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The Transport of Potassium Through Lipid Bilayer Membranes by the Neutral Carriers Valinomycin and Monactin

Experimental Studies to a Previously Proposed Model

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Summary. Stationary conductance experiments on neutral and negatively charged bilayer membranes in the presence of valinomycin or monactin agree with a recently proposed carrier transport model, which is common to both carrier types. This model assumes an interface reaction between a cation from the aqueous solution and a carrier molecule from the membrane phase to establish charge transport across the interface. The transport across the membrane interior is described by some kind of "Eyring model". The discussion of the current-voltage characteristic, the dependence of membrane conductance on the carrier and K^+ concentrations, and the comparison with appropriate experiments allow correlation of the different rate constants of the transport model. The results show that the rate constants partly depend on the surface charge of the membranes. This dependency can be described by introducing the Gouy-Chapman theory for charged surfaces into the transport model.

It was found that the carrier molecules could be added either to the aqueous phase or to the membrane-forming solution. The quantitative treatment of this phenomenon gives an evaluation of the partition coefficient of the carrier molecules between the membrane bulk phase and water.

The carrier hypothesis plays an important role for the understanding of the mechanism of ion transport in biological membranes. Until a few years ago, however, no molecule was known to function as an ion carrier. In 1964, certain streptomyces metabolites, such as valinomycin, the macrotetralide actins and the enniatins were found to enhance K^+ transport across mitochondrial membranes and to show all properties of an ion carrier (Pressman, 1968). All these molecules are also able to increase the cation permeability of artificial lipid membranes by many orders of magnitude (Mueller & Rudin, 1967; Andreoli, Tieffenberg & Tosteson, 1967; Tosteson, Andreoli, Tieffenberg & Cook, 1968; Eisenman, Ciani & Szabo, 1968; Liberman & Topaly, 1968). Common to these macrocyclic compounds is the

structural peculiarity of a hydrophilic interior and a hydrophobic exterior. Within a lipid membrane, such a molecule offers to an ion a polar environment similar to the normal environment of the ion in aqueous solution. An alkali ion which is extremely insoluble in a lipid membrane may cross the membrane in the form of a complex with the lipid-soluble macrocyclic compound. Although there is general agreement about these basic facts, little is known about the detailled transport mechanism. Ciani, Eisenman and Szabo (1969) gave a treatment of membrane conductivity in the limit of zero current. Markin *et al.* (1969) examined the carrier transport of ions on the basis of an electrodiffusion model. Recently, we published a carrier transport model which is based on an "Eyring treatment" of the membrane (Läuger & Stark, 1970). A "microscopic" theory like this should be better adapted to the real situation than a "macroscopic" theory such as the electrodiffusion model, because the diameter of the carrier molecules is only about a factor 4 smaller than the membrane thickness. One can show that in the limit of small currents both models give the same results.[1] However, the Eyring model describes the current-voltage characteristic better than the electrodiffusion model, as the latter predicts a supralinear J–V curve. This is not true in general, as we shall see. The carrier model of the preceding paper (Läuger & Stark, 1970) was tested mainly by comparison with the carefully performed experiments of Szabo, Eisenman and Ciani (1969) with macrotetralide actins. There is a lack of similar studies with valinomycin. The present paper contains stationary conductance measurements using neutral and negatively charged lipid membranes in the presence of valinomycin and monactin. A detailed comparison between the experiments and the theoretical expectations from the Eyring model has been performed. Since the experiments for both antibiotics were done under identical conditions, their carrier properties could be compared within the frame of our transport model.

Description of the Transport Model

We consider a bilayer membrane, which is on both sides in contact with aqueous solutions of an univalent cation M^+ of concentration c_M and of a neutral carrier S of concentration c_S (Fig. 1). Carrier S and metal M^+ may form a complex MS^+ in the aqueous phase (concentration c_{MS}) with an association-dissociation constant

$$K = \frac{c_{MS}}{c_M \cdot c_S}. \tag{1}$$

1 Läuger, P., Stark, G., *unpublished data.*

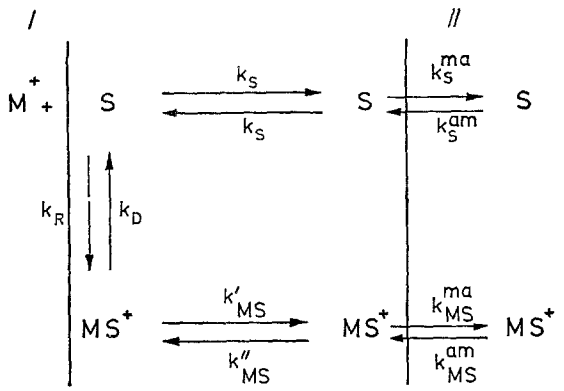

Fig. 1. Transport of the cation M^+ mediated by a neutral carrier

As the electrical conductivity of lipid bilayer membranes is extremely low in the absence of carriers, we may assume that the uncomplexed ion M^+ is almost excluded from the membrane. However, S and MS^+ may be exchanged between aqueous phase (*a*) and membrane (*m*) according to

$$S(a) \underset{k_S^{ma}}{\overset{k_S^{am}}{\rightleftharpoons}} S(m) \tag{2}$$

$$MS^+(a) \underset{k_{MS}^{ma}}{\overset{k_{MS}^{am}}{\rightleftharpoons}} MS^+(m). \tag{3}$$

