J. Membrane Biol. 48, 181–204 (1979)

Reversible Electrical Breakdown of Lipid Bilayer Membranes: A Charge-Pulse Relaxation Study

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Received 28 November 1978; revised 27 February 1979

Summary. Charge-pulse experiments were performed with lipid bilayer membranes from oxidized cholesterol/*n*-decane at relatively high voltages (several hundred mV). The membranes show an irreversible mechanical rupture if the membrane is charged to voltages on the order of 300 mV. In the case of the mechanical rupture, the voltage across the membrane needs about 50–200 μsec to decay completely to zero. At much higher voltages, applied to the membrane by charge pulses of about 500 nsec duration, a decrease of the specific resistance of the membranes by nine orders of magnitude is observed (from 10^8 to $0.1\,\Omega\,cm^2$), which is correlated with the reversible electrical breakdown of the lipid bilayer membrane. Due to the high conductance increase (breakdown) of the bilayer it is not possible to charge the membrane to a larger value than the critical potential difference V_c. For 1 M alkali ion chlorides V_c was about 1 V. The temperature dependence of the electrical breakdown voltage V_c is comparable to that being observed with cell membranes. V_c decreases between 2 and 48 °C from 1.5 to 0.6 V in the presence of 1 M KCl.

Breakdown experiments were also performed with lipid bilayer membranes composed of other lipids. The fast decay of the voltage (current) in the 100-nsec range after application of a charge pulse was very similar in these experiments compared with experiments with membranes made from oxidized cholesterol. However, the membranes made from other lipids show a mechanical breakdown after the electrical breakdown, whereas with one single membrane from oxidized cholesterol more than twenty reproducible breakdown experiments could be repeated without a visible disturbance of the membrane stability.

The reversible electrical breakdown of the membrane is discussed in terms of both compression of the membrane (electromechanical model) and ion movement through the membrane induced by high electric field strength (Born energy).

The effect of high electric fields on cell membranes has been studied in a number of recent publications [11, 12, 25, 28, 35, 38, 40–43, 45]. If the membrane is taken rapidly within nsec or μsec, to a critical voltage of

0022-2631/79/0048-0181/$04.80

about 1 V, a dramatic increase of the conductance and permeability of cell membranes is observed. This field-induced increase has been termed electrical (dielectric) breakdown. In contrast to analogous effects in solid-state physics, the electrical breakdown of cell membranes is reversible, i.e., the original cell membrane and its functions are restored after removal of the transmembrane field [37, 38, 44].

The electrical breakdown has been used both as a tool for membrane research and for the encapsulation of molecules normally impermeable to cell membranes [32, 37, 39]. In particular, the electrical breakdown technique is of great interest in loading red blood cells and lymphocytes with drugs. Such carrier systems can be used for controlled drug release in time and space in organisms of both animals and human beings without any immunological response [33, 37, 39, 44].

Because of the relevance of the application of the electrical breakdown technique to clinical therapeutics and diagnosis and to membrane research, more detailed information is required concerning its mechanism. Especially of interest is the question, in which parts of the membrane (lipid, protein, lipoprotein) does the electrical breakdown occur. Indeed, some evidence has been presented that it occurs in the lipid or lipoprotein region of the cell membrane [23, 35]. On the other hand, similar experiments described so far for lipid bilayer membranes have been found to be completely different from the electrical breakdown in cell membranes [13, 31]. Artificial lipid bilayer membranes exhibit a mechanical rupture at voltages of about 200–400 mV, a voltage considerably lower than the electrical breakdown voltage (1 V) of cell membranes. The reason for the mechanical rupture of the lipid bilayer membranes may be that the membrane could not be charged very rapidly to high voltages.

An elegant way of applying a high voltage to the membrane within nsec is offered by the charge pulse technique [7, 8, 14, 15]. This method has also the additional advantage of a high time resolution (150 nsec), which is provided by the recording system and is not limited by the membrane conductance and capacitance [3, 5, 7, 8]. So far, charge pulse relaxation studies have only been performed in order to study the transport kinetics of carrier molecules and of lipophilic ions [3, 5, 7, 8, 14, 15]. For most of these investigations small voltages have been applied in order to avoid perturbations of the bilayer membrane [3, 5, 7, 8]. However, we could also show that, with the same experimental set-up, lipid bilayer membranes can be polarized to voltages larger than 300 mV within 50 nsec [10].

In this paper we describe experiments in which lipid bilayer membranes were charged to much higher voltages with charge pulses of 400 nsec pulse length. For an injected charge, Q, smaller than 10^{-8} A sec (membrane capacitance $C \sim 10$ nF) the initial resulting voltage V_m at the membrane is given by

$$V_m = Q/C.$$

For larger values of the injected charge Q, this equation is no longer met and the voltage across the membrane does not exceed a critical voltage of approximately 1 V, which is defined as breakdown voltage. In this case no mechanical rupture of the bilayer membrane is observed. Moreover, a reversible increase of the membrane conductance occurs in these experiments which appears to correspond to the electrical breakdown of cell membranes. The absolute value of the breakdown voltage of bilayer membranes is influenced by temperature and apparently by the valency of the ions.

Materials and Methods

Optically black lipid bilayer membranes were obtained in the usual way [9] from a 1–2% (wt/vol) solution of different lipids in *n*-decane (Fluka, Buchs, Switzerland, purum). The chamber used for bilayer formation was made from Teflon; the membrane area was about 2 mm^2 (diameter of the circular hole, about 2 mm). The chamber was attached to a thermostated metal block, allowing the temperature in the chamber to be varied between 0 and 50 °C. Unless stated otherwise, it was kept at 25 °C.

The different salts (Merck, Darmstadt, G.F.R., analytical grade) were dissolved in twice-distilled water. The pH of the solutions was about 6. Only in two sets of experiments was a buffer of the following composition used: 1 M KCl, 10^{-3} M Tris, pH 9, and 1 M KCl, 10^{-3} citrate, pH 3.

Oxidized cholesterol was prepared by boiling a 4% (wt/vol) suspension of cholesterol (Eastman, reagent grade) in *n*-octane (Merck, analytical grade) for 4 hr under reflux and by bubbling oxygen through the suspension [27]. Three different samples of oxidized cholesterol prepared according to this procedure gave identical results. The oxidized cholesterol contained about 65% pure cholesterol and about 35% derivatives exhibiting larger polarity. The membranes from oxidized cholesterol/*n*-decane had a specific capacity of 555 nF cm^{-2}; assuming a dielectric constant of 2.1, the thickness is calculated to be 3.3 nm. The specific resistance in the presence of 1 M salt was about $10^8\ \Omega\,cm^2$ (R. Benz, *unpublished results*).

