# EVIDENCE FOR A DISCRETE CHARGE EFFECT WITHIN LIPID BILAYER MEMBRANES

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ABSTRACT A high amplitude voltage step technique has been used to measure the surface density of dipicrylamine anions adsorbed at the surfaces of lipid bilayer membranes. Accompanying low amplitude measurements have determined the relaxation time for transient current flow across the membranes, a parameter governed by the height of the central energy barrier which dipicrylamine anions must cross in moving from one membrane surface to the other. Measured relaxation times and surface charge densities have been related by a quasi-continuum model of the discrete charge effect, which predicts that the membrane central barrier height will increase with increasing density of adsorbed surface charge. The experimentally determined relationship is in satisfactory agreement with the predictions of the model. The model does not provide a complete description of the membrane/solution interface, however, because it cannot be applied to the description of previously measured isotherms for the adsorption of dipicrylamine anions onto bilayer membrane surfaces. Possible reasons for this discrepancy are discussed.

## INTRODUCTION

Ketterer et al (1971) have shown that hydrophobic anions of dipicrylamine ($DpA^-$) and of sodium tetraphenylborate ($TphB^-$) adsorb strongly at the interfaces between aqueous solutions and lipid bilayer membranes. They have described charge transport in these systems by a three-step process of anion adsorption at one membrane/solution interface, translocation over a central energy barrier to the opposite interface, and desorption into the adjacent aqueous phase. They identified exponentially decaying current transients, observed upon application of transmembrane voltage pulses, with the membrane translocation step. Thus the relaxation time of the transient, $\tau_0$, may be taken to be proportional to a Boltzmann factor incorporating the height of the central energy barrier, provided the amplitude of applied voltage is low ($<kT/e$). Here $k$ is the Boltzmann constant, $T$ is the absolute temperature, and $e$ is the magnitude of the electron charge.

Subsequent experiments using high amplitude transmembrane voltage steps ($>kT/e$) have shown that the surface density of adsorbed $DpA^-$ (Bruner, 1975) or $TphB^-$ (Andersen and Fuchs, 1975) may be measured directly. This is possible because high

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applied voltage will completely remove anions from the higher potential energy interface. Integration over time of the resulting current density transient yields the surface charge density initially residing on the depleted interface.

In the experiments to be described here we have combined low and high amplitude techniques to explore the relationship between the relaxation time, $\tau_0$, and the magnitude, $|\sigma_s|$, of the density of DpA$^-$ ions adsorbed on the surfaces of bilayer membranes. This study builds upon earlier investigations which have shown that $\tau_0$ increases with adsorbate concentration in the aqueous phases adjacent to bilayers, both for DpA$^-$ (Bruner, 1975; Benz et al., 1976; Wulf et al., 1977) and for TphB$^-$ (Andersen and Fuchs, 1975; Andersen et al., 1978). Alternating current admittance measurements on bilayers have revealed equivalent behavior for both anion species (de Levie et al., 1974; de Levie and Vukadin, 1975). It has further been established, again for both anion species, that $|\sigma_s|$ increases with aqueous phase adsorbate concentration (Bruner, 1975; Andersen and Fuchs, 1975; Wulf et al., 1977; Wang and Bruner, 1978*a*; Andersen et al., 1978). The proximal relationship of the membrane interior to the anion adsorption planes argues strongly for the existence of a direct relationship between the parameters $\tau_0$ and $|\sigma_s|$.

Andersen et al. (1978) have measured changes of surface potential produced by adsorbed TphB$^-$, both on monolayers and bilayer membranes formed from bacterial phosphatidylethanolamine (PE). In both cases they reported changes of surface potential greater in magnitude than 2.3 $kT/e$ per decade of TphB$^-$ added to the adjacent aqueous phases. They have interpreted their findings in terms of the discrete charge effect (Barlow and MacDonald, 1967; Levine et al., 1967). Consideration of this effect leads to the conclusion that the magnitude of the potential change experienced by an ion moving from the aqueous phase onto an adsorption plane containing dispersed discrete charges (the micropotential) will be lower than the corresponding potential change (the macropotential), deduced on the assumption that the same density of charge is uniformly smeared over the adsorption plane. Andersen and co-workers have pointed out that, although the micropotential governs the adsorption of TphB$^-$, their experiments are sensitive only to the macropotential. Using the same considerations, they have also presented a qualitative explanation for their observations that the relaxation time, $\tau_0$, measured for PE bilayers, increases with aqueous phase concentration of TphB$^-$.

In this paper we show first that the potential change experienced by an ion moving from an interfacial adsorption plane to the interior of a membrane can, under appropriate conditions, include a component attributable to the discrete charge effect. A quasi-continuum model is used to demonstrate that this component is directly proportional to the density of adsorbed charge. This leads to a relation between $\tau_0$ and $|\sigma_s|$ which does not depend explicitly upon this component of potential difference. The validity of the relation is then tested by comparison with experimental data on these two parameters, using DpA$^-$ as adsorbate. A preliminary report on this work has appeared elsewhere (Wang and Bruner, 1978*c*).

## THEORY

Anions adsorbed at a membrane/solution interface will generate a positively charged diffuse counterion atmosphere in the adjacent aqueous phase, the counterions being provided by an indifferent electrolyte. The result is a double layer which is electrically neutral overall. Any model of this structure which treats the adsorbed charges as a uniformly smeared distribution would, by Gauss's law, lead to the conclusion that the double-layer electric field is confined entirely to the solution side of the adsorption plane. In this case the double-layer field could not contribute to the potential change experienced by an ion moving from the adsorption plane to the center of the membrane. When account is taken of the discreteness of the adsorbed charges, however, this conclusion is no longer valid. An ion moving into the membrane will be influenced by fringing fields generated by nearby adsorbed ions.

