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Valinomycin-Mediated Ion Transport through Neutral Lipid Membranes: Influence of Hydrocarbon Chain Length and Temperature

R. Benz, G. Stark, K. Janko and P. Läuger

Fachbereich Biologie, Universität Konstanz, D-7750 Konstanz, Germany

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Summary. Stationary electrical conductance experiments together with nonstationary relaxation experiments allow a quantitative determination of rate constants describing carrier-mediated ion transport. Valinomycin-induced ion transport across neutral lipid membranes was studied. The dependence of the transport parameters on the chain length of the lipid molecules, on the kind of alkali ion, and on the temperature was determined. The relaxation time τ of the current following a voltage jump shows a marked increase with decreasing temperature or with increasing chain length of the lipid molecules. This variation of τ is interpreted on the basis of a varying membrane fluidity. It is shown that under favorable circumstances the equilibrium constant of complex formation in the aqueous phase may be obtained from membrane experiments. Furthermore, the kinetics of exchange of valinomycin between membrane and water was studied. We found a marked influence of the torus surrounding the black film on the kinetics as well as on the total amount of valinomycin molecules in the membrane. The problem of location of the free carrier molecules inside the membrane is discussed.

Compounds such as valinomycin, monactin or gramicidin have been shown to increase the permeability of natural and artificial lipid membranes for alkali metal ions by many orders of magnitude (Andreoli, Tieffenberg & Tosteson, 1967; Lev & Buzhinsky, 1967; Mueller & Rudin, 1967; Eisenman, Ciani & Szabo, 1968; Liberman & Topaly, 1968; Pressman, 1968). Whereas gramicidin seems to form fixed structures through thin hydrophobic permeability barriers such as artificial black films, valinomycin and monactin act as mobile molecules within those membranes (Krasne, Eisenman & Szabo, 1971). Their action has been explained by carrier models (Ciani, Eisenman & Szabo, 1969; Markin, Kristalik, Liberman & Topaly, 1969; Läuger & Stark, 1970). On the basis of such a model we performed a quantitative analysis of valinomycin-mediated potassium transport through negatively charged phosphatidyl inositol membranes by using stationary

and nonstationary conductance experiments (Stark & Benz, 1971; Stark, Ketterer, Benz & Läuger, 1971). This analysis has now been applied to neutral membranes, which show smaller relaxation amplitudes. In this case better agreement between theory and experiments was obtained since surface charge effects could be neglected. The temperature dependence of the model parameters, their variation with the chain length of the lipid molecules and their dependence on the kind of alkali cation was determined. Besides that, the time course of the exchange of valinomycin between membrane and aqueous solutions was studied. A pronounced influence of the membrane area on the kinetics of exchange as well as on the number of valinomycin molecules per unit area of the film was found. It was interpreted as an influence of the membrane torus.

Materials and Methods

Black lipid membranes were formed from four monounsaturated diacyl-L-α-phosphatidylcholines (lecithins) with different chain lengths of their fatty acid residues: dipalmitoleoyl-, dioleoyl-, dierucoyl- and dinervonoyllecithin [di-(16:1)-, di-(18:1)-, di-(22:1)-, and di-(24:1)-PC]. They were synthesized according to a modified version of the method of Baer and Buchnea (1959), purified across column chromatography and checked by thin-layer chromatography. The palmitoleic-, oleic- and erucoic acid used for the synthesis were commercial products, while the nervonic (cis-tetracos-15-enoic) acid was synthesized according to the electrolytic method of Kolbe (Bounds, Linstead & Weedon, 1954), in which the stereospecificity at the double bond is preserved. The purity of all fatty acids was checked by gas chromatography (in the form of the methylesters) and found to be greater than 98%. We used cells made from Teflon for membrane formation with a glass window to observe the membrane. The wall separating the two aqueous compartments contained a circular hole of varying diameter. By using different cells, membranes were formed with areas ranging from 5×10^{-3} cm^2 to 0.3 cm^2. The cell was adapted to a thermostated metal block. We used a 0.5–1% solution of lipid in *n*-decane for membrane formation.

Valinomycin was obtained from Calbiochem. Monactin was generously supplied by Ciba. These substances were added either to the lipid phase or to the aqueous solution. In the latter case the antibiotic was used as a concentrated ethanolic solution. The pH of the unbuffered aqueous solutions was about 6. The ionic strength of the salt solutions was kept constant at 1 M by adding LiCl.

Stationary conductance measurements were performed using two current- and two voltage electrodes (silver-silver chloride or platinized platinum electrodes). If valinomycin was added to the aqueous phase the time after which the conductance reached a stationary level ranged between 10 min and 1 hr, dependent on the equilibration time between membrane and water (*see* later section). The electrical relaxation experiments were performed as described by Ketterer, Neumcke and Läuger (1971). A voltage pulse (rise-time <1 μsec) from a generator was applied to the membrane. The current was measured by a Tektronix storage oscilloscope (type 549/1A7A) via the voltage drop across an external resistor. To keep the charging time of the membrane capacity short, the membrane area was reduced to 5×10^{-3} cm^2 (Stark *et al.*, 1971). Using the compensation voltage at the input of the oscilloscope, relaxation amplitudes could be meas-

ured down to 0.05. Depending on the external resistor (i.e., on the sensitivity of current measurement), relaxation times down to about 2 μsec could be resolved under optimal conditions.

The shape of the stationary current-voltage curves and also the magnitude of the relaxation time somewhat depend on the age of the membrane. Immediately after the "blackening process" the membrane still contains a considerable amount of decane, which becomes less with increasing time. This process is accompanied by an increase of the membrane capacity (White, 1970; Fettiplace, Andrews & Haydon, 1971). After 15 min, however, the measurements were sufficiently constant.

Description of the Transport Model

The model, which has been described in full detail previously (Läuger & Stark, 1970; Stark & Benz, 1971) assumes that a mobile, positively charged 1:1 complex MS^+ between a neutral carrier molecule S and a monovalent ion M^+ is responsible for charge transport across the membrane. The formation of the complexes which cross the membrane preferentially occurs at the interfaces where carrier molecules S from the membrane combine with cations M^+ (concentration c_M) from the aqueous phases (*see* Fig. 1). Both species S and MS^+ are assumed to be located near the interface (interfacial concentrations N_S', N_S'', N_{MS}', N_{MS}'' in moles/cm^2) and have to surmount a potential barrier in order to cross the membrane. For the positively charged complex MS^+ an electrical field produces an unsymmetrical barrier in contrast to species S which are neutral and not influenced by a voltage. If we neglect the movement of S and MS^+ across the interfaces for a moment, the kinetics of ion transport induced by the carrier S can be described by the interfacial reaction rate constants k_R and k_D and by the translocation rate constants k_S and k_{MS} (rate constant at zero voltage), using the assump-

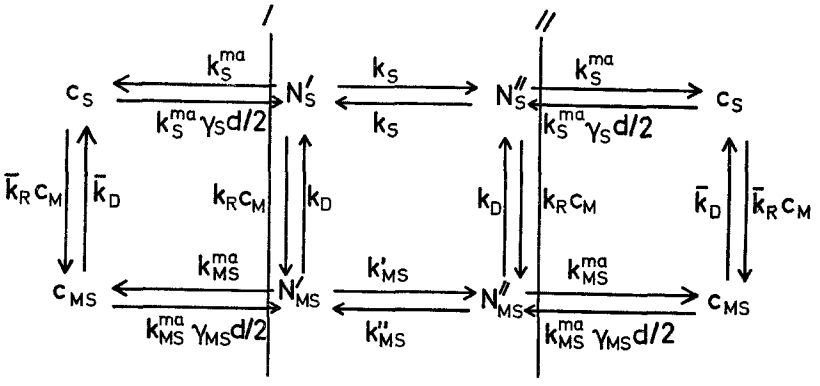

Fig. 1. Reaction scheme of ion transport mediated by a neutral carrier. *See text* for explanation of symbols

tion that the voltage dependence of k_{MS} is approximately given by an Eyring expression.

In principle, charge transfer across the interface could also proceed by association of M^+ and S in the aqueous phases (rate constants $\bar{k}_R$, $\bar{k}_D$) and diffusion of the complex across the interface. It turned out, however, that in the case of valinomycin and monactin the interface reaction is mainly responsible for the observed currents (Stark & Benz, 1971).

