BBA 75354

TETRAPHENYLBORATE CONDUCTANCE THROUGH LIPID BILAYER MEMBRANES

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(Received July 28th, 1969)

SUMMARY

At low concentrations of the anion in the water solutions on either side of the membrane (<0.3 mM), the membrane permeability is so great that transport is limited primarily by diffusion in the water. At higher concentrations the anion flux is independent of concentration; evidently there is an upper limit to the number of anions which can enter the membrane. Recognizing space charge as the probable cause of the latter phenomenon enables one to estimate the membrane–water partition coefficient (approx. 1) and the anion diffusion constant within the membrane (approx. 10^{-9} cm^2/sec).

INTRODUCTION

Conductance studies in lipid bilayer membranes[1,2,22] have mainly been concerned with the biologically important process in which permeability of small inorganic ions (K^+, H^+, *etc.*) is induced through complexing them with lipid-soluble carriers[3–8]. Despite the great interest in this process, no wholly satisfactory theory of it has evolved (in the sense of an explanation of the shapes of current–voltage characteristics or dependence upon concentrations of ion and carrier, for example). Part of the difficulty is that the experimental situation is complicated. There is almost certainly simultaneous transport of both the ion–carrier complex and the uncomplexed carrier through the membrane. Displaced chemical equilibria between these species and the carried ion must occur in the aqueous solutions on either side of the membrane[6]. Thus there are several microscopic processes and parameters that determine the macroscopic observations, and it is not clear how to abstract the latter from the former.

A much simpler experimental situation is one in which a well-defined, lipid-soluble ion is the sole species transported through the membrane; LIBERMAN AND TOPALY[5] report some examples. In this paper, the transport of one ion, the tetraphenylborate anion, which they demonstrated will readily permeate through lipid bilayers is examined in quantitative detail. Hopefully the phenomena observed may ultimately be of use in clarifying the mechanisms involved in the more interesting carrier–ion process.

Saturating current–voltage characteristics, as illustrated in Fig. 1, are found at lower aqueous tetraphenylborate concentrations (<0.3 mM). Analysis shows that,

whenever this occurs, one can immediately obtain from the data important transport parameters. At higher concentrations (>0.3 mM), a new phenomenon is found which is interpreted in terms of space-charge-limited intramembrane currents. This enables a rough estimate of further permeability parameters.

MATERIALS AND METHODS

Lecithin was extracted from hen eggs by the method of PANGBORN[9] (N, 1.9; P, 4.0). Pierce cholesterol was recrystallized from ethanol. Phillips 99 % *n*-decane was used as received. The membrane-forming solution consisted of an equimolar mixture of lecithin and cholesterol dissolved to 10–20 % by wt. in decane. Sodium tetraphenylborate was a gift from Dr. L. V. Interrante; it was Baker Reagent Grade which he recrystallized from ethanol.

The conductance apparatus was similar to that of HANAI *et al.*[10]. A Teflon pot was machined to a 0.01-cm thick wall in one area, and an hole 0.2 cm in diameter was drilled through at that point. After immersing the pot in the aqueous solution to be studied, membrane formation was initiated by spreading a drop of the lipid solution over the hole, using an injection syringe tipped with Teflon tubing[11]. Black film areas were determined visually.

The aqueous solutions were 0.1 M NaCl (indifferent electrolyte), 0.03 M Tris buffer (pH 7.2, to minimize tetraphenylborate decomposition), *plus* various concentrations of sodium tetraphenylborate. The temperature was regulated at $24 \pm 1°$. The low frequency (400 cycles/sec) black film capacitance was 0.4 $\mu F/cm^2$.

Separate Ag–AgCl pairs were used for current and voltage electrodes. These were impedance matched into an X–Y recorder or an oscilloscope with Keithley 200B electrometers, *plus* appropriate shunt resistors ($1 \cdot 10^9$–$1 \cdot 10^{10}\ \Omega$ for V, $1 \cdot 10^3$–$1 \cdot 10^6\ \Omega$ for J) for measuring steady-state characteristics. For the current transient experiments, a step voltage was imposed with a simple voltage clamp circuit constructed with a Tektronix Type O oscilloscope preamplifier.

