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Transport Mechanism of Hydrophobic Ions through Lipid Bilayer Membranes

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Summary. Evidence is presented that the transport of lipid-soluble ions through bilayer membranes occurs in three distinct steps: (1) adsorption to the membrane-solution interface; (2) passage over an activation barrier to the opposite interface; and (3) desorption into the aqueous solution. Support for this mechanism comes from a consideration of the potential energy of the ion, which has a minimum in the interface. The formal analysis of the model shows that the rate constants of the individual transport steps can be determined from the relaxation of the electric current after a sudden change in the voltage. Such relaxation experiments have been carried out with dipicrylamine and tetraphenylborate as permeable ions. In both cases the rate-determining step is the jump from the adsorption site into the aqueous phase. Furthermore, it has been found that with increasing ion concentration the membrane conductance goes through a maximum. In accordance with the model recently developed by L. J. Bruner, this behavior is explained by a saturation of the interface, which leads to a blocking of the conductance at high concentrations.

Lipid bilayer membranes are extremely good insulators in aqueous solutions of small ions such as Na^+ or K^+. For instance, in 0.1 M NaCl, resistance values as high as $10^9\ \Omega \cdot cm^2$ are observed (Hanai, Haydon & Taylor, 1965), corresponding (with a membrane thickness of 70 Å) to a bulk resistivity of the order of $10^{15}\ \Omega \cdot cm$. The reason for the low conductivity is the high amount of energy required for the transfer of small ions from the aqueous phase into the hydrocarbon medium of the membrane. The membrane conductivity increases by many orders of magnitude, however, if certain organic ions, such as picrate, tetraphenylborate or the dipicrylamine anion, are added to the aqueous solutions (Mueller & Rudin, 1967; Liberman & Topaly, 1968, 1969; Le Blanc, 1969). In the presence of these lipid-soluble ions, the conductance is determined by the transport of a single ion species. Such a system is therefore very suitable for the study of ion transport mechanism in lipid membranes (Le Blanc, 1969).

In this paper we present evidence that the transport of a lipid-soluble ion through the membrane involves three distinct steps: (1) the adsorption of the ion to the membrane-solution interface; (2) the translocation of the ion across an energy barrier to the other interface; and (3) the desorption from the interface into the aqueous solution. Evidence for this mechanism is derived from the potential energy curve of the ion in the membrane, which shows a minimum in the membrane-solution interface. A consequence of this potential curve is the occurrence of an electrical relaxation after a sudden change of the voltage. A relaxation of the electric current after a "field-jump" is indeed found in experiments with tetraphenylborate and the dipicrylamine anion. As the relaxation time and the conductivity are related to the rate constants of the individual transport steps, the latter may be calculated from the observed time course of the electric current.

Theoretical Considerations

Potential Energy Curve of a Hydrophobic Ion in the Membrane

The potential energy W of an ion in the membrane may be represented as the sum of two terms. The first term W_e accounts for the electrostatic interaction of the ion with the membrane. In a first approximation, the membrane may be considered as a thin homogeneous liquid film of dielectric constant ε_m, which is in contact with aqueous media of dielectric constant ε. If the membrane were of macroscopic thickness, W_e would be independent of position and would simply be given by the Born energy of the ion in the membrane. For a bilayer membrane with a thickness of the order of 100 Å, however, the electrical image forces acting on the ion near the membrane-solution interface have to be taken into account (Neumcke & Läuger, 1969). This leads to a potential curve $W_e(x)$ which is depicted in Fig. 1. As ε_m is much smaller than ε, $W_e(x)$ makes a large positive contribution to $W(x)$ in the center of the membrane. The second term $W_n(x)$ contains all other interactions except electrical polarization; i.e., $W_n(x)$ is the potential energy of the hypothetical neutral particle which is generated when the ion is discharged. For hydrophobic ions such as $\mathrm{B(phenyl)}_4^-$, $W_n(x)$ has a large negative value within the membrane. As $W_n(x)$ is determined chiefly by short-range interactions, the change of $W_n(x)$ in the interface is rather steep. In other words, when the ion moves across the interface, its solvation state abruptly changes so that the main decrease of $W_n(x)$ occurs within a distance roughly equal to the diameter of the ion. When $W_e(x)$ and $W_n(x)$ are added to give the total potential energy, the resulting function $W(x)$ shows two

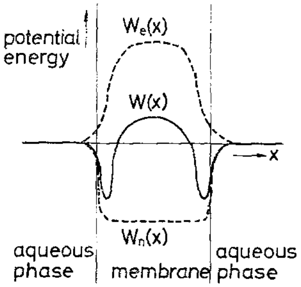

Fig. 1. Potential energy curve $W(x)$ of a lipid-soluble ion in the membrane. $W(x)$ is the sum of an electrostatic term $W_e(x)$, and a term $W_n(x)$ which accounts for the interaction of the equivalent neutral particle with the membrane

deep minima in the interfaces, which are separated by a broad barrier in the center of the membrane (Fig. 1). The occurence of a potential minimum in the interface is characteristic for the interaction between hydrophobic ions and a lipid membrane and is an essential assumption of the model which is outlined in the following paragraph. No explicit assumption, however, is required about the shape of $W(x)$ between the potential minimum and the aqueous phase; in particular, there may be an additional energy barrier which has to be surmounted when the ion is adsorbed from the aqueous solution to the interface.

Mathematical Analysis of the Model in the Limit of Low Ion Concentration

We assume that the only charge carrier in the membrane is the lipid-soluble ion X of valency z, which is present in the same concentration c on both sides of the membrane. We further assume that the aqueous solutions contain in addition to X an inert electrolyte whose ions are excluded from the membrane, so that the ionic strength can be varied independently of c. The concentrations of X in the left-hand and right-hand potential minima are denoted by N' and N'', respectively (expressed in moles per cm^2). The rates of change of N' and N'' are determined by the different transport steps indicated in Fig. 2. The rate constant for the jump from the potential

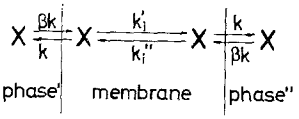

Fig. 2. Elementary steps in the transport of the lipid-soluble ion X through the membrane

minimum into the aqueous phase is denoted by k, the rate constant for the reverse jump by βk. Thus, β has the meaning of a partition coefficient:

$$\beta = \frac{N}{c} \tag{1}$$

where $N = (N')_{\text{eq}} = (N'')_{\text{eq}}$ is the interfacial concentration in the equilibrium state, i.e., in the absence of an external voltage.