The rate constants k_{MS}^{ma} and k_{MS}^{am} are connected with the partition coefficient γ_{MS} of the complex between both phases [Läuger & Stark, 1970, Eq. (16)]. As the exterior of the complex MS^+ is hydrophobic, γ_{MS} will be very much greater than the partition coefficient γ_M of the metal ion M^+. Therefore, the charge-carrier concentration in the membrane will be very much enhanced through the presence of the carrier molecules. The charge transfer across the membrane interior can be described by some kind of "Eyring mechanism". One can regard both interfaces to be separated by a symmetrical energy barrier. MS^+ may jump with a rate constant k_{MS} from one interphase over the barrier to the other interphase. The same holds for the uncomplexed carrier S (rate constant k_S). If a voltage is applied, k_S remains unaltered, as S is uncharged. However, k_{MS} is enlarged in one direction and reduced in the other under the influence of the electric field. The charge transfer across the interface could in principle be accomplished by reaction (3). But one can show that this mechanism is not sufficient to explain the experimentally

observed current across the membrane (*see* Appendix). This may be qualitatively understood, if one considers the fact that the concentration c_{MS} in the aqueous solution is very small so that polarization effects at the interface would limit the current to a value much smaller than the observed one. However, one can assume that in addition to mechanism (3) a chemical reaction takes place at the interface between an ion M^+ from the solution and a carrier S in the membrane. This heterogeneous recombination-dissociation reaction is described by rate constants k_R and k_D:

$$M^+(a)+S(m)\underset{k_D}{\overset{k_R}{\rightleftarrows}}MS^+(m). \tag{4}$$

From the absence of polarization effects, we conclude that charge transport through the interface proceeds mainly by reaction (4). This also follows from experiments described below.

The formal analysis of the model including the interfacial mechanisms (3) and (4) was given recently. It leads to the following expression for the electrical current J [Läuger & Stark, 1970, Eq. (18)]:

$$J=\frac{F\,d\,k_{MS}\,K\,c_M\,c_0\,\gamma_{MS}}{K\,c_M+1}\,\frac{\sinh(u/2)}{1+A\cosh(u/2)} \tag{5}$$

with

$$A=\frac{2k_{MS}(k_R\,c_M+2k_S+k_S^{ma})}{(k_D+k_{MS}^{ma})(k_R\,c_M+2k_S+k_S^{ma})-k_R\,c_M\,k_D} \tag{6}$$

and the notations: d = membrane thickness; F = Faraday constant; c_0 = total carrier concentration in the aqueous phase; $u=\frac{FU}{RT}$ = reduced voltage; U = voltage; R = gas constant; and T = absolute temperature.

From Eq. (5), the following expression for the membrane conductance λ_0 in the limit of small voltages is obtained:

$$\lambda_0=\frac{F^2\,d\,k_{MS}}{2RT}\,\frac{K\,c_M\,c_0\,\gamma_{MS}}{(K\,c_M+1)(1+A)}=\frac{F^2\,d\,k_{MS}}{2RT}\,\frac{c_{MS}^m}{1+A}. \tag{7}$$

c_{MS}^m is the concentration of the carrier complex in the membrane:

$$c_{MS}^m=\frac{K\,c_M\,c_0}{K\,c_M+1}\,\gamma_{MS}. \tag{8}$$

The parameter A represents the influence of the interface to charge transport. This will become clear if one considers the conductance λ_0 in the limit

$A \ll 1$. One can see from the definition of the translocation rate k_{MS} that $\tau = 1/k_{MS}$ means the time necessary for a complexed carrier molecule to cross the membrane. This diffusion time can be related to the diffusion coefficient D_{MS} by

$$\tau = \frac{d^2}{2D_{MS}} = \frac{1}{k_{MS}}. \tag{9}$$

Combining Eqs. (7) and (9) and neglecting A against 1:

$$\lambda_0 = \frac{F^2}{RT} \frac{D_{MS}}{d} c_{MS}^m. \tag{10}$$

Eq. (10) is identical with the conductivity (per unit area) of a macroscopic phase. Thus, in the limit $A \ll 1$, a thin lipid membrane with a thickness d of about 70 Å at small voltages behaves like a macroscopic phase. The factor $1/1+A$ then allows for the hindrance of charge transport through the interface.

If one neglects the ion transport contributed by mechanism (3) against the surface reaction mechanism (4) (i.e., if $k_S^{ma} \ll k_R c_M + 2k_S$ and $k_{MS}^{ma} \ll k_D$), expression (6) for A reduces to:

$$A = \frac{2k_{MS}}{k_D} + \frac{k_{MS}}{k_S} \frac{k_R c_M}{k_D}. \tag{11}$$

Eq. (5) contains some predictions which can be tested experimentally. The current J in a definite way depends on the total carrier concentration c_0, on the metal ion concentration c_M and on the voltage u. For convenience, the concentration dependencies will be studied using the conductivity in the ohmic region λ_0 instead of the current density J [Eq. (7)]. For the same reason, we consider the ratio λ/λ_0 to discuss the current-voltage characteristic. λ is defined as an integral conductivity ($\lambda = J/U$). Combining Eqs. (5) and (7) gives an expression for λ/λ_0, which depends only on the parameter A and on the reduced voltage u:

$$\frac{\lambda}{\lambda_0} = \frac{2(1+A)\sinh(u/2)}{u[1+A\cosh(u/2)]}. \tag{12}$$

The expressions for J and λ_0 contain the metal concentrations c_M instead of their corresponding activities a_M, because the latter are not known with sufficient accuracy in the proximity of an interface.

Materials and Methods

Membranes were formed from the following lipids:

(1) neutral dioleoyllecithin (dioleoyl phosphatidylcholine) (PC), which was synthesized according to a method described by Läuger, Lesslauer, Marti and Richter (1967); (2) phosphatidyl inositol (PI) from brain (General Biochemicals and Supelco, Inc.); and (3) phosphatidyl serine (PS) (Koch-Light-Laboratories). The last two lipids bear one negative charge per molecule. In some cases we used a lipid which had been extracted from sheep red cell membranes and was a gift of D.C. Tosteson (Duke University, Durham). Valinomycin was obtained from Calbiochem. It should be noted that different batches gave somewhat different membrane conductivities. The data presented in this paper refer to the batch with maximum "activity". Monactin was generously supplied by Ciba. Aqueous solutions of the antibiotics were prepared on the day of their use from small volumes of stock solutions in ethanol. The ethanol content generally was 0.1% and never exceeded 1%, a concentration we found to have no detectable effects on the electrical properties of bilayers. The pH of the aqueous solutions was about 6. The antibiotics could also be added to the lipid-decane phase from which the membranes were formed. The consequence of this experimental finding will be presented in Section 6. Membranes were formed from a 0.3 to 0.6% (w/v) lipid in n-decane solution on a circular aperture (3 mm in diameter) in the wall of a Teflon cell (Läuger *et al.*, 1967). The temperature was held at 25 °C.