In addition to oxidized cholesterol, a variety of other lipid were investigated with respect to their breakdown behavior. Dioleoyl phosphatidylcholine was synthesized in the Konstanz's laboratory [6, 18], egg phosphatidylethanolamine was isolated according to standard methods [26] and monolein was obtained from Nu Check Prep, Elysian, Minn. Other experiments were performed with membranes from dioleoyl phosphatidylcholine/cholesterol mixtures.

In the charge-pulse experiments the membrane capacity was charged to voltages between 10 mV and 2 V by injecting short current pulses. Two different experimental

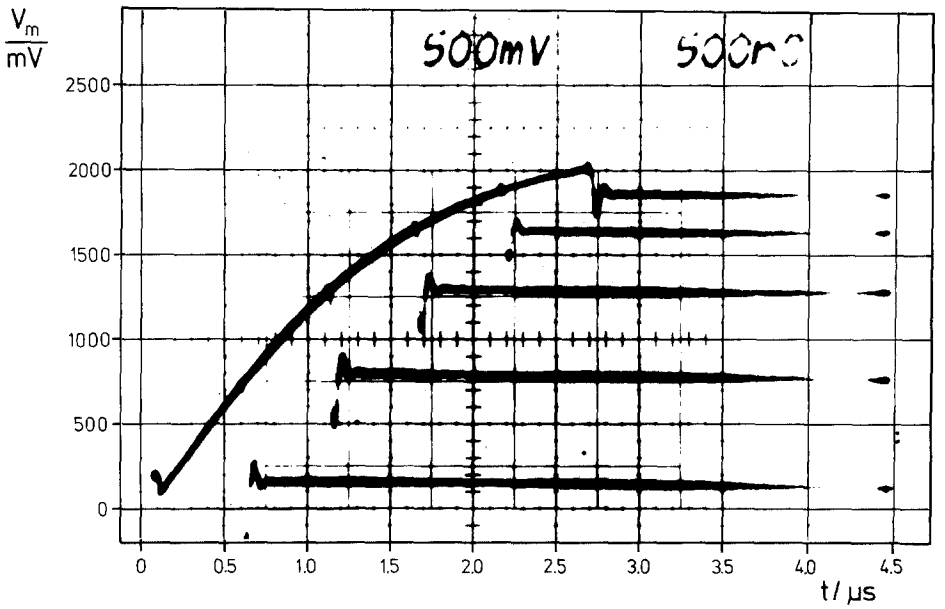

Fig. 1. Oscilloscope record of a control experiment using the FET switch. In series with the cell (resistance of electrolyte (1 M KCl) and electrodes were about 30 Ω) a parallel combination of a resistor (10^9 Ω) and a capacitor (10 nF) was used. The length of the charge pulses increased from 500 nsec to 2.5 μsec. The injected charge was between 1.5 $\times 10^{-9}$ A sec and 1.86×10^{-8} A sec. The bandwidth of the oscilloscope was about 40 MHz (*see* text for further explanation)

arrangements were used. The first set-up consisted of a voltage source (output voltage 10 mV–5.4 V) which was connected with the membrane cell through a fast FET-switch (2 N 5653, Pan Elektronik, Taufkirchen, G.F.R.). The impedance of the switch in the "open" position was larger than 10^{12} Ω while in the "closed" position the impedance was less than 100 Ω. The rise time of the current pulse was about 5 nsec, and the pulse length could be varied in steps of 50 ns, 500 ns and 5 μs. The switch was triggered with a separate battery-operated pulse generator. With this set-up, charge pulses of defined length could be applied to the membrane. It was also used in combination with a high impedance voltage follower for measurements of the membrane resistance and capacity during the experiments [8]. A typical experiment using this switch is given in Fig. 1. Charge pulses of different length, 500 nsec to 2.5 μsec (corresponding to injected charges between 1.5×10^{-9} A sec and 1.86×10^{-8} A sec) are applied to an electrical analogous circuit (10 nF, 10^9 Ω) in series with cell and electrodes but without a membrane. The trace on the left-hand side represents the superimposed charge pulses of different lengths, i.e., it shows the voltage at the output of the switch, whereas the five different tracings on the right-hand side represent the relaxation curves of the voltage in the electrical analogous circuit induced by these pulses. The voltage across this electrical analogous circuit is nearly constant within the given time range and reflects therefore the very long time constant of the electrical analogous circuit. The second set-up consisted of a fast commercial pulse generator (Philips, PM 5712) with a rise time of 4 nsec and an output impedance of 50 Ω. This generator was connected with the membrane cell through a diode with a reverse voltage resistance of about 10^9 Ω. This set-up was used for charging up the membranes to voltages of more than 1 V in about 400 nsec.