It should be pointed out that in the most general case β is not a constant but depends on the concentration c. This is a consequence of the surface charge which is built up by the adsorbed ions. In the following, however, we restrict ourselves to systems in which a large excess of an inert electrolyte is present in the aqueous phases. In this case the absolute value of the surface potential is low so that the electrostatic contribution to the free energy of adsorption may be neglected (*see* appendix A).

The transport of the ion X across the potential energy barrier in the interior of the membrane is described by rate constants k_i' and k_i'' (Fig. 2). In the presence of an external voltage U, the potential energy barrier is modified by an electrostatic term. As the permeable ions are mainly localized in the membrane surfaces, the space charge within the membrane is low and, accordingly, the electrical field independent of position (Walz, Bamberg & Läuger, 1969). We may therefore introduce the usual assumption that the change in the barrier height is equal to $zFU/2$ (F is the Faraday constant). If we denote the electrical potential in the left-hand and right-hand aqueous phases by ψ' and ψ'', respectively, we obtain:

$$k_i' = k_i e^{zu/2}, \quad u = \frac{U}{RT/F} = \frac{\psi' - \psi''}{RT/F}, \tag{2}$$

$$k_i'' = k_i e^{-zu/2} \tag{3}$$

(Zwolinsky, Eyring & Reese, 1949). R is the gas constant, T the absolute temperature, and k_i the rate constant in the absence of an external voltage.

If, at time $t=0$, a voltage U is suddenly applied across the membrane, the interfacial concentrations shift from the equilibrium values $N' = N'' = N$ toward new stationary levels $\bar{N}'$ and $\bar{N}''$. The rate of change of N' and N'' is given by:

$$\frac{dN'}{dt} = \beta k c - kN' - k_i' N' + k_i'' N'', \tag{4}$$

$$\frac{dN''}{dt} = \beta k c - kN'' - k_i'' N'' + k_i' N'. \tag{5}$$

Implicit in Eqs. (4) and (5) is the assumption that the probability of a jump from left to right depends only on N', not on N''. This is a good approximation in the limit of low interfacial concentrations where the ion always has the chance to jump into a free binding site. At high concentrations, however, saturation phenomena have to be expected; these are treated in the next section. The solution of Eqs. (4) and (5) reads:

$$N'(t)=\bar{N}'+(N-\bar{N}')e^{-t/\tau}, \tag{6}$$

$$N''(t)=\bar{N}''+(N-\bar{N}'')e^{-t/\tau}, \tag{7}$$

$$\tau=\frac{1}{k+k_i'+k_i''}. \tag{8}$$

$\bar{N}'$ and $\bar{N}''$ are the stationary concentrations which are reached in the limit $t \gg \tau$:

$$\bar{N}'=N\tau(k+2k_i'') \tag{9}$$

$$\bar{N}''=N\tau(k+2k_i'). \tag{10}$$

$\bar{N}'$ and $\bar{N}''$ are directly obtained from Eqs. (4) and (5) introducing $dN'/dt=dN''/dt=0$.

For the electrical current density

$$J=zF(k_i'N'-k_i''N''), \tag{11}$$

the following relation is obtained [using Eqs. (1)–(3) and (6)–(10)]:

$$J(t)=2zF\beta ck_i\sinh(zu/2)\frac{2k_i\cosh(zu/2)e^{-t/\tau}+k}{2k_i\cosh(zu/2)+k}. \tag{12}$$

For small voltages ($|zu/2| \ll 1$), this equation reduces to

$$J(t)\approx z^2F\beta ck_iu\frac{2k_ie^{-t/\tau_0}+k}{2k_i+k}, \tag{13}$$

$$\tau_0=\frac{1}{2k_i+k}. \tag{14}$$

For the representation of experimental results, it is convenient to introduce the ohmic conductivity

$$\lambda_0=\left(\frac{J}{U}\right)_{U\approx 0}. \tag{15}$$

At times $t=0$ and $t\to\infty$:

$$(\lambda_0)_{t=0}\equiv\lambda_{00}=\frac{z^2F^2}{RT}\beta ck_i, \tag{16}$$

$$(\lambda_0)_{t\to\infty}\equiv\lambda_{0\infty}=\frac{z^2F^2}{RT}\beta c\frac{kk_i}{2k_i+k}. \tag{17}$$

The main result is contained in Eq. (13) and may be interpreted in the following way. Immediately after the application of the voltage, an initial current $J(0)=z^2 F\beta c k_i u$ flows which is solely due to a redistribution of the adsorbed ions between the two potential minima. The current decays exponentially with a relaxation time τ_0 toward a stationary value

$$J(\infty)=\frac{k}{2k_i+k}\,J(0)\,. \tag{18}$$

If the internal barrier is relatively low, i.e., if $k_i \gg k$, the initial current is much larger than the stationary current. As λ_{00}, $\lambda_{0\infty}$, and τ_0 may be obtained from the time-course of the current, Eqs. (14), (16) and (17) may be used to calculate the two rate constants k and k_i as well as the partition coefficient β.

Saturation Phenomena

In experiments with picrate, tetraphenylborate, and dipicrylamine, it was found that the membrane conductivity reaches a maximum with increasing ion concentration c and thereafter decreases (*see* below). Such a behavior is easily understood on the basis of this model, if we take into account that the number of ions which may be adsorbed to the interface is limited. An ion may jump from left to right only if there is a free "site" on the right-hand interface. As a consequence, the ion transport is inhibited at high concentrations. A theory of this blocking phenomenon has recently been developed by L. J. Bruner (1970) for the stationary state of the membrane.