The partition coefficients γ_S and γ_{MS} are defined as dimensionless parameters in the following way (*see* Fig. 1). At equilibrium the fluxes across the interface are equal, i.e. $k_S^{ma} N_S = k_S^{ma} \gamma_S d c_S/2$ ($N_S' = N_S'' = N_S$, d = membrane thickness, c_S, c_{MS} concentrations of S or MS^+, respectively, in the aqueous phase) or

$$\gamma_S = 2N_S/d\,c_S. \tag{1}$$

Correspondingly,

$$\gamma_{MS} = 2N_{MS}/d\,c_{MS}. \tag{2}$$

The equilibrium constant of complex formation in the aqueous phase K is given by

$$K = \frac{c_{MS}}{c_M c_S} = \frac{\bar{k}_R}{\bar{k}_D}. \tag{3}$$

Because of the existing reaction cycle including interface and aqueous phases, K is correlated with the equilibrium constant K_h of the interface reaction (Läuger & Stark, 1970):

$$K_h = \frac{k_R}{k_D} = \frac{\gamma_{MS} K}{\gamma_S}. \tag{4}$$

The transport model, therefore, consists of three kinds of parameters. The rate constants k_R, k_D, k_S and k_{MS} account for the kinetics of ion transport induced by carrier molecules and will be treated in the following section. k_S^{ma}, k_{MS}^{ma}, γ_S and γ_{MS} are responsible for the exchange rate of S and MS^+ between membrane and water. $\bar{k}_R$ and $\bar{k}_D$ describe the complexation reaction in the aqueous phase.

The Rate Constants of Ion Transport

The rate constants k_R, k_D, k_{MS} and k_S may be determined by appropriate interpretation of stationary and nonstationary electrical experiments (Stark *et al.*, 1971).

Within the frame of the presented model the shape of stationary current-voltage curves may be super- or sublinear depending on the relative magni-

tude of the individual rate constants. The analysis shows that the shape only depends on one parameter A (Stark & Benz, 1971):

$$A = 2z + vc_M \quad \text{with } z = k_{MS}/k_D, \quad v = k_{MS}\,k_R/k_S\,k_D. \tag{5}$$

At small ion concentrations c_M, A is given by the ratio k_{MS}/k_D. If $k_D \gg k_{MS}$, the current-voltage curve is completely determined by the diffusion barrier of the membrane interior for MS^+. The exact shape of the barrier is not known and has been approximated by an Eyring expression assuming a steep energy maximum in the middle of the membrane. There has been a reasonable good fit to the experimental data up to voltages of about 100 mV. Above this limit large deviations occur which might reflect the approximative treatment of the energy barrier, but could as well be produced by structural changes of the membrane at high voltages. They could be caused by the pressure exerted by the charged membrane capacity (Stark, Benz, Pohl & Janko, 1972). For $k_D \leqq k_{MS}$ the current-voltage curve becomes sublinear, as the complexes at the negative interface do not dissociate fast enough. A saturating shape may also occur at high ion concentrations c_M, if the translocation rate k_S is comparably low (i.e., v not too small).

From a study of the concentration dependence of A the two independent combinations of rate constants z and v may be determined.

Another independent determination of v is based on the measurement of the conductance in the limit of small voltages λ_0 as a function of ion concentration c_M. In the case of negatively charged lipids, the two methods led to large differences for v (Stark & Benz, 1971). For the present neutral lipids, however, both values agree within reasonable limits (*see* next section).

Additional information about the rate constants is obtained from electrical relaxation experiments. On applying a voltage jump to the membrane, the time course of the current is measured. After the loading of the membrane capacity there is a further decrease of the current down to the stationary value. The physical basis of this process rests in a redistribution of both species S and MS^+ between the interfaces induced by the electrical field. The time course of the current has been given by Stark *et al.*, 1971:

$$J(t) = J_\infty (1 + \alpha_1 e^{-t/\tau_1} + \alpha_2 e^{-t/\tau_2}) \tag{6}$$

where J_∞ is the stationary current.

The relaxation times τ_1 and τ_2 and the relaxation amplitudes α_1 and α_2 are functions of the rate constants k_R, k_D, k_{MS} and k_S [*see* Appendix A, Eqs. (A.1)–(A.7)]. In the experiments presented here, the measured time

course of the current, $J(t)$, could be described by a single time constant τ. Within the frame of the transport model, τ had to be assigned to the longer relaxation time τ_1 from the following reasoning: from Eqs. (A.3) and (A.4) there follows for the sum of both relaxation amplitudes

$$\alpha_1 + \alpha_2 = A \cosh u/2 \tag{7}$$

where $u = FU/RT$ is the reduced voltage (U = voltage across the membrane, R = gas constant, F = Faraday constant, T = absolute temperature).

Using the value of A as obtained from stationary $J-U$ curves (*see above*), it was found that if τ was set equal to the shorter relaxation time τ_2 (and α to α_2), then also α_1 (and therefore the longer relaxation time τ_1) would have been easily measurable within the experimental error (*see* Tables 1 and 3). From the experimental finding of only one relaxation time we had to assign: $\tau = \tau_1$ and $\alpha = \alpha_1$.

The experimental quantities z, v, α and τ in principle allow the calculation of k_R, k_D, k_{MS} and k_S. The values of the rate constants obtained in this way can be checked by comparing the voltage dependences of α and τ with theoretical predictions according Eqs. (A.1) and (A.3). The numerical analysis was performed using a Hewlett-Packard calculator 9820 A with plotter 9862 A. Some details of the procedure are given in Appendix A, which also contains an estimate of the error involved in the analysis. It is shown that, because of the experimental error and the particular structure of the mathematical equations, a complete analysis is only possible within a limited region of values of z, v, τ and α. With the present system two rate constants (k_R and k_S) could be determined with reasonable accuracy, while for the two others (k_D and k_{MS}) only a lower limit could be derived in most cases. With the knowledge of k_R, z and v, the partition coefficient γ_S is obtained from the conductance λ_0 at small voltages, using Eq. (10) at small ion concentrations c_M (where $Kc_M \ll 1$).

As an example Fig. 2 contains the data for valinomycin/Rb and dipalmitoleoyllecithin membranes at 5 °C. From the value of the heterogeneous equilibrium constant $K_h = k_R/k_D \approx 0.4\ \text{M}^{-1}$, one concludes that saturation appears in the vicinity of $c_M \approx 1$ M. As in the case of negatively charged phosphatidylinositol membranes (Stark *et al.*, 1971), the translocation rate constants for S and MS^+ are nearly identical. Table 1 contains a comparison of the experimental data for potassium, rubidium and cesium as transported ions at 25 °C, and Table 2 the calculated model parameters. Since γ_S and k_S should be independent of the cation M^+, their values give a hint to the accuracy of the method. The association rate constant k_R is

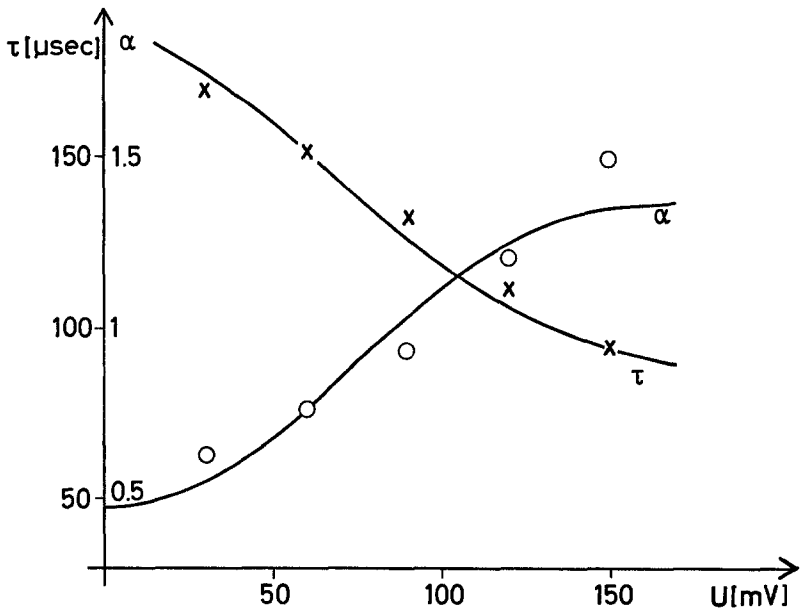

Fig. 2. Kinetic analysis of valinomycin-induced Rb-transport across dipalmitoleoyllecithin membranes at 5 °C. The figure contains experimental values of relaxation amplitude (o) and time (×) after a voltage jump of varying amplitudes obtained from a single membrane ($c_M = 1$ M; valinomycin concentration in the lipid phase was 10^{-3} M). The analysis was performed using (a) $z = 0.15$; $v = 0.4$ (from stationary current-voltage characteristics); (b) $\tau = 150$ μsec and $\alpha = 0.76$ (values at 60 mV). With the resulting rate constants, $k_R = 7.1 \times 10^3$ M^{-1} sec^{-1}, $k_D = 1.9 \times 10^4$ sec^{-1}, $k_{MS} = 2.9 \times 10^3$ sec^{-1}, $k_S = 2.7 \times 10^3$ sec^{-1}, the full lines according Eqs. (A.1) and (A.3) were drawn