This discrete charge effect at the membrane/solution interface will be modeled as illustrated in Fig. 1. The diffuse counterion atmosphere is replaced by a perfectly conducting mirror plane parallel to and situated at a distance, $l$, to the solution side of the anion adsorption plane. The adsorbed anions induce positive charges on the mirror plane. The resultant electric fields on the membrane side of the mirror plane are dipole fields. They are identical to those which would be present in the membrane half-space if the mirror plane were replaced by the symmetrically distributed positive image charges shown in Fig. 1. The dipole field is illustrated for one anion. In this

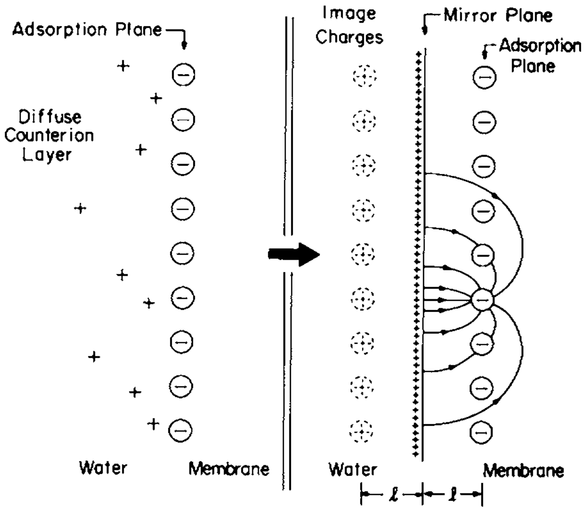

FIGURE 1 The diffuse aqueous layer adjacent to the adsorption plane for hydrophobic anions, illustrated on the left, is represented in the discrete charge model as a perfectly conducting mirror plane located a distance $l$ to the solution side of the adsorption plane. Adsorbed charges are imaged as illustrated on the right, where the dipole field produced by a representative anion and its positive image charge is shown.

model the anions will be treated as point charges; the entire medium to the membrane side of the mirror plane will be treated as a homogeneous dielectric with relative permittivity $\epsilon$. This model represents the imaging possibility characterized as "O—C" by Barlow and MacDonald (1967) involving single imaging at full magnitude. In our case, however, the interplanar spacing, $l$, cannot necessarily be equated to the separation between inner and outer Helmholtz planes. Consideration of the physical significance of $l$ will be deferred to the Discussion. Finally, we assume the membrane to be thick enough, in comparison with $l$, so that there is no need to introduce multiple imaging on account of finite membrane thickness (Brown, 1974).

The dipoles represented by each anion and its image charge are aligned parallel, hence are subject to mutual electrostatic repulsion. To take account of this we will assume that any given anion, which we will designate the central ion, will be at the center of a circular zone in the adsorption plane of radius $r_e$, the exclusion radius, within which no other anion will be found. We assume further that anions will be found at equal average density anywhere in the plane outside the exclusion zone. Therefore the charge density at radii $r \geq r_e$ will be considered uniform and equal in magnitude to $|\sigma_s|$. Now, with reference to Fig. 2, we calculate the potential change experienced by the central ion in moving a distance $D$ to the center of the membrane along a path perpendicular to the adsorption plane. In so doing we consider only the potential change, $\Delta V$, contributed by the dipole sheet surrounding the central ion. Using classical electrostatics (Lorrain and Corson, 1970), we form the integral,

$$\begin{aligned} \Delta V &= -\int_{r_e}^{\infty} \frac{2l\,|\sigma_s|}{4\pi\epsilon\epsilon_0[r^2 + D^2]}\,\frac{D}{(r^2 + D^2)^{1/2}}\,(2\pi r)\,\mathrm{d}r \\ &\simeq -\frac{l\,|\sigma_s|}{\epsilon\epsilon_0} \end{aligned} \qquad (1)$$

provided $r_e \ll D$. Admissibility of the expression for the dipole potential appearing in the integrand of Eq. 1 also requires that $l \ll r_e$. The validity of these approximations will be established below. The only quantity in Eq. 1 not previously defined is $\epsilon_0$, the permittivity of free space. For anions crossing the membrane this potential change will elevate the central barrier by an amount $(-e\Delta V)$. The transient current relaxation time in the presence of adsorbed surface charge, $\tau_0(|\sigma_s|)$, will then be related to the relaxation time in the limit of zero surface charge, $\tau_0(0)$, by the expression,

$$\begin{aligned} \frac{\tau_0(|\sigma_s|)}{\tau_0(0)} &= \exp\left[-\frac{e\Delta V}{kT}\right] \\ &\simeq \exp\left[\frac{el\,|\sigma_s|}{\epsilon\epsilon_0 kT}\right]. \end{aligned} \qquad (2)$$

This predicted relation between $\tau_0$ and $|\sigma_s|$ will be compared with the experiment below.

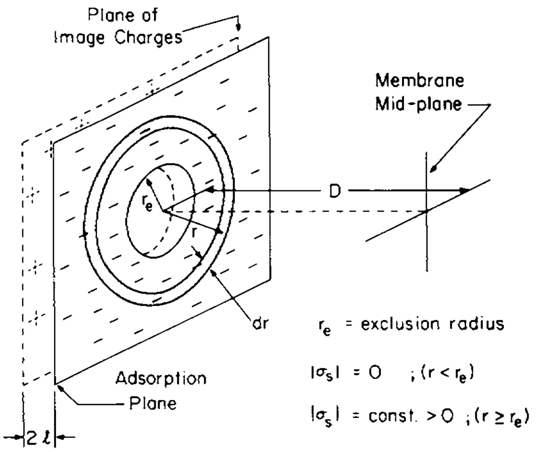

FIGURE 2 The quasi-continuum model of the discrete charge effect is illustrated. An adsorbed ion centered in an otherwise charge-free disk of radius $r_e$ experiences a change of potential in moving to the center of the membrane, determined in part by its interaction with other adsorbed ions. Integration over the equivalent dipole sheet representing the other ions and their images gives this interaction potential as illustrated above and carried out in Eq. 1.