The current-voltage curves were measured using two current- and two voltage electrodes (silver-silver chloride or platinized platinum electrodes) to avoid polarization effects. The measurements were performed under steady-state conditions. This means that, in the presence of valinomycin in the aqueous solution, the stationary membrane conductivity could only be measured at least 15 min after the membrane had been in the black state. Up to this time, the conductivity increased continuously. This time interval extended to about 1 hr if the solution was not stirred. The increase of current results from the very high partition coefficient of valinomycin and the limited diffusion rate across the phase boundary (*see* p. 148). Since the partition coefficient of monactin is smaller, the equilibrium between membrane and aqueous solution is established earlier. If the antibiotics were added to the bulk lipid phase, almost no increase of conductivity was observed after the membranes had blackened. This agrees with our conception of carrier transport presented earlier in this paper.

The Electrical Conductivity as a Function of K^+ and Carrier Concentrations

In Fig. 2 the membrane conductance in the ohmic region ($u \to 0$) is plotted as a function of the concentration of antibiotics. These were dissolved either in the aqueous phase or in the solution from which the membranes were formed. The experimental points agree with lines of unity slope. Each point is a mean value of at least five membranes. The deviations from the mean value generally were less than 50%. The saturation effect at high concentrations of valinomycin (10^{-6} to 10^{-5} M) in the aqueous solution results from its limited solubility. In this region of concentration, only a part of the added valinomycin dissolved.

The linear relationship between membrane conductance and antibiotic concentration indicates that the ion transport is mediated by single anti-

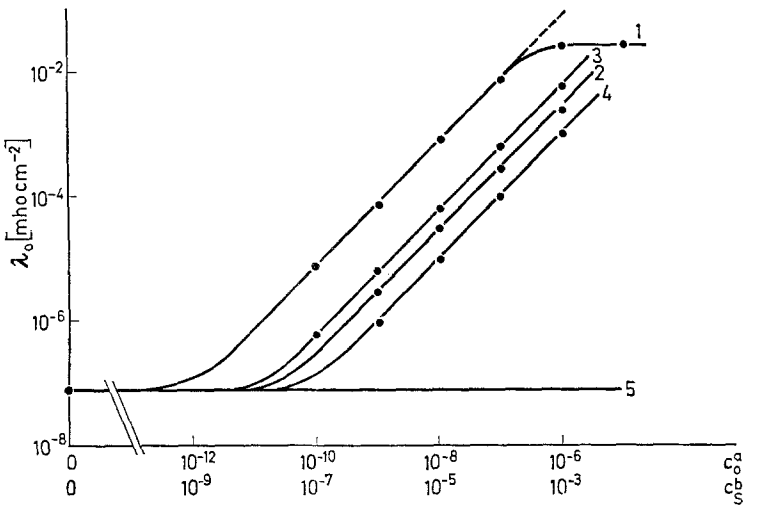

Fig. 2. Conductivity of dioleoyllecithin membranes as a function of the concentration of valinomycin and monactin with 1 M KCl in the aqueous solution. The antibiotics were added either to the aqueous phase (c_0^a) or to the membrane phase (c_S^b). Valinomycin added to the aqueous phase (curve *1*) and to the membrane phase (curve *2*). Monactin added to the aqueous phase (curve *3*) and to the membrane phase (curve *4*). Conductivity in the absence of antibiotics (curve *5*)

biotic molecules. Some kind of pore mechanism resulting from an interaction of several molecules can therefore be excluded. This finding agrees with that reported by Szabo *et al.* (1969) for monactin and by Tosteson *et al.* (1968) for valinomycin. It also agrees with the assumptions of the carrier model described above, as Eq. (7) predicts a proportionality between λ_0 and c_0.

The dependence of the conductivity on alkaline ion concentration c_M in the presence of monactin was studied by Szabo *et al.* (1969). They found a linear relationship, if they kept the ionic strength constant. This proved to be necessary, because they used lipids with a net negative charge. As Lesslauer, Richter and Läuger (1967) pointed out, a negatively charged interface gives rise to a diffuse double layer which influences the transport properties of bilayers. The Gouy-Chapman theory for the diffuse double layer relates the potential difference ψ between the interface and the bulk aqueous phase to the ionic strength of the latter. As the carrier complex of the alkaline ions is positively charged, its partition coefficinet depends on ψ according to:

$$\gamma_{MS} = \gamma_{MS}^0 \exp(-F\psi/RT) \tag{13}$$

where γ_{MS}^0 is the partition coefficient for a neutral interface. γ_{MS} doesn't depend on the external voltage U because of the high ionic strength of the aqueous solutions (Walz, Bamberg & Läuger, 1969).

In this way, the current density J [Eq. (5)] and the membrane conductance λ_0 [Eq. (7)] depend on the surface charge of the membrane. For a constant ionic strength, γ_{MS} does not depend on the alkali ion concentration, as ψ remains constant. McLaughlin, Szabo, Eisenman and Ciani (1970) showed that the conductance behavior of membranes with different charged head groups in the presence of nonactin and some other ion carriers is described by setting λ_0 proportional to γ_{MS} [Eq. (13)]. However, as we shall see, this is only an approximation which fails in certain cases. One can understand this as a direct consequence of the surface reaction (4) which adjusts the equilibrium between carrier and carrier-ion complex in the membrane and is responsible for the charge transport across the interface.

