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Temperature-Jump Experiments on Thin Lipid Membranes in the Presence of Valinomycin

W. Knoll and G. Stark

Fachbereich Biology, University of Konstanz, D-7750 Konstanz, Germany

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Summary. Temperature jump relaxation experiments on planar lipid membranes in the presence of valinomycin were performed using the absorption of a strong light flash as an energy source for the generation of the T-jump. The relaxation of the current carried by valinomycin/Rb^+ complexes was measured. The results were interpreted on the basis of a transport model which was also analyzed by voltage jump relaxation experiments. The study shows that the application of the T-jump technique provides valuable information about transport kinetics as well as the dynamics of the membrane structure. At the given experimental conditions the relaxation of the current is believed to reflect a temperature-dependent transition of the membrane to a new conformational state of lower order. The relaxation could be resolved with the present technique only at low temperatures and for membranes of high microviscosity.

Fast relaxation methods are widely used at present for the study of reaction mechanisms of complex chemical and biochemical systems (Eigen & De Maeyer, 1963; Eigen, 1968). They have increasingly been applied to transport phenomena in membranes, too. Ion transport induced by macrocyclic ionophores, such as valinomycin or the macrotetrolides, has been studied by transient and stationary relaxation techniques in artificial lipid vesicles (Grell, Funck & Eggers, 1975). The action of these compounds on the permeability properties of membranes is most easily demonstrated with planar lipid membranes via the change of the electrical conductance. The mechanism underlying the effect of ionophores has been successfully examined by the application of a voltage jump-current relaxation technique. This transient method uses the stepwise perturbation of the system by an electrical voltage difference and detects the time course of the current induced by the voltage. The rates of complex formation and dissociation of ion carriers like valinomycin and their translocation rates across lipid membranes have been determined by this method (Stark, Ketterer, Benz & Läuger, 1971;

Gambale, Gliozzi & Robello, 1973; Laprade, Ciani, Eisenman & Szabo, 1975; Knoll & Stark, 1975). Similarly, the kinetics of dimerization of the pore-former gramicidin has been studied (Bamberg & Läuger, 1973). The voltage-jump method as well as the related charge pulse method (Feldberg & Kissel, 1975; Benz & Läuger, 1976) are, however, confined to voltage dependent phenomena in membranes. Since all molecular events have a more or less pronounced temperature dependence, the temperature-jump method, at least in principle, should have a broader range of possible applications. A slow version of this method has been used to separate the temperature dependence of the partition coefficient from that of the rate constants at valinomycin-induced ion transport (Stark, Benz, Pohl & Janko, 1972).

This paper reports the first use of a fast temperature-jump method to investigate ion transport through planar lipid films. Again valinomycin-induced rubidium transport is studied.

Materials and Methods

Temperature jumps are often accomplished by the heat accompanying the discharge of a capacitor through the system under study. This method is, however, restricted to systems of low electrical resistance and is not applicable to planar lipid membranes. Therefore, the fast increase of the temperature was generated by absorption of a strong light flash. This technique has been developed for homogeneous solutions (Staerk & Czerlinski, 1965; Strehlow & Kalarickal, 1966; Hoffmann, Yeager & Stuehr, 1968; Caldin, Crooks & Robinson, 1971; Ameen, 1975), but was recently also applied to study the mechanism of ionic conductance in nerve fibers (Moore, Holt & Lindley, 1972; Moore, 1975). Both pulsed lasers and flash lamps have been used as sources of a high power flash. For a first application on lipid membranes a flashlamp was used which was constructed according to Hoppe (1972): Two tungsten electrodes are mounted at a variable distance in a brass casing filled with argon. The flash is focused on the membrane by a hollow mirror close to the electrodes and by an adjustable lens in the front part of the casing (quartz optics). The energy of the flash is stored in one to four high power condensers (8.4 μF each) fed by a high power supply (Heinzinger, HN 30000-20). The ignition voltage at an electrode distance of 2–4 mm and an argon pressure of 5–7 atm was about 6–10 kV. The emission spectrum of an argon discharge extends over the complete *UV* and visible region. To increase light absorption a dye was added to the aqueous phases on both sides of the lipid film. Best results were obtained with commercial ink (Geha, Brillant Schwarz) consisting of various triphenylmethane dyes. This mixture, despite being badly characterized, was favored to other dyes which were tried. It shows strong absorption over the whole visible spectrum. Besides, it did not interfere with valinomycin induced Rb^+-transport. Neither the steady state conductance of lipid membranes nor the voltage jump relaxation data described below were influenced by the presence of these dyes, which were added in rather low concentrations. The distance of the membrane from the glass window of the cuvette was kept small (2 mm) to avoid a loss of light intensity. The amplitude of the temperature jump generated in this way varied