Table 1. Stationary current-voltage and relaxation data for valinomycin-induced ion transport across dipalmitoleoyllecithin membranes at 25 °C; comparison between different alkali metal ions[a]

Ion	z	v [M^{-1}]	α (60 mV)	τ in μsec (60 mV)	λ_0 [Ω cm^2]$^{-1}$
K	0.15	0.1	0.21	32.5	$1 \cdot 10^{-5}$
Rb	0.15	0.5	0.65	24.5	$3.2 \cdot 10^{-5}$
Cs	0.1	0.06	0.12	30	$7 \cdot 10^{-6}$

[a] z and v were determined from the concentration dependence of current-voltage characteristic (10^{-2} M $\leqq c_M \leqq 1$ M). The stationary conductance data λ_0 were measured with $c_M = 10^{-2}$ M (+1 M LiCl) and 10^{-8} M valinomycin in the aqueous phases, whereas the relaxation experiments were performed at $c_M = 1$ M. For the definition of symbols *see text.*

largest with rubidium, but the differences are rather small and reflect the situation in methanol, which was studied by Grell, Funck and Eggers (1972). The essential difference between complex formation in a homogeneous

Table 2. Kinetic analysis of valinomycin-induced ion transport across dipalmitoleoyllecithin membranes at 25 °C; comparison between different alkali metal ions[a]

Ion	k_R [M^{-1} sec^{-1}]	k_D [sec^{-1}]	k_{MS} [sec^{-1}]	k_S [sec^{-1}]	γ_S	k_{on} [M^{-1} sec^{-1}]	k_{off} [sec^{-1}]
K	$9.9 \cdot 10^3$	$\geqq 2.4 \cdot 10^4$	$\geqq 3.6 \cdot 10^3$	$1.5 \cdot 10^4$	$9.3 \cdot 10^4$	$3.5 \cdot 10^7$	$1.2 \cdot 10^3$
Rb	$4.5 \cdot 10^4$	$\geqq 4.5 \cdot 10^4$	$\geqq 6.7 \cdot 10^3$	$1.4 \cdot 10^4$	$6.1 \cdot 10^4$	$5.5 \cdot 10^7$	$7.5 \cdot 10^2$
Cs	$9.8 \cdot 10^3$	$\geqq 3 \cdot 10^4$	$\geqq 3 \cdot 10^3$	$1.6 \cdot 10^4$	$4.6 \cdot 10^4$	$2 \cdot 10^7$	$2.2 \cdot 10^3$

[a] The data were calculated from the experimental values given in Table 1. k_{on} and k_{off} (corresponding to k_R and k_D, respectively) refer to the complexation reaction in methanol and were taken from Grell, Funck and Eggers (1972).

phase such as methanol and a membrane-water interface lies in the absolute value of k_R, which is about 3 orders of magnitude smaller for the membrane. There are quite a number of possible reasons for this difference. Shemyakin *et al.* (1969) suggested a special conformation of valinomycin at the interface with its hydrophobic groups directed towards the membrane and its hydrophilic groups directed towards the aqueous phase. Such a conformation generated and stabilized by the particular energetic situation of a membrane interface could be less favorable for complexation. On the other hand, Grell *et al.* (1972) concluded from circular dichroism studies of valinomycin in media of different polarity and in lipid vesicles that valinomycin in a membrane assumes a conformation similar to that in hexane, which should be favorable for complexation. Another reason for the low association rate constant could be the reduced fluidity of the membrane compared with a methanolic solution. During the complexation process the molecule has to undergo a conformational rearrangement, the rate of which might be influenced by the viscosity of the medium. As far as we know, no data exist at the moment as to the influence of viscosity on reaction rate constants of molecules like valinomycin in homogeneous solution. Besides, the access of ions to the place where complexation occurs could be hindered by the molecular interaction of the lipid molecules. Taking the study of Grell *et al.* (1972) as a basis, the complexes should be formed at the unpolar side of the interface, so that an ion would have to penetrate the region of the polar headgroups of the lipid molecules. This in turn could lead to a reduced ion concentration (or activity) at the place of complex formation. Since in all mathematical equations of the analysis, the concentration c_M only occurs in the combination $k_R c_M$, a reduction in c_M would automatically give a corresponding increase in k_R. Therefore, the introduction of an additional partition equilibrium between ion concentrations c_M^* at the place of complex

Table 3. Stationary current-voltage and relaxation data for membranes made from lecithins with different chain lengths in the presence of valinomycin and RbCl[a]

Lipid	z	v [M^{-1}]	α (60 mV)	τ in μsec (60 mV)	λ_0 [Ω cm^2]$^{-1}$
di-(16:1)-PC	0.15	0.5	0.65	24.5	$3.2 \cdot 10^{-5}$
di-(18:1)-PC	0.15	0.3	0.4	36	$1.8 \cdot 10^{-5}$
di-(22:1)-PC	0.3	0.2	0.2	109	$7 \cdot 10^{-6}$
di-(24:1)-PC	0.3	0.1	0.1	600	$1.1 \cdot 10^{-6}$

[a] z and v were determined from the concentration-dependence of current-voltage characteristic (10^{-2} M $\leqq c_M \leqq 1$ M). The stationary conductance λ_0 was measured with 10^{-2} M RbCl (+1 M LiCl) and 10^{-8} M valinomycin in the aqueous phases, whereas the relaxation experiments were performed with 1 M RbCl. The temperature was 25 °C.

Table 4. Kinetic analysis of valinomycin-induced Rb-transport across membranes made from lecithins with different chain lengths at 25 °C[a]

Lipid	γ_S	k_R [M^{-1} sec^{-1}]	k_D [sec^{-1}]	k_{MS} [sec^{-1}]	k_S [sec^{-1}]
di-(16:1)-PC	$6.5 \cdot 10^4$	$4.5 \cdot 10^4$	$\geqq 4.5 \cdot 10^4$	$\geqq 6.7 \cdot 10^3$	$1.3 \cdot 10^4$
di-(18:1)-PC	$7.2 \cdot 10^4$	$2.1 \cdot 10^4$	$\geqq 2.7 \cdot 10^4$	$\geqq 4 \cdot 10^3$	$1.1 \cdot 10^4$
di-(22:1)-PC	$1.4 \cdot 10^5$	$2.6 \cdot 10^3$	$\geqq 5 \cdot 10^3$	$\geqq 1.5 \cdot 10^3$	$4 \cdot 10^3$
di-(24:1)-PC	$1.7 \cdot 10^5$	$2.6 \cdot 10^2$	$\geqq 8.7 \cdot 10^2$	$\geqq 2.6 \cdot 10^2$	$7.7 \cdot 10^2$

[a] The data were calculated from the experimental values given in Table 3.

formation and c_M in the bulk aqueous phase (with $c_M^*/c_M \approx 10^{-3}$) would also explain the differences in the k_R values. That the association rate constant indeed is strongly influenced by membrane properties is shown by experiments with membranes made from lipid molecules with different chain lengths of their fatty acid residues. Table 3 shows a more or less pronounced dependence of the experimental quantities z, v, α and τ on the chain length. The analysis revealed a strong increase of k_R with decreasing chain length of the lipid molecules (Table 4) and thus confirms and extends a previous study, which was only based on stationary experiments (Stark *et al.*, 1972). It remains an open question whether the effect on k_R is produced by the action of the membrane structure on valinomycin itself or on the access of ions to the place of complex formation. In both cases, a decreasing membrane fluidity with increasing chain length could be the basis of the observed phenomena.