As we pointed out (Läuger & Stark, 1970), the heterogeneous rate constants k_R and k_D are connected with the partition coefficients γ_S and γ_{MS} by the relation

$$K_h = \frac{k_R}{k_D} = \frac{\gamma_{MS}}{\gamma_S} K. \tag{14}$$

The partition coefficient γ_S for the uncomplexed carrier molecules and the equilibrium constant K in the bulk solution are assumed to be independent of the surface charge. Therefore Eqs. (13) and (14) yield:

$$\frac{k_R}{k_D} = \left(\frac{k_R}{k_D}\right)_0 \exp(-F\psi/RT) \tag{15}$$

with the subscript 0 for a neutral interface.

Thus, the membrane conductance λ_0 in a double manner depends on the surface charge of the membrane, via the partition coefficient γ_{MS} and via the constant A [Eq. (11)].

Fig. 3 shows a linear dependence of the membrane conductance of neutral dioleoyllecithin membranes on the K^+ concentration c_K. The ionic strength was kept at 1 M by adding LiCl. This is possible because the membrane conductance in the presence of monactin and valinomycin is not appreciably influenced by LiCl. A proportionality between membrane conductance and K^+ concentration with monactin present has been also reported by Szabo *et al.* (1969). Tosteson (1968), Liberman and Topaly (1968), and Liberman, Pronevich and Topaly (1970) found a departure from linearity in the case of valinomycin. However, their results cannot be used to test the described carrier model, because they used a mixture of lipids for membrane formation which probably contained negative headgroups and were measured at variable ionic strength.

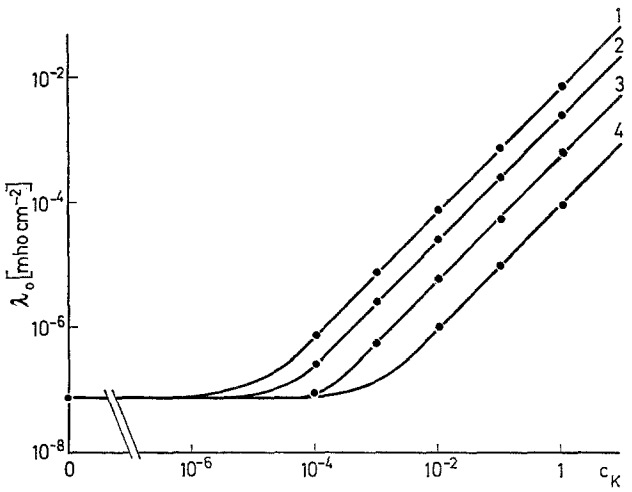

Fig. 3. Conductivity of dioleoyllecithin membranes as a function of KCl concentration in the aqueous phase. The ionic strength was held constant at 1 M with LiCl. Curve *1*, 10^{-7} M valinomycin in the aqueous solution; *2*, 10^{-3} M valinomycin in the membrane bulk phase; *3*, 10^{-7} M monactin in the aqueous solution; *4*, 10^{-4} M monactin in the membrane bulk phase

According to Eq. (7), a linear relaionship between λ_0 and c_M indicates that the following relations hold (up to at least $c_M = 1$ M):

(a) $Kc_M = \frac{c_{MS}}{c_S} \ll 1$; and

(b) $1+A$ independent of c_M.

Condition (a) means that the extent of complexation between the antibiotics and K^+ in aqueous solution is small. This is already known from measurements of the equilibrium constant K in ethanol-water mixtures by Shemyakin *et al.* (1969). They could not detect any complexes at all in pure aqueous solution within the experimental error. From the data presented in Fig. 3, we may conclude that $K \leqq 0.1$ M^{-1} for monactin and valinomycin.

If only reaction (4) contributes to the charge transport across the interface, it follows from condition (b) and Eq. (11) that:

$$\frac{k_{MS} k_R}{k_S k_D} \ll 1 + \frac{2k_{MS}}{k_D} \tag{16}$$

(k_R/k_D expressed in M^{-1}).

We shall also see from the discussion of the current-voltage characteristic that this condition is satisfied at least in the case of neutral lipids.

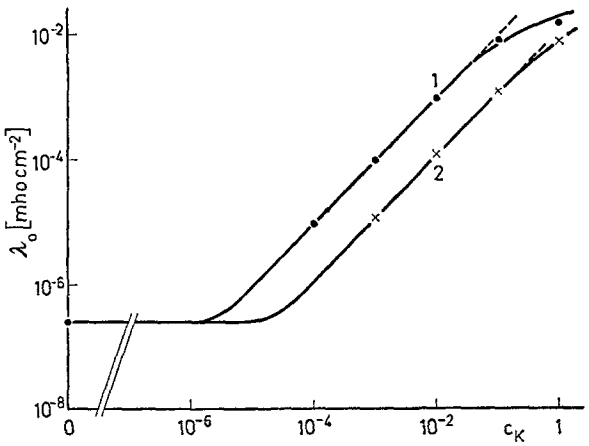

Fig. 4. Conductivity of phosphatidyl inositol membranes as a function of KCl concentration in the aqueous phase. The ionic strength was held constant at 1 M with LiCl. Full lines according to Eq. (7) (*see* also Table 1). Curve *1*, 10^{-7} M valinomycin in the aqueous solution; *2*, 10^{-7} M monactin in the aqueous solution

On the other hand, neglecting the surface reaction (4), this would result in $A=2k_{MS}/k_{MS}^{ma}$, which is independent of c_M. In this case, condition (b) would be automatically satisfied, and a linear dependence of λ_0 on c_M at constant ionic strength would be expected independently of whether the membrane is charged or not. Since this is in contradiction to the results with negatively charged membranes (*see* below and also the next section), where a saturation effect is apparent, additional evidence is obtained that reaction (4) is the main mechanism for the charge transport across the interface.

If the inequality (16) does not hold, a nonlinear saturation behavior of the conductance λ_0 results at high concentrations c_M [compare Eq. (7)].

Therefore, an enlargement of k_R/k_D should lead to deviations from the linear dependence of conductance on c_K. Such an increase is predicted by Eq. (15) for negatively charged membranes.