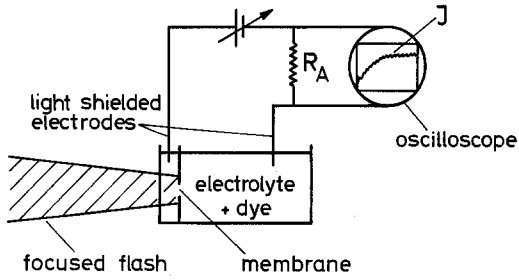

Fig. 1. Schematic diagram of the set-up used for the flash-lamp temperature-jump studies on thin lipid membranes

between 0.1–0.5 °C (with the *UV*-part of the spectrum cut off) depending on the experimental conditions. It was measured in three different ways: via the known temperature dependence of the electrolyte resistance (using two platin electrodes in the plane of the membrane in combination with a high frequency bridge), via the known temperature dependence of the steady-state membrane conductance of valinomycin/Rb^+, and by using a miniature thermocouple. The time course of the flash was measured with a photocell. The main part of the light emission occurred during the first 10 µsec, which, therefore, should correspond to the heating time of the system. The time resolution of the detection system, however, was limited by the HF-noise accompanying the discharge (duration ca. 100 µsec) and/or by the charging time of the circuit.

The thermal equilibration between a 50 Å thick bimolecular membrane and the adjacent aqueous phases occurs in the nanosecond region, as may be shown by a simple estimate. After the absorption of the flash there was a temperature gradient between the front and the rear side of the cuvette. By using an appropriate concentration of the dye, the temperature at the place of the membrane was, however, constant up to several seconds. For an experimental test of this constancy, membrane experiments were performed using conditions where the relaxation phenomena described in the next section were fast compared to the time resolution of the detection system.

The relaxation of the membrane conductance following the temperature jump was studied using the set-up shown in Fig. 1. A constant voltage is applied to the membrane via two light shielded Ag-AgCl-electrodes. The time dependence of the current was measured via the voltage drop across an external resistor. The current sensitivity of this arrangement depends on the magnitude of the external resistor R_A relative to the membrane resistance R_M (with a maximum at $R_A = R_M$). One disadvantage of this simple method consists in a small voltage jump, which accompanies a change in R_M (induced by a temperature jump). The magnitude of this side effect was minimized by using values of R_A as small as possible. A simple estimate shows that for $R_A \leqq 0.1\, R_M$ (as used in many cases) the contribution of the voltage jump to the total effect is less than 10 % and not of experimental significance. Experiments with an "ideal" voltage clamp are under way.

Black lipid membranes were formed from diphytanoyl-lecithin (di(16:4CH_3)−PC) synthesized by K. Janko in our laboratory. The branched fatty acid residues of this lipid give rise to an increased microviscosity of the membrane and to a concomitant slower kinetics of valinomycin induced Rb^+-transport (Pohl, Knoll, Gisin & Stark, 1976).

For other experimental details, such as the technique of the voltage-jump current-relaxation method, the reader is referred to previous publications (Knoll & Stark, 1975; Pohl *et al.*, 1976).

Results and Discussion

If the equivalent circuit of a lipid membrane is described as a resistor R_M parallel to a capacitor C_M, an ideal stepwise decrease of R_M (with C_M remaining constant) would produce the following time dependence of the current at constant voltage U:

$$J(t) = J_\infty(2) - (J_\infty(2) - J_\infty(1))\, e^{-t/\tau_c}. \tag{1}$$

$J_\infty(2)$ and $J_\infty(1)$ represent the steady state currents after and before the stepwise change of R_M and the characteristic charging time τ_c is given by

$$\frac{1}{\tau_c} = \left(\frac{1}{R_{\text{ext}}} + \frac{1}{R_M}\right)\frac{1}{C_M}. \tag{2}$$

The external resistor R_{ext} is the sum of R_A and the solution resistance R_S between the two electrodes (usually $R_S < R_A$).

Fig. 2 shows that the time course of the current J across a membrane doped with valinomycin, following a temperature jump, may, indeed, be fitted according to Eq. (1). The corresponding experimental time constant τ_T is, however, considerably larger (about 10 times) than τ_c (as calculated from Eq. (2)). Such a behavior is expected, if the temperature jump is not accompanied by a simultaneous decrease of R_M, i.e., if temperature dependent relaxation phenomena inside the membrane give rise to a delay. That this delay is, indeed, generated by membrane phenomena and is not an artifact (possibly arising from a thermal equilibration process inside the cuvette) is demonstrated by the experimental results of Figs. 3 and 4. The relaxation time τ_T depends on the Rb^+-concentration in the aqueous phase and is also different, if normal valinomycin is replaced by a dansylated analogue. In all cases the geometry of the cell was identical. Besides, τ_T decreases with increasing temperature. At 25 °C, in the presence of normal valinomycin, τ_T was found equal to the charging time τ_c according to Eq. (2). This indicates that the membrane processes responsible for the relaxation get faster with increasing temperature and can only be resolved under the present experimental conditions at rather low temperatures and for membranes with a rather high microviscosity. With membranes formed from dioleoyllecithin (di(18:1) – PC), which has two unsaturated fatty acid residues, the identity of the experimentally determined relaxation time τ_T with the time limit of the detection system was found also at low temperatures. In contrast to the clear influence of the temperature, τ_T was largely independent from the applied voltage. Only a small decrease was found at higher voltages (above 100 mV, using