The probability that an ion jumping from left to right will find a free adsorption site at the right interface is equal to $1-N''/N_s$, where N_s is the maximum number of ions which may be adsorbed per unit area. The same consideration applies for a jump from the aqueous solution to the interface. Thus, Eqs. (4), (5) and (11) have to be generalized in the following way:

$$\frac{dN'}{dt}=\beta c k\left(1-\frac{N'}{N_s}\right)-kN'-k_i'N'\left(1-\frac{N''}{N_s}\right)+k_i''N''\left(1-\frac{N'}{N_s}\right), \tag{19}$$

$$\frac{dN''}{dt}=\beta c k\left(1-\frac{N''}{N_s}\right)-kN''-k_i''N''\left(1-\frac{N'}{N_s}\right)+k_i'N'\left(1-\frac{N''}{N_s}\right), \tag{20}$$

$$J=zF\left[k_i'N'\left(1-\frac{N''}{N_s}\right)-k_i''N''\left(1-\frac{N'}{N_s}\right)\right]. \tag{21}$$

At zero voltage ($t<0$), the interfacial concentrations on both sides are equal: $N'=N''=N$. The equilibrium at the interface is then described by the relation $\beta c k(1-N/N_s)=kN$ which gives

$$N=\frac{\beta c}{1+\beta c/N_s}. \tag{22}$$

The stationary state concentrations $\bar{N}'$ and $\bar{N}''$ in the limit $t\to\infty$ are obtained by introducing $dN'/dt=dN''/dt=0$ into Eqs. (19) and (20). In the ohmic case in which $k_i'\approx k_i(1+zu/2)$, $k_i''\approx k_i(1-zu/2)$ holds and terms proportional to u^2 may be neglected, $\bar{N}'$ and $\bar{N}''$ are found to be

$$\bar{N}'=N\left[1-\frac{k_i z u}{k(1+\beta c/N_s)^2+2k_i(1+\beta c/N_s)}\right], \tag{23}$$

$$\bar{N}''=N\left[1+\frac{k_i z u}{k(1+\beta c/N_s)^2+2k_i(1+\beta c/N_s)}\right]. \tag{24}$$

In order to calculate the initial ohmic conductance λ_{00}, we introduce $N'=N''=N$ from Eq. (22) into Eq. (21) and obtain:

$$\lambda_{00}=\frac{z^2F^2}{RT}\,\frac{\beta c k_i}{(1+\beta c/N_s)^2}. \tag{25}$$

If, on the other hand, $N'=\bar{N}'$, $N''=\bar{N}''$ are introduced into Eq. (21), the stationary ohmic conductance is found to be (neglecting terms proportional to u^2):

$$\lambda_{0\infty}=\frac{z^2F^2}{RT}\,\beta c\,\frac{k k_i}{k(1+\beta c/N_s)^2+2k_i(1+\beta c/N_s)}. \tag{26}$$

In the limit $\beta c/N_s \ll 1$, Eqs. (25) and (26) reduce to Eqs. (16) and (17), respectively. The maximum of λ_{00} is reached at the concentration

$$c_{0,\,\max}=\frac{N_s}{\beta} \tag{27}$$

and is equal to

$$\lambda_{00,\,\max}=\frac{z^2F^2}{4RT}\,k_i N_s. \tag{28}$$

Correspondingly, for $\lambda_{0\,\infty}$:

$$c_{\infty,\,\max}=\frac{N_s}{\beta}\sqrt{1+2k_i/k}, \tag{29}$$

$$\lambda_{0\infty,\,\max}=\frac{z^2F^2}{2RT}\,\frac{k k_i N_s\sqrt{1+2k_i/k}}{(k+k_i)(1+\sqrt{1+2k_i/k})+k_i}. \tag{30}$$

It is seen from Eqs. (27)–(30) that a measurement of λ_{00} and $\lambda_{0\infty}$ at high ion concentrations c may provide additional information about the parameters of the model. In practice, the number of "adsorption sites", N_s, is most easily obtained from $c_{0,\max}$ [Eq. (27)]. Eqs. (28)–(30) may then be used for a check of the internal consistency of the model.

Materials and Methods

Dipicrylamine (Fluka, puriss.) and sodium tetraphenylborate (Merck, zur Analyse) were used without further purification. Dioleoyllecithin was synthesized after the method of Robbles and van den Berg (1969) and purified twice on a silicic acid column; the product gave a single spot on the thin-layer chromatogram.

Bimolecular "black" membranes were formed as described previously (Läuger, Lesslauer, Marti & Richter, 1967) from a solution of dioleoyllecithin in n-decane on a Teflon support with a circular hole 3 mm in diameter. Ag:AgCl or platinized platinum electrodes of area 1 cm² were used throughout.

The arrangement for the electrical relaxation measurements is shown in Fig. 3. A voltage U could be applied to the membrane through an electronic switch with a rise time of about 1 μsec. The internal resistance of the voltage source and the switch in the "on"-position was a few ohms. The time-course of the current was measured with a Tektronix 549/1 A 7 A storage oscilloscope as a voltage-drop across the external resistor R_e. The cell with the membrane is represented in Fig. 3 by an equivalent circuit consisting of a resistance R_s (electrodes plus solutions) in series with a parallel combination of the membrane resistance R_m and capacitance C_m. In most experiments the aqueous solutions contained 0.1 M KCl or NaCl which gave a cell resistance of $R_s \simeq 100\ \Omega$. In all cases the condition $R_e + R_s \ll R_m$ was fulfilled, so that the time constant of the circuit was given by:

$$\tau_c = R_m C_m \frac{R_e + R_s}{R_e + R_s + R_m} \approx C_m (R_e + R_s). \tag{31}$$

As $C_m \simeq 4 \times 10^{-8}$ F (membrane area $\simeq 0.1$ cm²) and $R_e + R_s \geqq 200\ \Omega$, τ_c is equal to 8 μsec in the most favorable case. The effective time resolution of the system, i.e., the minimum relaxation time which could be measured with an accuracy of a few percent, was about $10\,\tau_c$. The general case in which τ is no longer large compared with τ_c is considered in appendix B.

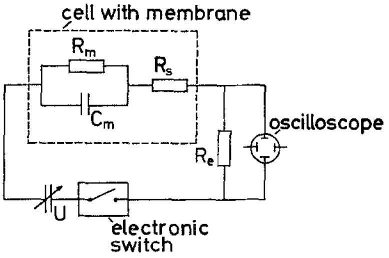

Fig. 3. Arrangement for electrical relaxation measurements. The cell with the membrane is represented by a simplified equivalent circuit. R_s is the combined resistance of the electrodes and the aqueous solutions; R_m and C_m are the resistance and capacitance, respectively, of the membrane

In order to check that the observed relaxation phenomena are not influenced by electrode polarization, the following procedure was used. After the normal relaxation experiment, the membrane was destroyed and the equivalent components $(R_m)_{t=0}$ and C_m in parallel were inserted between one electrode and the external circuit in place of the membrane (*compare* Fig. 3). The measurement was then repeated. In all cases the time course of the current was found to be equal to that expected from the equivalent circuit of Fig. 3. Therefore, polarization phenomena at the electrodes could be excluded.

