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INFLUENCE OF MEMBRANE STRUCTURE ON THE KINETICS OF CARRIER-MEDIATED ION TRANSPORT THROUGH LIPID BILAYERS

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Summary

Charge-pulse relaxation experiments of valinomycin-mediated Rb^+ transport have been carried out in order to study the influence of membrane structure on carrier kinetics. From the experimental data the rate constants of association (k_R) and dissociation (k_D) of the ion-carrier complex as well as the rate constants of translocation of the complex (k_{MS}) and of the free carrier (k_S) could be obtained. The composition of the planar bilayer membrane was varied in a wide range. In a first series of experiments, membranes made from glycerolmonooleate dissolved in different *n*-alkanes (*n*-decane to *n*-hexadecane), as well as solvent-free membranes made from the same lipid by the Montal-Mueller technique were studied. The translocation rate constants k_S and k_{MS} were found to differ by less than a factor of two in the membranes of different solvent content. Much larger changes of the rate constants were observed if the structure of the fatty acid residue was varied. For instance, an increase in the number of double bonds in the C_{20} fatty acid from one to four resulted in an increase of k_S by a factor of seven and in an increase of k_{MS} by a factor of twenty-four. The stability constant $K = k_R/k_D$ of the ion-carrier complex as well as the translocation rate constants k_S and k_{MS} were found to depend strongly on the nature of the polar headgroup of the lipid. The incorporation of cholesterol into glycerolmonooleate membranes reduced k_R, k_{MS} and k_S up to seven-fold.

Introduction

Planar lipid bilayer membranes have been widely used as models for biological membranes and as tools for the physicochemical investigation of transport processes. By incorporating specific transport systems such as macrocyclic ion carriers or channel-forming peptides detailed information on ion permeation mechanisms could be obtained. In most experiments the membranes have

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been formed using the technique developed by Mueller et al. [1] in which a lipid solution in a solvent, such as a *n*-alkane, is spread across a hole in a plastic sheet. In the optically black state these membranes still contain a certain amount of solvent and their thickness is larger than the thickness of a pure bilayer [2—11]. If a series of *n*-alkanes is used as solvents it is found that the membrane thickness decreases with increasing chain length of the *n*-alkane [7,11,26]. Virtually solvent-free membranes can be made by the more recent Montal-Mueller technique, starting from lipid monolayers at the air/water interface [12].

Very little is known on the influence of solvent on the transport properties of the membrane, for instance on the transport kinetics of lipophilic ions or of ion carriers. The question to what extent transport rates are determined by the nature of the lipid and to what extent they are influenced by the solvent present in the membrane, is, of course, of some importance for the use of black lipid films as models for biological membranes. For this reason we have carried out a study on the kinetics of valinomycin-mediated cation transport through membranes made from glycerolmonooleate in a series of *n*-alkane solvents (*n*-decane to *n*-hexanedecane) and through solvent-free (Montal—Mueller) membrane of the same lipid. The use of an ion carrier as a "probe" for the membrane structure [13,14] seems particularly useful because carrier-mediated ion transport occurs in several distinct steps (complexation, translocation of the loaded carrier, dissociation, back-transport of the unloaded carrier) and each of these steps is affected differently by changes in the structure of the membrane. A kinetic analysis of the carrier system yielding the rate constants of the four transport steps has been performed in the past using the voltage-jump relaxation technique [15] and, more recently, by the charge-pulse relaxation technique [16]. The latter method has been used throughout in this study.

We have also investigated the dependence of transport kinetics on the structure of the lipid. This part of the paper is an extension of previous studies [14,17,18] on the influence of hydrocarbon chain length on the rate constants of carrier-mediated ion transport. The translocation rate constant of the ion-carrier complex depends on several factors such as the shape of the dielectric barrier, the viscosity of the membrane, and the dipolar potential in the membrane-solution interface. The relative importance of these factors is difficult to assess at the moment. Studies with lipids of different structures seem therefore interesting. We have used monoglycerides with fatty acids differing in the hydrocarbon chain length as well as in the number and position of double bonds. Furthermore, the polar head group and the nature of the linkage between hydrocarbon chain and polar residue (ester and ether bonds) were varied. In a third series of experiments, membranes made from a mixture of glycerolmonooleate and cholesterol were studied.

Description of the transport model

The model which was previously described in full detail [15,19] is based on the assumption that the association between the ion M^+ (aqueous concentration c_M) and the carrier S takes place in the membrane-solution interface, the rate constant of association and dissociation of the complex being k_R and k_D,

respectively. The translocation steps of the complex MS^+ and of the free carrier S are described by rate constants k_{MS} and k_S. It is assumed that only k_{MS} is voltage-dependent. This is of course an approximation since it is known that only part of the applied voltage drops across the central barrier [20—22]. The resulting voltage-dependence of k_R and k_D, however, is rather small [23]. Furthermore, the influence of barrier shape on the voltage-dependence of k_{MS} is neglected; this is permissible as long as the voltage is small [24,25]; in our experiments the voltage was about 10 mV. At $t = 0$ the membrane capacity is charged up virtually instantaneously to a voltage V_m^0. For $|V_m^0| \ll 25$ mV the decay of the voltage is given by [16]:

$$V_m(t) = V_m^0[a_1 \exp(-\lambda_1 t) + a_2 \exp(-\lambda_2 t) + a_3 \exp(-\lambda_3 t)] \quad (1)$$

$$a_1 + a_2 + a_3 = 1 \quad (2)$$

If all relaxation times $\tau_i = 1/\lambda_i$ and all relative relaxation amplitudes a_i are known, the rate constants k_R, k_D, k_{MS} and k_S as well as the total carrier concentration N_0 in the membrane may be calculated in the following way: defining the quantities:

$$P_1 = \lambda_1 + \lambda_2 + \lambda_3 \quad (3)$$

$$P_2 = \lambda_1\lambda_2 + \lambda_1\lambda_3 + \lambda_2\lambda_3 \quad (4)$$

$$P_3 = \lambda_1\lambda_2\lambda_3 \quad (5)$$

$$P_4 = a_1\lambda_1 + a_2\lambda_2 + a_3\lambda_3 \quad (6)$$

$$P_5 = a_1\lambda_1^2 + a_2\lambda_2^2 + a_3\lambda_3^2 \quad (7)$$

the rate constants and N_0 are given by [16]:

$$k_{MS} = \tfrac{1}{2}\left(\frac{P_5}{P_4} - P_4\right) \quad (8)$$

$$k_D = \frac{1}{2k_{MS}}\left[\frac{P_1P_5}{P_4} - P_2 + \frac{P_3}{P_4} - \left(\frac{P_5}{P_4}\right)^2\right] \quad (9)$$