Fig. 4 shows the dependence of λ_0 on c_K for negatively charged phosphatidyl inositol membranes. Indeed, saturation behavior begins at high potassium concentrations, which is more pronounced for valinomycin. The full lines are theoretical curves calculated from Eqs. (7) and (11). Apart from the experimental parameters c_M and c_0, λ_0 [Eq. (7)] depends on three combinations of constants ($Kc_M \ll 1$). Table 1 contains two of the three with their optimal values for adapting the concentration dependence of λ_0. The values for PC and PI membranes correspond to Figs. 3 and 4.

However, one needs additionally the values of $2k_{MS}/k_D$, which were obtained by analysis of the current-voltage characteristic (Table 2).

Table 1. *Two combinations of rate constants derived from concentration dependencies of conductivity [Eq. (7), $d=70$ Å, $T=25$ °C*

Membrane forming lipid	$(k_{MS}\,K\,\gamma_{MS})_{val}$ in $[M\,sec]^{-1}$	$\left(\frac{k_{MS}}{k_S}\frac{k_R}{k_D}\right)_{val}$ in $[M]^{-1}$	$(k_{MS}\,K\,\gamma_{MS})_{mon}$ in $[M\,sec]^{-1}$	$\left(\frac{k_{MS}}{k_S}\frac{k_R}{k_D}\right)_{mon}$ in $[M]^{-1}$
PC	7.5×10^7	<0.1	5×10^6	<0.1
PI	1.2×10^9	7	1.2×10^8	0.7
PS	1.1×10^9	3	1.5×10^8	0.8

Since K is independent of membrane properties, one derives from Table 1 the ratio $(k_{MS}\gamma_{MS})_{PI}/(k_{MS}\gamma_{MS})_{PC}$. Assuming that the translocation rate k_{MS} is equal for PI and PC membranes and remembering that PC is a neutral lipid one gets with Eq. (13):

$$\frac{\gamma_{MS}(PI)}{\gamma_{MS}(PC)}=\exp(-F\psi/RT). \tag{17}$$

The numerical values for this ratio are: valinomycin 16.5, and monactin 23.6.

Eq. (17) demands that both values should agree. The difference may be due to variations of k_{MS} for the two types of bilayer membranes, because the inner structure of the membranes may somewhat depend on the lipids. From this we may conclude that the application of Eq. (13) is an approximation too, because it demands that the only difference between the membranes consists in the charge of the polar headgroups. Nevertheless, one can use Eq. (17) as an approximation to calculate the voltage ψ. From ψ one can derive the surface charge density σ of the PI membranes by applying the Gouy-Chapman theory (*see*, e.g., Neumcke, 1970). This theory introduces another approximation, as it is not strictly applicable at high ionic strengths (formation of an immobile layer of gegenions). Being aware of these objections, one gets a density of one charge per 71 Å^2 from the valinomycin data and one charge per 58 Å^2 from the monactin data. This last value agrees with a similar approach of McLaughlin *et al.* (1970), who found one charge per 50 Å^2. The same magnitude of charge densities is known from monolayer studies.

Discussion of the Current-Voltage Characteristic

A survey of the literature for current-voltage curves of bilayer membranes in the presence of monactin and valinomycin reveals a rather contradictory picture. A number of authors (Andreoli *et al.*, 1967; Buzhinsky, 1968;

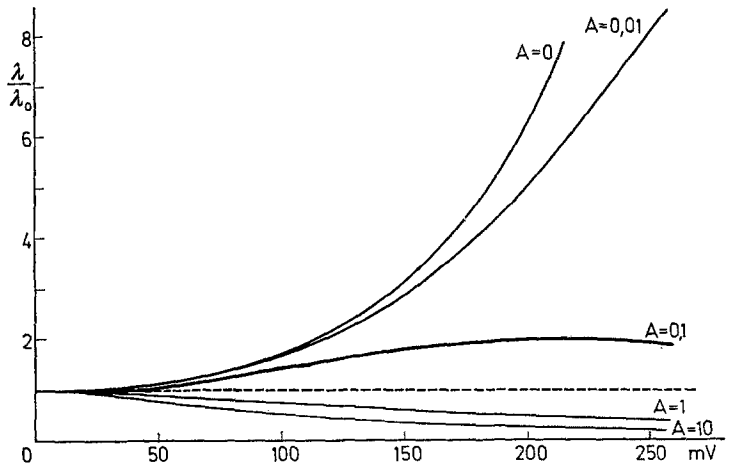

Fig. 5. Conductance ratio λ/λ_0 [Eq. (12)] as a function of voltage with A as parameter

Szabo *et al.*, 1969) report that the current rises faster than the voltages does (superlinear behavior). Liberman and Topaly (1968*a*, *b*) and Liberman *et al.* (1970), on the other hand, found that the current rose more slowly than the voltage and even reached a saturating value at high voltages. We shall refer to such behavior as supralinear. We shall show that these two different current-voltage characteristics result from different properties of the lipids used for membrane formation and can be interpreted on the basis of the proposed carrier model.

In the following, we consider the ratio λ/λ_0 instead of J. We define:

(a) a superlinear behavior by $\frac{d(\lambda/\lambda_0)}{dU} > 0$, and

(b) a supralinear behavior by $\frac{d(\lambda/\lambda_0)}{dU} < 0$.

Fig. 5 illustrates that, depending on the parameter A, both super- and supralinear J–U characteristics can be obtained from Eq. (12). With $A \ll 1$, a superlinear behavior results in the usual voltage range $0 \leqq U \leqq 250$ mV. For $A \approx 0.1$, a maximum occurs in the λ/λ_0 curve (at about 200 mV) which corresponds to a point of inflection in the J–U curve and represents a transition from super- to supralinear behavior. If $A > 0.4$, a supralinear curve is observed. Since A depends on the concentration c_M [Eq. (11)], in favorable cases one should observe a transition from super- to supralinear behavior with increasing c_M. Therefore, a study of λ/λ_0 as a function of c_M allows the separate determination of the concentration-dependent part $k_{MS} k_R c_M / k_S k_D$ and of the concentration-independent part $2k_{MS}/k_D$ of A.