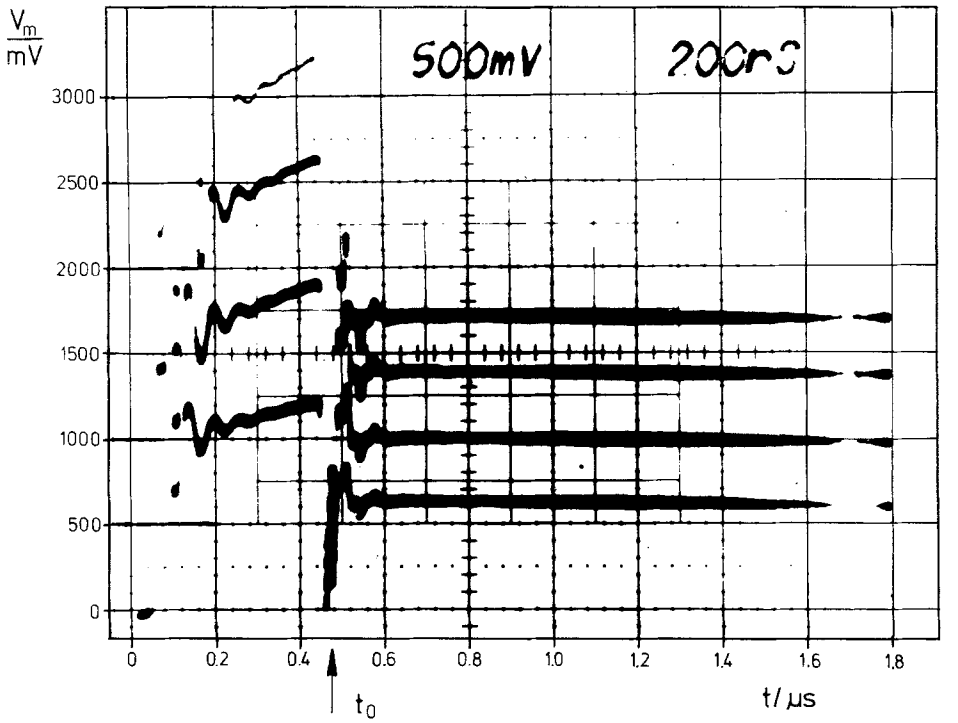

Fig. 2. Oscilloscope record of a control experiment using the diode charge-pulse generator. In series with the cell (resistance of electrolyte (1 M KCl) and electrodes were about 30 Ω) a parallel combination of a resistor (10^9 Ω) and a capacitor (10 nF) simulating the membrane was used. The length of the charge pulses was about 400 nsec. Four charge pulses of increasing charge (6.3×10^{-9}, 1.0×10^{-8}, 1.4×10^{-8}, and 1.7×10^{-8} A sec) were applied to the electrical analogous circuit. The bandwidth of the detecting system was about 40 MHz

In control experiments, the set-up was carefully tested with electrical analogous circuits. One of these experiments is given in Fig. 2. Charge pulses of 400 nsec and different voltages (injected charge between 6.3×10^{-9} and 1.7×10^{-8} A sec) are applied to an electrical analogous circuit (10 nF, 10^9 Ω) in series with the cell and electrodes but without a membrane. The traces at the left-hand side of Fig. 2 show the charge pulses, whereas the traces at the right-hand side show the slow discharging of the electrical analogous circuit. The charge pulses were applied to the membranes via Ag/AgCl platinum black electrodes (Annex Instruments, Santa Ana, Calif.). The voltage transient across the membranes was monitored with a Tektronix 7633 storage oscilloscope. The bandwith of the plug-in amplifier 7A13 was reduced from 80 to about 40 MHz during the experiments in order to damp the oscillations. The time resolution of the whole set-up, including charge-pulse generator and oscilloscope, was about 30–40 nsec. Photographs of some oscillographic records were digitized with a Summagraphic digitizer (HV-2-20) and a semilogarithmic plot of the voltage *vs.* time was performed. The data were fitted by the least square method (HP-9820A calculator with a 9862A plotter) and the straight line so obtained was extrapolated to zero in order to obtain the initial membrane potential difference at the time the switch was opened.

Results

Mechanical (Irreversible) Breakdown

The mechanical rupture of lipid bilayer membranes has been described in previous publications [13, 31]. This kind of breakdown is observed if voltages between 150 and 400 mV are applied to a membrane for a relatively long time (msec to sec). This irreversible breakdown also occurs at the end of the lifetime of a bilayer which seems to be dependent on the composition of a membrane [6, 30]. These and other aspects of the mechanical breakdown have been published elsewhere [30]. The irreversible rupture of the membrane induced by a voltage is also observed with membranes made from oxidized cholesterol, if the membrane is charged to a voltage of the order of 300 mV in a charge-pulse experiment.

Figure 3 shows a typical oscilloscope record of such an experiment. The lower trace corresponds to an experiment in which the membrane is charged to 180 mV. In this case no mechanical rupture is observed. In a second experiment (upper trace) the membrane is initially charged to about 360 mV. After a slow decay of the voltage during about 250 μsec, possibly caused by a capacity relaxation [6], the mechanical breakdown begins and the membrane potential drops to zero within about 150 μsec. From the result given in Fig. 3, it is evident that the rupture of the membrane does not immediately lead to a high conductance state. Presumably a small hole appears at a distinct point of the membrane caused by a lateral shift of the solvent due to the electric field [6, 24]. As indicated by Fig. 3, it takes some time until the conductance of the increasing hole is large enough to discharge the membrane completely. These findings are so far consistent with the optical observation that the mechanical rupture of a membrane begins from one single point of the membrane.

If the membrane is charged to a somewhat larger voltage (400–500 mV), the discharge of the membrane due to the mechanical rupture occurs during a shorter time. However, the time required for this process was never found to be less than 50 μsec.

Electrical (Reversible) Breakdown

When much higher voltages are applied to the membranes by current pulses of 100–400 nsec duration, an effect was observed which is completely different from the mechanical rupture described above. A typical