$$k_S = \frac{1}{2k_D}\frac{P_3}{P_4} \quad (10)$$

$$k_R = \frac{1}{c_M}(P_1 - P_4 - 2k_S - 2k_{MS} - k_D) \quad (11)$$

$$N_0 = \frac{2RTC_m}{F^2}\frac{P_4}{k_{MS}}\left(1 + \frac{k_D}{k_R c_M}\right) \quad (12)$$

with C_m being the specific capacity of the membrane. The interfacial concentration of the free carrier S and the complex MS^+ may be calculated using the relations for N_S and N_{MS} as published earlier [16]. The partition coefficients γ_S and γ_{MS} are defined as dimensionless parameters:

$$\gamma_S = \frac{2N_S}{dc_S} \tag{13}$$

$$\gamma_{MS} = \frac{2N_{MS}}{dc_{MS}} \tag{14}$$

d is the membrane thickness and c_S and c_{MS} are the concentrations of the carrier S and the complex MS^+ in the aqueous phase ($c_o = c_S + c_{MS}$ is the total carrier concentration in the aqueous phase). Because of the small equilibrium constant K for valinomycin and Rb in water ($K \leqslant 0.1\ M^{-1}$ [17]), γ_S may be calculated using the approximation $c_S \approx c_o$. For γ_{MS} only a lower limit can be calculated from Eqn. 14.

Materials and Methods

Optically black lipid bilayer membranes were formed in the usual way [17] from a 1—2% (w/v) solution of the lipids in *n*-alkanes (Merck, standard for gas chromatography). The circular hole in the Teflon wall between the two aqueous compartments had a diameter of 2 mm. The temperature in the cell was kept at 25° C. The aqueous phase contained 1 M RbCl (Merck, analytical grade) dissolved in twice destilled water and 10^{-7} M valinomycin (Calbiochem, San Diego, Calif.). The aqueous solutions were unbuffered and had a pH of about 6. The measurements were performed 10—20 min after the membrane had turned completely black.

Bilayer membranes made from monolayers (solvent-free membranes) were formed as described earlier [11,12]. A 12.5-μm thick plastic septum (Yellow Springs Instrument, Membrane Kit No. 5937 for oxygen electrodes) with a hole of 0.2—0.3 mm in diameter was clamped between two Teflon troughs.

After filling the troughs with the aqueous solution and cleaning the water surface, the two water levels were adjusted below the hole by means of two syringes. The lipid was then added to the surface as a 0.1% (w/v) solution in *n*-hexane (Merck, Uvasol grade). The amount of lipid on the surface was in large excess compared with the amount needed for a monolayer. After evaporation of the solvent the water level on one side was adjusted just above the hole with the syringe. Then the level on the other side was also raised. If there was no membrane formed across the hole, the procedure was repeated. It cannot be excluded that small amounts of hexane were still present in the membrane [11,42].

Membranes were formed from monoglycerides (Nu Check Prep, Elysian, Minn.) with the following fatty acid residues: palmitoleoyl (Δ^9-$C_{16:1}$), petroselinoyl (Δ^6-$C_{18:1}$), vaccenoyl (Δ^{11}-$C_{18:1}$), elaidinoyl (*trans*Δ^9-$C_{18:1}$), linoleoyl ($\Delta^{9,12}$-$C_{18:2}$), linolenoyl ($\Delta^{9,12,15}$-$C_{18:3}$), eicosenoyl (Δ^{11}-$C_{20:1}$), eicosadienoyl ($\Delta^{11,14}$-$C_{20:2}$), eicosatrienoyl ($\Delta^{11,14,17}$-$C_{20:3}$), arachidonoyl ($\Delta^{5,8,11,14}$-$C_{20:4}$) erucoyl (Δ^{13}-$C_{22:1}$). The lipids contained about 98% of the 1-isomer and gave a single spot in a thin-layer chromatogram. The purity of the fatty acids was checked by mass spectroscopy and gas chromatography; the results indicated that the fatty acids contained trace amounts of other fatty acids. The content was greater than about 90—95%. L-1,2-dipalmitoleoyl-3-phospha-

tidylcholine was synthesized in our laboratory by Benz and Janko [29]. 1-Oleylglycerol was purchased from Serdary (London, Ontario N6G 2R7, Canada).

The purity of both lipids was checked by thin-layer chromatography and was found to be greater than 99%.

The charge pulse experiments were performed as described in a previous publication [16]. In the meantime the time-resolution of the method was increased to about 200 ns and the noise-level of the detecting system substantially reduced. The membrane capacitance was charged up to a voltage of about 10 mV. For this purpose a voltage source was connected with the cell by means of an FET switch during a time between 20 ns and 100 ns. The impedance of the switch in the "open" position was larger than $10^{12}\Omega$. The switch was triggered repetitively by a separate battery-operated pulse generator with pause periods which were at least 20-times longer than the longest relaxation time of the membrane. An oscillographic record of a charging pulse is given in Fig. 1. The transients of the membrane voltage were recorded with a Tektronix 7633/7A13 storage oscilloscope. If not otherwise indicated the detecting system had a bandwidth of about 10 MHz. The original photographs of the oscillograms were enlarged to 15 × 20 cm in order to match the resolution of the digitizer (Hewlett Packard 9864A). The digitized data from the curves were further analyzed with a Hewlett Packard 9820A calculator and a plotter 9862A.

The experiments were performed with a pair of carefully selected platinized platinum electrodes of large surfaces (about 2 cm^2 for each electrode) with an asymmetry of less than 0.5 mV. The performance of the whole set-up was carefully checked with resistors in parallel to an unmodified membrane and with dummy circuits replacing cell and membrane. An example is given in Fig. 2. A

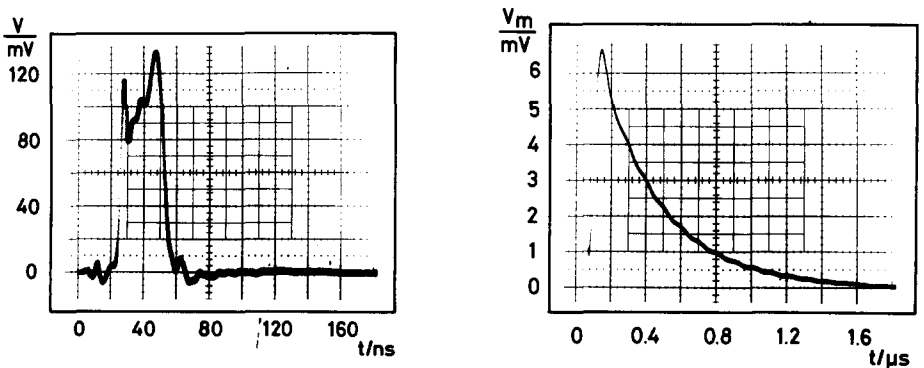

Fig. 1. Oscillographic record of a charging pulse of about 30 ns duration which was applied to a resistance of 100Ω. The measurement was performed with a Tektronix 7633 oscilloscope/7A13 amplifier at a bandwith of 80 MHz.