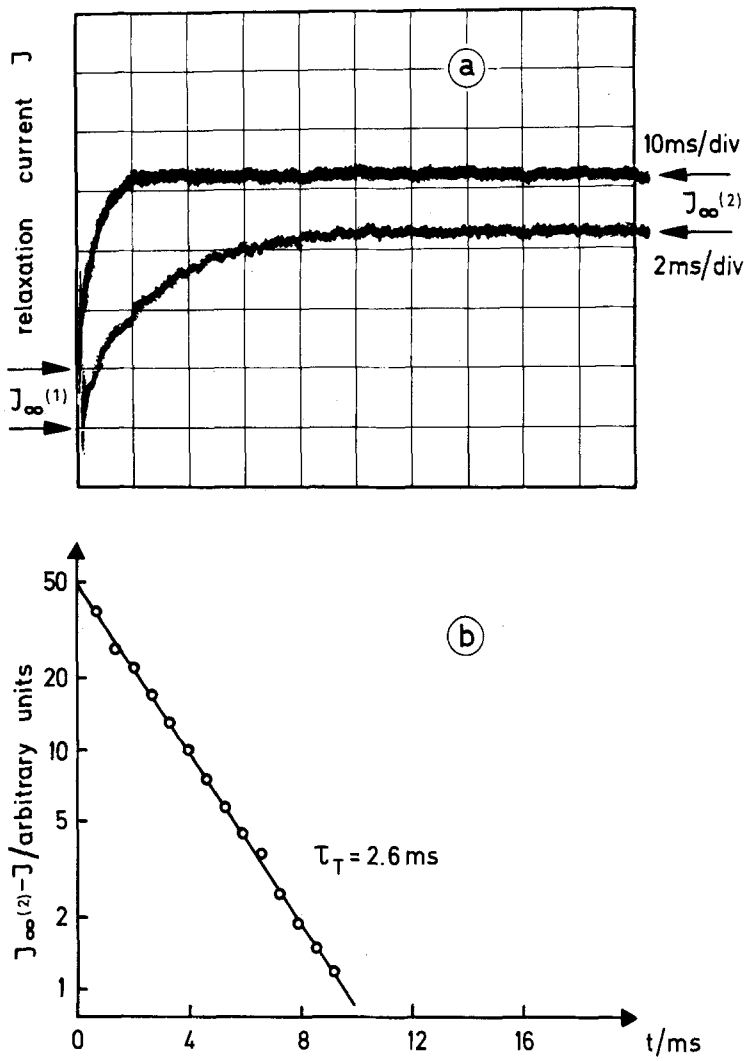

Fig. 2. Typical current relaxation of a di(16:4 CH_3)–PC membrane (doped with 10^{-3} M valinomycin in the membrane-forming solution) following a temperature jump of about 0.2 K. Experimental conditions: 2 M RbCl, 0 °C, $R_M = 1.1 \times 10^5\,\Omega$, $R_A = 9.7 \times 10^3\,\Omega$, applied voltage $U = 100$ mV, charging time $\tau_c = 250\,\mu$sec. (*a*): Original curves of a double beam oscilloscope with two different time scales: upper trace 10 msec/div., lower trace 2 msec/div., current sensitivity 5.2 nA/div. (*b*): Semilogarithmic plot of the data from (*a*)

di(16:4 CH_3)–PC and low temperatures). Besides the same values for τ_T were obtained, if KCl was used instead of RbCl.

Relaxation phenomena associated with membrane processes in the presence of valinomycin have been found already with the voltage-jump relaxation method. There, the initial current J_0 following a voltage jump