The background conductance (with all solutes present except tetraphenylborate) was less than $1 \cdot 10^{-7}\ \Omega^{-1} \cdot cm^{-2}$. In one experiment, a gradient of tetraphenylborate concentration across the membrane was generated by exchanging the aqueous solution on one side of the bilayer membrane for a solution one-tenth as concentrated in tetraphenylborate; a Harvard infusion-withdrawal pump was used. The predicted Nernst potential was observed, dilute side negative. In all other experiments, tetraphenylborate concentration was the same on either side of the membrane.

RESULTS

Low concentrations

Experimental results. For an aqueous tetraphenylborate concentration $\lesssim 0.3$ mM, the steady-state transmembrane current saturated with increasing voltage (Fig. 1). A similar behavior for several ion–carrier systems was reported by LIBERMAN AND TOPALY[5]. This behavior was observed down to a tetraphenylborate concentration of 1 μM, where the currents began to approach background. Throughout this range the current obtained at any voltage was directly proportional to tetraphenylborate

concentration; otherwise the shape of the current–voltage characteristic was independent of tetraphenylborate concentration.

There was a long delay (20–30 sec) in reaching the steady state after a change in voltage[5], so long as the current was not already saturated; if the voltage was greater than about 150 mV, a further increase produced only a capacitive spike. These long current decays were investigated further by applying step increases in voltage (away from $V = 0$) and following the subsequent current decay, as in Fig. 2. Voltage steps of 20–100 mV were used. Within the experimental error the time scales for the observed transients were independent of tetraphenylborate concentration. The current decays were approximately exponential for $t \gtrsim 10$ sec, with a time constant of 7 ± 1 sec, but deviated from this dependence at shorter times.

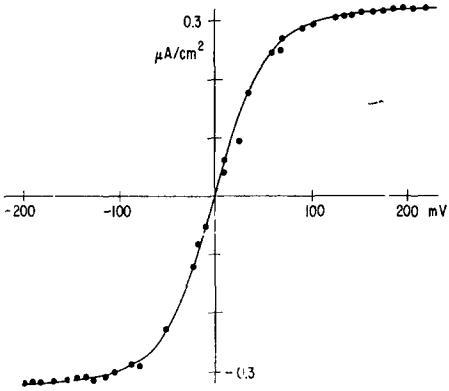

Fig. 1. A typical steady-state J–V characteristic at low concentration; in this case [tetraphenylborate] = 10 μM. The data points were taken 30 sec after setting the voltage. The theoretical curve is from Eqn. 6 with $eJ_{sat_n} = 0.33$ μA/cm², $R = 0.3$.

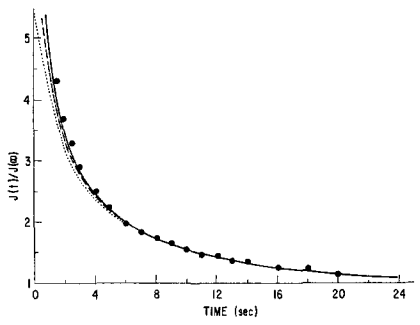

Fig. 2. Current decay subsequent to a voltage step of 52 mV applied at $t = 0$; [tetraphenylborate] = 4.5 μM. The theoretical curves, Eqn. 18, were each computed by assuming a value for $LD_w/\delta D_m K_p$ (———, 0.1; – – – –, 0.2; ·····, 0.3) then choosing t_0 so that the data were fit exactly at $t = t_0$ (t_0 = 7.2, 6.9, 6.6 sec, respectively).

Also observed were the N-type negative resistance characteristics reported by LIBERMAN AND TOPALY[5] when ramp voltage sweeps were applied. This is a real but trivial phenomenon. It results from the superposition of large, decaying current transients such as in Fig. 2 on top of a saturating steady-state current–voltage characteristic as in Fig. 1.

In view of the interpretation of these data presented in the following section, the effect of stirring the electrolyte on the steady-state current–voltage characteristics was tested. A short length of nickel wire was placed in the outer aqueous compartment and rotated at speeds up to 700 rev./min. No effect was found. This is in agreement with the observations of EVERITT *et al.*[12] in that they were unable to remove unstirred layers with a considerably more efficient stirring arrangement.