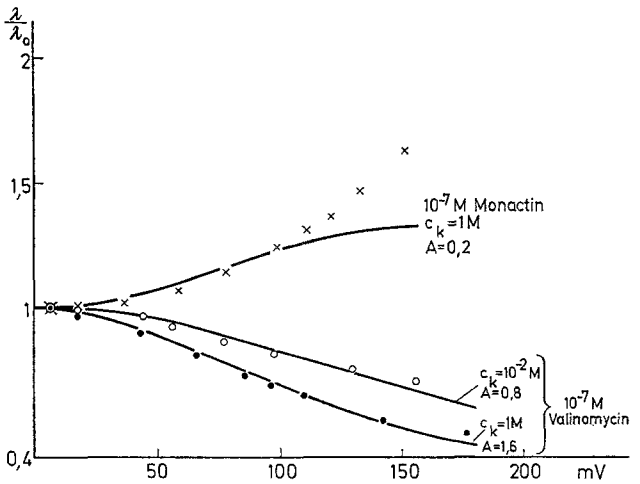

Fig. 6. Conductance ratio λ/λ_0 as a function of voltage for phosphatidyl inositol membranes in the presence of 10^{-7} M valinomycin or monactin at different KCl concentrations c_K. The ionic strength was held constant at 1 M with LiCl. Full lines calculated from Eq. (12) with the indicated values for A

With neutral PC membranes, both carrier types gave a superlinear current-voltage characteristic. Fig. 6 illustrates measurements on negatively charged PI membranes. Whereas valinomycin showed supralinear behavior for $10^{-4}\,\text{M} \leqq c_K \leqq 1\,\text{M}$, monactin gave superlinear characteristics even with negative lipids. These experimental results explain the discrepancies mentioned in the beginning and can be interpreted with the presented carrier model. An example of transition from super- to supralinear behavior with increasing c_K is plotted in Fig. 7. The membranes were formed from erythrocyte lipids (kindly supplied by D.C. Tosteson), which contained a certain amount of negative lipids. A similar result was obtained by G. Eisenman (*personal communication*).

Figs. 6 and 7 show a departure of the experimental values from the theoretical curve [Eq. (12)] at high voltages. One has to conclude from this fact that at high voltages the conductivity is influenced by additional effects. In the frame of the presented model, one could imagine that the rate constants k_R and k_D are voltage-dependent. Since the carrier molecules are very large, they should "see" a considerable part of the voltage drop across the membrane. The superposition of the electrostatic energy with the binding energy of the carrier complex leads to an enhancement of k_D on one interface and a reduction on the other interface in such a way that an increase in the current results. A quantitative study of this effect will be carried out in the future. One should add at this point that the influence of image forces

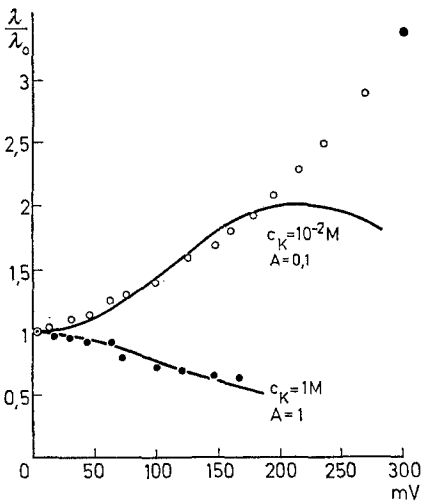

Fig. 7. Conductance ratio λ/λ_0 as a function of voltage for membranes formed from erythrocyte lipids (D.C. Tosteson) with 10^{-7} M valinomycin in the aqueous solution at two different KCl concentrations. The ionic strength was held constant at 1 M with LiCl. Full lines calculated from Eq. (12) with the indicated values for A

acting on an ion near the interface is included in the considered transport model. These image forces, which give rise to an additional potential barrier for the charged carrier complex, lead to a reduction of k_{MS} (thus the approximation $k_{MS} \approx k_S$, which has been used previously, has to be abandoned). The treatment of the image forces by Neumcke and Läuger (1969) shows, however, that their influence should not be too big for large molecules.

Although there are greater deviations of the theory from the experimental data at high voltages, there is a reasonable agreement for $U \leqq 100$ mV. The quantitative conclusions resulting from the discussion of the concentration dependence of conductance in the ohmic region should therefore contain a higher degree of reliability than those based on the discussion of current-voltage characteristics. Nevertheless, one can try to get some information about the rate constants from the concentration dependence of the J–U characteristic. However, the numbers of Table 2 should be considered only as approximate values. This applies also to the values in Table 1, because the values of $2k_{MS}/k_D$ (Table 2) were used in their calculation. The values of $k_{MS}k_R/k_Sk_D$, which are optimal for adapting the data of Fig. 4 (Table 1), differ from those given in Table 2 up to a factor 8. The reason for this deviation may be the voltage dependence of k_R and k_D mentioned above.

Table 2 again shows that condition (b) of the preceding section and Eq. (16) are satisfied in the case of neutral lipids, as $k_{MS}k_R/k_Sk_D \ll 1$ M^{-1}.

Table 2. *Approximate evaluation of ratios of rate constants from the current-voltage characteristic*

Membrane forming lipid	$\left(\frac{2k_{MS}}{k_D}\right)_{val}$	$\left(\frac{k_{MS}k_R}{k_S k_D}\right)_{val}$ in $[M]^{-1}$	$\left(\frac{2k_{MS}}{k_D}\right)_{mon}$	$\left(\frac{k_{MS}k_R}{k_S k_D}\right)_{mon}$ in $[M]^{-1}$
PC	0.3	<0.1	0.1	<0.1
PI	0.8	1	0.3	<0.1
PS	0.7	0.6	0.3	<0.1

The Partition Coefficient of the Carrier Molecules Between Bulk Lipid Phase and Water

Although valinomycin and monactin are usually added to the aqueous phase (Mueller & Rudin, 1967; Szabo *et al.*, 1969; Markin *et al.*, 1969), we found that they could be dissolved as well in the membrane-forming solution. This was done by adding a small amount of an ethanol solution of the antibiotics to the lipid-decane phase and evaporating the ethanol. The membrane conductance remained approximately at a constant value over a period of at least 1 hr, when the membrane was in the black state, although the aqueous phases which originally contained no antibiotics were stirred. From this fact one has to conclude that the carrier concentration in the membrane remains constant and that molecules which enter the aqueous solution across the interface are replaced by molecules from the surrounding membrane torus. Experiments have been started to measure the rate constants of exchange of carrier molecules between both phases.