Eq. 1 takes no account of the fact that the central ion, upon moving into the membrane, must overcome the attraction of its own image charge. This image force makes a major contribution to the height of the membrane central barrier (Neumcke and Läuger, 1969). This force is, however, independent of the presence of other adsorbed charges, being a major determinant of $\tau_0(0)$. In this analysis we are concerned only with the modification of the membrane central barrier height due to interaction between adsorbed ions.

It should be emphasized that, although $r_e$ does not appear in Eq. 2, the introduction of this parameter is essential to the quasi-continuum model of the discrete charge effect presented here. As the exclusion radius is increased (decreased), the fringing field experienced by the central ion becomes weaker (stronger) but extends to a greater (lesser) perpendicular distance from the adsorption plane. This explains the insensitivity of $\Delta V$ to $r_e$, provided the conditions $l \ll r_e \ll D$ are always met.

An estimate of the exclusion radius may be made by approximating to $kT$ the interaction energy between a pair of the previously described parallel aligned dipoles. The magnitude of each dipole moment is $p = 2el$. The estimate is,

$$\frac{1}{2}\left[\frac{p^2}{4\pi\epsilon\epsilon_0 r_e^3}\right] \approx kT \tag{3}$$

from which it follows that,

$$r_e \approx \left[\frac{1}{2\pi}\left(\frac{\epsilon}{\epsilon_0}\right)\frac{e^2}{kT}\left(\frac{l}{\epsilon}\right)^2\right]^{1/3} . \tag{4}$$

The factor 1/2 on the left of Eq. 3 enters because the dipole fields extend only over the half-space on the membrane side of the mirror plane. The Bjerrum length (Harned and Owen, 1958) can be defined for our case as the separation, $q$, at which two univalent ions of like charge in an infinite medium of relative permittivity $\epsilon$ will have a mutual electrostatic energy of $2kT$. In terms of $q$, Eq. 4 becomes,

$$r_e \approx l[4q/l]^{1/3}. \tag{5}$$

A numerical estimate of $r_e$ will be given below.

The model developed here resembles that of Grahame (1958), who was concerned with the discrete charge effect upon the relationship between micro- and macropotentials in the electric double layer. Grahame, however, related the exclusion radius to the distance between adsorbed ions (assumed to occupy a hexagonal array on the inner Helmholtz plane) whereas we assume that parameter to be independent of the surface density of adsorbed ions. Further justification of our assumption is presented in the Appendix, together with a more detailed comparison of our analysis with that of Grahame.

## MATERIALS AND METHODS

A discussion of experimental methods used, with emphasis upon high field measurement of the surface density of adsorbed $DpA^-$ ions, has been published elsewhere (Wang and Bruner, 1978*a*). In addition to the dioleyl phosphatidylcholine (PC-Analabs Inc., North Haven, Conn.) used in both that work and this, we have made use here of bacterial phosphatidylethanolamine (PE-Analabs), glycerol 1 monooleate (GMO-Nu Chek Prep, Elysian, Minn.), and cholesterol (Sigma Chemical Co., St. Louis, Mo.). Dipicrylamine was obtained from Aldrich Chemical Co., Milwaukee, Wisc., and phloretin from K & K Laboratories Inc. (Plainview, N.Y.).

In this work both high and low field measurements were always made on the same membrane, i.e., both $|\sigma_s|$ and $\tau_0(|\sigma_s|)$ were always measured on the same membrane. All low-field data were taken using applied potential pulses of 20 mV amplitude. Current transients were recorded on a storage oscilloscope, photographed, and subsequently measured for semilog plots of current amplitude vs. time. The relaxation time $\tau_0$ was given by the slope of such plots.

## RESULTS

All graphic data are presented as semilog plots, the quantity $\log[\tau_0(|\sigma_s|)/\tau_0(0)]$ being plotted vs. $|\sigma_s|$ on the linear scale. Data are presented in Fig. 3 for the aqueous electrolytes NaCl, $BaCl_2$, and $MgSO_4$. For each salt three concentrations, 0.01, 0.1, and 1.0 M, were used in the aqueous phases. As an illustration we consider the data obtained using 1.0 M $BaCl_2$ in the aqueous phases. In this case, for aqueous phase $DpA^-$ concentrations in the range $10^{-9}$ to $10^{-8}$ M, the measured low field relaxation time, $\tau_0$, remained constant and equal to 0.56 ms. This then was the value assigned to $\tau_0(0)$. High field measurements of $|\sigma_s|$ in this range of $DpA^-$ concentration yielded values between $1.65 \times 10^{-2}$ and $1.5 \times 10^{-1}$ $\mu C/cm^2$. Above $10^{-8}$ M $DpA^-$, measured values of $\tau_0$ increased sharply. At $5 \times 10^{-7}$ M $DpA^-$, the measured value of $\tau_0$ was 17.5 ms. High field measurement of $|\sigma_s|$, carried out in the same experiment, yielded the value of 2.75 $\mu C/cm^2$. The ratio of measured relaxation times is then found to be