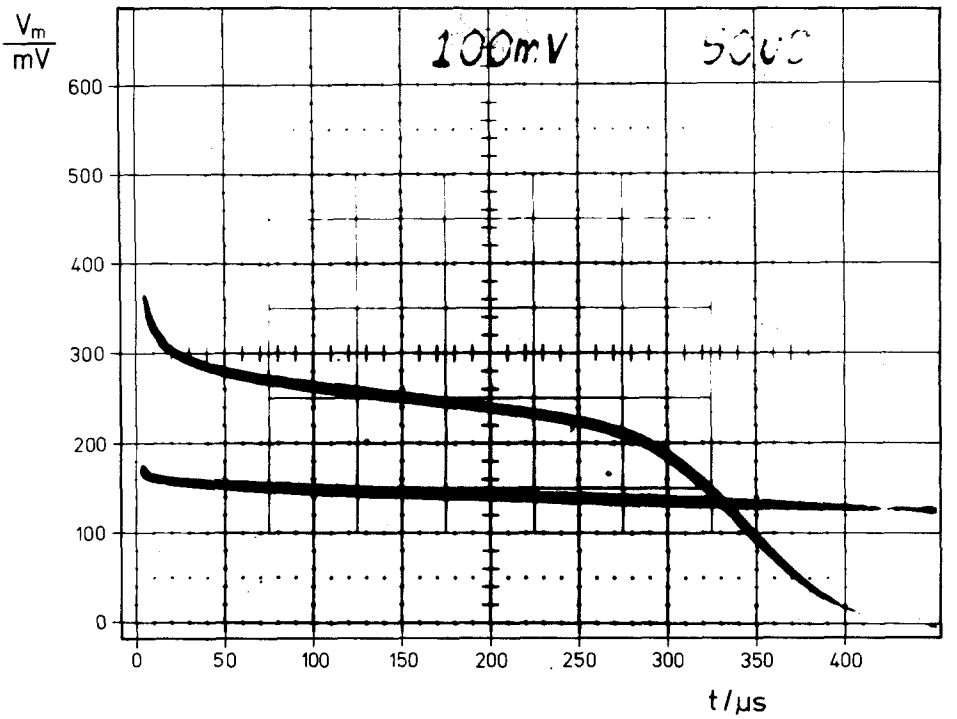

Fig. 3. Oscilloscope record of two charge-pulse experiments performed on a membrane made from oxidized cholesterol/*n*-decane (membrane capacity 9.6 nF). In the first experiment the membrane was charged by a charge pulse (injected charge 1.7×10^{-9} A sec) of 100 nsec duration to a voltage of 180 mV (lower trace). In the second experiment the membrane was charged to 360 mV by a charge pulse of 250 nsec duration (injected charge 3.5×10^{-9} A sec). Mechanical rupture occurs about 250 μsec after the application of the charge pulse. The decrease of the voltage observed both in the first and second experiment before rupture has occurred, is caused mainly by capacitance relaxations [6]. $T = 25\,°C$, 1 M KCl

experiment is presented in Fig. 4 with a time scale of 1 μsec. A membrane from oxidized cholesterol/*n*-decane bathed in 1 M KCl is charged to voltages between 600 mV and 1 V within 400 nsec. A single trace of one of the charge pulses is seen at the left-hand side of Fig. 4. After the charge pulses, the outer circuit is switched so as to have a high resistance, the voltage across the membrane decaying then only by charge movements within the membrane. As indicated by Fig. 4, there is a rapid drop of the voltages to values between 0 and 220 mV, considered to be too low to cause a mechanical rupture. The membrane is not destroyed by these high voltages, and the five successive charge-pulse experiments given in Fig. 4 are taken from one single membrane. The fast decay of the voltage is caused by a high conductance state of the membrane, comparable to

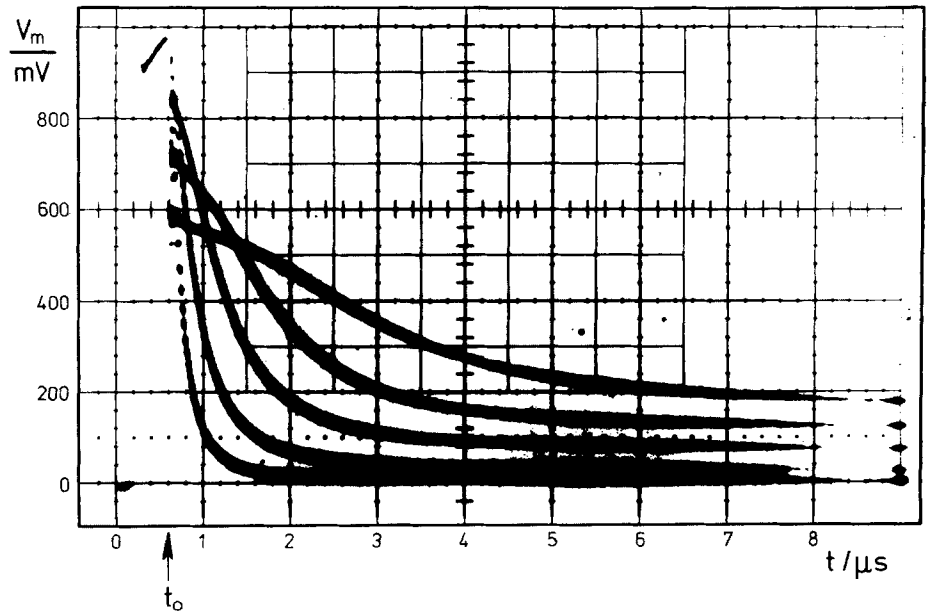

Fig. 4. Charge-pulse experiments performed on a membrane made from oxidized cholesterol/*n*-decane (membrane capacitance 10.6 nF). Five charge pulses of 400 nsec duration and increasing charge (trace 1, 6.2×10^{-9} A sec to trace 5, 1.5×10^{-8} A sec) were applied to the same membrane using the FET charge pulse generator; $T = 25$ °C, 1 M KCl. The charge pulse of trace 1 is seen on the left-hand side of the record. Note that for times longer than 5 μsec, the four lower traces are approximately parallel to the time axis

the breakdown processes in cell membranes [11, 12, 25, 35, 38, 40–43, 45]. It is also evident from Fig. 4 that the high conductance state does not last longer than a couple of μsec. After this time the original resistance of the membrane is restored.

Analogous to living cells, the breakdown phenomena observed on lipid bilayer membranes are reversible and reproducible. In more than 20 successive runs, each following the other with a time interval of several seconds, the conductance increase was the same and the traces on the screen of the oscilloscope coincided. In addition, after the 20 runs, the original conductance and capacitance of the lipid bilayer membrane was restored.

In order to study this strong conductance increase, the time scale of the oscilloscope records was expanded. An example of such an experiment is given in Fig. 5. Charge pulses of different charges but with constant duration (400 nsec) were applied to a membrane from oxidized cholesterol/*n*-decane (not shown in Fig. 5). The magnitude of the charge