As one knows the concentration c_S^b of carrier molecules in the membrane bulk phase, it is possible to determine the partition coefficient Γ_S of the carrier molecules between bulk lipid phase and water by bilayer experiments. However, the structure of bilayer membranes differs from the bulk lipid phase (membrane-forming solution), so that one has to introduce a new partition coefficient γ_S^{bm} between bulk phase and membrane. The concentration c_S^m is correlated to the corresponding bulk concentration c_S^b by

$$c_S^m = \gamma_S^{mb} c_S^b. \tag{18}$$

From Eq. (1) and from the definition of the partition coefficients, we obtain (using $c_{MS} + c_S = c_0$):

$$c_{MS}^m = \frac{\gamma_{MS}}{\gamma_S} K c_M c_S^m. \tag{19}$$

Combining Eqs. (7), (18) and (19):

$$\lambda_0 = B \frac{K c_M}{K c_M + 1} \gamma_{MS} c_0^a = B \frac{\gamma_{MS}}{\gamma_S} K c_M \gamma_S^{mb} c_S^b \tag{20}$$

with

$$B = \frac{F^2 d k_{MS}}{2RT(1+A)}.$$

If $\lambda_0(a)$ and $\lambda_0(b)$ are the membrane conductivities, when the antibiotics are added to the aqueous phase and to the bulk lipid phase, respectively, it follows from Eq. (20) by using $\Gamma_S = \gamma_S / \gamma_S^{bm}$:

$$\frac{\lambda_0(a)}{\lambda_0(b)} = \frac{1}{K c_M + 1} \frac{c_0^a}{c_S^b} \Gamma_s. \tag{21}$$

Therefore, by performing two independent experiments, adding the antibiotics first to the aqueous phase (concentration c_0^a) and then to the bulk lipid phase (concentration c_S^b), and measuring the corresponding membrane conductivities, gives the partition coefficient $\Gamma_S (K c_M \ll 1)$.

Fig. 3 illustrates the appropriate experiments.

From a comparison of curves *1* and *2* and curves *3* and *4*, respectively, one gets for the solution of the neutral lipid PC in decane (0.3%, W/V): Γ_S (valinomycin) $= 2.5 \times 10^4$, and Γ_s (monactin) $= 6 \times 10^3$.

These values for the partition coefficients Γ_S were proven correct by testing them in the following way. Valinomycin (or monactin) was added to the aqueous phase as well as to the bulk lipid phase in such concentrations that the equilibrium between both phases was established according to Γ_S. The membrane conductivity then reached the same value as if the antibiotics were only added to one of the two phases. It should be noted that when the antibiotics were added to the membrane-forming solution alone, their concentration in the aqueous phase at the end of a membrane experiment was far from the equilibrium value predicted by Γ_S. The carrier concentration was determined by measuring the conductivity of membranes in these aqueous solutions using a new Teflon cuvette and utilizing the linear relationship between λ_0 and c_0. Summarizing these experimental results, one may conclude that the diffusion of valinomycin and monactin from the membrane into the aqueous phase is slow. Details of these experiments will be published elsewhere.

Comparison of the Carrier Properties of Valinomycin and Monactin

Figs. 2–4 show there is a higher membrane conductance with valinomycin than with monactin at equal concentrations in the aqueous or membrane bulk phase. Assuming that there are no greater differences in the

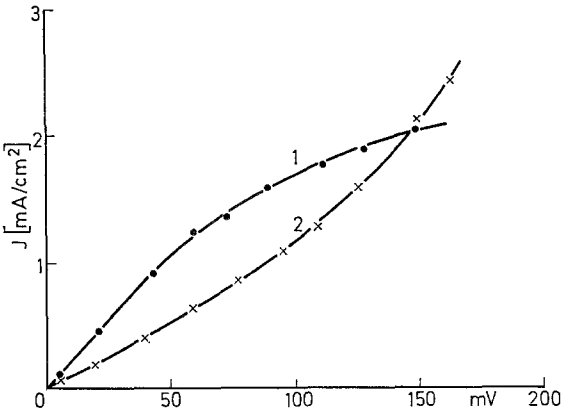

Fig. 8. Current-voltage characteristic for phosphatidyl-serine membranes in the presence of 1 M KCl in the aqueous solution. Curve *1*, 10^{-7} M valinomycin in the aqueous solution; *2*, 10^{-7} M monactin in the aqueous solution

translocation rate k_{MS} of either antibiotic, one concludes from Table 1:

$$(K\gamma_{MS})_{val} > (K\gamma_{MS})_{mon}.$$

This is equivalent with the statement that the membrane contains more charge carriers MS^+ in the case of valinomycin at equal c_M and c_0 [Eq. (8)]. From $\Gamma_S(val) > \Gamma_S(mon)$, one may assume that also $\gamma_S(val) > \gamma_S(mon)$. This means that there are more free carrier molecules S in the membrane in the case of valinomycin. One concludes from Table 1 that the ratio of complexed to free carrier molecules is also larger for valinomycin. By comparing columns 3 and 5 of Table 1, and assuming $(k_{MS}/k_S)_{val} \approx (k_{MS}/k_S)_{mon}$, one gets:

$$(k_R/k_D)_{val} > (k_R/k_D)_{mon}.$$

This is the reason why valinomycin shows saturation effects at lower concentrations (c_K) than monactin does (*compare* the two sections following Methods).