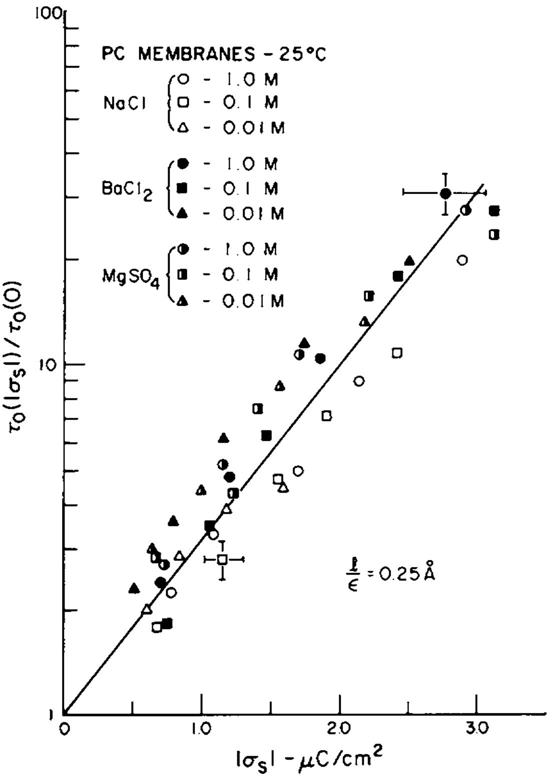

FIGURE 3 The ratio of time constants for current transients measured at low field, expressed as $\log[\tau_0(|\sigma_s|)/\tau_0(0)]$, is plotted vs. surface charge density, $|\sigma_s|$, determined by high field measurements. The discrete charge parameter, $l/\epsilon$, appearing in Eq. 2, is determined from the slope of the line drawn through the data points. The value of the parameter is seen to be insensitive to variation of the ionic strength of the aqueous phases adjacent to the membrane. Representative error bars are shown.

equal to 31. These values yielded a single point which appears as a solid circle to the upper right in Fig. 3. Affixed error bars indicate the range of estimated error in the measured quantitites. Complete tabular data upon which Fig. 3 is based will be presented elsewhere (Wang, 1978).

The data of Fig. 3, considered in toto, support the model upon which Eq. 2 is based. The slope of the single straight line we have drawn through the data points yields the model parameter value, $l/\epsilon$ = 0.25 Å, the value being insensitive to aqueous phase ionic strength.

In Fig. 4, we present similar data for different bilayer lipid compositions, all at a fixed aqueous phase electrolyte composition of 0.1 M NaCl. Though the data show substantial scatter, no marked variation of slope is evident. As in Fig. 3, a single line corresponding to $l/\epsilon$ = 0.25 Å has been drawn through the points. We note in particular that the addition of cholesterol to PC in the membrane-forming solution does not produce a significant change of slope.

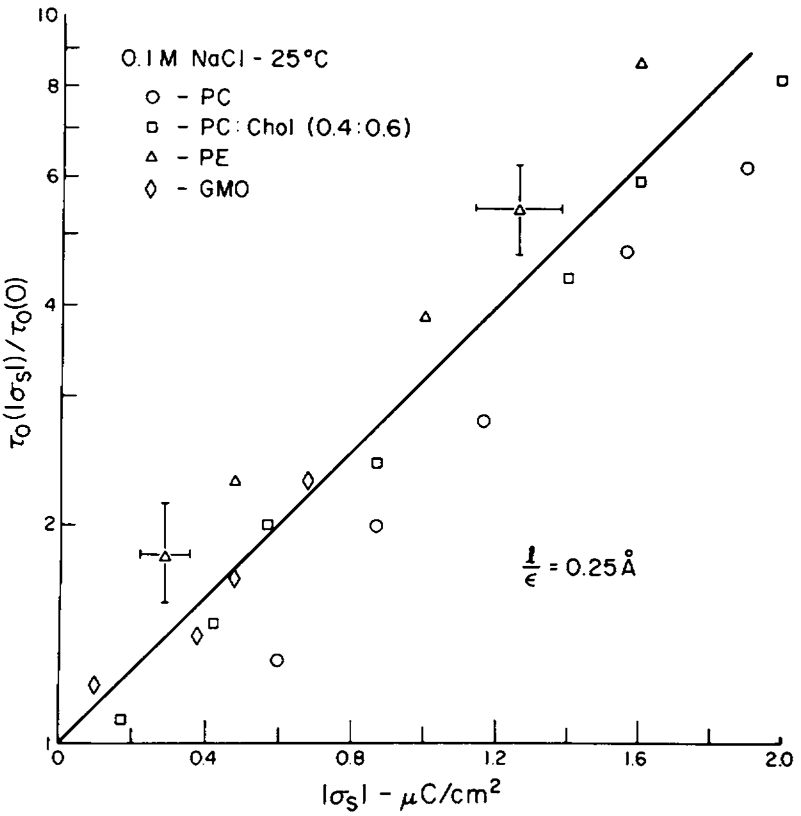

FIGURE 4 $\text{Log}[\tau_0(|\sigma_s|)/\tau_0(0)]$ is plotted vs. $|\sigma_s|$ for the lipids indicated. In the case of the mixture, PC: cholesterol, the mole fraction of each component in the membrane-forming solution is indicated. Though scatter is evident, the data suggest that the model parameter, $l/\epsilon$, does not vary significantly among the lipids indicated.

Experimental conditions have been found, however, in which significant variability in plots of $\log[\tau_0(|\sigma_s|)/\tau_0(0)]$ vs. $|\sigma_s|$ is observed. In Fig. 5 the effect of addition of cholesterol to GMO membrane-forming solutions is illustrated. Though scatter is again considerable, any fit to the model presented here would clearly require an increase in the value of the parameter $l/\epsilon$ with increasing addition of cholesterol. The opposite effect is indicated for addition of phloretin to the aqueous phases surrounding PC bilayer membranes (Fig. 6).

