative coefficient in the resulting expression for the membrane conductance. This coefficient, a diffusion constant for the Nernst-Planck analysis or an equivalent expression from rate theory including an exponential dependence upon an activation energy, would be the source of the expected pressure-dependent conductance in either case.

Our observations could be consistent with diffusive flow in the transient case if the environment of the moving hydrophobic ions was more water-like than hydrocarbon-like. This follows from the minimal pressure dependence of the viscosity of water (see Table II; also the work of Bett and Cappi [32]). Nevertheless, these ions must translocate the membrane during transient flow. If the ion current resulted from exterior flows on and off the membrane interfaces, with continuity requirements being met by displacement current flowing across the membrane itself, then the membrane specific capacitance would have to be related to the transient flow parameters by

$$C' = \lambda_{00}\tau_0 \tag{9}$$

This relation simply states that, under the conditions assumed, the membrane is displaying the transient '*RC*' response of a true capacitor. Using values appropriate to $DPA^-$ ($\lambda_{00} \cong 10^{-2}\ \Omega^{-1} \cdot cm^{-2}$; $\tau_0 \cong 10^{-3}$ s) gives $C' \cong 10\ \mu F \cdot cm^{-2}$. This value is two orders of magnitude larger than measured values (Table I). Thus the hydrophobic ions must cross the membrane.

Given that hydrophobic ions do indeed cross the membrane during the transient response, the possibility of rate limiting by a non-diffusive step should be considered. One such step could be the breaking of a weak chemical bond. In the case of hydrogen bonds, the associated activation volume would be approx. 4 $Å^3$ per bond [33], small enough not to be resolved in our experiments.

The picture for hydrophobic ions is further complicated by the range of pressure dependent conductances encountered at higher concentrations for different ionic species. The anion, $DPA^-$, shows a quasi-stationary conductance characterized by a positive activation volume of 20 $Å^3$ per ion (Fig. 6) which is doubled in the presence of valinomycin (Fig. 8). Anionic $BPh_4^-$ shows a much smaller but resolvable positive activation volume of 7 $Å^3$ per ion (Fig. 7), while cationic $AsPh_4^+$ shows no measurable response to pressure (data not shown). It appears that these responses are dominated by structural details of the probes and of their interactions with the membrane which are beyond the scope of the concepts applied here.

As noted in the preceding section, hydrostatic compression of the solvent-containing membranes used in this study revealed a compressibility comparable to that of bulk alkanes. It is of interest to compare this with electrocompression studies on similar membranes [34]. These indicate a specific capacitance increase of approx. 10% under applied transmembrane voltage of approx. 150 mV. The equivalent Maxwell pressure is

$$P_{el} = \frac{1}{2}\varepsilon\varepsilon_0\left(\frac{V}{d}\right)^2 \tag{10}$$

or about $2.6 \cdot 10^3$ $N \cdot m^{-2}$ for a membrane of thickness, $d = 50$ Å. This pressure is about 0.025 atm above ambient. A compressibility calculated from Eqn. 8 using the equivalent electrical pressure would yield a result more than four orders of magnitude larger than that determined under hydrostatic pressure. The uniaxial character of electrocompression, together with its negligible effect on the thicker torus and microlenses, permits 'squeeze out' of solvent, thereby accounting for the much higher apparent compliance of solvent-containing membranes. Nominally solvent free membranes, on the other hand, show a much lower increase of specific capacitance with applied voltage [34,35]. Equivalent compressibilities calculated in this case approach those obtained under hydrostatic pressure. The analysis in the preceding section established that the percent decrease of membrane thickness should equal one-third the increase of membrane capacitance. Thus we expect at most about a 3% decrease of membrane thickness, which cannot significantly affect our results.

In summary, the pressure studies described here support the view of carrier mediated transport which depicts the carrier-ion complex as a charged particle moving through a fluid membrane medium having hydrodynamic properties comparable to those of bulk hydrocarbons. This simple picture cannot however, be extended to a description of charge transport by hydrophoic ions.

It has been suggested that the large positive activation volumes which we have observed to characterize carrier-mediated potassium ion conductance in planar bilayers may be due to the loss of electrostrictively compressed water of solvation, released when the ion is complexed by the ionophore. It was further suggested that this contribution to the activation volume would be lacking in the case of hydrophobic ion transfer from water into the membrane, presumably because charge delocalization and the otherwise nonpolar character of the hydrophobic ion would minimize the formation of electrostricted, solvated water in its vicinity when in the aqueous phase. Chemical reactions involving the release of ions into water are indeed invariably accompanied by a volume contraction on the order of 20 $cm^3$/mol or 33 $Å^3$ per ion pair [36]. A system of particular interest here is that provided by the complexation of $K^+$ ions by the bicyclic ligand, cryptand 2,2,2. The volume of complexation in water is reported [37] to be 18 $cm^3$/mol (30 $Å^3$ per complex). While the sign and magnitude of the observed complexation volume in this system could be correlated with a comparable volume attributable to complexation in the case of carrier-mediated $K^+$ transport, a quantitative accounting is not provided (Fig. 2). Turning to the case of hydrophobic ions, we observe that dissociation of the weak acids, phenol and *p*-nitrophenol, is accompanied by ionization volumes of $-18$ $cm^3$/mol and $-10$ $cm^3$/mol, respectively [36]. The formation of solvated water around these rather hydrophobic anions is thus seen to be substantial, arguing against any expectations to the contrary in the case of the hydrophobic anions studied in our experiments. Thus, while the release of solvated water from ions entering a membrane may contribute in part to observed activation volumes, it does not appear to us to be possible to formulate a complete explanation of our findings on the basis of such considerations alone.

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