The translocation rate constant k_S varies much less than k_R. Its reduction with increasing chain length may also result from a variable fluidity of the

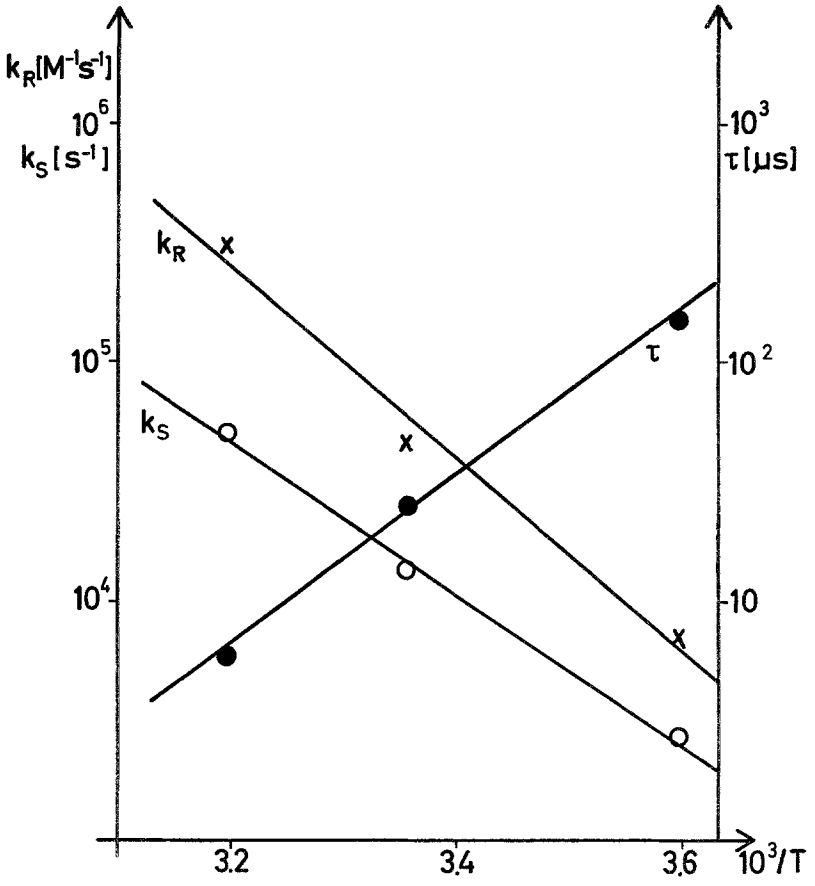

Fig. 3. Temperature dependence of the experimental relaxation time (right-hand ordinate scale), of the computed association rate constant k_R and the translocation rate constant k_S (left-hand ordinate scale) for dipalmitoleoyllecithin membranes, valinomycin and Rb. The relaxation times were measured at 60 mV with 1 M RbCl in the aqueous phases (mean values from five membranes). The deviations from the mean value were less than 20%

membrane and/or by varying the distance between the minima of the energy profile for the free carrier S. It has been shown that the thickness of black films made from lecithin in *n*-decane increases much less than proportional to the chain length of the lipid molecules (Stark *et al.*, 1972). For the calculation of γ_S a membrane thickness of 50 Å was therefore assumed throughout.

Figs. 3 and 4 show the temperature dependence of valinomycin-induced Rb transport. Experiments were performed at 5, 25 and 40 °C. While z, v and α are rather temperature-independent (not shown in the figures), there is a strong decrease of the relaxation time τ with increasing temperature.

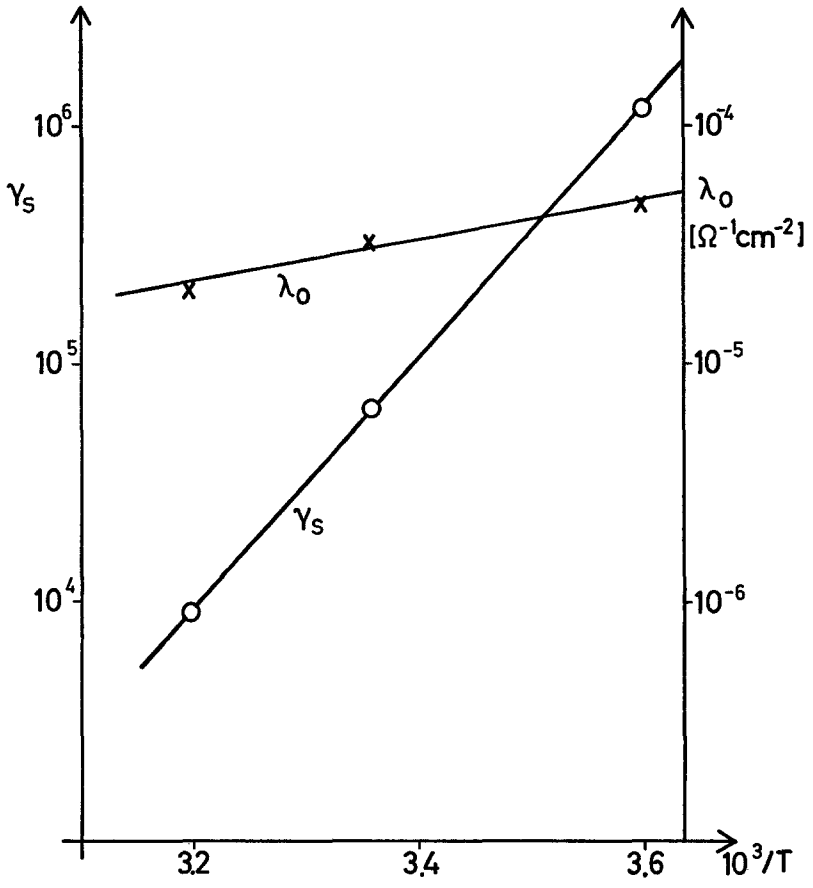

Fig. 4. Temperature dependence of conductivity λ_0 (right-hand ordinate scale) and partition coefficient γ_S (left-hand ordinate scale) for dipalmitoleoyllecithin membranes, valinomycin and Rb. The conductance data represent mean values from five membranes (deviations from the mean value were less than a factor of 2) and were measured at $c_{Rb} = 10^{-2}$ M and $c_{val} = 10^{-8}$ M in the aqueous phase

As the analysis showed, this comes from a pronounced increase of k_R and k_S with temperature (corresponding to activation energies of about 19 and 14 kcal/mole, respectively). It seems reasonable to suppose that these effects are produced via the temperature dependence of membrane fluidity. There is only a small change of conductance λ_0 with temperature, which may be positive or negative (*see also* Fig. 5). This occurs because the increase in k_R with temperature is cancelled by a concomitant decrease of the partition coefficient γ_S [Eq. (10)]. The temperature dependence of conductance (and therefore also of the calculated γ_S, however, may be somewhat influenced by the temperature dependence of the exchange of valinomycin between membrane and torus (*see* later section).

The Conductance as a Function of Ion Concentration

We have shown previously [Stark & Benz, 1971, Eq. (7)] that the conductance λ_0 in the limit of small voltages may be written as

$$\lambda_0 = \frac{F^2 d}{2RT} \frac{k_{MS} c^m_{MS}}{(1+A)} \tag{8}$$

where c^m_{MS} is the mean concentration of complexes inside the membrane, which is connected to the interfacial concentration N_{MS} via $c^m_{MS} = 2N_{MS}/d$. [F = Faraday constant, R = gas constant, T = absolute temperature, d = thickness of membrane; for the definition of A *see* Eq. (5).]

Valinomycin can be added either to the aqueous phase or to the membrane-forming solution (Stark & Benz, 1971). In case of addition to the water, c^m_{MS} is written [using Eqs. (3) and (4) and $c_0 = c_S + c_{MS}$]:

$$c^m_{MS} = c_{MS}\gamma_{MS} = \gamma_S \frac{k_R c_M}{k_D} \frac{c_0}{(K c_M + 1)}. \tag{9}$$

Therefore, from Eqs. (5), (8) and (9):

$$\lambda_0 = \frac{F^2 d}{2RT} \frac{z\gamma_S k_R c_M c_0}{(K c_M + 1)(1 + 2z + v c_M)}. \tag{10}$$

If valinomycin is added to the lipid-decane solution and if its transfer rate from the membrane torus to the black film is fast compared with the transfer rate from the membrane to the aqueous phase, one can assume equilibrium between membrane and torus with respect to the uncomplexed carrier S (*see* next section). Then the concentrations of S in the membrane c^m_S and in the lipid bulk phase c^b_S are connected via the partition coefficient γ^{mb}_S:

$$c^m_S = \gamma^{mb}_S c^b_S. \tag{11}$$

In the limit of small voltages there is also equilibrium between the species S and MS^+:

$$c^m_{MS} = \frac{k_R}{k_D} c_M c^m_S. \tag{12}$$

With Eqs. (8), (11) and (12), the conductance in the case of an addition of valinomycin to the lipid phase is written:

$$\lambda_0 = \frac{F^2 d}{2RT} \frac{z\gamma^{mb}_S k_R c_M c^b_S}{(1 + 2z + v c_m)}. \tag{13}$$