Assuming a membrane dielectric constant, $\epsilon \sim 2$, characteristic of a hydrocarbon, the experimentally determined value of $l/\epsilon$ yields immediately, $l \sim 0.5$ Å. Using these estimates in either Eq. 4 or 5, ($q \sim 140$ Å for $\epsilon \sim 2$), we obtain for the exclusion radius, $r_e \sim 5$ Å. Inasmuch as a typical bilayer membrane half-thickness would be $D \sim 25$ Å, we see that the condition, $l \ll r_e \ll D$, necessary for the validity of our simplified treatment of the discrete charge effect, is reasonably well satisfied. Factors governing the choice of a value for $\epsilon$ will be considered further in the Discussion.

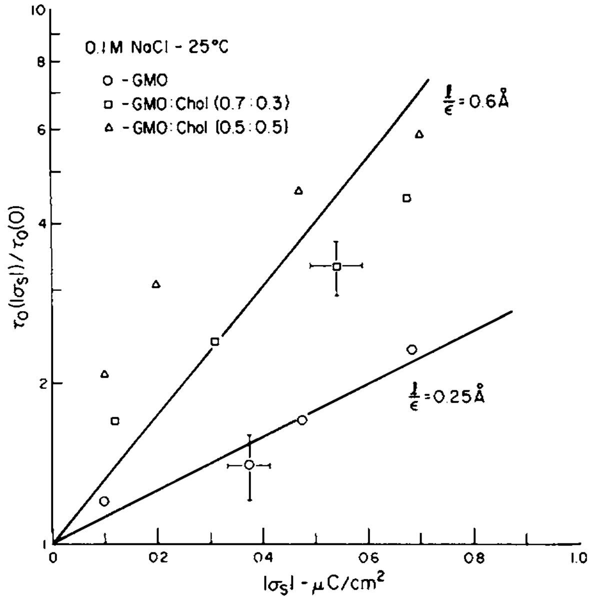

**FIGURE 5** This plot of $\log[\tau_0(|\sigma_s|)/\tau_0(0)]$ vs. $|\sigma_s|$ indicates that the addition of cholesterol to GMO membrane-forming solutions produces a significant increase in the model parameter, $l/\epsilon$. Note the expanded scale on the abscissa of this figure, when making comparisons with Figs. 4 and 6.

Our estimate for the exclusion radius should be compared with the mean distance between adsorbed ions deduced from the known surface charge density. The maximum surface charge density measured in these experiments is $|\sigma_s|_{\text{max}} \approx 3\ \mu\text{C/cm}^2$. The corresponding minimum average distance between adsorbed ions would be

$$<d>_{\text{min}} \approx [e/|\sigma_s|_{\text{max}}]^{1/2} \approx 20\ \text{Å}. \tag{6}$$

This establishes that the constraints of packing do not impose a smaller mean distance between adsorbed ions than the estimated exclusion radius.

In the Introduction it was stated that the attraction between a hydrophobic anion and its own image charge would make a major contribution to $\tau_0(0)$. Studies of the temperature dependence of $\tau_0$ (Bruner, 1975) have shown for dioleyl PC that the activation enthalpy for $\text{DpA}^-$ translocation is 14 kcal/mol. This result implies a barrier height of about 600 mV, roughly half the height to be expected if the potential change due to image force alone were operative in a membrane with a hydrocarbon core of dielectric constant $\epsilon \sim 2$ (Neumcke and Läuger, 1969). These considerations suggest that the central barrier height is determined by a superposition of the image potential with another of different origin, the most likely being that generated by dipole layers inherent to the structure of the membrane/solution interface.

The experimental evidence for membrane dipole potentials has been presented in recent reviews (McLaughlin, 1977; Andersen, 1978). The evidence indicates that these potentials make the membrane interior positive with respect to the exterior by several

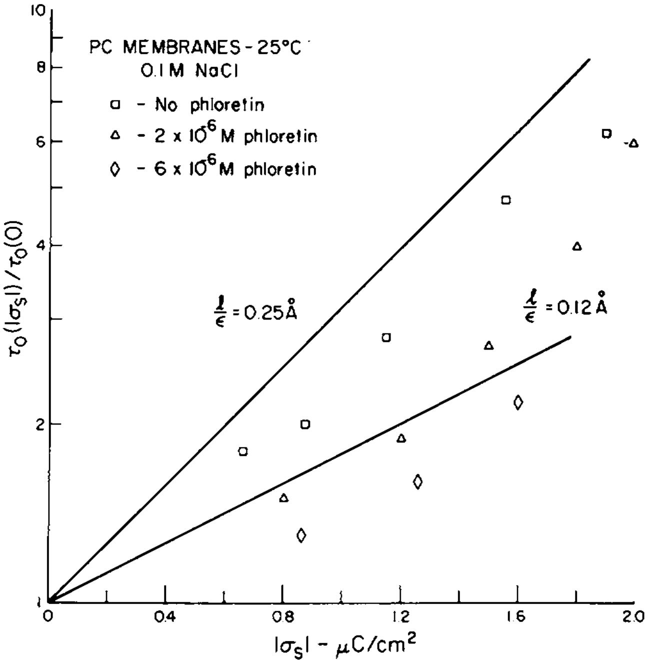

FIGURE 6 This plot of $\log[\tau_0(|\sigma_s|)/\tau_0(0)]$ vs. $|\sigma_s|$ illustrates the effect of phloretin addition to the aqueous phases adjacent to PC bilayer membranes. The trend toward lower values of the model parameter, $l/\epsilon$, is indicated.