Results

Electrical Relaxation of the Membrane

In the presence of N(picryl)$_2^-$ or B(phenyl)$_4^-$, very pronounced relaxation effects are observed. A typical example is shown in Fig. 4 where the current J is plotted as a function of time after a voltage jump of $U = 33$ mV. The solution contained 5×10^{-8} M dipicrylamine at pH 6; with a pK of 2.66 (Gaboriaud, 1966), the dipicrylamine is fully dissociated at this pH value. After the charging of the membrane capacitance, the current decays with a time constant τ of about 1 msec. The initial current $J(0)$ is obtained

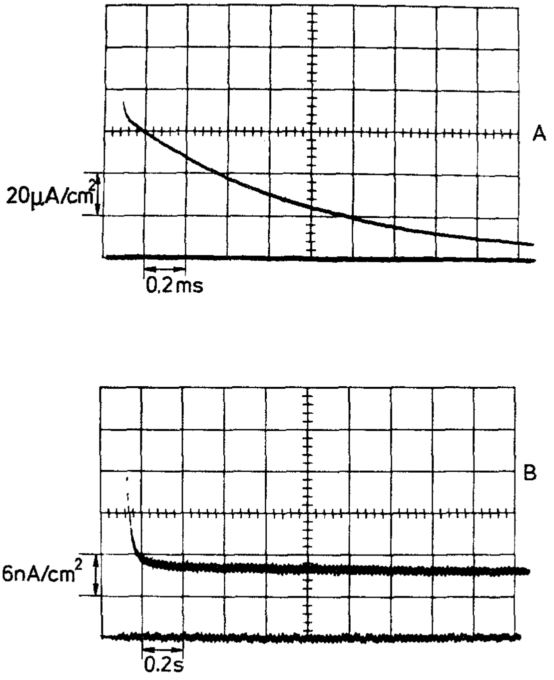

Fig. 4. Time-course of the current density after the application of a voltage step of $U = 33$ mV across the membrane. Both measurements were made with the same membrane (note the largely different current- and time-scales). The capacitative time constants τ_c of the circuit [Eq. (31)] were 8 μsec (A) and 4 msec (B). 5×10^{-8} M dipicrylamine in 0.1 M KCl; pH 6; 25 °C

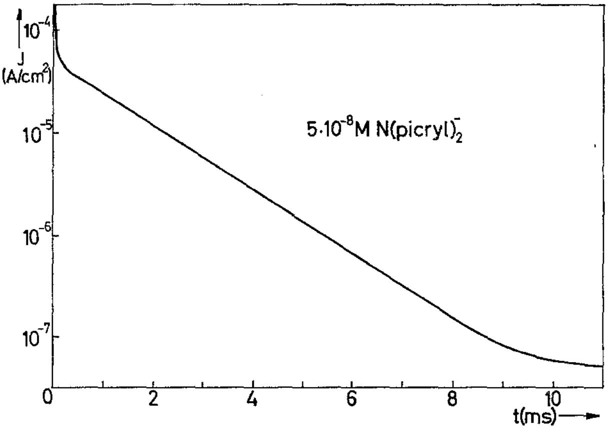

Fig. 5. Semi-logarithmic plot of the current density J as a function of time t under the same experimental conditions as in Fig. 4. The curve has been constructed from several oscillograms with different scales of current and time, obtained from the same membrane

by extrapolation to zero time and is equal to ~70 μamp/cm^2.[1] Ideally, the current should approach a stationary value $J(\infty)$ for $t \gg \tau$ [*see* Eq. (18)]. This is not strictly the case, as Fig. 4B shows. The slow decrease of the current for $t \gg \tau$ is caused by diffusion polarization in the aqueous phases (Le Blanc, 1969; Neumcke, 1971). The occurrence of diffusion polarization has the consequence that the exact value of $J(\infty)$ cannot be obtained from the experimental $J(t)$ curve. However, it may be concluded from Fig. 4 that $J(\infty) \ll J(0)$, a result which will be used below.

In Fig. 5 the current is plotted on a logarithmic scale as a function of time. After the initial charging of the membrane capacitance, log J becomes a linear function of t, as predicted by Eq. (13) in the limit $k \ll k_i$ [the condition $k \ll k_i$ is fulfilled because $J(\infty)$ is much smaller than $J(0)$ – *see* Eq. (18)]. The exponential decay of the current is a characteristic property of the "internal" relaxation process which consists of a redistribution of hydrophobic ions between the two membrane surfaces. On the other hand, diffusion polarization results from a shift of the ion concentrations in the external phases near the membrane. In the presence of pure diffusion polarization, the time-course of the current cannot be described by an exponential function (Neumcke, 1971); instead, $J(t)$ is given by $A - B\sqrt{t}$ for short t and by $C/\sqrt{t}$ for long t (A, B and C being constants). The experimentally observed shape of $J(t)$ may therefore be used to discriminate between diffusion polarization and the internal relaxation process. Results

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[1] By using different external resistances R_e, it has been checked that the extrapolated value of $J(0)$ as well as the time constant τ are independent of the charging time τ_c.

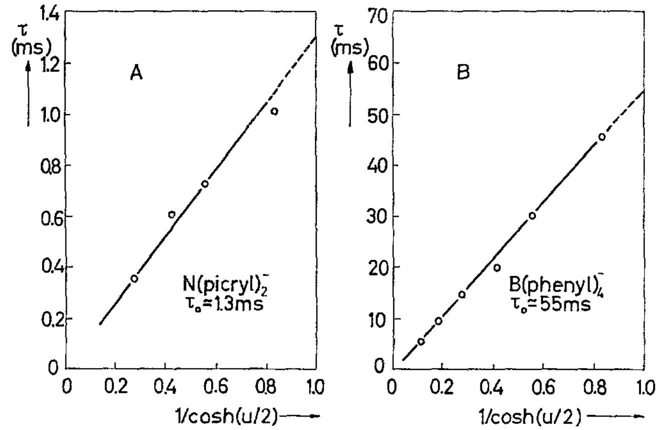

Fig. 6. Voltage dependence of the relaxation time τ. The experimental values of τ are plotted as a function of 1/cosh $(u/2)$. u is the reduced voltage [*compare* Eq. (2)]. Extrapolation to $\cosh(u/2) = 1$ gives the relaxation time, τ_0, in the ohmic limit $u \approx 0$. (A) 5×10^{-8} M dipicrylamine in 0.1 M KCl; pH = 6; 25 °C. (B) 10^{-7} M tetraphenylborate in 0.1 M NaCl; pH = 6; 25 °C

qualitatively similar to those with dipicrylamine are obtained in the presence of tetraphenylborate. In both cases $J(\infty)$ is much smaller than $J(0)$, i.e., $k \ll k_i$. However, with B(phenyl)$_4^-$, the relaxation time is about 40 times longer than with N(picryl)$_2^-$.