At small concentrations of c_M ($Kc_M \ll 1$ and $1+2z \gg vc_M$) Eqs. (10) and (13) both show proportionality between λ_0 and c_M. At high c_M, Eq. (13) leads to a saturating conductance, whereas Eq. (10) even shows a maximum conductance at $c_M = ((1+2z)/vK)^{1/2}$. Such a maximum in conductance has been reported for trinactin by Ciani, Laprade, Eisenman and Szabo (1973). The physical basis of this difference rests in the fact that in the case of addition of valinomycin to the water, equilibrium between water and membrane is established with respect to S and MS^+. If valinomycin is added to the lipid phase, however, the equilibrium between torus and membranes is assumed only for S, since the torus contains no positively charged complexes MS^+ (in the absence of a lipid-soluble anion). For valinomycin and potassium we found at 25 °C $K \ll 1$ M^{-1} (Stark & Benz, 1971). The same also holds for rubidium at 25 °C, since the curvature of the concentration dependence of λ_0 is the same whether valinomycin is added to the lipid or aqueous phase (*see* Fig. 5). For $c_M K \ll 1$, the deviation from linearity at high concentrations is determined by the values of z and v, which were obtained from the concentration dependence of the shape of the current-voltage curves. Fig. 5 (curve 3) shows a satisfactory agreement between the theoretical curve according to Eq. (10) (with $Kc_M \ll 1$) or Eq. (13) (with the known values of z and v) and the experimental data. For membranes with a net negative charge this test of consistency was less convincing (Stark & Benz, 1971). The observed discrepancies in the case of charged membranes may be explained by changes of the surface charge accompanying substitution of lithium by potassium or rubidium *(unpublished result)*.

At 5 °C the influence of K in Eq. (10) can no longer be neglected, since at $c_M = 1$ M there occurs a difference between both kinds of addition of valinomycin. The data may be explained assuming $K = 1$ M^{-1} for rubidium. Since the deviations are rather small, this value should be considered, however, only with some restrictions. This also applies to the partition coefficient γ_{MS} which is obtained from Eq. (4), if K, k_R/k_D and γ_S are known. For dipalmitoleoyllecithin, valinomycin and rubidium one gets at 5 °C (data of Fig. 2):

$$\gamma_S \approx \gamma_{MS}.$$

This is consistent with an earlier estimate for negatively charged membranes, where we found $\gamma_{MS} > \gamma_S$ (Stark *et al.*, 1971). The Gouy-Chapman potential enhances the concentration of the positive complexes inside the membrane. To explain the approximate equality of the partition coefficients, one has to assume that for the complex the large negative electrostatic energy (Born energy) must be compensated by a positive contribution. This could

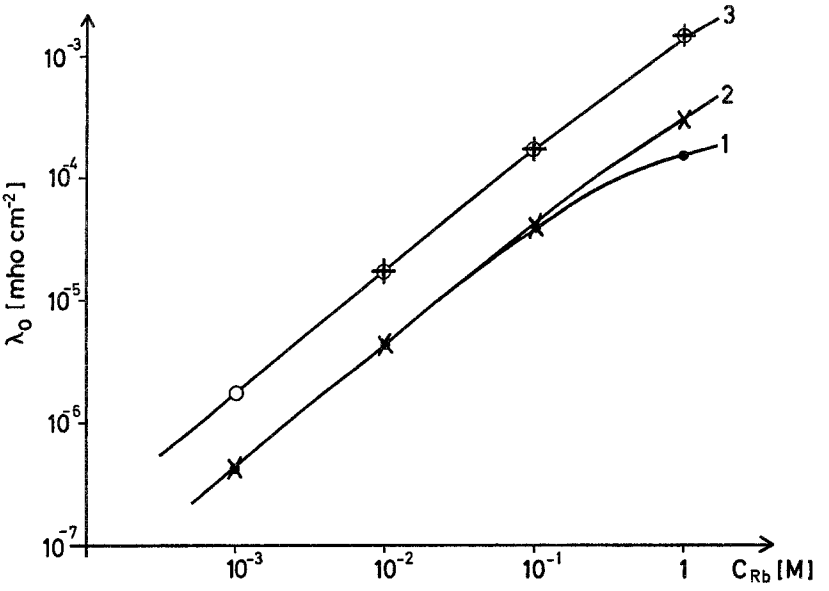

Fig. 5. Conductance of dioleoyllecithin membranes as a function of RbCl concentration in the aqueous phase. The ionic strength was held constant at 1 M with LiCl. •: 10^{-8} M valinomycin in the aqueous phase, temperature 5 °C. ×: 10^{-3} M valinomycin in the membrane-forming solution, temperature 5 °C; the real conductance values were divided by the constant factor 3.37. ○: 10^{-8} M valinomycin in the aqueous phase, temperature 25 °C. +: 10^{-3} M valinomycin in the membrane-forming solution; temperature 25 °C; the real conductance values were divided by the constant factor 1.79. Each experimental value represents a mean of at least five membranes. The deviations from the mean values were less than a factor of 2. The ground level conductance (without rubidium) was about 5×10^{-8} [mho cm^{-2}]. Full line 1 according to Eq. (10) with $k_R\,\gamma_S=3.9\times10^8$ [M^{-1} sec^{-1}]; $K=1$; $z=0.15$; $v=0.5$. Full line 2 according to Eq. (13) with $k_R\,\gamma_S^{mb}=1.3\times10^4$ [M^{-1} sec^{-1}]; $z=0.15$; $v=0.5$. Full line 3 according to Eq. (10) with $k_R\,\gamma_S=1.6\times10^9$ [M^{-1} sec^{-1}]; $K=0$; $z=0.15$; $v=0.3$. The values for z and v were derived from the concentration dependence of the current-voltage curve

arise from an interaction of the more hydrophobic exterior of the conformation of MS^+ with the membrane interior in comparison to S (Haydon & Hladky, 1972).

Similar experiments with potassium instead of rubidium gave $K_K\approx 0.3$ M^{-1} at 5 °C. Another possibility is to derive the equilibrium constant K for other ions from that of Rb by comparing the zero voltage conductances at equal valinomycin- and ion concentrations. If we assume identical geometry and charge distribution of all ion-complexes (i.e., if γ_{MS} and k_{MS} are not dependent on the ion M^+) one derives from Eqs. (4) and (10):

$$\frac{\lambda_0^i}{\lambda_0^k}=\frac{K_i}{K_k}\,\frac{(K_k c+1)}{(K_i c+1)}\,\frac{(1+2z+vc)_k}{(1+2z+vc)_i} \tag{14}$$

with $c_M^i=c_M^k=c$.

At small concentrations c one gets from Eq. (14) ($Kc \ll 1$; $vc \ll 1+2z$)

$$\frac{\lambda_0^i}{\lambda_0^k} = \frac{K_i}{K_k} \frac{(1+2z)_k}{(1+2z)_i}. \tag{15}$$

If the rate-limiting step of ion transport is transport of MS^+ across the membrane (i.e. if $2z \ll 1$), one directly obtains the selectivity ratio K_i/K_k from the conductance ratio. This was used by Eisenman, Szabo, McLaughlin and Ciani (1972) to determine the selectivity pattern of various carrier systems. In the more general case one has to use Eq. (14) or Eq. (15), where z and v are obtained from the shape of the current-voltage curve. At 5 °C we found (by using $K_{Rb} = 1\ M^{-1}$) $K_K = 0.3\ M^{-1}$ and $K_{Cs} = 0.1\ M^{-1}$ for valinomycin in water. Eq. (14) expresses the fact that the selectivity of ion transport is not always given by the selectivity of complex formation.