Steady-state analysis. The fundamental assumption made is that tetraphenylborate is the only species transported through the membrane to any significant extent. Specifically this means that the permeation of all cations and all anions except tetraphenylborate is ignored. It represents application of Occam's razor to the observations: (1) tetraphenylborate is the only species whose presence is necessary and

sufficient to produce the conductances observed and (2) it is unnecessary to invoke other assumptions, *i.e.*, the present analysis will explain the experimental data. Water permeation is also ignored; its activity is virtually identical on the two sides of the membrane, anyway.

One possible cause of current saturation is diffusion polarization[13], in which transport is actually limited by diffusion in the water. Another is limitation of transport by the rate of entrance of the ion into the membrane from the water. Consider both possibilities for the moment, although the numerical results finally obtained will indicate that diffusion polarization was the process operative in these experiments.

Let L represent the membrane thickness and $c(0)$, $c(L)$ the concentrations of tetraphenylborate in the water immediately at the membrane–water interfaces; [tetraphenylborate] is the nominal concentration in the bulk. With current flow, exhaustion and enrichment regions form on either side of the membrane. For a large excess of indifferent electrolyte[13]

$$c(0),\ c(L) = [\text{tetraphenylborate}] \mp J\delta/D_w \tag{1}$$

where J is the steady-state anion flux in the direction $0 \rightarrow L$, δ the thickness of the aqueous diffusion layer, D_w the diffusion constant of tetraphenylborate in water.

Let k_{wm}, k_{mw} be the rate constants for entrance and exit of tetraphenylborate into and out of the membrane, respectively. Then the usual linear boundary conditions at the water–membrane interfaces read[14]

$$\pm J = k_{wm}c(0) - k_{mw}n(0),\ k_{wm}c(L) - k_{mw}n(L) \tag{2}$$

where $n(0)$ and $n(L)$ are the tetraphenylborate concentrations immediately inside the membrane at the interfaces. Eqns. 1 and 2 can be solved for $n(0)$ and $n(L)$ in terms of J and [tetraphenylborate],

$$n(0),\ n(L) = K_p[\text{tetraphenylborate}]\ (1 \mp J/J_{satn}) \tag{3}$$

Here $K_p \equiv k_{wm}/k_{mw}$ is the equilibrium membrane–water partition coefficient for the anion, and the flux at saturation is identified as

$$J_{satn} = [\text{tetraphenylborate}]\ (k_{wm}^{-1} + \delta/D_w)^{-1} \tag{4}$$

Thus, J saturates at $\pm J_{satn}$ when $n(0)$ or $n(L)$ vanishes.

The values of $n(0)$ and $n(L)$ serve as boundary conditions for the continuity-of-current equation inside the membrane. Because of the large excess of indifferent electrolyte in the experiments, all the applied voltage was dropped across the membrane. Assume that the membrane is homogeneous and that the anion concentration inside it is sufficiently small that space charge can be neglected (for the moment). Then the electric field within the membrane is everywhere equal to $-V/L$, where $V \equiv \psi(L) - \psi(0)$ is the applied potential difference. The continuity-of-current equation can be expressed as[15]

$$n(L) = n(0) \exp(eV/kT) + (1 - \exp(eV/kT))JkTL/eVD_m \tag{5}$$

where D_m is the diffusion constant of tetraphenylborate within the membrane. Using the results of Eqns. 3 and 4, we have finally an equation for the steady-state flux–voltage characteristic

$$J_{satn}/J = \coth(eV/2kT) + (kT/eV)R \tag{6}$$

where the parameter R is defined as

$$R \equiv (k_{wm}^{-1} + \delta/D_w)^{-1} L/K_p D_m \tag{7}$$

Eqn. 6 predicts that a change of [tetraphenylborate] will effect the J–V characteristic only by changing the scale of J, *i.e.*, through a change in J_{satn} (Eqn. 4). This is in accord with the experimental data. Furthermore, Eqns. 4, 6 and 7 show that the experimental J–V characteristics must yield values for the important permeability parameters $(k_{wm}^{-1} + \delta/D_w)$ and $K_p D_m/L$.