From Eqs. (2), (3) and (8), it is predicted that the relaxation time τ depends on the reduced voltage u:

$$\begin{aligned} \tau &= \frac{1}{k + 2k_i \cosh(zu/2)} \qquad (\beta c \ll N_s) \\ &\approx \frac{1}{2k_i \cosh(zu/2)} \qquad (k \ll k_i)\,. \end{aligned} \tag{32}$$

Thus, if τ is plotted as a function of 1/cosh $(zu/2)$, a straight line should result. This is indeed the case for N(picryl)$_2^-$ as well as for B(phenyl)$_4^-$, as Fig. 6 shows. By extrapolation to $\cosh(zu/2) = 1$, the relaxation time τ_0 in the ohmic limit $u \approx 0$ is obtained.

Membrane Conductance as a Function of Concentration

The initial membrane conductance λ_{00} in the ohmic limit is shown in Fig. 7 as a function of the concentration c of both dipicrylamine and tetraphenylborate. In both cases, λ_{00} increases linearly with c at low c, but goes through a maximum at higher c. Similar behavior is found with picrate

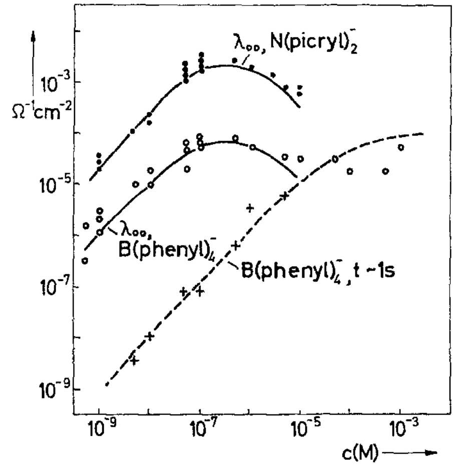

Fig. 7. Membrane conductance as a function of ion concentration c. Upper curves: conductance λ_{00} at time $t=0$. •, 0.1 M KCl plus various concentrations of dipicrylamine (25 °C, pH 6); ∘, plus various concentrations of tetraphenylborate; ——, theoretical curve according to Eq. (33); +, conductance after $t=1$ sec (tetraphenylborate); ------, conductance measurements of Liberman and Topaly (1969) (tetraphenylborate)

as a permeable ion (E. Bamberg, *unpublished*). According to the model described above, the occurrence of a maximum in the $\lambda_{00}(c)$-curve is a consequence of the limitation in the number of ions which may be adsorbed to the membrane surface. It is seen from Eqs. (25), (27) and (28) that the following relation holds for $\lambda_{00}(c)$:

$$\lambda_{00}=\frac{4c/c_{0,\,\mathrm{max}}}{(1+c/c_{0,\,\mathrm{max}})^2}\,\lambda_{00,\,\mathrm{max}}\,. \tag{33}$$

This function is plotted in Fig. 7 together with the measured values of λ_{00}. The agreement between the theoretical curve and the experimental results is satisfactory for $\mathrm{N(picryl)}_2^-$; however, the deviation which is observed with $\mathrm{B(phenyl)}_4^-$ at higher concentrations indicates that the blocking of the conductance is not complete in this case.

It is interesting to note that the conductance maximum is only observed in the initial current. If the conductance is recorded several seconds after the voltage has been applied to the membrane, only an asymptotic behavior of λ_0 at high concentrations is obtained (Liberman & Topaly, 1969; Le Blanc, 1969). This is easily explained. Under conditions where the ratio λ_0/c is high, and after times of the order of seconds, the current is determined essentially by diffusion in the external phases and is largely independent of the properties of the membrane (*see* below). However, at high c, the ratio λ_0/c is diminished as a consequence of the saturation, and the diffusion polarization becomes less pronounced. Accordingly, the static conductance approaches λ_{00} at high ion concentrations. This is shown in Fig. 7 in which the experimental conductance values for B(phenyl)$_4^-$, taken at $t=1$ sec from the oscillograms are plotted together with the $\lambda_0(c)$-curve from the paper of Liberman and Topaly (1969). It is seen that the conductance measured at $t=1$ sec approximately agrees with the values of Liberman and Topaly, which were presumably recorded after a time of one to several seconds.

At long times, where the current J is limited by diffusion in the aqueous phases, J is simply given by (Neumcke, 1971)

$$J(t)=\frac{1}{2}z^2Fcu\sqrt{\frac{D}{\pi t}} \qquad (|u|\ll 1) \tag{34}$$

where D is the diffusion coefficient of the permeable ion in the aqueous phases. With the known diffusion coefficient of B(phenyl)$_4^-$ in water, $D=5.2\times 10^{-6}$ cm^2 sec^{-1} (Skinner & Fuoss, 1964), the conductance after $t=1$ sec is calculated from Eq. (34) to be $2.5\times 10^{-8}\ \Omega^{-1}$ cm^{-2} for $c=10^{-8}$ M. This value is approximately equal to the static conductance reported by Liberman and Topaly (1969) and also found in our measurements ($10^{-8}\ \Omega^{-1}$ cm^{-2}).

In the case of N(picryl)$_2^-$, the current at long times ($t\sim 1$ sec) exceeds the value predicted by Eq. (34) by a factor of about two to three. As the current in this time range has not been used for the evaluation of the parameters of the model (*see* below), the origin of the discrepancy has not been studied further. A possible reason may be that a second charge-transport mechanism (for instance, transport of H$^+$ or K$^+$) becomes predominant after the decay of the current carried by N(picryl)$_2^-$. However, as λ_{00} and τ are independent of the pH and the K$^+$ concentration, it may be concluded that the initial relaxation process is determined only by the transport of N(picryl)$_2^-$.