The Exchange of Valinomycin between Membrane and Water

A small volume of an ethanolic solution of valinomycin was added with stirring to the aqueous solutions on both sides of a membrane which was in the black state. Simultanously the membrane conductance was checked by measuring the current at a constant voltage. Without valinomycin the current was given by the low intrinsic conductance of the membrane in the presence of KCl. On addition of valinomycin at time $t = 0$ the current rose and finally reached a stationary value after a time interval which was influenced by temperature, chain length of the lipid molecules and especially by the membrane area. With membranes formed from dierucoyllecithin having an area of 0.1 cm^2, the steady state was reached after about 50 min. This time interval decreased with the membrane area (being about 10 min at an area of 5×10^{-3} cm^2). Besides, the equilibration time increased with the chain length of the lipid molecules and decreased with increasing temperature. Since time effects produced by the kinetics of ion transport in the presence of valinomycin occur in the microsecond region, it seems obvious to suppose that the time course of the experiments just described, which is at least 6 orders of magnitude slower, reflects the transport of valinomycin from water into the membrane. Eq. (8) shows a proportionality between conductance λ_0 and concentration c_{MS}^m of complexes in the membrane. Since this concentration of MS^+ is proportional to the total concentration of carrier molecules in the membrane (S plus MS^+), the current is a measure for the total number N_0 of carrier molecules in the membrane. Fig. 6 shows the results obtained with dierucoyllecithin membranes. The

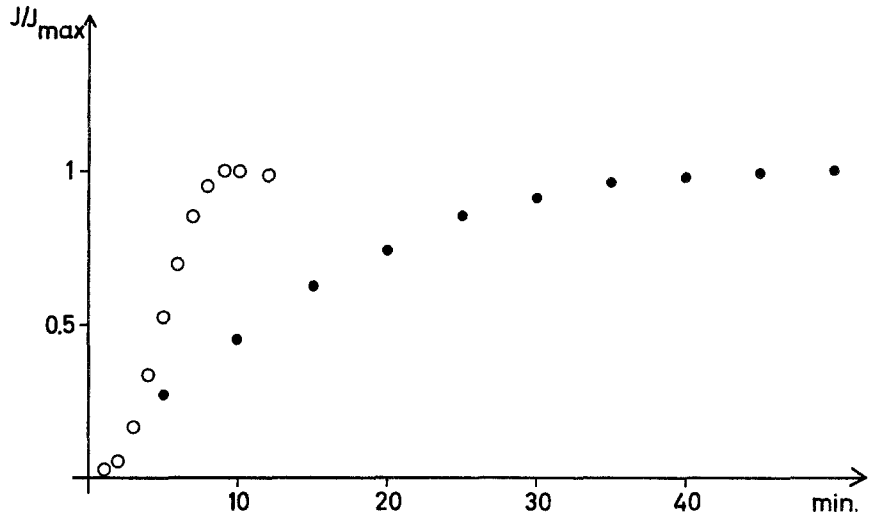

Fig. 6. Time course of the current at constant voltage across dierucoyllecithin membranes following the addition of valinomycin to the aqueous phases at time t=0 (final concentration 10^{-8} M). The aqueous solution contained 1 M KCl. Ordinate: current J divided by the maximum current reached at long times. Full circles: membrane area 0.1 cm^2. Open circles: membrane area 5×10^{-3} cm^2

dependence of apparent equilibration time on membrane area may be understood by taking into account the torus which surrounds the black membrane. There is a continuous exchange of valinomycin between membrane and torus generated by diffusion and by convection. The latter process includes a permanent partial renewal of the membrane from the bulk phase of torus, which is enhanced by stirring. Therefore, if valinomycin is added to the aqueous phase, there is a permanent flow of valinomycin from the membrane to the torus. In this case, the torus represents a large reservoir of approximately zero concentration for valinomycin. Each valinomycin molecule in the membrane possesses a certain probability per second to be transferred into the torus. The mean rate constant k_S^{mt} describing this process will depend on the membrane area (*see* Appendix B). Because of the permanent flow of valinomycin from the membrane into the torus, an approximate equilibrium between membrane and water will exist only at large areas, where the flow into the torus is small, compared with the total valinomycin flux from the aqueous phase into the membrane. For large areas the valinomycin concentration only in the vicinity of the torus is influenced, whereas at small areas the whole membrane is concerned (the circumference of the membrane is proportional to the radius, whereas the area depends on the square of the radius). The time dependence of the

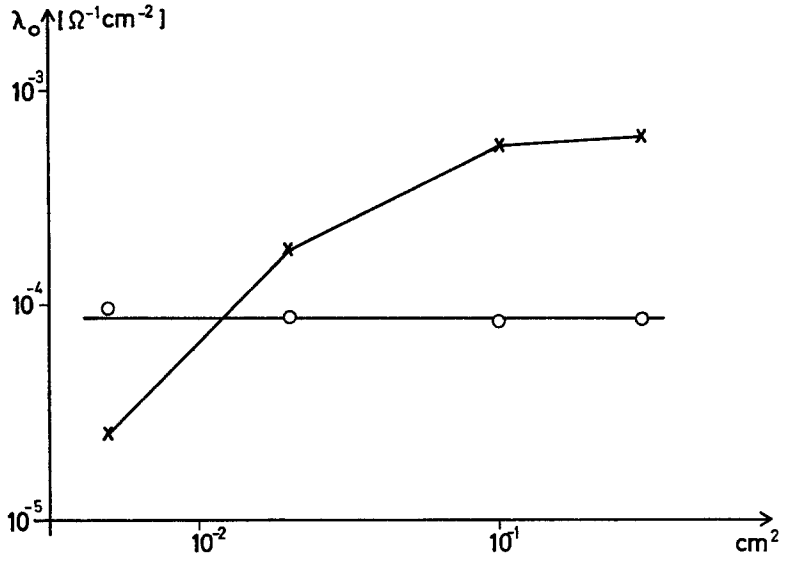

Fig. 7. Dependence of conductivity from membrane area (double logarithmic plot). The experiments were performed with dioleoyllecithin membranes at 25 °C and 1 M KCl in the aqueous solutions. ×: 10^{-8} M valinomycin in the aqueous phases. ○: 10^{-8} M monactin in the aqueous phases. The data represent mean values of at least five membranes. The deviations from the mean values were less than a factor of 2

conductance and its final value reached at long times are thus determined by the relative magnitude of two total fluxes, namely from water to the membrane and from the membrane into the torus. Fig. 7 shows that the mean number of valinomycin molecules per unit area (or λ_0) in the stationary state indeed increases by more than one order of magnitude between areas of 5×10^{-3} cm^2 and 0.3 cm^2. The true value of the partition coefficient γ_S, which is derived from λ_0, is obtained only for membrane areas of at least 0.1 cm^2. For the values of γ_S given in this and earlier papers membrane areas of about 0.1 cm^2 were used throughout. In contrast to valinomycin, the exchange of the ion carrier monactin between membrane and water is fast enough to compensate for the flow into the torus, since the conductance is independent of the membrane area within the studied region (Fig. 7) (*see also* Szabo, Eisenman & Ciani, 1969).

The experiments shown in Figs. 6 and 7 may be understood on the basis of a relatively slow diffusion of valinomycin across the membrane interface taking into account the influence of the torus (*see* Appendix B, part 1). Instead of assuming a slow diffusion across the interface, Fig. 6, however, may also be explained by unstirred layer effects. Since the partition coefficient γ_S is very high in favor of the membrane, a great amount of valino-

mycin has to diffuse across the unstirred layers, which takes a rather long time (*see* Appendix B, part 2). The dependence of the equilibration time on factors like temperature or chain length of the lipid molecules may then be understood on the basis of a varying γ_S. A careful mathematical treatment of the exchange phenomena including unstirred layer effects has to be worked out in order to separate diffusion across the interface from that across unstirred layers. An experimental method to distinguish between both possibilities is to look for an influence of the stirring rate of the aqueous solutions on the conductance of the membrane. Recently, an increase of conductance with the stirring rate was reported by Hladky (1973) and interpreted as an unstirred layer-controlled valinomycin-diffusion into the membrane. With our lipids and experimental conditions we were unable, however, to confirm his observation.

Concluding Remarks

The carrier transport model presented in this and previous papers allows one to understand the essential points of all kinds of experiments, which were performed with black lipid films in the presence of valinomycin and monactin. Nevertheless some important questions remain open, which should be studied in the future.

First of all, the description of the current-voltage curves at high voltages is inadequate. There are several possible reasons for those deviations. The energy barrier for the charged species MS^+, described by an Eyring expression, is only an approximation. Recently, Hall, Mead and Szabo (1973) tried to calculate the exact shape of the energy profile from an analysis of current-voltage curves of symmetrical and asymmetrical systems for nonactin-K. Since they neglected the influence of the interface reaction as a possible reason for the observed rectification effects (Stark, 1973), their results remain doubtful. Besides, there are other phenomena which could also contribute to the observed discrepances. As the size of the carrier molecule is not negligible compared with the membrane thickness, the carrier can "see" a considerable part of the voltage drop across the membrane (Stark & Benz, 1971). This would lead to voltage-dependent rate constants k_R and k_D. The effect was studied in some detail by Hladky (1972). In addition, structural changes of the membrane at high voltages could deform the energy barrier, which would modify the voltage dependence of the translocation rate constant k_{MS}.