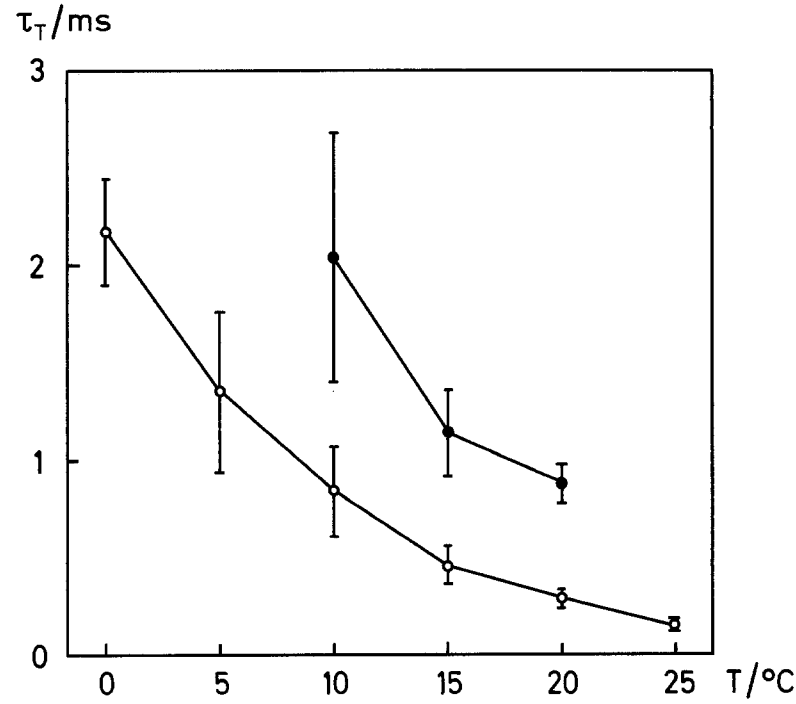

Fig. 3. Temperature dependence of the relaxation time τ_T. At 25 °C τ_T is identical with the charging time τ_C of the electronic circuit, which was held approximately constant at all temperatures. (Di(16:4 CH_3)—PC-membranes doped with 10^{-3} M valinomycin (open circles) or 10^{-3} M dansyllysine-valinomycin (full circles), 1 M RbCl in water). Each point represents the mean value of at least 5 membranes, the bars indicate the SE

is larger than the steady-state current J_∞ (Stark *et al.*, 1971; Gambale *et al.*, 1973; Laprade *et al.*, 1975; Knoll & Stark, 1975; Pohl *et al.*, 1976). The time course of J could be fitted by two exponential terms according to

$$J(t)=J_\infty(1+\alpha_1 e^{-t/\tau_1}+\alpha_2 e^{-t/\tau_2}). \tag{3}$$

Eq. 3 also adequately describes the current relaxation of valinomycin induced Rb^+-transport across membranes formed from diphytanoyllecithin (Fig. 5). As in the case of the temperature-jump relaxation time τ_T, both relaxation times τ_1 and τ_2 increase with decreasing temperature (Table 1).

The voltage-jump relaxation experiments on lipid membranes containing ion carriers like valinomycin have been hitherto interpreted on the basis of a simple model shown in Fig. 6 (Stark *et al.*, 1971). This "reaction scheme" has turned out to be a good first order approximation. If it is assumed that an electric voltage difference across the

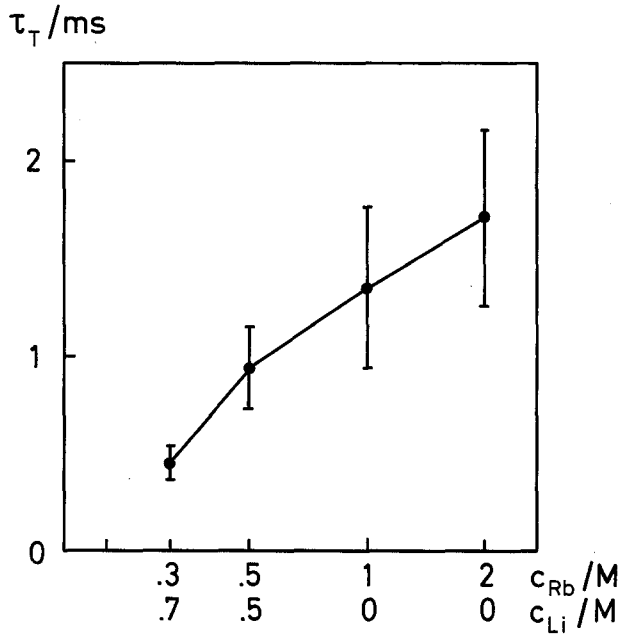

Fig. 4. Dependence of the relaxation time τ_T on the Rb^+-concentration. (Rb^+ is replaced by Li^+). Each point represents the mean value of at least 5 membranes. Bars indicate the SE. (Di(16:4 CH_3) – PC membranes doped with 10^{-3} M valinomycin, T = 5 °C)

membrane only affects the translocation of the positively charged carrier complexes MS^+ across the membrane (i.e. the rate constant k_{MS}), the time course of the current after a voltage jump in fact obeys Eq. (3) (the amplitude of a third relaxation time being zero). Then, the four rate constants of the model may be calculated from the two relaxation times τ_1, τ_2 and from the two relaxation amplitudes α_1, α_2, since these experimental quantities are only functions of the rate constants and of the ion concentration c_M in the aqueous phase (Knoll & Stark, 1975). The quantitative knowledge of all model parameters allows one to predict the result of other kinetic experiments such as the temperature-jump experiments described above. A comparison of these deductions from the voltage-jump experiments with the actual experimental results of the temperature-jump experiments will then either confirm the assumptions of the model or, in case of discrepancies, provide new information about carrier-mediated ion transport through lipid membranes.