Fig. 2. Oscillographic record of a test experiment with a glycerolmonooleate/*n*-decane membrane in 1 M KCl (resistance of solutions and electrodes about $R_S = 40\Omega$). The membrane capacity was $C = 4.9$ nF (membrane area $A = 1.23$ mm^2) and the membrane resistance of the order of $10^8\Omega$. In order to simulate the carrier-induced conductance an external resistance $R_e = 33\Omega$ was introduced parallel to C and R_S. At time $t = 0$ a charging pulse of about 20 ns duration was applied to the membrane. For time $t > 200$ ns the decay of the membrane voltage is purely exponential with a time constant of 369 ns which nearly agrees with the calculated time constant $(R_e + R_s)C = 358$ ns.

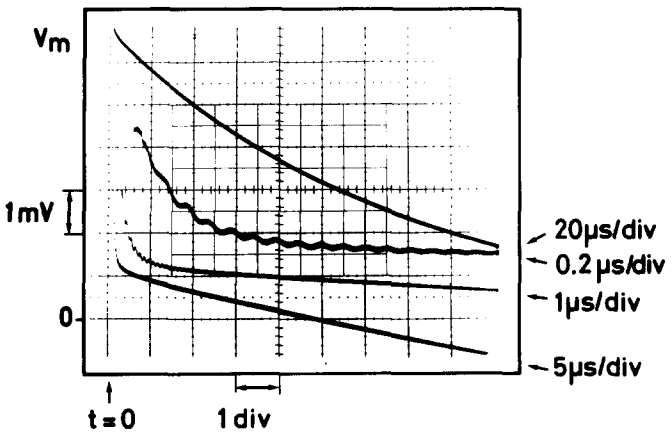

Fig. 3. Decay of the membrane voltage V_m after a charge pulse. Glycerol monoeicosatrienoate/*n*-decane membrane in a aqueous solution of 10^{-7} M valinomycin and 1 M RbCl; T = 25° C, membrane area about 2 mm^2. At time t = 0 the membrane capacitance was charged up by a current pulse of about 20 ns duration to a voltage V_m^0 = 11.5 mV. A repetitive pulse sequence was used with pause intervals of 1 ms between two single pulses. The deacy of V_m was recorded with different sweep times, as indicated on the right side of the oscillogram. The base lines of the different records were: 0 mV (20 µs/div), −5.56 mV (5 (5 µs/div), −5.53 mV (1 µs/div), and −5.14 mV (0.2 µs/div).

glycerolmonooleate/*n*-decane membrane with a capacity of C = 4.9 nF (area about 1.26 mm^2) was formed in 1 M KCl. Parallel to the electrodes on both sides of the membrane an external resistor of R_e = 33Ω was introduced. From R_e and C together with the resistance of electrolyte and electrodes of about 40Ω the RC-time constant of the circuit is calculated to be 358 ns. From Fig. 2 a time constant of 369 ns for the exponential decay is obtained. The time resolution of the whole set-up is limited by oscillations of the FET-switch, the geo-

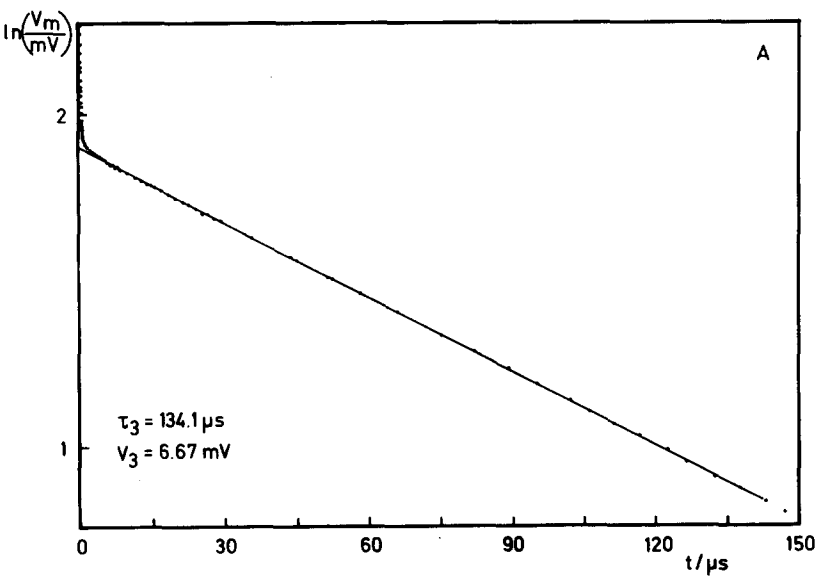

Fig. 4. For legend see opposite page.