Membrane Conductance as a Function of Voltage

A theoretical expression for the voltage dependence of the initial conductance $(\lambda)_{t=0}\equiv(J/U)_{t=0}$ is obtained from Eqs. (12) and (16):

$$\left(\frac{\lambda}{\lambda_0}\right)_{t=0}=\frac{2}{zu}\sinh\left(\frac{zu}{2}\right). \tag{35}$$

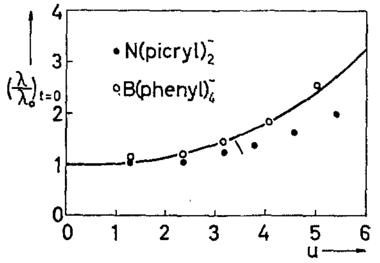

Fig. 8. Initial membrane conductance as function of the reduced voltage u. •, 5×10^{-8} M dipicrylamine in 10^{-1} M KCl (25 °C, pH 6); ∘, 5×10^{-8} M tetraphenylborate in 10^{-1} M NaCl (25 °C, pH 6); ——, theoretical curve according to Eq. (35)

This function and the experimental values of $(\lambda/\lambda_0)_{t=0}$ are plotted in Fig. 8. It may be seen that the agreement between the theoretical curve and the experimental values is fairly good. This simple form of the conductance function is obtained because only the voltage at the top of the activation energy barrier enters into the expressions for k_i' and k_i'' [Eqs. (2) and (3)]. A more detailed treatment of λ/λ_0 based on an electrodiffusion model, in which the shape of the activation barrier is calculated from the image force, has been given previously (Neumcke & Läuger, 1969).

Evaluation of the Parameters β, k, k_i, N_s

It has been found experimentally that the relation $J(\infty) \ll J(0)$ holds for N(picryl)$_2^-$ as well as for B(phenyl)$_4^-$; therefore, according to Eq. (18), $k \ll k_i$. This means that the rate-determining step in the transport of the ion across the membrane is the jump across the interface. With $k \ll k_i$, Eq. (14) reduces to $\tau_0 \approx 1/2\, k_i$, so that the rate constant k_i can be directly obtained from the relaxation time τ_0. Using Eq. (16), the partition coefficient β is then calculated from the experimental value of λ_{00}/c taken from the linear part of the $\lambda_{00}(c)$-curve. Likewise, the maximum number, N_s, of adsorption sites in the interface is obtained from $c_{0,\,\mathrm{max}}$ [Eq. (27)].

For the determination of the rate constant k, the stationary conductance $\lambda_{0\,\infty}$ [Eq. (17)] would be needed. However, as pointed out above, the evaluation of $\lambda_{0\,\infty}$ from $J(t)$ is not possible because the current is determined by diffusion polarization at times $t \gg \tau_0$. Therefore, only upper and lower limits for k can be estimated. An upper limit is given by the inequality $k \ll k_i$. On the other hand, a lower limit may be estimated from the current which is observed after the end of the exponential phase of $J(t)$. For instance, in the case of N(picryl)$_2^-$, the exponential component of $J(t)$

Table 1. *Summary of experimental data*

Ion	τ_0 (sec)	λ_{00}/c [a] ($\Omega^{-1}\,\mathrm{cm}^{-2}\,\mathrm{M}^{-1}$)	$c_{0,\max}$ (M)	$\lambda_{00,\max}$ ($\Omega^{-1}\,\mathrm{cm}^{-2}$)	ϑ
$\mathrm{N(picryl)}_2^-$	1.3×10^{-3}	3×10^{4}	3×10^{-7}	3×10^{-3}	0.8
$\mathrm{B(phenyl)}_4^-$	55×10^{-3}	1×10^{3}	3×10^{-7}	7×10^{-5}	1.1

[a] Taken from the linear part of $\lambda_{00}(c)$.

Table 2. *Calculated values of the parameters* β, k, k_i, *and* N_s

Ion	k_i (sec^{-1})	k (sec^{-1})	β (cm)	N_s (cm^{-2})
$\mathrm{N(picryl)}_2^-$	380	$1<k\ll400$	2×10^{-2}	4×10^{12}
$\mathrm{B(phenyl)}_4^-$	9	$0.1<k\ll10$	3×10^{-2}	5×10^{12}

decays to the basic membrane conductivity ($\sim10^{-9}\,\Omega^{-1}\,\mathrm{cm}^{-2}$) after $t^*\simeq20$ msec (this is seen from Fig. 5 by extrapolation). At this time t^*, the diffusion current as calculated from Eq. (34), is smaller than the stationary current $J(\infty)$ which would be observed in the absence of polarization. Thus, if $J(\infty)>J(t^*)$ is introduced into Eq. (18), a lower limit of k is obtained.

A summary of the experimental data is given in Table 1. For a test of the internal consistency of the data, the table also contains calculated values of the dimensionless quantity ϑ defined by

$$\vartheta \equiv \frac{c_{0,\max}}{4\lambda_{00,\max}} \left(\frac{\lambda_{00}}{c}\right)_{c\ll c_{0,\max}}. \tag{36}$$

As may be seen from Eq. (33) the theory requires that $\vartheta\equiv1$. This is approximately the case, as Table 1 shows. The calculated values of β, k, k_i, and N_s are presented in Table 2.

Discussion

The transport of lipid-soluble ions through bilayer membranes has been treated previously, to a first approximation, as a diffusion process in a continuous medium, using the Nernst-Planck formalism (Ciani, Eisenman & Szabo, 1969; Walz *et al.*, 1969). In this paper we propose a more detailed model which is based on the potential energy function of an ion

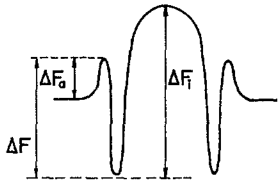

Fig. 9. Activation energies involved in the ion transport through the membrane

in the membrane and which describes the ion transport as a passage over activation barriers. Furthermore, it is shown that the rate constants associated with the different transport steps can be evaluated (under favorable conditions) from the relaxation of the electric current which is observed after a sudden change of the voltage.