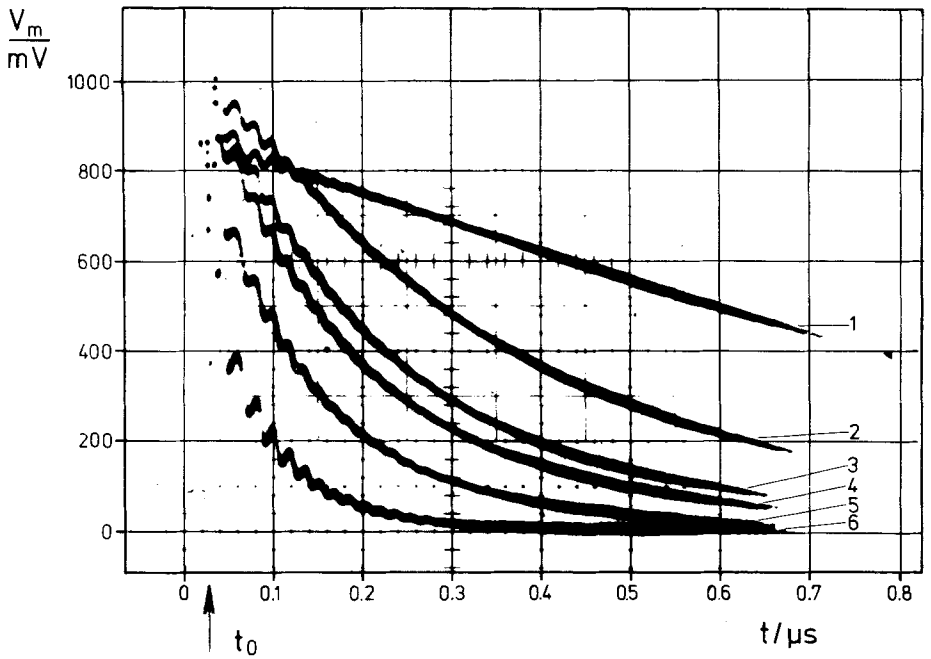

Fig. 5. Oscilloscope record charge-pulse experiments performed on a membrane made from oxidized cholesterol/*n*-decane (membrane capacitance 11.5 nF). Six charge pulses of 400 nsec duration and increasing charge (trace 1; 8.5×10^{-9} A sec to trace 6: 3.4 $\times 10^{-8}$ A sec) were applied to the same membrane; diode charge pulse generator; $T = 25$ °C, 1 M KCl in D_2O. Note that trace 6 corresponds to the charge pulse with the maximum charge

pulses increased from 8.5×10^{-9} A sec (trace 1) to 3.4×10^{-8} A sec (trace 6). Whereas from the first to the second trace the initial voltage across the membrane after the end of the charge pulse increased a little, charge pulses with larger injected charge did not cause a further increase. This results from the electrical breakdown which occurs during the charging of the membrane. The membrane resistance drops from $5 \times 10^9\ \Omega$ (specific resistance about $10^8\ \Omega$, membrane area 2×10^{-2} cm^2) to at least $5\ \Omega$ (*see* below) in less than 100 nsec. Due to the drop of the membrane resistance to this low value in comparison to the high total value of the other resistances in the circuit of about $80\ \Omega$ (output resistance of the charge-pulse generator, resistance of the electrodes, and the electrolyte), it is not possible to keep the membrane charged at the high voltage of 1 V. The membrane is already discharged during the charge pulse, and therefore only a maximum voltage of 1 V can be built up across the membrane.

The resistance of the membrane during the voltage relaxation follow-

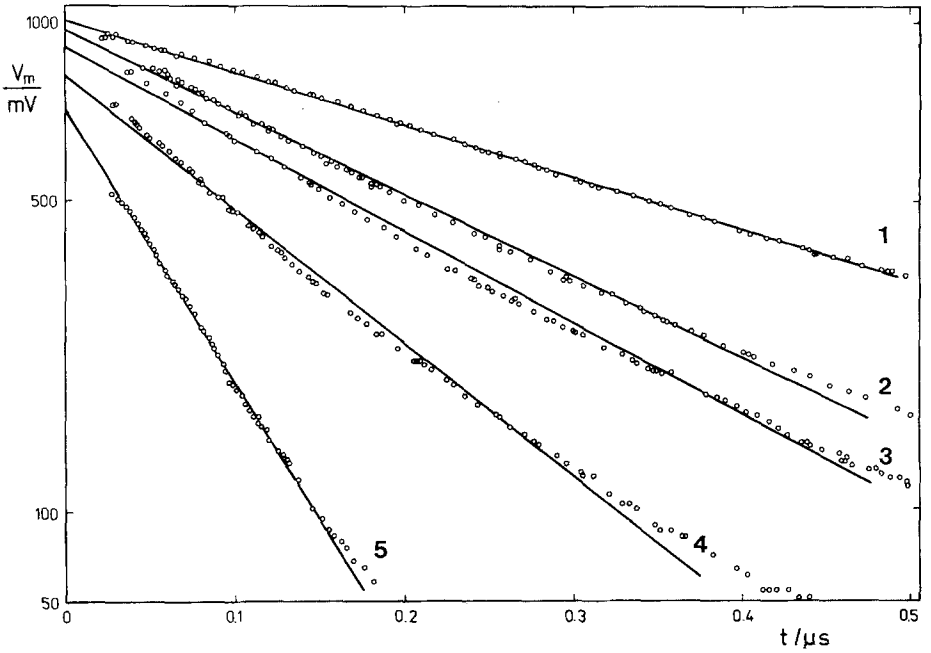

Fig. 6. Semilogarithmic plot of the membrane voltage V_m *vs.* time after the end of the charge pulses. The data was taken from the experiments similar to those presented in Fig. 5. The initial voltage V_0 and the time constant were calculated using the least squares method. The membrane capacitance was 9.6 nF.

trace 1: $Q = 9.8 \times 10^{-9}$ A sec; $V_0 = 1000$ mV; $\tau = 600$ nsec
trace 2: $Q = 1.3 \times 10^{-8}$ A sec; $V_0 = 960$ mV; $\tau = 350$ nsec
trace 3: $Q = 1.8 \times 10^{-8}$ A sec; $V_0 = 840$ mV; $\tau = 300$ nsec
trace 4: $Q = 2.3 \times 10^{-8}$ A sec; $V_0 = 780$ mV; $\tau = 160$ nsec
trace 5: $Q = 3.0 \times 10^{-8}$ A sec; $V_0 = 660$ mV; $\tau = 70$ nsec

ing a supercritical charge pulse is approximately constant during one µsec. This is shown in Fig. 6, where semilogarithmic plots of voltage *vs.* time of five typical traces similar to those in Fig. 5 are presented. These curves are approximately straight and were fitted using the least squares method yielding time constants between 70 and 400 nsec. The extrapolation of these curves to the end of the charge pulse ($t = 0$) gives the intitial voltage, V_0, which can be calculated with an accuracy of 50 mV.

From the exponential decays the resistance of the membranes can be estimated. At low voltages the specific capacity of the membranes from oxidized cholesterol/*n*-decane is 555 nF/cm^2. So far, it is not clear if the capacitance value is changed during the time in which the high voltage is applied; nevertheless, the value for the capacity may be considered as a lower limit. The time constant τ for the discharge of a capacitor through

a resistor is given by

$$\tau = R_m \cdot C_m \tag{1}$$

where R_m and C_m are the specific resistance and the specific capacity of the membrane. With a minimum value of about 50–70 nsec for τ, R_m is calculated to be $0.1\,\Omega\,\mathrm{cm}^2$. Since the area of the membrane is about $2 \times 10^{-2}\,\mathrm{cm}^2$ the upper limit for the membrane resistance in the high conductance state is about $5\,\Omega$.