The mathematical treatment of the time dependence of the current J after a temperature jump is outlined in the Appendix. On the basis of the model of Fig. 6 a biphasic behavior is predicted. The early phase is due to the temperature dependence of the charge carrier translocation across

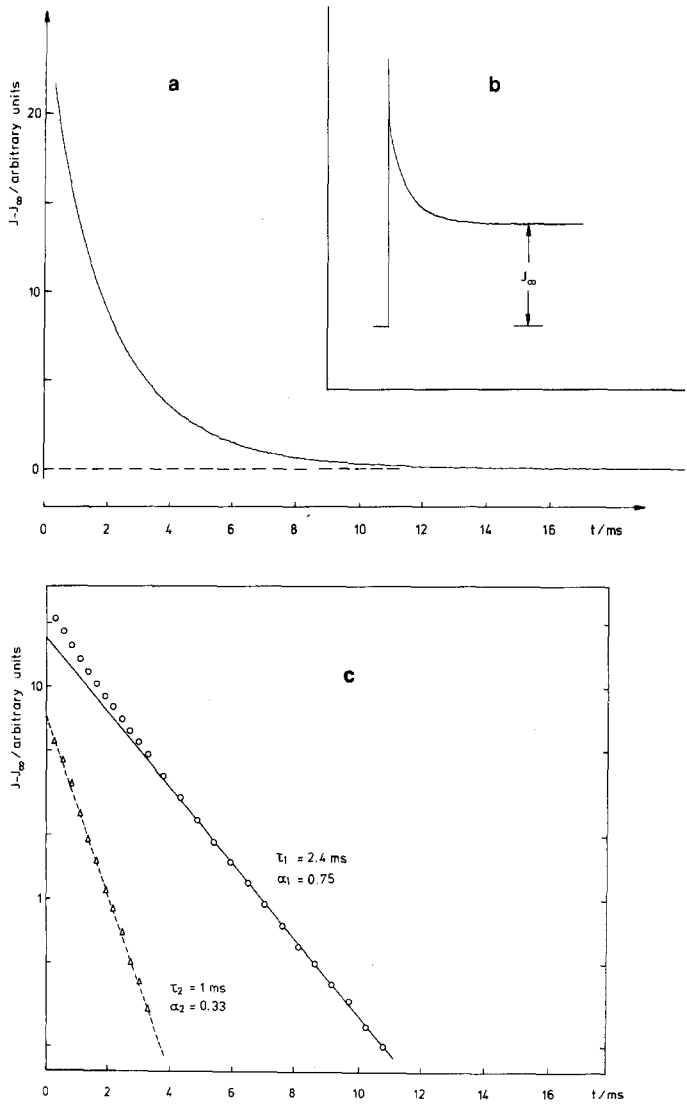

Fig. 5. Typical current relaxation of a di(16:4 CH_3)−PC membrane (doped with 10^{-3} M VAL in the membrane-forming solution following a voltage jump of 50 mV (1 M RbCl, 5 °C, membrane area 5×10^{-3} cm^2, $R_A = 10^3\,\Omega$). (*a*): Original curve of the $X-Y$-plotter (average of 512 pulses, compensated for J_∞). (*b*): The inset shows the same experiment without compensation (average of 128 pulses) using a 3.5 times smaller time scale and a reduced current sensitivity. The initial current spike is mainly due to the loading of the membrane capacity (charging time $\tau_c = 2\,\mu$sec, $J_\infty = 70$ nA). (*c*): Semilogarithmic plot of the data from (*a*) and evaluation of the parameters of Eq. (3) using J_∞ from (*b*). (○) original data; (△) after subtraction of the extrapolated slower process. A simple calculation shows that $J - J_\infty$ in (*a*) is not influenced by the loading of the membrane capacity (100 μsec = 50 τ_c). For details of the technique, *see* Pohl *et al.* (1976)

Table 1. Voltage jump relaxation data of valinomycin-mediated Rb^+-transport through di(16:4 CH_3)–PC membranes at 3 different temperatures (mean values of at least 5 membranes and SE; 1 M RbCl, 10^{-3} M VAL, 30 mV)