According to the theory of Eyring, the rate constant of a transport process is equal to a frequency factor f times the exponential of the free energy, ΔF, of activation. Thus,

$$k_i = f_i \cdot e^{-\Delta F_i/RT}, \tag{37}$$

$$k = f \cdot e^{-\Delta F/RT}, \tag{38}$$

$$\beta k = f_a l_a \cdot e^{-\Delta F_a/RT} \tag{39}$$

(*compare* Figs. 2 & 9). l_a is the jump length for a jump from the aqueous solution into the potential minimum at the interphase. As the passage across the interfacial barrier in either direction may be assumed to occur in a single jump, the frequency factors f and f_a are of the order of $RT/hN_A \simeq 6 \cdot 10^{12}\ \mathrm{sec}^{-1}$ (h = Planck constant; N_A = Avogadro's number). The passage across the barrier in the interior of the membrane, however, involves in reality a series of jumps over smaller activation barriers which are superimposed on the smooth energy curve of Fig. 9. For the purpose of a crude estimation, we neglect this complication and set $f \simeq RT/hN_A$. We then obtain, with $l_a \simeq 10$ Å, the following approximate values of the activation energies (*see* Table 3). ΔF_i should be roughly equal to the dielectric energy of the ion in the middle of the membrane. In the case of the spherical

Table 3. *Calculated free energies of activation (kcal/mole)*

Ion	ΔF_i	$\Delta F - \Delta F_a$	ΔF	ΔF_a
N(picryl)$_2^-$	14.0	7.3	$14.0 < \Delta F < 17.5$	$6.7 < \Delta F_a < 10.2$
B(phenyl)$_4^-$	16.2	7.5	$16.2 < \Delta F < 18.9$	$8.7 < \Delta F_a < 11.4$

tetraphenylborate ion with a radius of 4.2 Å (Grunwald, Baughman & Kohnstam, 1960), the image force calculation (Neumcke & Läuger, 1969) gives a dielectric energy of 17.4 kcal/mole (using $\varepsilon_m = 2$ and $d_m = 50$ Å for the dielectric constant and the thickness, respectively, of the membrane). This value is indeed not very different from $\Delta F_i = 16.2$ kcal/mole, as calculated from the measured rate constant.

An essential feature of the proposed model is the limitation in the number of ions which can be adsorbed to the membrane-solution interface, and the resulting decrease of the conductance at high ion concentrations. Previously, the asymptotic behavior of the conductance at high values of c observed in static conductance measurements had been tentatively explained by the assumption that the ion concentration in the membrane is space-charge limited (Le Blanc, 1969; Neumcke & Läuger, 1970). The experiments presented above have shown, however, that the static conductance is strongly influenced by polarization effects in the aqueous phases, and, therefore, is not a very meaningful parameter. On the other hand, the initial conductance which is a real property of the membrane does not exhibit an asymptotic behavior, but decreases at high ion concentration. This finding is not consistent with the former continuum treatment involving space charges; it can be easily understood, however, if the number of adsorption sites in the interface is limited.

An important question, of course, is the nature of this limitation. Again, one may suppose that the electric charge built up in the interface in the course of the ion adsorption may prevent the adsorption of further ions. However, application of the Gouy-Chapman theory (Neumcke, 1970) shows that in the limit of high ion concentration, the interfacial density of adsorbed ions does not become a constant but increases with $c^{1/3}$. Therefore, the saturation must be of non-coulombic origin. The observed number of adsorption sites, $N_s \simeq 5 \times 10^{12}$ cm^{-2}, corresponds to a mean distance of about 45 Å. Considering the number of lecithin molecules (about 2.5×10^{14} cm^{-2} on each side of the membrane, if dense packing is assumed), there is one adsorption site for every 50 lecithin molecules. Therefore, any well-defined stoichiometry between adsorbed ions and lipid molecules seems unlikely. However, another explanation is feasible. From the considerations mentioned at the beginning of this paper, it is probable that the adsorbed ions are localized in the layer of the polar head groups of the lipid molecules. While the hydrocarbon tails have a tendency to remain as closely packed as possible for energetic reasons, the head groups will be pushed aside by the adsorbed ions. The resulting strain will therefore limit the number of ions which can be incorporated into the two-dimensional "lattice" of the polar head groups. A similar phenomenon is well known from three-dimensional crystals in which the solubility of foreign substances is severely restricted by steric constraints.

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Appendix A

Electrical Potential at the Membrane-Solution Interface Owing to Ion Adsorption

According to the Gouy-Chapman theory, the relation between the electrical potential ψ_s at the membrane surface and the charge density σ owing to the adsorbed ions is given by (Neumcke, 1970)

$$\psi_s = \frac{2RT}{F} \operatorname{arc\,sinh} \frac{\sigma}{\sigma_0} \equiv \frac{2RT}{F} \ln \left[\frac{\sigma}{\sigma_0} + \sqrt{\left(\frac{\sigma}{\sigma_0}\right)^2 + 1} \right] \qquad (40)$$

$$\sigma_0 = \frac{\varepsilon \kappa RT}{2\pi F} \quad (z = \pm 1) \qquad (41)$$

(ε = dielectric constant of water; $1/\kappa$ = Debye length). The maximum possible charge density at the membrane surface is equal to $-e_0 N_s$ (e_0 = elementary charge). With $N_s = 5 \times 10^{12}$ ions/cm^2, $\varepsilon = 78.5$, $1/\kappa = 9.6$ Å (for an ionic strength of 0.1 M, as used in the experiments), we obtain $\psi_s = -11$ mV. The influence of surface charges on the properties of the membrane is small as long as the absolute value of ψ_s is less than $RT/F \simeq$ 26 mV. We may therefore conclude that under the conditions of our experiments Gouy-Chapman effects may be neglected.

Appendix B

Alternating-Current Impedance of the Membrane

Besides relaxation experiments, an alternative method for the determination of the rate constants consists in a measurement of the alternating current impedance of the membrane. For this purpose an alternating voltage

$$U = U_0 \sin \omega t \qquad (42)$$

of frequency $\omega/2\pi$ is applied to the membrane. The resulting current is then given by

$$J = J_0 \sin(\omega t + \varphi). \qquad (43)$$

The information about the rate constants is contained in the *a-c.* impedance $|Z| \equiv U_0/J_0 = f(\omega)$ and in the phase shift $\varphi(\omega)$. In the following, we calculate φ and $|Z|$ as functions of the rate constants.