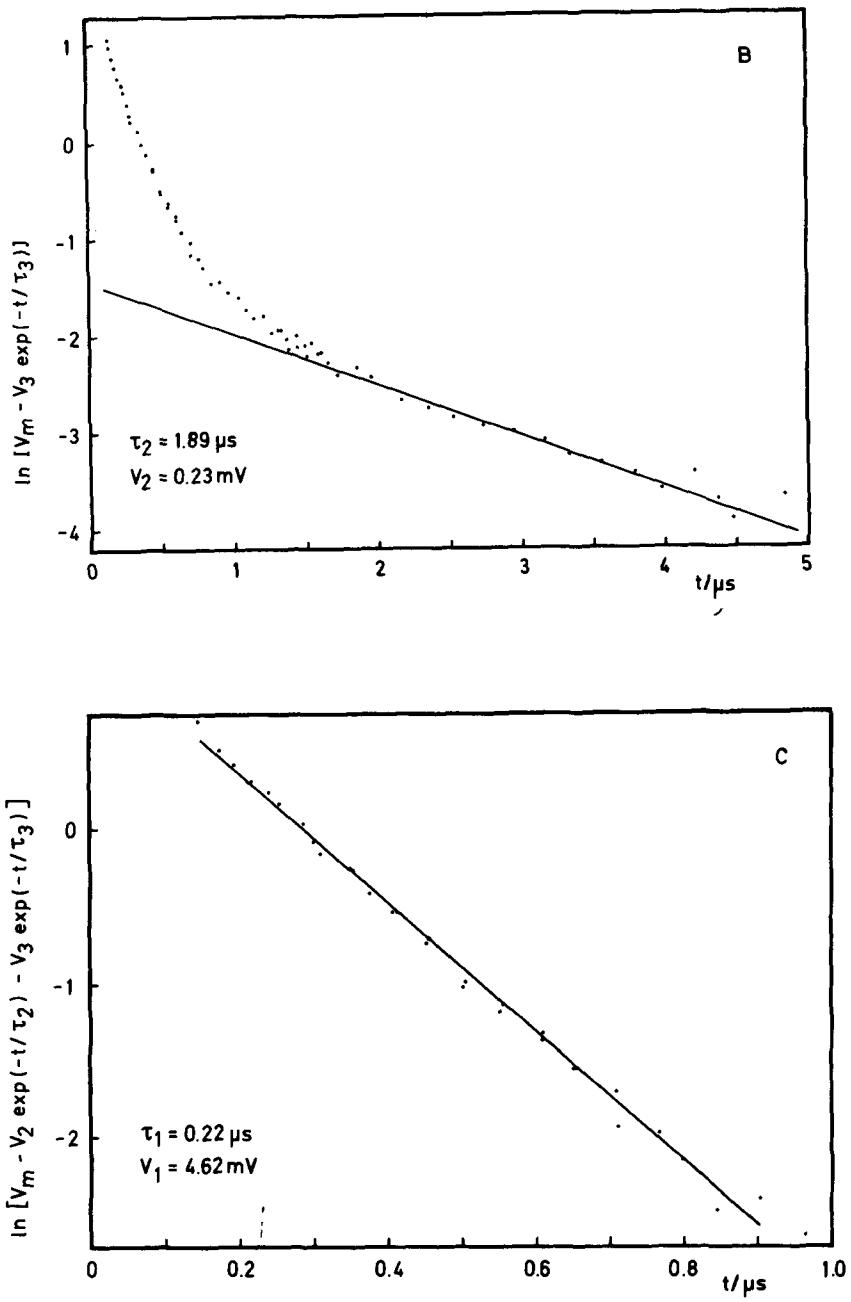

Fig. 4. Analysis of the data of Fig. 3. The relaxation times τ_i and the voltage amplitudes V_i (Eqn. 15) were evaluated from the three successive plots, A, B, and C. In each case te regression line was drawn which gave the best fit to the plotted points in the limit of long times t. The correlation coefficients were calculated to be $r = 0.9999$ (plot A, 5 µs $< t <$ 150 µs), $r = 0.9919$ (plot B, 1.5 µs $< t <$ 5 µs) and $r = 0.9968$ (plot C, 0.146 µs $\leq t <$ 1 µs). From the data given in A, B and C, the following values for the rate constants and for N_O were calculated from Eqns. 3—12. $k_R = 3.32 \cdot 10^5$ $M^{-1} \cdot s^{-1}$; $k_D = 5.26 \cdot 10^4$ s^{-1}; $k_{MS} = 1.34 \cdot 10^6$ s^{-1}; $k_S = 9.36 \cdot 10^4$ s^{-1}; $N_O = 4.56 \cdot 10^{-13}$ mol · cm^{-2}.

metry of the cell and the area of the hole in the cell wall. Under optimal conditions a time resolution of 200 ns was obtained. Relaxation processes with time constants of about 150 ns and an amplitude of 1 mV (total amplitude ≈ 10 mV) could be measured with the above described experimental set-up. In test experiments with undoped membranes of the different lipids in 1 M KCl or 1 M RbCl solution and an external resistance in parallel to the membrane only one time constant was observed in general, which agreed with the calculated RC time of the circuit. Only glycerolmonooleate/*n*-hexadecane membranes showed an additional early voltage decay with a time constant of about 150 ns and a relative amplitude of 0.1 (membrane voltage $V_m^0 \approx 10$ mV). The origin of this fast relaxation process is not yet clear. The early voltage-decay may be caused by the impedance of the polar-layer which may be represented by a large capacity in parallel with a small resistance [27]. Or the transient may reflect a reorientation of dipoles in the membrane [28]. The experimental data obtained from glycerolmonooleate/*n*-hexadecane membranes were not influenced by this additional fourth relaxation process which cannot be explained by the carrier model.

As discussed in the previous section, the decay of the membrane voltage V_m (t) after a charge pulse is governed by three relaxation processes ($\tau_1 < \tau_2 < \tau_3$):

$$V_m(t) = V_1 \exp(-t/\tau_1) + V_2 \exp(-t/\tau_2) + V_3 \exp(-t/\tau_3) \quad (15)$$

The relaxation times τ_i and the voltage amplitudes V_i were evaluated from the record of $V_m(t)$ as described earlier [16]. An example is given in Figs. 3 and 4. In each case the regression line was calculated using a least-squares fitting program.

From Eqn. 12 it is seen that for the calculation of the total carrier concentration in the membrane the value of the specific membrane capacity C_m is needed. For most of the systems studied here the specific capacity was given in previous papers [11,29]. For the others C_m was measured as described earlier [29]. Rectangular pulses with a voltage of $V_m = 10$ mV were applied to the membrane. The current was measured as a voltage drop across an external resistance with a 5115 Tektronix storage oscilloscope. The membrane capacity C was calculated using the relation $V_m C = \tau I_o$, where I_o is the current extrapolated to zero time and τ the time constant of the exponential decay. The area of the membranes was measured using an eyepiece micrometer. The values of the calculated specific capacity varied within 6% around the mean value.

Results

(a) Influence of the solvent

The experimental results obtained with membranes made from glycerolmonooleate dissolved in four different *n*-alkanes and with solvent-free membranes of the same lipid are given in Table I. In addition to the mean values of the experimental data of τ_1, τ_2, τ_3, a_1 and a_2 (a_3 is equal to $1-a_1-a_2$) the specific membrane capacity C_m is given. In contrast to the voltage clamp experiments, where relaxation times and amplitudes are independent of the carrier concentration in the membrane, the relaxation data from the charge-pulse experiments are strongly dependent on the total carrier concentration N_o in the

TABLE I

Relaxation times τ_i and relative relaxation amplitudes a_i obtained from charge-pulse experiments with membranes made from glycerolmonooleate dissolved in different *n*-alkanes and with solvent-free membranes from the same lipid. The aqueous phase contained 1 M RbCl and 10^{-7} M valinomycin; $T = 25°C$. The values of the specific capacity C_m were taken from ref. 11 and the experimental data for *n*-decane from ref. 16. The values of τ_i and a_i are mean values obtained from different membranes (compare Table II).