It is interesting to note that such low values of the membrane resistance can only be measured using the charge pulse method. Due to the high resistance of the outer circuit, no voltage drop occurs in the electrolyte and at the electrodes, which have a higher total resistance than the membrane.

As already mentioned, a similar phenomenon as described here, has been reported for biological membranes [11, 12, 25, 35, 38, 40–43, 45). In these experiments the membrane potential did not exceed a certain value in response to ever increasing current (or voltage) pulses applied to the cell membrane. This critical voltage has been defined as the breakdown voltage, V_c.

It is a little difficult to define a similar voltage in the experiments presented here, because relaxation experiments performed with the charge-pulse method cannot be simply compared with the cell membrane breakdown experiments. Due to this difficulty, the maximum voltage to which a membrane can be charged in charge-pulse experiments is defined as the breakdown voltage V_c. V_c is an intrinsic quantity and does not depend on the actual capacity of the membrane and on the injected charge (if the charge is large enough and breakdown occurs). The breakdown voltage is defined in analogy to solid-state physics.

Figure 7 shows oscilloscope traces of six charge-pulse experiments with increasing duration from 0.5 to 3 μsec. The traces of the six charge pulses coincide, indicating the excellent reproducibility of the measurements. The upper traces correspond to the voltages at the output of the charge-pulse generator. The lower traces correspond to the discharge of the membrane after the charge-pulse generator is switched off. The conductance increase of the membrane (i.e., electrical breakdown) happens at the small hump on the upper trace (*compare* Fig. 1).

The current density in a membrane is considerable when the electrical breakdown occurs. In the experiment presented in Fig. 7, a voltage of 5.4 V is applied to a total resistance (membrane, electrodes, electrolyte, and inner resistance of voltage source and the FET) of about $100\,\Omega$. This

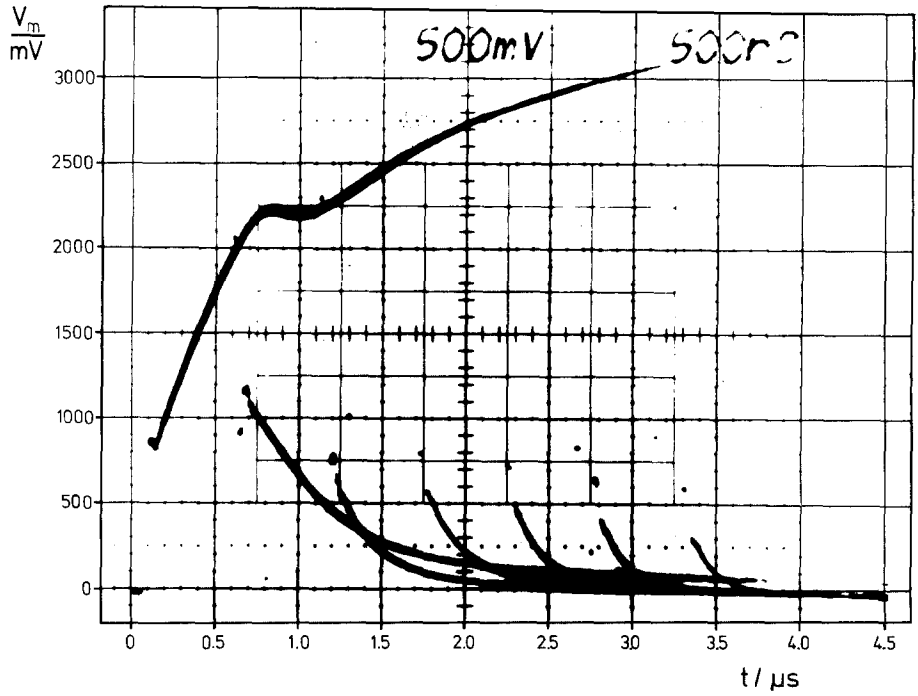

Fig. 7. Oscilloscope record of charge-pulse experiments at high voltage performed on a membrane made from oxidized cholesterol/*n*-decane. Six charge pulses of increasing durations (0.5–3 µsec) were applied to one membrane; FET charge pulse generator; $T = 25\,°C$, 1 M KCl. The upper trace corresponds to the voltage at the output of the charge-pulse generator and represents a superposition of seven single pulses

corresponds to a current of about 54 mA or to a current density (membrane area 2×10^{-2} cm^2) of about 2.7 A cm^{-2}. A current density of such a magnitude has so far not been reported in experiments with artificial or biological membranes [2, 9, 11, 17, 34].

To study the influence of ions on the absolute value of V_c, breakdown measurements were performed in the presence of various salts in the aqueous phase bathing the membranes. The results are given in Table 1. In a first set of experiments different alkalichlorides were investigated. As can be seen from Table 1, no change of the breakdown voltage in the series Li^+, Na^+, K^+, Rb^+, and Cs^+ was observed. Replacing the chloride by nitrate also did not influence V_c. The same is true for salts with monovalent cations and divalent anions and vice versa. Only in the case of a 2 : 2 salt (0.5 M $MgSO_4$) an increase of V_c from 1000 to 1700 mV was found. This may be a hint that rapid movements of ions through

Table 1. Dielectric breakdown voltage, V_c, as a function of salt and pH

Salt		C_M/M	V_c/mV
LiCl		1	980
NaCl		1	990
KCl	(pH 3)	1	1000
KCl	(pH 6)	1	1000
KCl	(pH 9)	1	950
KCl	(D_2O)	1	950
KCl		3	800
KCl		0.3	1300
KCl		0.1	>1500
RbCl		1	1020
CsCl		1	950
KNO_3		1	1000
$MgCl_2$		0.5	1000
$CaCl_2$		0.5	1050
$BaCl_2$		0.5	950
K_2SO_4		0.5	1000
$MgSO_4$		0.5	1700

[a] If not stated otherwise, the pH of the solutions was 6; $T=25$ °C.

Note that the breakdown increases significantly in the presence of $MgSO_4$. Data are average values obtained from at least five membranes. The scatter of the breakdown voltage is about 100 mV.

the membrane may be involved in the breakdown phenomena for conductance.