Even more in question is the energy profile of the free carrier molecules S in the membrane. For the species MS^+ the physical basis for the assumption

of an energy maximum in the middle of the membrane are the electrical image forces acting on an ion near the interface of two media of different dielectric constants (Neumcke & Läuger, 1969). The superlinear shape of the current-voltage curve obtained under certain conditions is also consistent with this fact. For the species S a barrier in the middle of the membrane would follow from a specific interaction with the interfaces, as was proposed by Shemyakin *et al.* (1969). In the meantime, Grell, Funck and Eggers (1972) interpreted their circular dichroism studies on valinomycin in lipid vesicles in such a way that the molecules assume a conformation similar to that in hexane. They concluded from this that valinomycin is not located at the interfaces but preferentially in the hydrophobic interior of the membrane. Their experiments were performed at extremely high valinomycin concentration, where a perturbation of the membrane structure appears possible. Besides there is no unequivocal conclusion from a CD-spectrum to the place of minimum energy of the carrier inside the membrane. Nevertheless, the exact energy profile of S is an open question at present.[1]

As an alternative to our original model we therefore considered also the case that the free carrier S is mainly located in an energy minimum in the middle of the membrane, from which it may jump to the interface and react with an ion M^+ from the aqueous solution (Appendix C). The expressions which are obtained from this model for the stationary and nonstationary membrane current are formally identical with those derived from the original model. The only change is that the translocation rate constant and the partion coefficient of the free carrier have now to be interpreted in a different way (Appendix C). The rate constants k_R and k_D, however, appear as invariant parameters which may be calculated from the experimental data irrespective of the assumed location of S.

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Appendix A

Calculation of the Rate Constants from the Experimental Data

From the shape of the stationary current-voltage curves two combinations of rate constants are obtained [*see* Eq. (5)]: $z = k_{MS}/k_D$ and $v = k_{MS}\, k_R/k_S\, k_D$. The relaxation of the current after a voltage jump is described

1 *Note Added in Proof.* Recently, M. Hsu and I. S. Chan [*Biochemistry* **12**:3872 (1973)] found by NMR-studies of unsonicated dipalmitoleoyllecithin liposomes that valinomycin is situated at the interface.

by Eq. (6), with (Stark *et al.*, 1971)

$$\tau_1=(a-b)^{-1} \tag{A.1}$$

$$\tau_2=(a+b)^{-1} \tag{A.2}$$

$$\alpha_1=\frac{A}{2}\cosh\frac{u}{2}+B \tag{A.3}$$

$$\alpha_2=\frac{A}{2}\cosh\frac{u}{2}-B \tag{A.4}$$

$$a=\frac{1}{2}\left(k_R c_M+k_D+2k_S+2k_{MS}\cosh\frac{u}{2}\right) \tag{A.5}$$

$$b=\frac{1}{2}\left[\left(k_R c_M-k_D+2k_S-2k_{MS}\cosh\frac{u}{2}\right)^2+4k_R c_M k_D\right]^{1/2} \tag{A.6}$$

$$B=\frac{\cosh(u/2)}{4b}\left[A\left(k_R c_M+k_D+2k_S-2k_{MS}\cosh\frac{u}{2}\right)-4k_{MS}\right]. \tag{A.7}$$

Eqs. (A.1) and (A.2) both fulfill the equation

$$k_D=\frac{-(1/\tau_{1,2})^2+ek_R/\tau_{1,2}}{-f/\tau_{1,2}+gk_R} \tag{A.8}$$

where $e=c_M+2z/v$, $f=1+2z\cosh\frac{u}{2}$ and $g=2z/v+2z\,e\cosh\frac{u}{2}$.

Eqs. (A.3) and (A.4) meet the equation

$$\left(\alpha_{1,2}-\frac{A}{2}\cosh\frac{u}{2}\right)^2=B^2. \tag{A.9}$$

By substituting k_{MS}, k_S and k_D by z, v and Eq. (A.8) in Eq. (A.9) one gets a 4th order equation for k_R, the coefficients of which are only functions of the experimental quantities z, v, α and τ. The four solutions k_R^i correspond to the four possibilities of attributing the experimental values α and τ to α_1, α_2, τ_1 and τ_2. For reasons explained earlier in this paper, the solution has to be chosen which attributes τ to the longer relaxation time τ_1 (and α to α_1). The other rate constants are then derived from z, v and Eq. (A.8).

An estimate of error shows that the procedure in many cases leads to a rather inaccurate determination of either k_D and k_{MS} or k_R and k_S. This is demonstrated for a special case in Fig. 8. For given values of z, v and τ the relaxation amplitude $\alpha=\alpha_1$ was varied. Its maximum value is derived from Eq. (7) as $\alpha_1^{max}=A\cosh\frac{u}{2}$ (one can show that $\alpha_{1,2}\geqq 0$). For $\alpha_1\approx\alpha_1^{max}$ a small change in α, i.e. a small experimental error, leads to a large change in k_R. If $\alpha_1<\alpha_1^{max}$, however, k_R is rather insensitive to variations of α_1. On the contrary, the dissociation rate constant k_D, which is calculated at given z,

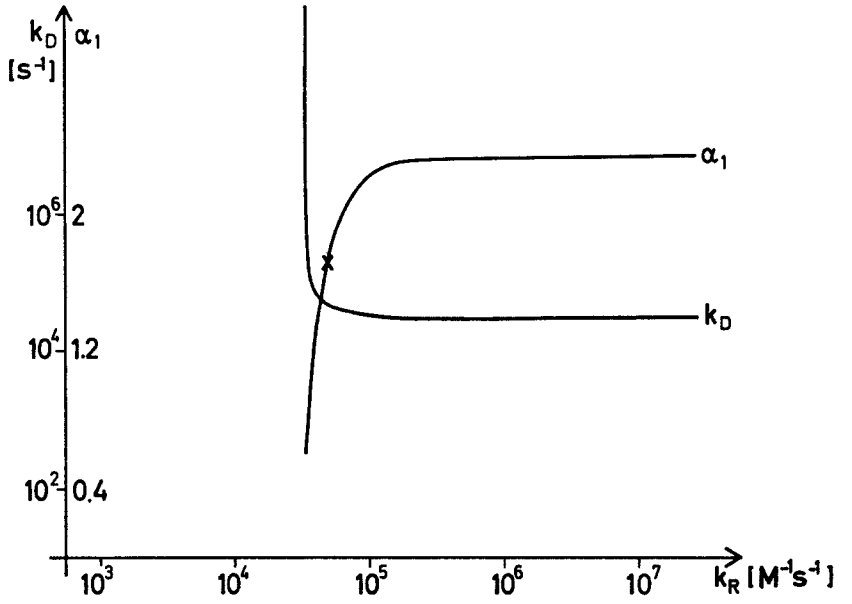

Fig. 8. Variation of k_R and k_D with α_1 at constants z, v and τ. $z=0.4$; $v=1$; $\tau=23$ µsec (data from Stark *et al.*, 1971). ×, measured value

v, τ and k_R from Eq. (A.8) shows a pole at k_R values corresponding to small α. Therefore, at very small amplitudes α_1 (not drawn in Fig. 8) negative k_D values appear, which are without a physical meaning. For a carrier system with a relatively high dissociation rate constant, a small experimental error may lead to negative values of k_D. Only within a limited region of α_1, both k_R and k_D may be obtained within reasonable accuracy (error less than a factor of 5). As Fig. 8 shows, this was the case with the experimental data given in a previous paper (Stark *et al.*, 1971). The data presented in the present paper always allow a rather exact determination of k_R, while the computed value of k_D sometimes shows variations of several orders of magnitude within the experimental error of z, v, α and τ. Tables 2 and 4 therefore contain only a lower limit of k_D (and of k_{MS}), which is derived from Eq. (A.8) at high k_R:

$$k_D^{\min} = e/g\tau \tag{A.10}$$

$$k_{MS}^{\min} = z\,k_D^{\min}. \tag{A.11}$$

Appendix B

Kinetics of Exchange of Carrier Molecules between Membrane and Water

1. Diffusion Across the Interface. We assume that diffusion of carrier molecules across the unstirred layers is fast compared with their rate of penetration into the membrane. We further assume that the latter process

is also slow compared with diffusion inside the membrane and with the process of equilibration between species S and MS^+ at the interface and in the water. This means that Eq. (3) is valid and that the interfacial concentrations N_S and N_{MS} are related by

$$\frac{N_S}{N_{MS}} = \frac{k_D}{k_R c_M}. \tag{B.1}$$

The total concentration of carrier molecules inside the membrane, N_0, is equal to $N_0 = 2N_S + 2N_{MS}$. With Eq. (B.1):

$$\begin{aligned} N_S &= N_0 k_D / 2(k_R c_M + k_D) \\ N_{MS} &= N_0 k_R c_M / 2(k_R c_M + k_D). \end{aligned} \tag{B.2}$$

The neutral carrier molecules inside the membrane can move into the torus surrounding the bimolecular film. If the carrier was added to the aqueous phase, the carrier concentration in the torus is close to zero. Each carrier molecule S in the membrane has a certain probability per second to be transferred into the torus. This probability is high for molecules near the border and is small in the middle of the membrane. We introduce a mean transfer probability k_S^{mt} into the torus for all carrier molecules of the membrane. k_S^{mt} will be high for small membrane areas and low for large areas. The carrier complexes MS^+ cannot move into the torus in the absence of a lipid-soluble anion.