Solvent	$\tau_1/\mu s$	$\tau_2/\mu s$	$\tau_3/\mu s$	a_1	a_2	C_m/nF cm^{-2}
n-Decane	0.696	2.04	63.1	0.357	0.236	390
n-Dodecane	1.049	3.42	80.7	0.407	0.147	416
n-Tetradecane	1.099	3.62	69.8	0.483	0.114	469
n-Hexadecane	0.763	4.11	63.2	0.558	0.112	585
Solvent-free	0.705	5.57	62.4	0.363	0.126	745

membrane [16]. As the black film is not always in a true partition equilibrium with the aqueous phase with respect to valinomycin [17], the value of N_o and therefore also the values of the relaxation times and amplitudes are subjected to relatively large variations from membrane to membrane. The rate constants, however, which are evaluated from τ_i and a_i (see below) show much smaller variations.

Table II contains the values of the rate constants and of N_o, which were calculated from $\tau_i = 1/\lambda$, a_i and C_m according to Eqns. 8—12. In addition, the partition coefficient γ_S of the free carrier is given.

γ_S was calculated using Eqn. 13. The thickness d of the hydrocarbon layer of the membrane may be obtained from $C_m = \epsilon\epsilon_o/d$, where $\epsilon_o = 8.85 \cdot 10^{-12}$ F/m is the permittivity of free space and $\epsilon = 2.1$ the dielectric constant of a hydrocarbon. From the results of Table II it is seen that the rate constants of translocation, k_{MS} and k_S are nearly independent of the nature of the solvent used for membrane formation and are almost the same for solvent-free membranes. The rate constants of the interfacial reaction k_R and k_D seem to decrease in the series *n*-decane to *n*-hexadecane to solvent-free membranes

TABLE II

Rate constants k_R, k_D, k_{MS}, k_S for valinomycin-mediated Rb^+-transport across membranes made from glycerolmonooleate dissvolved in different *n*-alkanes and solvent-free membranes, as calculated from the data of Table I. The partition coefficient γ_S was calculated according to Eqn. 13. For solvent-free membranes only a lower limit for γ_S could be given because the aqueous carrier concentration is decreased in this case by uptake of carrier into the excess lipid in the water surface. In addition to the standard deviations, the number *n* of membranes for each set of the experimental conditions is given. The results for *n*-decane were taken from ref. 16.

Solvent	$k_R/10^4$ M^{-1} · s^{-1}	$k_D/10^4$ s^{-1}	$k_{MS}/10^4$ s^{-1}	$k_S/10^4$ s^{-1}	N_0/pmol cm^{-2}	$\gamma_S/10^3$	*n*
n-Decane	37 ± 12	24 ± 6	27 ± 3	3.5 ± 0.4	0.68 ± 0.30	5.6	7
n-Dodecane	21 ± 5	10 ± 3	20 ± 1.5	3.9 ± 0.5	0.70 ± 0.32	5.1	9
n-Tetradecane	18 ± 5	9 ± 2	18 ± 1.5	4.0 ± 0.4	0.93 ± 0.28	7.8	9
n-Hexadecane	15 ± 3	9 ± 2	23 ± 3	3.0 ± 0.6	1.70 ± 0.5	20	11
Solvent-free	8 ± 1.5	12 ± 4	41 ± 8	3.2 ± 0.4	1.1 ± 0.3	>26	9

whereas the heterogeneous stability constant $K_h = k_R/k_D$ remains approximately constant. An interesting result is the strong influence of the solvent on the partition coefficient γ_S.

(b) Variation of the structure of the fatty acid residue

Tables III and IV summarize the experimental results obtained from monoglycerides with different fatty acid residues. All membranes were formed from a solution of the lipid in *n*-decane. In a first series of experiments the chain length of the mono-unsaturated fatty acid, as well as the position of the double bond was varied. The two *cis-trans* isomers of the Δ^9-$C_{18:1}$ fatty acid residue were also included. It is seen from Table IV that k_R and k_D vary only within a factor of about two within the range of the mono-unsaturated fatty acid residues studied here. A much larger dependence on the structure of the hydrocarbon chain was found for the translocation rate constants k_{MS} and k_S, both constants decreasing 8- to 10-fold between $C_{16:1}$ and $C_{22:1}$.

In a second series of experiments the number of double bonds was varied. Again, it is found that k_R and k_D are not very sensitive to a change in the structure of the hydrocarbon chain (Table IV). On the other hand, k_{MS} increases about 9-fold from $C_{18:1}$ to $C_{18:3}$ and more than 20-fold from $C_{20:1}$ to $C_{20:4}$. A similar but smaller variation is observed for k_S. This means that in highly unsaturated monoglycerides the dissociation reaction becomes more and more rate limiting in the overall transport reaction.

TABLE III

Relaxation times τ_i and relative relaxation amplitudes a_i obtained from charge pulse experiments with membranes from monoglycerides with different fatty acid residues. The lipids were dissolved in *n*-decane. The aqueous phase contained 1 M RbCl and 10^{-7} M valinomycin, 25°C. The values for the specific capacity denoted by an asterisk (*) were taken from ref. 11. The experimental data for glycerolmonooleate were taken from ref. 16.