The curve through the data points of Fig. 1 was constructed using $R = 0.30$ and $eJ_{satn} = 3.3 \cdot 10^{-7}$ A/cm^2. The fit is excellent, which means that the theory presented above is adequate to account for the experimental results. On the other hand, the J–V curves are rather insensitive to R, since it is small (see Eqn. 6), so that the error in determining this quantity is large. A "best fit" for J_{satn} and R was obtained as follows. For each data point (J and V known) Eqn. 6 defines a linear relation between J_{satn} and R. Thus, plotting J_{satn} *vs.* R for all data points on one page yields a multitude of lines which intersect near the "best" values of these parameters. Proceeding in this way I found $J_{satn}/[\text{tetraphenylborate}] = 3.3 \pm 0.3 \cdot 10^{-2}$ A$\cdot$cm$^{-2}\cdot$M^{-1} and $R = 0.2 \pm 0.1$. Eqns. 4 and 7 then gave $(k_{wm}^{-1} + \delta/D_w)^{-1} = 3.4 \pm 0.3 \cdot 10^{-4}$ cm/sec and $K_p D_m/L = 1.7 \pm 0.8 \cdot 10^{-3}$ cm/sec.

One is tempted to identify the latter figure as the membrane permeability of tetraphenylborate, but that would be true only if electrodiffusion through the membrane, rather than the rate constants k_{wm} or k_{mw}, limits transport. This is not established.

Transient analysis. The situation in this case is very complicated, and an exact mathematical analysis has not yet been found. An approximate treatment was obtained as follows. Assume that the response for equilibration across the membrane is fast compared with the diffusion processes in the aqueous unstirred layers, *i.e.*,

$$D_m/L^2 \gg D_w/\delta^2,\ k_{mw} \gg D_w/\delta \tag{8}$$

Then the tetraphenylborate concentration and flux within the membrane will be determined entirely by diffusion processes in the water.

Let $c\,(x,t)$ be the tetraphenylborate concentration as a function of distance, x, and time, t, after application of a step voltage, V, at $t = 0$. Consider the region to the left of the membrane, $-\delta \leqslant x \leqslant 0$. Here $c\,(x,t)$ is a solution of

$$\partial c/\partial t = D_w \partial^2 c/\partial x^2 \tag{9}$$

with boundary conditions

$$c(x,0) = [\text{tetraphenylborate}], \tag{10}$$

$$c(-\delta,t) = [\text{tetraphenylborate}], \tag{11}$$

$$c(x,\infty) = [\text{tetraphenylborate}]\,(1 - (1 + x/\delta)J(\infty)/J_{satn}) \tag{12}$$

where $J(\infty)$ is the steady-state flux at voltage V. This is given by Eqn. 6 but with the term in k_{wm} omitted for self consistency.

One additional boundary condition is needed to establish a solution. The flux must be continuous at the water–membrane interface. At $t = 0$ the tetraphenylborate concentration within the membrane is uniform and equal to K_p [tetraphenylborate],

so that the flux within the membrane is also uniform and equal to that quantity times the tetraphenylborate membrane mobility times the electric field. Hence, the final boundary condition reads

$$-D_w(\partial c/\partial x)_{x=-t=0} = (K_p D_m/L)\,(eV/kT)\,[\text{tetraphenylborate}] \tag{13}$$

The solution for $-\delta \leqslant x \leqslant 0$ is

$$c(x,t)/[\text{tetraphenylborate}] = 1 - (1 + x/\delta)J(\infty)/J_{satn} + \frac{J(\infty)}{J_{satn}}\frac{2(\delta+h)^2}{h\delta\pi^2}\sum_{m=1}^{\infty}\frac{1}{m^2}\sin\left(\frac{m\pi\delta}{\delta+h}\right)\sin\left(\frac{m\pi(\delta+x)}{\delta+h}\right)\exp\left(-\frac{m^2t}{t_0(1+h/\delta)^2}\right) \tag{14}$$

where

$$t_0 = \delta^2/\pi^2 D_w \tag{15}$$

$$\delta/h = 1 + (K_p D_m \delta/D_w L)\,(eV/kT)\coth(eV/2kT) \tag{16}$$

By symmetry the solution to the right of the membrane, $L \leqslant x \leqslant L + \delta$, is

$$c(x - L, t) = 2[\text{tetraphenylborate}] - c(-x,t) \tag{17}$$

The general behavior of $c\,(x,t)$ is illustrated in Fig. 3; this shows in a graphic way the appearance of exhaustion and enrichment layers on either side of the membrane.