We denote the total charge density (coulombs/cm^2) of the left-hand interphase at time t by q (the charge density of the right-hand interface is then equal to $-q$). As q

varies with time, the electric current is discontinuous in the interphase:

$$J-J_m=\frac{dq}{dt} \tag{44}$$

where J and J_m are the current densities in the aqueous phases and in the membrane, respectively. [In the previous analysis, we have assumed that the membrane capacitance is already charged at the beginning of the relaxation process ($\tau_c \ll \tau$), so that $J=J_m$.] The charge density q is related to the voltage U and to the geometrical capacitance C_m per unit area of the membrane:

$$q=C_m U=\frac{\varepsilon_m}{4\pi d_m} U \tag{45}$$

where ε_m and d_m are the dielectric constant and the thickness of the membrane, respectively. In the following, we restrict ourselves to the ohmic limit ($|U| \ll RT/F$) and assume that the membrane is far from saturation ($\beta c \ll N_s$). J_m is then given by [*compare* Eq. (11)]:

$$J_m=zF(k_i' N'-k_i'' N''). \tag{46}$$

$N'(t)$ and $N''(t)$ are obtained by solving Eqs. (4) and (5) under the conditions

$$k_i' \approx k_i\left(1+\frac{zu}{2}\right)=k_i\left(1+\frac{zu_0}{2}\sin\omega t\right) \tag{47}$$

$$k_i'' \approx k_i\left(1-\frac{zu}{2}\right)=k_i\left(1-\frac{zu_0}{2}\sin\omega t\right) \tag{48}$$

($u_0 \equiv U_0 F/RT$). The asymptotic solution ($t \to \infty$) reads

$$N'+N''=2\beta c \tag{49}$$

$$N'-N''=-\frac{2\beta c k_i \tau_0 z u_0}{\sqrt{1+\omega^2\tau_0^2}}\sin(\omega t+\varphi_m) \tag{50}$$

$$\operatorname{tg}\varphi_m=-\omega\tau_0 \tag{51}$$

where $1/\tau_0=2k_i+k$, as in Eq. (14). Eqs. (45)–(51) may now be introduced into Eq. (44). If the result is compared with Eq. (43), the following relations are obtained:

$$\operatorname{tg}\varphi=\omega\tau_0\frac{1+(1+\omega^2\tau_0^2)C_m/a^2 C_i}{(k/2k_i)+\omega^2\tau_0^2/a}, \tag{52}$$

$$\frac{1}{|Z|}=2k_i C_i\sqrt{1+\frac{a^2}{1+\omega^2\tau_0^2}+\left(\frac{\omega\tau_0 C_m}{a C_i}\right)^2-2\frac{a-\omega^2\tau_0^2 C_m/C_i}{1+\omega^2\tau_0^2}} \tag{53}$$

with

$$C_i \equiv \frac{z^2F^2}{2RT}\beta c; \quad a \equiv \frac{2k_i}{2k_i+k}.$$

Using Eqs. (52) and (53), we can show by a lengthy but straightforward calculation that the membrane may be represented by the equivalent circuit indicated in Fig. 10, if C_i

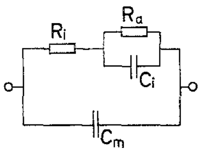

Fig. 10. Equivalent circuit of the membrane

is defined as above and the following values are assigned to R_i and R_a:

$$R_i = \frac{RT}{z^2 F^2} \frac{1}{\beta c k_i}, \tag{54}$$

$$R_a = \frac{RT}{z^2 F^2} \frac{2}{\beta c k}. \tag{55}$$

Comparison with Eqs. (16) and (17) shows that $R_i = 1/\lambda_{00}$ and $R_i + R_a = 1/\lambda_{0\,\infty}$, as expected.

The foregoing calculation demonstrates that the redistribution of ions between the potential minima at the interfaces gives rise to an additional capacitance C_i. For instance, at a concentration c where βc becomes equal to $N_s/10 = 4 \times 10^{11}$ ions/cm^2 (dipicrylamine), C_i is about 1.3 μF/cm^2, a value about three times larger than the geometrical capacitance of the membrane. Especially if k_i becomes very large, R_i goes to zero, so that C_i can no longer be separated from C_m in an *a-c.* bridge measurement. In other cases, impedance measurements may be used for the evaluation of the parameters β, k, and k_i. However, as a comparison between Eqs. (13), (52), and (53) shows, the field-jump experiment is much easier to interpret.

References

Bruner, L. J. 1970. Blocking phenomena and charge transport through membranes. *Biophysik* **6**:241.

Ciani, S., Eisenman, G., Szabo, G. 1969. A theory for the effects of neutral carriers such as the macrotetralide actin antibiotics on the electric properties of bilayer membranes. *J. Membrane Biol.* **1**:1.

Gaboriaud, R. 1966. Sur le comportement des acides non chargés dans les milieux eau-méthanol. *Compt. Rend. Acad. Sci. (Paris) C* **263**:911.

Grunwald, E., Baughman, G., Kohnstam, G. 1960. The solvation of electrolytes in dioxane-water mixtures, as deduced from the effect of solvent change on the standard partial molar free energy. *J. Amer. Chem. Soc.* **82**:5801.

Läuger, P., Lesslauer, W., Marti, E., Richter, J. 1967. Electrical properties of bimolecular phospholipid membranes. *Biochim. Biophys. Acta* **135**:20.

Le Blanc, Jr., O. H. 1969. Tetraphenylborate conductance through lipid bilayer membranes. *Biochim. Biophys. Acta* **193**:350.

Liberman, E. A., Topaly, V. P. 1968. Selective transport of ions through bimolecular phospholipid membranes. *Biochim. Biophys. Acta* **163**:125.

— — 1969. Permeability of bimolecular phospholipid membranes for lipid-soluble ions. *Biophysics* **14**:477.

Mueller, P., Rudin, D. O. 1967. Development of K$^+$–Na$^+$ discrimination in experimental bimolecular lipid membranes by macrocyclic antibiotics. *Biochem. Biophys. Res. Commun.* **26**:398.

Neumcke, B. 1970. Ion flux across lipid bilayer membranes with charged surfaces. *Biophysik* **6**:231.

– 1971. Diffusion polarization at lipid bilayer membranes. *Biophysik* **7**:95.

– Läuger, P. 1969. Nonlinear electrical effects in lipid bilayer membranes. II. Integration of the generalized Nernst-Planck equations. *Biophys. J.* **9**:1160.

– – 1970. Space charge-limited conductance in lipid bilayer membranes. *J. Membrane Biol.* **3**:54.

Robles, E. C., Van den Berg, D. 1969. Synthesis of lecithins by acylation of O-(sn-glycero-3-phosphoryl) choline with fatty acid anhydrides. *Biochim. Biophys. Acta* **187**:520.

Skinner, J. F., Fuoss, R. M. 1964. Conductance of triisoamylbutylammonium and tetraphenylboride. *J. Phys. Chem.* **68**:1882.

Walz, D., Bamberg, E., Läuger, P. 1969. Nonlinear electrical effects in lipid bilayer membranes. I. Ion injection. *Biophys. J.* **9**:1150.

Zwolinsky, B. J., Eyring, H., Reese, C. 1949. Diffusion and membrane permeability. *J. Phys. Colloid Chem.* **53**:1426.