Fig. 8 demonstrates the characteristic differences between both carrier types by a selected example (high K^+ concentration, negative lipids). With equal carrier concentrations in the aqueous phase, there is a larger current in the case of valinomycin at small voltages. This results from the larger charge-carrier concentration. With increasing voltage, however, the carrier complexes MS^+ are not discharged fast enough at the membrane surface; thus the current in the case of monactin exceeds that measured in the presence of valinomycin.

The translocation rate k_{MS} turned out to be smaller than the dissociation rate k_D by a factor of 2.5 to 20, depending on the carrier type and the lipid used for membrane formation (Table 2). Especially in the monactin-K^+ system, the rate-limiting step for the K^+ transport is the migration of the charge complex MS^+ across the membrane interior. However, in certain cases (valinomycin, negative lipids), the differences between both constants are small ($k_{MS} \approx k_D$).

Stationary conductance experiments supply only combinations of rate constants; to determine rate constants separately, relaxation methods must additionally be used.

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Appendix

Calculation of the Maximum Current Density According to the Interfacial Charge Transfer Mechanism Eq. (3)

Neglecting the interface reaction (4), we assume that charge transport through a membrane-water interface occurs only by diffusion of the carrier complex MS^+ [Eq. (3)]. Since the membrane resistance is very much higher than the resistance of the aqueous solution, the whole voltage drops across the membrane. Therefore, the complexes MS^+ in the aqueous phase are transported to and from the interface only by diffusion and not by an electrical field. If a current flows across the membrane, however, one has to consider also the chemical reaction (1), which contributes to the reestablishment of the original concentration c_{MS} in the aqueous phases near the interface. The maximum current, which can be carried across the membrane by the complexes MS^+, is given by the maximum rate with which these complexes are driven to the interface by diffusion and produced by reaction (1). We shall estimate both components describing: (a) the maximum current J_d^{max} carried by diffusion, and (b) the maximum current J_c^{max} maintained by the chemical reaction (1).

(a) J_d^{max} corresponds to a maximum flow $\phi_d^{max} = J_d^{max}/F$ of complexes MS^+. ϕ_d^{max} is given by the maximum diffusional flow across the unstirred layer of the aqueous phase near the membrane surface. With a thickness δ of this layer and a diffusion coefficient D_{MS} of the complex MS^+ in the aqueous phase, one finds:

$$\phi_d^{max} = D_{MS} c_{MS}/\delta .$$

Therefore

$$J_d^{max} = F \frac{D_{MS}}{\delta} c_{MS} . \tag{A.1}$$

(b) We assume that the deficit of molecules MS^+ in the water layer adjacent to the interface generated by a current is balanced by the production of new complexes. The production rate of these complexes is given by $\bar{k}_R c_M c_S$ [moles/cm^3 sec], $\bar{k}_R$ being the recombination rate in the aqueous solution. Neglecting any dissociation of complexes MS^+, the thickness $\bar{\delta}$ of the layer necessary to produce enough complexes in order to

carry a current J across the membrane, is calculated as:

$$\bar{\delta} = \frac{J}{\bar{k}_R c_M c_S F}. \tag{A.2}$$

However, the dissociation of the complexes can only be omitted, when their mean lifetime τ_Z is larger than the diffusion time τ_d across the layer:

$$\tau_z > \tau_d. \tag{A.3}$$

This condition delivers an approximation of the maximum current J_c^{max}. $\bar{k}_D$ being the dissociation rate of the complex in aqueous solution, τ_Z is given by:

$$\tau_z = 1/\bar{k}_D. \tag{A.4}$$

The diffusion time τ_d can be estimated by:

$$\tau_d = \bar{\delta}^2/2D_{MS}. \tag{A.5}$$

Combining Eqs. (A2)–(A.5), one finds, with $K = \bar{k}_R/\bar{k}_D$:

$$J < F c_M c_S (2K \bar{k}_R D_{MS})^{\frac{1}{2}}. \tag{A.6}$$

Assuming that there are enough uncomplexed molecules S available (this would be the case only if the back-diffusion of S across the membrane is unlimited), one can give a limit for J_c^{max} from Eq. (A.6) by taking $\bar{k}_R$ as diffusion-controlled ($\bar{k}_R^d$):

$$J_c^{max} < F c_M c_S (2K \bar{k}_R^d D_{MS})^{\frac{1}{2}}. \tag{A.7}$$

With the numerical values:

$D_{MS} < 10^{-5}$ cm^2/sec;
$\delta = 3 \times 10^{-2}$ cm (*unpublished experimental result*):
$K \leqq 10^2$ cm^3/mole (*see* section following Methods);
$c_0 = c_{MS} + c_S = 10^{-10}$ mole/cm^3;
$c_M = 10^{-3}$ mole/cm^3;
$k_R^d = 5 \times 10^{11}$ cm^3/mole sec (Diebler *et al.*, 1969)

one obtains from Eqs. (A.1) and (A.7):

$$J_d^{max} < 3 \times 10^{-10} \text{ amp/cm}^2;$$

and

$$J_c^{max} < 3 \times 10^{-4} \text{ amp/}cm^2.$$

J_d^{max} and J_c^{max} have to be compared with experimental values J_{exp} for neutral dioleoyl-lecithin membranes under the same conditions. For 10^{-7} M valinomycin or monactin and 1 M KCl in the aqueous solution, we got the following values for the electrical current:

$$J_{exp}(\text{val}) = 3.7 \times 10^{-3} \text{ amp/cm}^2 \quad (U = 250 \text{ mV}),$$

and

$$J_{exp}(\text{mon}) = 5 \times 10^{-4} \text{ amp/cm}^2 \quad (U = 250 \text{ mV}).$$

The experimental values J_{exp} are many orders of magnitude larger than J_d^{max}, but only slightly larger than the upper limit of J_c^{max}. As J_c^{max} approaches the value of 3×10^{-4} amp/cm^2 only by assuming an unlimited diffusion of free carrier molecules across the inter-

face, which proved to be slow (*see* last section of text), the difference between J_c^{max} and J_{exp} is in fact very much larger. Therefore we can conclude that charge transport across the interface will mainly proceed by the interface reaction (4).

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