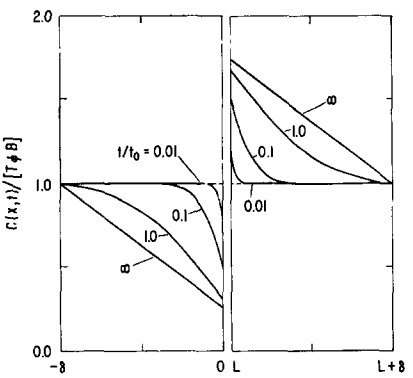

Fig. 3. Behavior of the tetraphenylborate ([TφB]) concentration in the unstirred water layers subsequent to the application of a voltage step at $t = 0$, according to Eqn. 14, showing the formation of exhaustion and enrichment regions. These profiles were computed for a step of 52 mV, right side positive, assuming $LD_w/\delta D_m K_p = 0.2$.

The external circuit current is proportional to the flux, $J(t)$, at the interfaces, $-D_w(\delta c/\delta x)_{x=0}$:

$$J(t)/J(\infty) = 1 - \frac{\delta+h}{h\pi}\sum_{m=1}^{\infty}\frac{1}{m}\sin\left(\frac{2m\pi\delta}{\delta+h}\right)\exp\left(-\frac{m^2t}{t_0(1+h/\delta)^2}\right) \tag{18}$$

In the limit $V \uparrow \infty$, or $h \downarrow 0$, these equations reduce exactly, as they must, to Cottrell's solution of the problem of electrode current transients at fixed diffusion overvoltage[16].

The experimental points of Fig. 2 are compared with several theoretical curves constructed from Eqn. 18 with several values of the parameter $K_p D_m \delta/D_w L$. In each case the theoretical curve was fit to the data at $t_0 = \delta^2/\pi^2 D_w$. The theoretical curves

are indistinguishable beyond about 5 sec, and each gives an excellent overall fit beyond this time. At shorter times the theoretical curves diverge, and it is clear that $D_m\delta/D_wL$ must be in the range of 0.2 ± 0.1, exactly as established from the steady-state analysis in the previous section.

Furthermore, the value of $t_0 = \delta^2/\pi^2 D_w$ found from analysis of these data and those for other voltage steps is 7 ± 1 sec. Under our present assumption that $k_{wm} \gg D_w/\delta$, the steady-state analysis must be interpreted to mean $D_w/\delta = 3.4 \pm 0.3 \cdot 10^{-4}$ cm/sec. Combining these figures yields $D_w = 8 \pm 1 \cdot 10^{-6}$ cm^2/sec and $\delta = 2.3 \pm 0.4 \cdot 10^{-2}$ cm. The first value is in satisfactory agreement with the known[17] diffusion constant of tetraphenylborate in water, $5.2 \cdot 10^{-6}$ cm^2/sec; the second is exactly in the range commonly found for unstirred aqueous layers[13].

The good agreement obtained ignoring k_{wm} means that this rate constant must indeed be considerably larger than D_w/δ (see Eqn. 4); thus, the current saturation observed in these experiments was primarily caused by diffusion polarization in the water.

High concentrations

Experimental results. At aqueous tetraphenylborate concentration > 0.3 mM, the current–voltage characteristics no longer saturated, but on the contrary were superlinear in voltage (Fig. 4). The long delays in reaching steady state that were observed in the low concentration regime were absent here, *i.e.*, the RC time constant of the system was the only factor determining the time response.

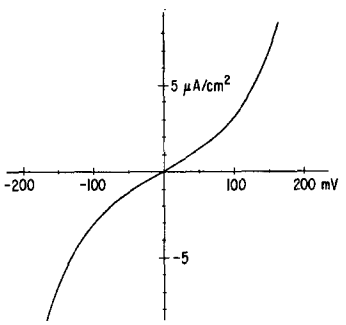

Fig. 4. The J–V characteristic at high concentration. These data were taken at [tetraphenylborate] = 1.3 mM, but the same curve was found for all [tetraphenylborate] > 0.3 mM.

The most significant observation was that the whole current–voltage characteristic was entirely independent of tetraphenylborate concentration throughout this range, approx. 0.3 mM up to the solubility limit, approx. 0.01 M. That is, to emphasize, the same characteristic (Fig. 4) was observed at 0.3 mM as at 0.01 M, to within experimental error.