T/°C	τ_1/μsec	α_1	τ_2/μsec	α_2
5	2040 (±100)	0.40 (±0.08)	830 (±30)	0.26 (±0.11)
25	204 (±20)	0.33 (±0.11)	53 (±10)	0.12 (±0.05)
40	40 (±6)	0.47 (±0.12)	11 (±2)	0.27 (±0.14)

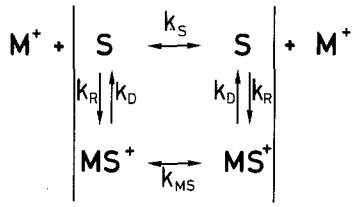

Fig. 6. Simple model of carrier mediated ion transport

the membrane. The late phase depends on the temperature dependence of the interfacial concentrations of the complexes MS^+ on both sides of the membrane. Redistribution processes of the complexes proceed with the same relaxation times, whether induced by a voltage- or temperature jump. The amplitude of this second process has been found, however, to be negligible compared to the first one, if calculated on the basis of the temperature dependence of the voltage-jump data (Tables 1, 2 and Fig. 7). Therefore, the increase of the current after a temperature jump should parallel a temperature-dependent change of the translocation rate constant k_{MS}. The experimental temperature-jump relaxation time τ_T was found to be more than 100 times larger than the rise time of the T-jump (for di(16:4 CH_3)–PC at 5 °C) and more than 10 times larger than the charging time constant τ_c. This means, in the frame of the present arguments, that k_{MS} does not increase simultaneously with the temperature as assumed in the calculation. It seems to follow a temperature jump with a delay, which depends on the structural properties of the membrane and also on the temperature itself. Before continuing the discussion about a possible molecular interpretation of the observed relaxation, one should ask whether the voltage jump data and their analysis are sufficiently accurate to support the conclusion obtained above. This ques-

Table 2. Analysis of the voltage jump data of Table 1 on the basis of the model of Fig. 6 (for details of the procedure *see* Knoll & Stark (1975))[a]

T/°C	$k_R c_M$/sec^{-1}	k_D/sec^{-1}	k_{MS}/sec^{-1}	k_S/sec^{-1}
5	2.0×10^2	6.1×10^2	1.1×10^2	3.0×10^2
25	4.5×10^3	1.1×10^4	1.2×10^3	3.1×10^3
40	2.1×10^4	5.2×10^4	9.1×10^3	1.4×10^4

[a] The resulting activation energies are $E_A(k_R c_M) \approx 23$ kcal/mole, $E_A(k_D) \approx 23$ kcal/mole, $E_A(k_{MS}) \approx 22$ kcal/mole, $E_A(k_S) \approx 19$ kcal/mole.

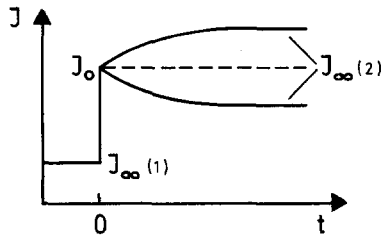

Fig. 7. Schematic representation of the time course of the current after a temperature jump on the basis of the model of Fig. 6. For a temperature jump from 5 to 5.2 °C at a constant membrane voltage of 100 mV, the following values were obtained: $J_\infty(1)/FN_0 = 34.25$; $J_0/FN_0 = J_\infty(2)/FN_0 = 35.23$ (*see* text)

tion should be asked in view of the fact that the model used for the analysis represents only an approximation. Modifications have been suggested, which mainly concern the voltage dependence of the rate constants and details of the complex formation at the membrane-water interface. Though these modifications must be confirmed by other experimental approaches and do not influence the basic statements of the model, they might be sufficient to change the analysis of the voltage jump data to a certain extent. Therefore, the prediction of a negligible small amplitude of the "second phase", which describes the redistribution of the complexes, might be questioned. Since the experimental values of the temperature-jump relaxation time τ_T are similar to the slower voltage-jump relaxation time τ_1 (compare Fig. 3 and Table 1), one might argue that it is the redistribution of the complexes ("second phase"), which is observed in the experiment. Then, however, the amplitude of the first process must be very small. This would mean that the temperature dependence of the rate constant k_{MS} must be very small (i.e., considerably

smaller than calculated in Table 2)[1]. The relaxation of the current could be well fitted by a simple exponential with no indication of a faster process which might be attributed to a fast increase of k_{MS}. Therefore, in the absence of any experimental evidence for a sufficiently small temperature dependence of k_{MS}, the interpretation based on the original carrier model is favored at present.