hundred millivolts. Thus, for negatively charged DpA⁻, the inherent dipole potential difference is of the appropriate sign and magnitude to account for the discrepancy between central barrier height inferred from the temperature dependence of $\tau_0$, and the height to be expected if the image potential alone were operative. As noted in the reviews cited above, the dipole potential is sensitive to the membrane lipid and to the presence of certain molecules, such as phloretin. Because this sensitivity should be reflected in differing values of $\tau_0(0)$, we present in Table I the values of this parameter observed for the different lipid systems studied. The values range over a factor of 200, implying an equivalent variation of membrane central barrier height of about 130 mV. The addition of cholesterol to both PC and GMO membranes lowers $\tau_0(0)$. This is consistent with previous findings that cholesterol increases the positive interior potential of bilayer membranes (Szabo, 1974, 1976). The addition of phloretin to the aqueous phases, on the other hand, increases $\tau_0(0)$ observed in PC membranes. This also agrees with the observations of others (Andersen et al., 1976; Melnik et al., 1977) that such phloretin addition decreases the positive interior potential of membranes. Although it thus appears that measurement of $\tau_0(0)$ for hydrophobic anions could serve as a probe of membrane dipole potential changes, any quantitative application would depend upon knowledge of the location of the dipole layer relative to the anion adsorption plane. This point has been discussed in greater detail elsewhere (Wang and Bruner, 1978*b*).

Application of the superposition principle of electrostatics establishes that the

TABLE I

LIMITING VALUES OF THE RELAXATION TIME

(0.1 M NaCl; $T = 25^\circ C$)

| Lipid | $\tau_0(0)$ | Lipid | $\tau_0(0)$ |
|---|---|---|---|
| | *ms* | | |
| PC | 0.86 | PE | 80 $\mu s$ |
| PC:Cholesterol (0.4:0.6)* | 0.27 | GMO | 0.52 ms |
| PC-Phloretin | | GMO:Cholesterol | |
| $(2 \times 10^{-6}$ M)‡ | 2.0 | (0.7:0.3)* | 60 $\mu s$ |
| $(6 \times 10^{-6}$ M)‡ | 4.7 | (0.5:0.5)* | 20 $\mu s$ |

*The mole fractions of lipid and cholesterol in the membrane-forming solutions are indicated.

‡The concentration of phloretin in the aqueous phase is indicated.

presence of a fixed structural dipole layer would not modify the description of the discrete charge effect presented here. Such modification would occur only if the dipole layer were significantly perturbed by an increasing surface density of adsorbed $DpA^-$ ions.

## DISCUSSION

For the current transients accompanying low amplitude applied voltage steps in the presence of $DpA^-$ and $TphB^-$, an initial membrane conductance, $\lambda_{00}$, can be defined (Ketterer et al., 1971). It is simply the initial current density (excluding that which charges the membrane capacitance) divided by the amplitude of applied transmembrane voltage. For both hydrophobic anion species they reported a striking maximum of $\lambda_{00}$, plotted as a function of aqueous phase concentration of adsorbed anion. They interpreted the observation in terms of an interfacial saturation effect.

Wulf et al. (1977) have subsequently pointed out that the model developed by Ketterer and co-workers predicts that the initial conductance, relaxation time, and surface charge density are related by,

$$\lambda_{00}\tau_0 = \frac{e}{2kT} \mid \sigma_s \mid , \tag{7}$$

using the notation of this paper. This result will be strictly correct only when ions moving between the membrane interfaces experience the full applied transmembrane potential difference. It is known that translocating ions experience a smaller potential difference (Bruner, 1975; Andersen and Fuchs, 1975); nevertheless Eq. 7 provides a clear understanding of the origin of the maximum of $\lambda_{00}$. Thus it has been found that, at aqueous phase concentrations of $DpA^-$ of $10^{-8}$ M or less, $\mid \sigma_s \mid$ increases linearly with $DpA^-$ concentration while $\tau_0$ is constant. Hence a linear increase of $\lambda_{00}$ with $DpA^-$ concentration is expected. As the $DpA^-$ concentration is further increased, however, the corresponding increase of $\mid \sigma_s \mid$ becomes sublinear (Wang and Bruner, 1978*a*, *b*). At the same time $\tau_0$ starts to increase rapidly because of the onset of the discrete charge effect. From Eq. 7, $\lambda_{00}$ must pass through a maximum and then decrease, this behavior being an indirect consequence of the onset of the discrete charge effect (see also Andersen et al., 1978).

In the preceding section we presented an estimate for the exclusion radius, $r_e \sim 5$ Å, based upon an assumed dielectric constant, $\epsilon \sim 2$, for the medium near the adsorbed ions. With the ratio, $l/\epsilon$, fixed by reference to experimental data, it follows from Eq. 4 that the calculated value of $r_e$ will vary in direct proportion to $(\epsilon)^{1/3}$. Thus the maintenance of the condition, $l \ll r_e \ll D$, required for the validity of the quasi-continuum model, is not critically sensitive to the choice of $\epsilon$. For values in the range $1 \leq \epsilon \leq 8$, we find 4 Å $\leq r_e \leq$ 10 Å and corresponding values 0.25 Å $\leq l \leq$ 2 Å. Hence the condition for validity is plausibly maintained over this range of $\epsilon$. A choice of value as large as $\epsilon \sim 80$, corresponding to bulk water, leads, on the other hand, to the conclusion that $l \sim r_e \sim 20$ Å, these values being comparable to the membrane half-thickness, $D \sim 25$ Å. The condition for validity of the model clearly cannot be maintained for such a high value of $\epsilon$. Our estimate of $r_e \sim 5$ Å, based upon an assumed low value of dielectric constant, is in good accord with coulombic hard-core radii calculated by Barlow and MacDonald (1967), although they consider different imaging conditions. Their calculations, like ours, are based upon a comparison with thermal energy of the screened electrostatic interaction energy between adsorbed ions.