Involvement of the field dissociation effect in the breakdown mechanism has been discussed in some publications concerning the breakdown phenomena in biological membranes [12, 20, 36]. Besides the possibility that ion pairs may dissociate in the membrane, it cannot be ruled out that water molecules dissociate into ions at this high field strength (3×10^6 V/cm at 1 V) [21]. Therefore, some experiments were performed in 1 M KCl dissolved in heavy water (D_2O) instead of ordinary water (H_2O). So far, no difference between the two experiments has been observed. The same is true for measurements at different pHs (Table 1), although the membranes from oxidized cholesterol were a little more fragile at pH 3.

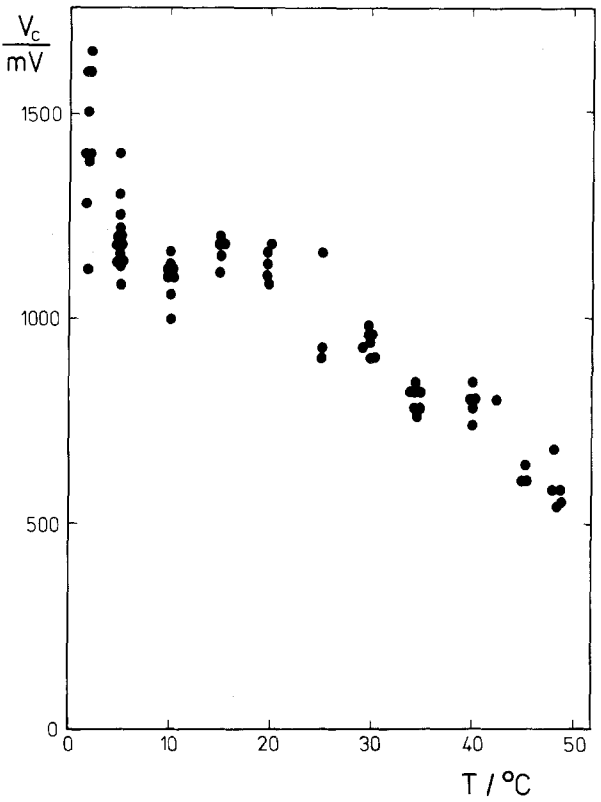

Fig. 8. Breakdown voltage V_c as a function of the temperature. V_c is defined as the maximum voltage to which a membrane from oxidized cholesterol/*n*-decane can be charged by a charge pulse of 400 nsec duration. Diode charge pulse generator; 1 M KCl

Breakdown experiments with algae membranes performed at different temperatures showed a pronounced temperature dependence of V_c [11, 12, 36]. Between 4 and 30 °C, V_c decreased from 1200 to 600 mV. To test if the breakdown voltage shows a similar temperature dependence in experiments with lipid bilayer membranes, the temperature was varied between 2 and 48 °C in the presence of 1 M KCl. As indicated in Fig. 8, V_c decreases from about 1500 mV at 2 °C to about 500 mV at 48 °C. This temperature dependence corresponds to that observed on biological membranes.

In addition to membranes from oxidized cholesterol/*n*-decane a number of other lipids was used for breakdown experiments. The voltage relaxations obtained with these lipids in the range of 100 nsec to 1 μsec are very similar to those measured on oxidized cholesterol membranes.

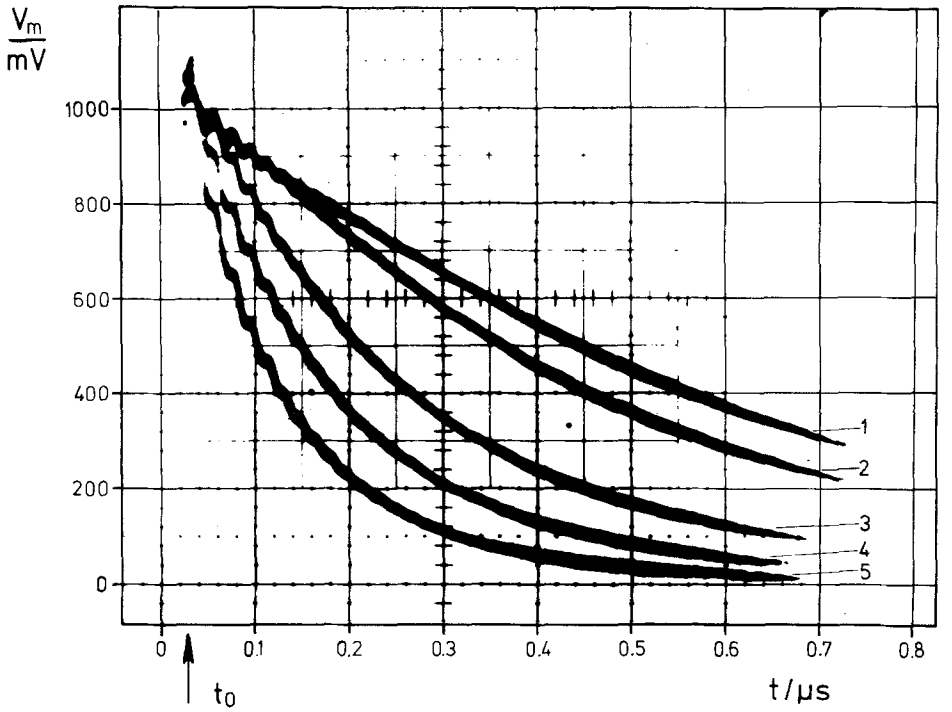

Fig. 9. Oscilloscope record of charge pulse experiments at high voltages performed on membranes made from a dioleoyl phosphatidylcholine/cholesterol mixture with a molar ratio of 1 : 1 (membrane capacitance 7.3 nF). The lipid was dissolved in *n*-decane. Five charge pulses of 400 nsec duration and increasing charge (trace 1: 7.5×10^{-9} A sec to trace 5: 2.9×10^{-8} A sec) were applied to five different membranes; diode charge pulse generator, $T = 25\,^{\circ}C$, 1 M KCl. Note that no membrane survived one single charge pulse

However, it should be noted that not a single membrane survived the breakdown. A typical example is given in Fig. 9. Whereas the oscilloscope traces presented in Fig. 5 were obtained from one single membrane, five membranes were needed to obtain the results of Fig. 9. In principle it cannot be excluded that mechanical rupture and electrical breakdown coincide at high voltages in this case because of comparable time constants of both processes. On the other hand, we feel that the strong similarity between the voltage relaxations observed with oxidized cholesterol membranes and membranes from other lipids like phosphatidylcholine, phosphatidylethanolamine, or monoolein suggests that the same process is responsible for the conductance increase at high voltages. It is quite possible that the membranes from oxidized cholesterol remain stable after the breakdown (presumably because of their rigid structure),

whereas membranes from other lipids become instable due to secondary processes caused by the high field leading to mechanical rupture.