As discussed above, the essential consequence of the analysis, outlined in the Appendix, is the finding of a delayed increase of the rate constant k_{MS} after a T-jump. Such a delay could be generated, if a sudden temperature rise (equivalent to a sudden increase of translational, vibrational and rotational energies) is not accompanied by a simultaneous equilibration of the "conformational state" of the lipid membrane. The structure of a lipid bilayer in the liquid crystalline state contains a considerable number of "defect structures". These are believed to be formed by rotational isomers of the fatty acyl chains of the lipid molecules (*see*, e.g., Sackmann, 1974; Seelig & Seelig, 1974), thus resembling polymers. The number of defect structures increases with the temperature. A pronounced increase has been observed at the transition from the gel to the liquid crystalline state of a bilayer membrane. But also above the transition temperature a continuous decrease of the hydrocarbon chain ordering with increasing temperature was reported (Seelig & Seelig, 1974). The observed relaxation of the current after a temperature jump could reflect the time which the system needs to attain a new equilibrium state (i.e., a state of lower structural order). In other words, the translocation of valinomycin complexes across a membrane might " sense" the dynamics of structural order inside the membrane. No experimental data concerning the time scale of these structural dynamics seem to be available for a direct comparison at present. Träuble (1971) and Tsong (1974) measured the kinetics of the phase transition from the gel to the liquid crystalline state for lipid vesicles made from "saturated lipids" (dipalmitoyllecithin and dimyristoyllecithin, respectively). The reported relaxation times range from milliseconds to more than one second. In agreement with the theory of cooperative phase transitions (Adam, 1973), a distinct maximum was found within the range of the phase transition. An extrapolation from these values to the situation of a

1 This statement would be largely independent from the details of the assumed model. Any model, which assumes an instantaneous rise of the mobility of the charge carriers inside the membrane after a T-jump, would show a stepwise jump of the current (experimentally observed rise time τ_c).

membrane in the fluid state made from a lipid with branched fatty acid residues does not seem reasonable. Besides, the structural dynamics will undoubtedly be influenced by the presence of a probe the size of valinomycin. The effect of an incorporation of large molecules on the membrane structure in their vicinity has been discussed recently (Ginsburg & Stark, 1976). Molecules, such as valinomycin, are probably surrounded by an "annulus" of disturbed lipid structure, which reflects the size and nature of the incorporated molecule. The slightly different relaxation times for the dansylated valinomycin analogue (Fig. 3) seem to be understandable in this context. The effect of a replacement of Rb^+ by Li^+ on the relaxation time τ_T (Tig. 4) could be interpreted on the basis of a different interaction of monovalent cations with the lipid membrane. Various membrane phenomena have been found to depend on the kind of ions present in water, e.g., the binding of fluorescent dyes (Träuble, 1971) or the temperature of the phase transition (Simon, Lis, Kauffman & MacDonald, 1975).

The interpretation of the present experimental results on the basis of a structural relaxation of the membrane is certainly premature at present in view of the limited knowledge about valinomycin-induced ion transport. The analysis shows, however, that the temperature-jump method provides supplementary information to the voltage-jump method and is a valuable tool for the study of the interdependence of transport kinetics and dynamics of the membrane structure.

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Appendix

Relaxation of the Membrane Current Following a T-Jump

The calculation is performed on the basis of the simple carrier model shown in Fig. 6, and is closely related to the procedure used at the voltage-jump relaxation (Stark *et al.*, 1971). Therefore, only a brief sketch is presented:

We assume that at the time $t=0$ an ideal temperature jump raises the temperature from T to $T+\Delta T$. It is further assumed that this increase in

temperature is accompanied by a simultaneous change of the temperature-dependent rate constants, i.e., $k_i(1) \rightarrow k_i(2)$ $(i = R,D,S,MS)$ where the denotions (1) and (2) refer to the situation before (temperature T) and after (temperature $T+\Delta T$) the T-jump. The time dependence of the interfacial concentrations N_S and N_{MS} of the species S and MS^+ is given by the same set of linear differential equations as in case of a voltage jump (Stark *et al.*, 1971, Eqs. 26–28)[2]. The initial conditions are, however, different, since a fixed voltage is applied throughout the experiment. The stationary concentrations of S and MS^+ at the left interface (′) are

$$\bar{N}'_S = N_0 \frac{k''_{MS} k_D (k_R c_M + k_S) + k_S k_D (k_D + k'_{MS})}{(k_R c_M + k_D)[(k'_{MS} + k''_{MS})(k_R c_M + 2k_S) + 2k_S k_D]} \tag{A1}$$

$$\bar{N}'_{MS} = N_0 \frac{k_R c_M [k''_{MS}(k_R c_M + 2k_S) + k_S k_D]}{(k_R c_M + k_D)[(k'_{MS} + k''_{MS})(k_R c_M + 2k_S) + 2k_S k_D]}. \tag{A2}$$