The black films formed most slowly and reluctantly in the high tetraphenylborate concentration regime; typically this required an hour or more. Unlike what is observed in, for example, 0.1 M NaCl solutions where the boundary between the thick film and the growing black film is sharp, here there was invariably a border of grey film of some intermediate thickness at the boundary. This seemed to impede the black film growth. The black films, once formed, also were uncommonly fragile.

Interpretation: space-charge-limited currents. In the usual treatments of membrane permeability[14] interaction between permeant molecules is ignored, and rightfully so. But in the present situation there is a powerful source of interaction: electrostatic forces. Under our fundamental assumption that the tetraphenylborate anion is the only species which enters the membrane proper, the neutralizing positive ions must be left behind in the aqueous layers near the water–membrane interfaces. Thus there is a negative potential inside the membrane which increases as more tetraphenylborate is dissolved, *i.e.*, as aqueous tetraphenylborate concentration is increased. At some point this potential must become so large that no more tetraphenylborate will enter the membrane regardless of further increase in tetraphenylborate concentration. From this point on the anion currents are space charge limited. This explanation of the observations is proposed.

Now granting that the data show evidence of permeant interaction, one might well question whether the interaction is electrostatic in origin. However, the Coulomb force is the longest range interaction possible between ions (a $1/r$ dependence), so it becomes important at ion densities well below those at which any other, shorter range interaction is significant. Hence, the observed interaction must be electrostatic.

Knowing the Coulomb force law between ions, we are able to estimate the ion density within the membrane at which space-charge effects set in. Consider the thermodynamic identity

$$K_p \equiv \exp(-\Delta G/kT) \tag{19}$$

where K_p is the membrane/water partition coefficient, and ΔG is the Gibbs free energy for the transfer of one tetraphenylborate anion from water into the membrane; both quantities are functions of activity, of course. Now if K_p at infinite dilution (negligible interaction) is not too many powers of ten different from one, then ΔG at infinite dilution must be of the general order of kT in magnitude (*e.g.*, $K_p = (2 \cdot 10^4)^{\mp 1}$ for $\Delta G = \pm 10\ kT$). Therefore, the ion–ion interactions must limit K_p when they reach the order of kT in magnitude, *i.e.*, when they approach ΔG at infinite dilution.

Naively one might expect this to occur when the average distance between ions, $<r>$, within the membrane is such that

$$e^2/\varepsilon <r> \approx kT \tag{20}$$

where ε is the dielectric constant of the membrane. Taking[18] $\varepsilon = 2$, one finds $<r>^{-1} = 0.3 \cdot 10^6$ cm^{-1}, or an ion density of roughly $<r>^{-3} = 4 \cdot 10^{16}$ cm^{-3}. This should be the maximum possible ion density in the membrane, ρ_{max}.

A more sophisticated approach, which leads to essentially the same result, is to calculate ρ_{max} as that ion density at which the Debye length is equal to the membrane thickness[19], *i.e.*,

$$\varepsilon kT/2\pi e^2 \rho_{max} = L^2 \tag{21}$$

Taking[18] $L = 50$ Å and $\varepsilon = 2$ one finds $\rho_{max} = 2 \cdot 10^{17}$ cm^{-3}.

Ideally one would rather measure than calculate the ion density within the membrane, but I have been unable to conceive a way to do this. The first thought was to measure black film destabilization with tetraphenylborate present through a change in apparent interfacial energy[21], but calculation shows the change in energy would be a small percentage of that of the film without tetraphenylborate, so this method would be inaccurate and unreliable. Until such time as a good method is

found, one must be content with rough estimates such as represented by Eqns. 20 and 21.

Both estimates give ρ_{max} as approx. $1 \cdot 10^{17}$ cm^{-3}. Accepting this figure and remembering that the transition to the space-charge-limited regime occurs at aqueous [tetraphenylborate] = 0.3 mM = $1.8 \cdot 10^{17}$ cm^{-3}, one sees that the partition coefficient at infinite dilution must be approx. 1.

Finally, this result can be combined with the low tetraphenylborate concentration conductance data to obtain an estimate of D_m, the membrane diffusion constant. Since $K_p D_m/L = 1.7 \pm 0.8 \cdot 10^{-3}$ cm/sec, K_p is approx. 1, and L is approx. 50 Å, therefore D_m is approx. $1 \cdot 10^{-9}$ cm^2/sec.

These estimates of K_p and D_m are surely no better than order of magnitude.