Fatty acid residue	Variation of chain length and structure					
	τ_1/μs	τ_2/μs	τ_3/μs	a_1	a_2	C_m/nF cm^{-2}
Palmitoleoyl (Δ^9-$C_{16:1}$)	0.419	1.72	80.7	0.273	0.068	445*
Petroselinoyl (Δ^6-$C_{18:1}$)	0.893	2.70	68.8	0.281	0.275	378
Oleoyl (Δ^9-$C_{18:1}$)	0.696	2.04	63.1	0.357	0.236	390*
Elaidinoyl (*trans* Δ^9-$C_{18:1}$)	1.37	3.81	90.0	0.123	0.496	375
Vaccenoyl (Δ^{11}-$C_{18:1}$)	0.684	2.10	92.3	0.292	0.273	396
Eicosenoyl (Δ^{11}-$C_{20:1}$)	1.62	3.53	125.9	0.376	0.306	345*
Erucoyl (Δ^{13}-$C_{22:1}$)	1.92	4.23	164.9	0.203	0.534	318
	Variation of the number of double bonds					
Oleoyl (Δ^9-$C_{18:1}$)	0.696	2.04	63.1	0.357	0.236	390*
Linoleoyl ($\Delta^{9,12}$-$C_{18:2}$)	0.202	1.26	95.9	0.407	0.063	464
Linolenoyl ($\Delta^{9,12,15}$-$C_{18:3}$)	0.124	1.10	137.1	0.366	0.023	576
Eicosenoyl (Δ^{11}-$C_{20:1}$)	1.615	3.53	125.9	0.376	0.306	345*
Eicosadienoyl ($\Delta^{11,14}$-$C_{20:2}$)	0.503	2.37	126.6	0.379	0.066	414
Eicosatrienoyl ($\Delta^{11,14,17}$-$C_{20:3}$)	0.202	1.73	123.0	0.478	0.018	539
Arachidonoyl ($\Delta^{5,8,11,14}$-$C_{20:4}$)	0.131	1.40	210.6	0.373	0.009	552

TABLE IV

Rate constants k_R, k_D, k_{MS}, k_S and total carrier concentration N_0 in the membrane for valinomycin-mediated Rb^+-transport across membranes from different monoglycerides dissolved in *n*-decane. The results were calculated from the data given in Table III. In addition to the standard deviations, the number *n* of membranes for each set of experimental conditions is given. γ_S was calculated according to Eqn. 13. The results for glycerolmonooleate were taken from ref. 16.

Fatty acid residue	$k_R/10^4$ $M^{-1} \cdot s^{-1}$	$k_D/10^4$ s^{-1}	$k_{MS}/10^4$ s^{-1}	$k_S/10^4$ s^{-1}	N_0/pmol cm^{-2}	$\gamma_S/10^3$	*n*
Variation of chain length and structure							
Palmitoleoyl (Δ^9-$C_{16:1}$)	43 ± 15	13 ± 3	74 ± 7	8.5 ± 0.9	0.27 ± 0.05	1.5	7
Petroselinoyl (Δ^6-$C_{18:1}$)	31 ± 6	22 ± 4	22 ± 4	2.9 ± 0.4	0.57 ± 0.22	4.8	13
Oleyl (Δ^9-$C_{18:1}$)	37 ± 12	24 ± 6	27 ± 3	3.5 ± 0.4	0.68 ± 0.30	5.6	7
Elaidinoyl (*trans*Δ^9-$C_{18:1}$)	29 ± 5	23 ± 5	12 ± 2	1.7 ± 0.2	0.73 ± 0.31	6.5	12
Vaccenoyl (Δ^{11}-$C_{18:1}$)	41 ± 7	25 ± 5	31 ± 4	2.5 ± 0.3	0.65 ± 0.22	5.2	8
Eicosenoyl (Δ^{11}-$C_{20:1}$)	23 ± 5	12 ± 3	10.1 ± 1.2	1.8 ± 0.2	0.89 ± 0.22	5.7	10
Erucoyl (Δ^{13}-$C_{22:1}$)	24 ± 4	13 ± 4	7.0 ± 2.1	1.1 ± 0.1	1.1 ± 0.4	5.4	8
Variation of the number of double bonds							
Oleoyl (Δ^9-$C_{18:1}$)	37 ± 12	24 ± 6	27 ± 3	3.5 ± 0.4	0.68 ± 0.30	5.6	7
Linoleoyl ($\Delta^{9,12}$-$C_{18:2}$)	67 ± 14	13 ± 3	143 ± 13	6.2 ± 0.8	0.41 ± 0.22	1.7	8
Linolenoyl ($\Delta^{9,12,15}$-$C_{18:3}$)	74 ± 16	8 ± 2	250 ± 28	9.6 ± 1.2	0.36 ± 0.17	1.1	5
Eicosenoyl (Δ^{11}-$C_{20:1}$)	23 ± 5	12 ± 3	10.1 ± 1.2	1.8 ± 0.2	0.89 ± 0.22	5.7	10
Eicosadienoyl ($\Delta^{11,14}$-$C_{20:2}$)	34 ± 7	9 ± 1	39 ± 4	3.2 ± 0.6	0.72 ± 0.32	3.4	7
Eicosatrienoyl ($\Delta^{11,14,17}$-$C_{20:3}$)	34 ± 6	5 ± 1	136 ± 3	9.4 ± 0.7	0.67 ± 0.27	2.5	6
Arachidonoyl ($\Delta^{5,8,11,14}$-$C_{20:4}$)	42 ± 5	3 ± 0.5	240 ± 15	12 ± 3	0.43 ± 0.18	0.8	5

TABLE V

Relaxation times τ_i and relative relaxation amplitudes a_i obtained from charge pulse experiments with membranes made from different lipids dissolved in *n*-decane. The aqueous phase contained 1 M RbCl and 10^{-7} M valinomycin; T = 25°C. The experimental data for glycerolmonooleate were taken from ref. 16.

Lipid	$\tau_1/\mu s$	$\tau_2/\mu s$	τ_3/μ	a_1	a_2	C_m/nF cm^{-2}
1,2-Dipalmitoleoyl-3-phosphatidylcholine	1.35	7.85	31.4	0.049	0.546	387 **
Glycerolmono-palmitoleate	0.419	1.72	80.7	0.273	0.068	445 *
Glycerolmono-oleate	0.696	2.04	63.1	0.357	0.236	390 *
1-Oleylglycerol	2.51	7.50	88.8	0.066	0.141	385

* From ref. 11.
** From ref. 29.

(c) Other lipids.

A number of experiments have been performed with a di-(16:1)-lecithin(1,2-dipalmitoleoyl-3-phosphatidylcholine) as well as with the ether analog of glycerolmonooleate, 1-oleylglycerol. The results obtained with these lipids are summarized in Tables V and VI. For comparison the kinetic data of glycerolmonopalmitoleate and of glycerolmonooleate (taken from Tables III and IV) are also included in Tables V and VI. The values of the rate constants for 1,2-dipalmitoleoyl-3-phosphatidylcholine/*n*-decane membranes agree with previous data [17] obtained with the voltage-jump relaxation method; the latter values are given in brackets (Table VI). It is seen from Table VI that the stability constant k_R/k_D of the valinomycin/Rb^+ complex is about 20-times higher in the glycerolmonopalmitoleate membrane as compared with the lecithin membrane with the same fatty acid residue. Furthermore, the translocation rate constants

TABLE VI

Rate constants k_R, k_D, k_{MS}, k_S and total carrier concentration N_0 in the membrane for valinomycin-mediated Rb^+-transport across membranes made from different lipids dissolved in *n*-decane. The results were calculated from the data of Table V. In addition to the standard deviations, the number *n* of membranes for each set of experimental conditions is given. γ_S was calculated according to Eqn. 13. The results for glycerolmonooleate were taken from ref. 16. The values for 1,2-dipalmitoleoyl-3-phosphatidylcholine given in brackets are from ref. 17.