The corresponding expressions at the right interface (″) are obtained, if the superscripts (′) and (″) are exchanged. At times $t \leqq 0$, the steady state concentrations $\bar{N}'_S(1)$ and $\bar{N}'_{MS}(1)$ are determined by the rate constants $k_i(1)$, while the steady-state concentrations $N'_S(2)$ and $N'_{MS}(2)$, reached at times long after the T-jump (at $T+\Delta T$), are calculated by inserting $k_i(2)$ into Eqs. (A1) and (A2). The solution of the problem may be written as

$$N_i(t) = \bar{N}_i(2) + \sum_{k=1}^{3} A_i^k e^{-t/\tau_k} \qquad i = S',\ S'',\ MS',\ MS''. \tag{A3}$$

The three relaxation times τ_k are identical to those describing the current relaxation after a voltage jump. Their dependence on the rate constants was given by Stark *et al.* (1971) (Eqs. 35–39). Since $N_i(t=0)$ is equal to $\bar{N}_i(1)$, Eq. (A3) may be also written as

$$N_i(t) = \bar{N}_i(1) - \sum_{k=1}^{3} A_i^k (1 - e^{-t/\tau_k}). \tag{A4}$$

Within the frame of the model the current J is given by

$$J = F(N'_{MS} k'_{MS} - N''_{MS} k''_{MS}) \tag{A5}$$

(F = Faraday constant)

2 The exchange of valinomycin molecules between membrane and water has been found to be very slow compared to the process considered in this paper (Stark *et al.*, 1972). Therefore, the total carrier concentration N_0 is assumed to be constant.

The introduction of Eq. (A4) into Eq. (A5) yields the time dependence of the current following an ideal temperature jump:

$$J(t) = J_0 \left[1 + \sum_{k=1}^{3} \alpha_k (1 - e^{-t/\tau_k})\right] \tag{A6}$$

with

$$J_0 = F[\bar{N}'_{MS}(1)\, k'_{MS}(2) - \bar{N}''_{MS}(1)\, k''_{MS}(2)] \tag{A7}$$

and

$$\alpha_k = \frac{F}{J_0}[A^k_{MS''}\, k''_{MS}(2) - A^k_{MS'}\, k'_{MS}(2)] \tag{A8}$$

being time independent quantities. The coefficients A^k_i are complicated functions of the rate constants k_i, which are not presented.

Fig. 7 shows a schematic diagram of the time course of J according to Eq. (A6). It may be interpreted in the following way: The steady-state current $J_\infty(1)$ before the T-jump is given by application of Eq. (A5):

$$J_\infty(1) = F[\bar{N}'_{MS}(1)\, k'_{MS}(1) - \bar{N}''_{MS}(1)\, k''_{MS}(1)]. \tag{A9}$$

At $t = 0$ there is a stepwise change from $J_\infty(1)$ to J_0. It results from the change of the translocation rate constant k_{MS}. This quantity describes the mobility of the charge carriers MS^+ inside the membrane and has been assumed to follow a temperature jump without any delay (Eqs. (A7) and (A9) differ only in the value of k_{MS}). The subsequent relaxation of the current is due to the fact that the interfacial concentrations N'_{MS} and N''_{MS} usually depend on the temperature. The change of these quantities after the T-jump takes place with the same relaxation times τ_k as in case of a voltage jump. The amplitude may be positive or negative depending on the sign of the temperature dependence. Finally a new steady state (Eqs. (A5) and (A6))

$$J_\infty(2) = F[\bar{N}'_{MS}(2) k'_{MS}(2) - \bar{N}''_{MS}(2) k''_{MS}(2)] = J_0 \left(1 + \sum_{k=1}^{3} \alpha_k\right) \tag{A10}$$

is reached.

We will now interpret the time course of J on the basis of the voltage jump data shown in Table 1. The rate constants of the model and their activation energies were calculated and are summarized in Table 2. The activation energies were used to predict the result of a temperature jump from 5 to 5.2 °C. The procedure was as follows: the interfacial concentrations $\bar{N}_i$ as fractions of the total carrier concentration N_0 were calculated from Eqs. (A1) and (A2) using the "steep barrier approxima-

tion" to describe the voltage dependence of k'_{MS} and k''_{MS} (*i.e.*, $k'_{MS} = k_{MS}e^{-u/2}$ and $k''_{MS} = k_{MS}e^{u/2}$, u = reduced voltage). Then, the normalized quantities $J_\infty(1)/FN_0$, J_0/FN_0 and $J_\infty(2)/FN_0$ were obtained from Eqs. (A9), (A7) and (A10). It was found that the amplitude of the initial fast jump which is associated with the temperature controlled change of the rate constant k_{MS}, by far exceeds the amplitude of the second slower process which is described by the three relaxation times τ_k (*see* legend to Fig. 7). Therefore, only the fast process should be visible in the experiment. This means that the current should either rise with the characteristic time constant of the detection system (charging time τ_c) or should be limited by the rise time of the T-jump, if the translocation rate constant k_{MS} changes simultaneously with the temperature.

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