We now imagine a black film without carrier and add carrier to the aqueous phase at time $t = 0$ up to concentration $c_0 = c_S + c_{MS}$. Since we assumed diffusion across the unstirred layer to be fast compared with the transport across the interface, the time derivative of the carrier concentration N_0 is given by the fluxes of S and MS^+ across the interface and by the transfer rate into the torus (*see* Fig. 1).

$$\frac{dN_0}{dt} = 2\left(c_S k_S^{ma} \gamma_S \frac{d}{2} - k_S^{ma} N_S\right) + 2\left(c_{MS} k_{MS}^{ma} \gamma_{MS} \frac{d}{2} - k_{MS}^{ma} N_{MS}\right) - 2k_S^{mt} N_S. \tag{B.3}$$

N_0, N_S and N_{MS} have to be considered as mean concentrations which are averaged over the total area of the film. Accordingly, Eq. (B.3) is only a rough approximation. By substituting N_S and N_{MS} by N_0 and c_{MS} by c_S, making use of Eqs. (B.1) and (B.3), Eq. (B.3) becomes

$$\frac{dN_0}{dt} = -aN_0 + b \tag{B.4}$$

with

$$a = \frac{(k_D k_S^{ma} + k_R c_M k_{MS}^{ma} + k_D k_S^{mt})}{(k_R c_M + k_D)} \tag{B.5}$$

and

$$b = c_S d(k_S^{ma} \gamma_S + K c_M k_{MS}^{ma} \gamma_{MS}). \tag{B.6}$$

Assuming $N_0 = 0$ at $t = 0$, the solution of Eq. (B.4) reads

$$N_0 = \frac{b}{a}[1 - \exp(-at)]. \tag{B.7}$$

The conductance follows the time course of Eq. (B.7), as λ_0 is proportional to N_0 [*see* Eqs. (1), (10), (B.2)]. Eq. (B.5) shows that the time constant of the conductance increase depends on the transfer rate constant of S into the torus, k_S^{mt}, and therefore on the membrane area, which indeed was observed (Fig. 6). The conductance reached at long times is also influenced by the membrane area ($N_0^\infty = b/a$). The theory therefore qualitatively explains the increase of the time constant $1/a$ with membrane area (Fig. 6) and the concomitant increase of λ_0 (Fig. 7).

2. Diffusion Across the Unstirred Layers. As complexation in the aqueous phases was always small under our experimental conditions, we consider only the free carrier molecules S and neglect species MS^+. With a concentration c_S in the aqueous phases, the amount of S in the membrane at equilibrium is given by $c_S \gamma_S d$ [moles/cm^2]. Half of this amount has to diffuse across each unstirred layer. The maximum flux across an unstirred layer of thickness δ is $\Phi_{max} = c_S D/\delta$. An estimate of the minimum time required to equilibrate the membrane with carrier S is obtained by neglecting back diffusion from the membrane to the aqueous phase:

$$t_{min} = \frac{c_S \gamma_S d}{2\Phi_{max}} = \frac{\gamma_S d \delta}{2D}. \tag{B.8}$$

With $\gamma_S = 10^5$, $d = 50$ Å, $\delta = 3 \times 10^{-2}$ cm, $D = 10^{-6}$ cm^2/sec, one gets from Eq. (B.8) $t_{min} \approx 12$ min, which compares well with the experimental data. The slightly sigmoidal increase of current at small times (Fig. 6) may also be well understood on the basis of diffusion across unstirred layers (*see* Carslaw & Jaeger, 1959).

Appendix C

A Modified Carrier Mechanism

As an alternative to our original model we now assume that the free carrier molecule S has an additional energy minimum in the middle of the membrane (Fig. 9). From this equilibrium position it may jump to the interface and combine with an ion M^+ from the aqueous solution. For the charged complex MS^+, however, we retain the original energy profile which has two minima in the interfaces and a barrier in the center of the membrane. A barrier shape of this kind accounts for the image forces acting upon MS^+. In the following we denote the concentration of S in the central minimum

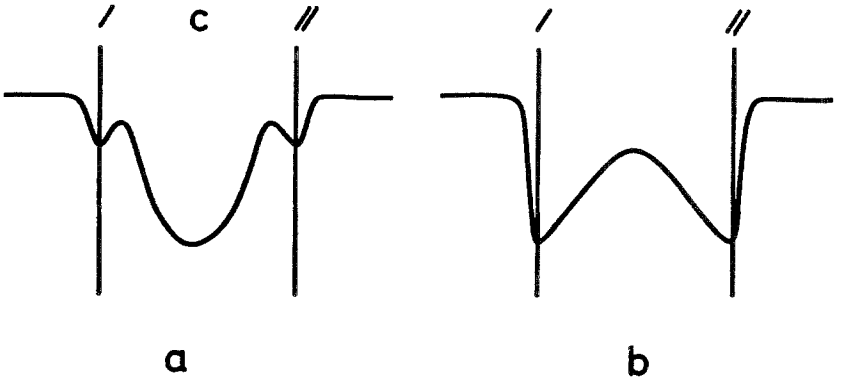

Fig. 9. Modified carrier mechanism. Energy profiles of the free carrier molecules (*a*) and of the ion carrier complexes (*b*) inside the membrane

by N_S^0; furthermore, the rate constant for the jump of S from the center to the interface is denoted by k_S^c and the rate constant for the reverse process by k_S^i (Fig. 9). All other notations remain the same. The rates of change of the concentrations are then expressed by

$$\frac{dN_S'}{dt} = -k_R c_M N_S' + k_D N_{MS}' - k_S^i N_S' + k_S^c N_S^c \tag{C.1}$$

$$\frac{dN_S''}{dt} = -k_R c_M N_S'' + k_D N_{MS}'' - k_S^i N_S'' + k_S^c N_S^c \tag{C.2}$$

$$\frac{dN_S^c}{dt} = k_S^i N_S' + k_S^i N_S'' - 2k_S^c N_S^c \tag{C.3}$$

$$\frac{dN_{MS}'}{dt} = k_R c_M N_S' - k_D N_{MS}' - k_{MS}' N_{MS}' + k_{MS}'' N_{MS}'' \tag{C.4}$$

$$\frac{dN_{MS}''}{dt} = k_R c_M N_S'' - k_D N_{MS}'' - k_{MS}'' N_{MS}'' + k_{MS}' N_{MS}'. \tag{C.5}$$

The current J through the membrane is given as before:

$$J = F(k_{MS}' N_{MS}' - k_{MS}'' N_{MS}''). \tag{C.6}$$

The solution of equations (C.1) to (C.6) in the stationary and non-stationary state may be obtained by the same procedure as used previously (Läuger & Stark, 1970; Stark *et al.*, 1971). It is then found that the expressions for all experimental parameters (z, v, α_1, α_2, τ_1, τ_2) in terms of the rate constants remain the same, provided that the translocation rate constant k_S is now replaced by $k_S^i/2$. In addition, the partition coefficient $\gamma_S \equiv 2N_S/c_S\, d$, which is obtained from the expression for the membrane conductance [Eq. (10)], now accounts only for that fraction of the carrier which is located

in the interfacial energy minima, whereas the total partition coefficient $\bar{\gamma}_S$ is given by

$$\bar{\gamma}_S \equiv \frac{2N_S + N_S^c}{c_S d} = \gamma_S \left(1 + \frac{k_S^i}{2k_S^c}\right). \tag{C.7}$$

The rate constant k_S^c does not enter into the expressions for z, v, α and τ but only appears in the relation (C.7) for the partition equilibrium.

An analysis of the experimental results on the basis of the modified carrier model would therefore lead to the same numerical values of k_R, k_D, and k_{MS} as in the original model. Instead of k_S, however, $k_S^i/2$ would be determined. The measured membrane conductance λ_0 [Eq. (10)] would give the value of the partition coefficient γ_S between interface and water; the total partition coefficient $\bar{\gamma}_S$ (and therefore also the rate constant k_S^c), however, could not be obtained from conductance data. The existing kinetic data do not allow an unambiguous distinction between the two alternative carrier models. A distinction would be possible in principle, if the concentration of S in the membrane could be determined by an independent analytical method. A simple estimate on the basis of the measured value of γ_S, however, shows that the amount of valinomycin in the middle of the membrane cannot be very large. Otherwise, a considerable disturbance of the membrane structure would result.

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