Lipid	$k_R/10^4$ M$^{-1}\cdot$s^{-1}	$k_D/10^4$ s^{-1}	$k_{MS}/10^4$ s^{-1}	$k_S/10^4$ s^{-1}	N_0/pmol cm^{-2}	$\gamma_S/10^3$	*n*
1,2-Dipalmitoleoyl-3-phosphatidyl-choline	8.2 ± 2.5 (4.5)	45 ± 4	9.1 ± 2	2.2 ± 0.4 (1.3)	1.6 ± 0.5	28 (65)	6
Glycerolmono-palmitoleate	43 ± 15	13 ± 3	74 ± 7	8.5 ± 0.9	0.27 ± 0.05	1.5	7
Glycerolmono-oleate	37 ± 12	24 ± 6	27 ± 3	3.5 ± 0.4	0.68 ± 0.30	5.6	7
1-Oleylglycerol	8.3 ± 1.8	15 ± 2	9.8 ± 2.6	4.1 ± 0.5	0.32 ± 0.16	4.3	4

TABLE VII

Relaxation times τ_i and relative relaxation amplitudes a_i obtained from charge-pulse experiments with membranes made from mixtures of glycerolmonooleate and cholesterol in *n*-decane. The mole fraction x of cholesterol (referred to total lipid) is given in the first column. The aqueous phase contained 1 M RbCl and 10^{-7} M valinomycin; T = 25°C. The experimental values for pure glycerolmonooleate were taken from ref. 16. The value of the specific capacity denoted by an asterisk (*) was taken from ref. 11.

x	$\tau_1/\mu s$	$\tau_2/\mu s$	$\tau_3/\mu s$	a_1	a_2	C_m/nF cm^{-2}
0	0.696	2.04	63.1	0.357	0.236	390 *
0.048	0.863	2.28	74.9	0.346	0.247	385
0.091	1.02	2.99	98.3	0.228	0.301	393
0.167	1.22	3.68	104.0	0.173	0.365	406
0.333	2.36	8.69	139.8	0.083	0.390	435
0.500	3.32	27.9	256.5	0.015	0.381	538

k_{MS} and k_S are considerably smaller in the lecithin membrane than in the monoglyceride membrane.

Glycerolmonooleate and its ether analog give similar values of k_D and k_S, whereas both k_R and k_{MS} are 3- to 4-times smaller in the ether compound (Table VI).

(d) Membranes containing cholesterol

The results from experiments made from a mixture of glycerolmonoleate/cholesterol in *n*-decane are summarized in Tables VII and VIII. The mole fraction x_{chol} of cholesterol (referred to total lipid) was varied between 0 and 0.5; at $x_{chol} \geqslant 0.67$ microcrystals appeared in the membrane-forming solution and in the border between torus and black film. As shown in Table VII, all three relaxation times strongly increase as x_{chol} is varied between 0 and 0.5. The corresponding variations in the rate constants are given in Table VIII. It is seen that, while k_D and γ_S remain virtually constant, the three other rate constants k_R, k_{MS} and k_S decrease about 7-fold if x_{chol} increases from 0 to 0.5.

TABLE VIII

Influence of cholesterol on the rate constants of valonomycin-mediated Rb^+-transport, as calculated from the experimental data of Table VII. The partition coefficient γ_S was calculated according to Eqn. 13. In addition to the standard deviations the number n of membranes for each set of experimental conditions is given.

x	$k_R/10^4$ M$^{-1}\cdot$s^{-1}	$k_D/10^4$ s^{-1}	$k_{MS}/10^4$ s^{-1}	$k_S/10^4$ s^{-1}	N_0/pmol cm^{-2}	$\gamma_S/10^3$	n
0	37 ± 12	24 ± 6	27 ± 3	3.5 ± 0.4	0.68 ± 0.30	5.6	7
0.048	39 ± 8	19 ± 3	23 ± 4	3.2 ± 0.3	0.73 ± 0.31	5.0	6
0.091	30 ± 3	20 ± 3	20 ± 2	2.1 ± 0.2	0.52 ± 0.22	4.4	8
0.167	27 ± 4	22 ± 4	18 ± 3	1.8 ± 0.2	0.62 ± 0.28	6.1	7
0.333	13 ± 3	14 ± 2	8 ± 2	1.2 ± 0.1	0.56 ± 0.19	6.8	8
0.500	5 ± 1	17 ± 3	4 ± 2	0.5 ± 0.1	0.72 ± 0.27	16	7

Discussion

In this study we have analyzed the kinetics of an ion-carrier system in a wide range of compositions of the lipid membrane. In all cases investigated here the three relaxation processes which are predicted by the theory of the charge-pulse experiment could be resolved. It was therefore possible to evaluate from the experimental data the rate constants of the four elementary transport steps of the valinomycin/Rb^+ system, as well as the partition coefficient of the carrier between water and the membrane.

We first studied the question of to what extent the solvent which is present in membranes of the Mueller-Rudin type affects the transport rates. With different *n*-alkanes as solvents the specific capacity of the glycerolmonooleate membrane varied between 390 nF cm^{-2} (*n*-decane) and 585 nF cm^{-2} (*n*-hexadecane). With a dielectric constant of $\epsilon = 2.1$, this corresponds to a variation in the thickness d of the dielectric layer between 4.27 nm (*n*-decane) and 3.18 nm (*n*-hexadecane). Solvent-free membranes of the same lipid made by the Montal-Mueller method had a specific capacity of 745 nF/cm^2, corresponding to a thickness of $d = 2.50$ nm. Despite this relatively large change in thickness and solvent content the translocation rate constant k_S of the free carrier remains virtually constant and the translocation rate constant k_{MS} of the loaded carrier increases only by a factor of about two between $d = 4.27$ nm and $d = 2.50$ nm. This small variation of k_{MS} is rather unexpected in view of the fact that the translocation of the charged complex is limited by the image-force barrier [30,31] which depends on the thickness of the dielectric layer. For the further discussion we introduce the energy $w(d)$ of an univalent ion located in the central plane of a membrane of thickness d.