DISCUSSION

The analysis presented here is based upon the fundamental assumption that the tetraphenylborate anion is the only species that permeates through the membrane to any significant degree. The rest of the analysis is a straightforward application of the classical ideas of permeability theory[14], electrodiffusion[15] and diffusion polarization[13]. The fit of the data to the theoretical curves is excellent so the analysis must be judged adequate and the fundamental assumption not incorrect.

A point to note is that the membrane is treated as a structureless, homogeneous film in the analysis, whereas the real lecithin-cholesterol–decane bilayers are surely not. It is simply not necessary to invoke an internal membrane structure to account for the data. Putting this in a negative way, the conductivity data in themselves are insufficient to test internal membrane structure.

The observation that the membrane parameter $K_p D_m/L$ is large compared to the aqueous diffusion velocity, D_w/δ, is most important. One suspects that tetraphenylborate is not unique in this respect. This will probably occur whenever large transmembrane conductivities are found. In particular, I imagine it will be necessary to take into account diffusion polarization in order to understand the mechanism of carrier-induced ion transport[3–8]. This point was already made by LIBERMAN AND TOPALY[5]; the present work adds some fairly hard, if circumstantial, evidence to their argument.

The remarkable behavior of the conductance at high tetraphenylborate concentration and its interpretation in terms of space-charge-limited currents is the new discovery of this work. Again, it is suspected that this is a general phenomenon that is important with efficient ion carriers at high concentrations.

The method of estimating K_p and D_m, albeit only to orders of magnitude, could be of general significance. A determination of these numbers is necessary if we are to achieve a general understanding of the microscopic mechanism of membrane transport. To my knowledge, the only other estimate of an intramembrane diffusion constant is that of COLE[20] for K^+ in squid axon; fortuitously, no doubt, the two values are about the same, approx. 10^{-9} cm$^2 \cdot$sec^{-1}.

Finally, I should point out some difficulties in the present analysis in order to suggest lines of improvement.

Consider first the current–voltage characteristics at high tetraphenylborate concentration (Fig. 4). The current is superlinear in voltage, and, given the inter-

pretation in terms of space-charge-limited currents, one's first idea is to attribute the superlinearity to a transition from a first-power V dependence to a Mott–Gurney V^2/L^3 dependence at high V (ref. 19). There are two things wrong with this. The rise of current at the highest voltages is faster than V^2; the characteristic looks more like an exponential dependence. Second, the steady-state space-charge-limited current equations[19] for an assumed $L = 50$ Å were solved, and it was found that up to $V = 10\ kT/e = 257$ mV, the current remains accurately linear in V at all ion concentrations; the transition to a V^2 dependence never occurred below about 1 V. This explanation is not tenable.

A remaining, plausible interpretation of these J–V characteristics is that the diffusion constant, or mobility, of tetraphenylborate within the membrane increases with increasing V. After all, even at $V = 50$ mV the average field inside a 50-Å membrane is already 10^5 V/cm, so such a V-dependent mobility at even higher fields is not surprising; that it would assume an exponential form is indeed reasonable. The J–V characteristic (Fig. 4) can thus be thought of as a mobility–V characteristic, according to this interpretation.

Now this, if true, is interesting in itself. It does, however, mean that the analysis of the low tetraphenylborate concentration conductance data was incorrect in treating D_m as independent of V. If it increases with V, then the current should approach its saturation value more rapidly with increasing V than described by Eqn. 6. However, given the experimental error in the data, *plus* the relative insensitivity of the theoretical curves to $K_p D_m/L$ in the present system, incorporation of a V-dependent D_m gives no improvement in the fit of theory with experiment. This may be necessary in other systems.

Another improvement that could be introduced into this analysis is a better treatment of the ion–ion interaction energy than is afforded by Eqns. 20 or 21. Approaches such as calculation of Madelung energies for various configurations of anions and compensating cations were used but these artificial approaches are even less satisfactory physically than the simple arguments in Eqns. 20 and 21. What is required is a sophisticated statistical mechanical treatment that does not ignore ion–ion spatial correlation, a many-body problem.

ACKNOWLEDGMENTS

I am greatly indebted to Charles P. Bean for his suggestions, criticisms and encouragement. Discussions with Melvin Calvin, John A. Bergeron, and William J. Ward were also invaluable.

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