Our assumption of $\epsilon \sim 2$ seems most appropriate because the membrane interior, into which the fringing fields of adsorbed anions extend, is a hydrocarbon phase. The resulting value of $l \sim 0.5$ Å, however, is sufficiently small in comparison to the dimensions of $\text{DpA}^-$ ions to obscure the spatial relationship between adsorbed ions and image charges. Two points to be made in this connection are: (*a*) as noted above, the choice of a larger value of $\epsilon$, and hence of $l$, is not precluded by the model, and (*b*) the $\epsilon$ value chosen must undoubtedly represent some form of weighted average over polar and nonpolar regions of the lipid membrane, as well as boundary layer water present in varying degrees of dielectric saturation depending upon distance from the adsorption plane.

The model developed here to take account of the discrete charge effect superficially resembles the three-capacitor model developed by Andersen et al. (1978) to account for their observed isotherms characterizing the adsorption of $\text{TphB}^-$ ions onto bilayer membranes. In fact our observed value of $l/\epsilon$ can be used (assuming the adsorbed charge to be smeared) to calculate an equivalent boundary layer-specific capacitance, $(\epsilon\epsilon_0/l)$, of 35 $\mu\text{F/cm}^2$. This value, for adsorbed $\text{DpA}^-$, may be compared with their value of 70 $\mu\text{F/cm}^2$, quoted for $\text{TphB}^-$ adsorbed onto PE membranes bathed in 1.0 M NaCl solutions. The two models are not equivalent, however, for reasons we now consider. The nonlinearity of adsorption isotherms is due to perturbation by adsorbed hydrophobic ions of the potential difference between the bulk aqueous phase and sites on the anion adsorption plane. We will refer to this perturbing potential difference as the outer potential. The increase of $\tau_0$ with $|\sigma_s|$, on the other hand, reflects a perturbation by adsorbed hydrophobic ions of the potential difference between sites on the adsorption plane and the center of the membrane. We label the latter perturbing potential difference the inner potential. The inner potential governs the discrete charge effect and, for the model developed here, is equal to $\Delta V$ of Eq. 1, subject to the condition, $l \ll r_e \ll D$. Under the same condition our model gives for the outer potential, $\Delta V' \approx \Delta V(l/r_e)$, which would be negligible. This potential is evaluated as Eq. A-1 in the Appendix. The three-capacitor model, however, assumes a smeared distribution of adsorbed charge, equivalent to taking $r_e = 0$, and hence $\ll l$. It concludes thereby that the outer potential is simply $\Delta V' = \Delta V$, unattenuated by the factor $(l/r_e)$ of Eq. A-1. In this limit the three-capacitor model is unable to account for the magnitude of the inner potential implied by the experimental data reported here. Thus we are forced to the conclusion that our proposed model for the discrete charge effect and the three-capacitor model for the adsorption isotherms are mutually exclusive. The only possibility for combining them into a unified description of both inner and outer potentials would appear to be by introducing an assumption to the effect that $r_e \sim l$.

Any such unified description of the potential profile experienced by hydrophobic ions in the membrane/solution interface must be able also to account for the fact that the measured adsorption isotherms for $DpA^-$ ions are clearly sensitive to aqueous phase ionic strength (Wang and Bruner, 1978*a*), whereas the data of Fig. 3 above show no such sensitivity. In other words, the outer potential is observed to depend upon the diffuse structure of the aqueous space charge layer adjacent to the membrane surface whereas the inner potential does not. The adsorption isotherms for $DpA^-$ ions at various aqueous phase ionic strengths have been interpreted by Wang and Bruner (1978*a*) in terms of the Gouy-Chapman theory of the diffuse layer, modified by the assumption of dielectric saturation of the water exposed to boundary layer electric fields of sufficient intensity. The assumption of dielectric saturation has the effect of increasing the magnitude of the surface potential associated with a given surface charge density, and thereby permits a quantitative interpretation of the observed isotherms. The measured isotherms for adsorbed $DpA^-$ may also be fit to the three-capacitor model[1] assuming a boundary layer-specific capacitance which varies with aqueous phase ionic strength. Thus the isotherm for 0.01 M NaCl is fit by an assumed specific capacitance of $30\,\mu F/cm^2$, whereas that for 1.0 M NaCl requires a capacitance of $65\,\mu F/cm^2$. The isotherm for 0.1 M NaCl is fit by a boundary layer capacitance of $40\,\mu F/cm^2$ whereas those for 0.1 M $MgSO_4$ and $BaCl_2$ require values of 75 and $80\,\mu F/cm^2$, respectively. These variations may be understood in terms of a boundary layer capacitance consisting of a series combination of a fixed Stern layer component and a diffuse layer component with capacitance inversely proportional to the solution Debye length. We conclude that both the dielectric saturation model and the three-capacitor model reflect the need to consider the diffuse aqueous layer in dealing quantitatively with the observed isotherms for adsorption of $DpA^-$ ions. Yet no corresponding sensitivity of the discrete charge model parameter, $l/\epsilon$, to aqueous phase ionic strength is observed, as would be expected if $l$ were related to the solution Debye length. The lack of such sensitivity is evident from Fig. 3. The maximum error in slope of the single line drawn through the combined data points is estimated to be no more

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[1] O. S. Andersen. Personal communication.

than ±20%. This suggests that imaging of adsorbed hydrophobic ions takes place primarily at a dielectric interface, and does not involve counterions in the aqueous phase. A satisfactory integration of models describing both the adsorption isotherms and the discrete charge effect will undoubtedly introduce a higher level of complexity to the assumed structure of the membrane/solution interface than has heretofore been considered.

Andersen (1978) has considered an equivalent problem in commenting upon the need to introduce the discrete charge effect, in the face of the successful application of continuum diffuse double layer theory to the description of membrane surface potentials (McLaughlin, 1977). He argues qualitatively that an ion in the low dielectric constant membrane phase will have a much stronger interaction with the adjacent interface than will an ion in the high dielectric constant aqueous phase. Thus interaction between ions, inherent to the structure of the diffuse layer, would be comparatively less important in the membrane phase than in aqueous solution. A satisfactory integration of models as discussed above must await a quantitative development of this or similar arguments.