If two membranes of thickness d_1 and d_2 are considered the difference in $w(d)$ is given by [31]:

$$\Delta w = w(d_1) - w(d_2) = h\left(\frac{1}{d_2} - \frac{1}{d_1}\right) \tag{16}$$

$$h = \frac{{e_0}^2}{4\pi\epsilon_0\epsilon_m kT} \ln\left(\frac{2\epsilon_w}{\epsilon_w + \epsilon_m}\right) 6 \simeq 17.8 \text{ nm} \tag{17}$$

($T = 298$ K)

e_o is the elementary charge, ϵ_o the permittivity of free space, $\epsilon_m \simeq 2.1$ the dielectric constant of the hydrocarbon layer, $\epsilon_w \simeq 78.5$ the dielectric constant of water, k the Boltzmann constant and T the absolute temperature; w is expressed in units of kT. For $d_1 = 4.27$ nm, $d_2 = 2.50$ nm, Δw becomes equal to 3.0.

This means that, if only the dielectric energy is taken into account, the translocation rate constants in a glycerolmonooleate/*n*-decane membrane and in a solvent-free glycerolmonooleate membrane should differ by a factor of approx. $\exp(\Delta w) \simeq 20$. (This factor becomes even larger, if allowance is made for the effects of barrier shape.)

There are at least two possibilities to explain the small influence of membrane thickness on k_{MS}. The solvent introduced into the bilayer may diminish

the effective viscosity of the membrane so that the increase in height of the dielectric barrier is partially compensated by an increase in mobility of the ion-carrier complex. The other possibility is based on the assumption that the solvent is inhomogeneously distributed in the membrane [32] such that small areas exist which are almost free of solvent. These areas would have a thickness which is smaller than the average thickness d and which is virtually independent of the nature of the solvent. Ion transport would then occur predominantly through these thinner regions of the membrane. This hypothesis would also provide an explanation for the peculiar observation that the partition coefficient γ_S of the free carrier increase with decreasing solvent content of the membrane (Table II).

In the framework of the kinetic analysis γ_S is related to the membrane concentration of carrier molecules which participate in the ion transport; this concentration would increase if the number and area of thin regions in the membrane increase.

In contrast to the small variation of k_{MS} and k_S with the solvent content of the membrane, a much more pronounced dependence of the translocation rate constants on the structure of the lipid from which the membrane has been formed was found. With increasing the chain length of the mono-unsaturated fatty acid residue of the monoglyceride from C_{16} to C_{22} k_S decreases about 8-fold and k_{MS} about 10-fold (Table IV). An even more drastic effect on k_S and k_{MS} was observed by varying the number of double bonds in the fatty acid. Between $C_{20:1}$ and $C_{20:4}$ k_S increases by a factor of about seven and k_{MS} by a factor of about twenty-four. Presumably this strong increase of the translocation rate constants originates in part from the reduced viscosity of the membrane. Also the difference in the translocation rate constants observed with the *cis-trans* isomers of the Δ^9-$C_{18:1}$ monoglyceride (Table IV) possibly result from a viscosity effect. Another factor which may influence k_{MS} is the somewhat larger dielectric constant and therefore the lower electrostatic energy barrier of a membrane made from highly unsaturated lipids. The increase of specific membrane capacity with increasing unsaturation of the lipid (Table III) may be partially caused by the change in the dielectric constant.

The results summarized in Table VI allow a comparison between lipids of identical fatty acid chains but different polar head groups. Dipalmitoleoyl phosphatidylcholine and glycerolmonopalmitoleate differ considerably in all four rate constants. The most striking difference occurs in the value of the stability constant $K_h = k_R/k_D$ of the complex, K_h being about 20-times larger in the case of the monoglyceride as compared with the lecithin. Recently, Hladky and Haydon [37] have studied the stationary membrane conductance λ_o of lecithin and monoglyceride membranes in the presence of a cationic carrier system (nonactin/K^+) and have presented arguments that the large difference in λ_o results from the existence of a dipolar potential in the membrane-solution interface which makes the interior of a lecithin membrane more positive than the interior of a monoglyceride membrane. Our finding that K_h is larger in a monoglyceride membrane than in a lecithin membrane is consistent with this picture. However, it is seen from Table VI that also the two translocation rate constants k_{MS} and k_S, as well as the partition coefficient γ_S differ considerably in membranes made from the two lipids. As γ_S and k_S change in opposite direc-

tions, these additional effects which are independent of the dipolar potential tend to cancel each other and do not greatly affect the stationary membrane conductance. The influence on k_S may be discussed on the basis of a higher fluidity of the monoglyceride membranes in contrast to membranes from lecithin. Furthermore, it is not clear to what extent a carrier can be influenced by an existing dipole potential. The valinomycin-cation complex has a diameter of 1.5 nm and a height of 1.2 nm; a molecule of this size necessarily creates a large local perturbation of the structure of the dipole layer.

Incorporation of cholesterol into a glycerolmonooleate membrane reduces k_R, k_{MS} and k_S up to about 7-fold (Table VIII). The decrease of the translocation rate constants upon incorporation of cholesterol is even more remarkable as, at the same time, the membrane thickness as determined from the specific capacity (Table VII) decreases. Such a reduction of membrane thickness with increasing cholesterol content has alreadey been observed by Hanai et al. [33]. Cholesterol may affect the structure of the membrane and the kinetics of carrier transport in different ways [37—41]. By interacting with the hydrocarbon chains of the lipid molecules cholesterol may reduce the fluidity of the membrane and thereby decrease the translocation rate of the carrier. On the other hand, there is experimental evidence that cholesterol changes the dipolar potential of the membrane so that the interior of the membrane becomes more positive [37—40]. Depending on the location of the absorbed ion-carrier complex with respect to the plane of the dipoles, a change in the dipolar potential may affect the stability constant K_h of the complex as well as the translocation rate constant k_{MS}. The observation that the effect of cholesterol on k_{MS} is about the same as on k_S seems to indicate that the translocation rate constant of the charged complex is not significantly influenced by the dipole potential. On the other hand it is seen from Table VIII that the stability constant $K_h = k_R/k_D$ decreases about 7-fold if the mole fraction x of cholesterol in increased from 0 to 0.5. Both findings taken together may in principle be interpreted by the asumption that the charged complex is located on the hydrocarbon side of the dipole layer. We consider this interpretation as tentative, however, in view of the difficulties discussed above in the quantitative application of the dipolar-layer model to carrier-mediated ion transport.

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