## APPENDIX

We consider first the relationship of the model presented here to that of Grahame (1958). He was concerned with the discrete charge effect upon the potential difference, which we will label $\Delta V'$, existing across the inner region of the double layer. Using the quasi-continuum model and the notation of this paper, $\Delta V'$ is given by replacing $D$ by $l$ in Eq. 1, thus

$$\begin{aligned}\Delta V' &= -\int_{r_e}^{\infty} \frac{2l\,|\sigma_s|}{4\pi\epsilon\epsilon_0[r^2+l^2]}\,\frac{l}{[r^2+l^2]^{1/2}}(2\pi r)\,\mathrm{d}r,\\ &\cong -\frac{l\,|\sigma_s|}{\epsilon\epsilon_0}\left(\frac{l}{r_e}\right) = \Delta V\left(\frac{l}{r_e}\right), \qquad \text{(A-1)}\end{aligned}$$

assuming $l \ll r_e$ and taking $\Delta V$ from Eq. 1. Note that $\Delta V'$ is the potential difference experienced by a test charge in moving a distance $l$ from the mirror plane to the adsorption plane, along a path passing perpendicularly through the center of the exclusion disk. By contrast, we note that the larger quantity, $\Delta V$, of Eq. 1 is the potential difference experienced by a test charge moving along the same path from the adsorption plane to the membrane mid-plane. Both $\Delta V'$ and $\Delta V$ are potential differences generated by adsorbed ions (and their images) which lie beyond the exclusion radius. Interaction between the central ion (or a test charge) and its own image is omitted from consideration for reasons discussed in the theory section.

If we now assume, as did Grahame, that $r_e$ is fixed by the mean distance between charges, then we could equate $r_e$ to $\langle d \rangle$ of Eq. 6, dropping the subscripts designating minimum and maximum values. It would then follow from Eqs. 6 and A-1 that $\Delta V'$ is proportional to $|\sigma_s|^{3/2}$, a conclusion reached by Grahame using a fixed lattice model. The potential difference of primary interest to our development, $\Delta V$, on the other hand, is insensitive to the value of $r_e$, provided $r_e \ll D$, the half-thickness of the membrane.

Yet Eq. 6 of the text and the accompanying discussion make it clear that if $r_e$ were determined by the mean distance $\langle d \rangle$ between adsorbed ions, then, under the experimental conditions encountered, the inequality $r_e \ll D$ would, in general, not be satisfied. To equate $r_e$ to $\langle d \rangle$, however, would be equivalent to adopting a lattice model for the distribution of anions on the adsorption plane, a procedure justifiable only when it is established that the magnitude of the repulsive interaction energy between anions separated by a distance $<d>$ is sufficient ($\geq kT$) to stabilize such a structure. The estimate of $r_e$ based upon this energy criterion and the model developed here are presented as Eq. 4 or 5. Numerical evaluation using either equation with the experimentally determined value of the model parameter $l/\epsilon$ establishes that $r_e$ is in fact significantly less than either the mean distance between adsorbed ions or the membrane half-thickness.

Finally, we present a more rigorous calculation of $\Delta V$, beginning with a generalization of Eq. 3. The magnitude of the pairwise interaction energy between parallel dipoles representing adsorbed ions and their images, the dipoles being separated by a distance $r$, is

$$|U(r)| = \frac{1}{2}\left[\frac{p^2}{4\pi\epsilon\epsilon_0 r^3}\right]. \tag{A-2}$$

The density of adsorbed charge at a distance $r$ from the central ion would then be modified by a Boltzmann factor as follows:

$$\begin{aligned} |\sigma_s(r)| &= |\sigma_s| \exp\left[-\frac{|U(r)|}{kT}\right] \\ &= |\sigma_s| \exp[-(r_e/r)^3] \end{aligned} \tag{A-3}$$

with $r_e$ defined by Eq. 3. With the charge distribution of Eq. A-3 replacing that of the exclusion disk model, Eq. 1 for $\Delta V$ is replaced by

$$\Delta V = -\int_0^\infty \frac{2l\,|\sigma_s| \exp[-(r_e/r)^3]}{4\pi\epsilon\epsilon_0[r^2 + D^2]} \frac{D}{[r^2 + D^2]^{1/2}} (2\pi r)\,\mathrm{d}r \tag{A-4}$$

which, upon integration once by parts, yields

$$\Delta V = -\frac{l\,|\sigma_s|}{\epsilon\epsilon_0} \int_0^\infty \frac{D}{[r^2 + D^2]^{1/2}} \left\{\frac{d}{dr}(\exp[-(r_e/r)^3])\right\} dr. \tag{A-5}$$

The term in curly brackets in the integrand will be sharply peaked near $r = r_e$, and, over the limits shown, will have the integral property of a $\delta$-function. If this term is replaced in Eq. A-5 by a $\delta$-function centered on $r = r_e$, tantamount to returning to the charge distribution assumed by the exclusion disk model, then Eq. A-5 becomes

$$\Delta V = -\frac{l\,|\sigma_s|}{\epsilon\epsilon_0}\left[\frac{D}{[r_e^2 + D^2]^{1/2}}\right]. \tag{A-6}$$

This is the exact result of the integral presented in Eq. 1, reducing to the approximate result cited there when $r_e \ll D$. Evidence supporting the validity of this inequality has been cited above.

Analysis of Eq. A-5 shows that the term in curly brackets will attain its maximum value when $r = (3/4)^{1/3} r_e = 0.91\, r_e$, and that >90% of the total contribution to the integral is generated over the range of integration $0.5\, r_e \leq r \leq 2.5\, r_e$.

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