Discussion

Evidence is presented that lipid bilayers made from oxidized cholesterol break down electrically when the membrane potential exceeds a value of about 1 V in nsec. The criteria for electrical breakdown [11, 12, 38] are fulfilled: The field-induced conductance increase is reversible, at least for bilayers made from oxidized cholesterol; in these experiments the breakdown occurs in the time range of 100 nsec; the critical voltage, V_c, is of the same order as with cell membranes, and its temperature dependence is analogous to that observed with living cells; in addition, the absence of any influence of pH and D_2O on the breakdown voltage of bilayer membranes is in agreement with similar findings on cell membranes (U. Zimmermann & F. Beckers, *unpublished results*). It is evident, however, that the electrical breakdown phenomenon has to be distinguished from the mechanical rupture of the bilayer membrane at voltages between 200 and 400 mV. The point is that the time constant of the discharging process in response to mechanical rupture is at least 50 μsec, whereas the discharging process as a result of electrical breakdown has a much faster time constant of 1 μsec or even less.

In the voltage range of 150 to 300 mV across cell membrane the so-called punch-through effect [12, 34] is observed, provided the pulse length of the applied current is of the order of msec to sec. The punch-through effect is also reversible, possibly because of repair mechanisms in the membrane, but it is associated with changes of the ion concentration profiles within and of the ionic current through the membrane. It is not unlikely that similar processes are involved in the mechanical rupture of a bilayer membrane. In any case, the reversible increase in the bilayer conductance is not related to this punch-through effect. On the basis of the evidence presented above, this field-induced effect on the conductance of a bilayer membrane reflects a true electrical breakdown.

Under the assumption that ionic channels are generated in the bilayer membrane when breakdown has occurred, we can calculate the breakdown area, provided that the potassium ions have the same mobility in the channels as in the bulk aqueous phases. The surface area of the channels is calculated to be about 5×10^{-7} cm^2 at a membrane resistance of 5 Ω measured instantaneously after breakdown. This area is

about five orders of magnitude lower than the total area of the bilayer membrane ($2 \times 10\,cm^{-2}$). With the same assumptions the number of the channels can be estimated. The resistance of an aqueous channel of 1 and 10 nm in diameter is $3.5 \times 10^8\,\Omega$ and $3.5 \times 10^6\,\Omega$, respectively, while total measured resistance of the bilayer membrane is $5\,\Omega$. Thus it follows that the number of channels has to be 7×10^7 and 7×10^5 per membrane area, respectively. These values correspond to a channel density between 3.5×10^7 and 3.5×10^9 channels/cm^2.

The mechanism of the electrical breakdown of cell membranes has been interpreted in terms of an electro-mechanical compression of the membrane transverse to the membrane plane. Crowley [13] and White [31], on the other hand, suggested that the mechanical rupture of lipid bilayers at 200 to 400 mV results from an electro-mechanical instability within the membrane. Indeed, the capacity of artificial lipid bilayers is voltage dependent with the capacity-voltage relationship being given by the following equation:

$$\frac{\Delta C}{C_m} = \alpha V_m^2 \tag{2}$$

where ΔC is the change in membrane capacity in response to a voltage V_m, C_m is the specific capacity of the membrane at $V_m = 0$. The proportionality factor α ranges between $15\,V^{-2}$ (solvent containing bilayers [6]) and $0.02\,V^{-2}$ (solvent-free bilayers obtained by the Montal-Müller method [1]). Although it was originally suggested that the increase in capacity with increasing voltage results from a decrease in the membrane thickness [13, 31], it is now well established that the measured voltage dependence of the capacity is caused by the squeezing of solvent into microlenses [6, 24]. The latter interpretation has been confirmed by measurements of both the time course of the capacity change [6] and by the extremely low capacity change in response to increasing voltage when using solvent-free membranes [1, 4]. The lateral shift of solvent induced at low voltages may be another process involved in the mechanical rupture of the membrane. Thus, it seems quite clear that the real electro-mechanical compression (neglecting the lateral shift of solvent) of the bilayer membrane does not play a dominant role in the mechanical rupture of the bilayer membrane.

On the other hand, compression of the membrane by electric fields may be involved in the electrical breakdown of cell membranes and lipid bilayers. Zimmermann *et al.* [42] proposed an electro-mechanical model

which was later extended by Coster and Zimmermann, (quoted in ref. 34), based on the assumption that the thickness, d, for a certain finite membrane area depends on the voltage across the membrane, V_m, and the mechanical pressure P. The magnitude of the expected change in membrane thickness depends also on the compressibility and the dielectric constant of this finite membrane area.

The finite membrane area is considered to be a capacitor filled with an isotropic elastic dielectric. At equilibrium the electrical compressive force, P_e, arising from the voltage across the membrane and the mechanical compressive force, P, arising either from the turgor pressure inside the cell or from the external hydrostatic pressure are counterbalanced by the elastic restoring force, P_m, generated by the compression of the membrane material:

$$P + P_e + P_m = 0. \tag{3}$$

In the absence of mechanical pressure Eq. (3) reduces to

$$P_e = P_m = 0. \tag{4}$$

A quantitative description of the electrical compressive forces and the elastic restoring forces on a molecular scale is difficult. In order to proceed we assume that the macroscopic laws of electrostatic and elasticity (Hook's law for the one-dimensional case) are applicable. Therefore, it follows that:

$$P_e = \frac{\varepsilon_m \cdot \varepsilon_0 \cdot V_m^2}{2d^2} \tag{5}$$

$$P_m = Y_m \ln \frac{d}{d_0} \tag{6}$$

where ε is the dielectric constant of the membrane material, ε_0 the electric permittivity of the free space, V_m the membrane voltage, d the thickness of the stressed membrane, and d_0 the thickness of the unstressed membrane ($P=0$, $V_m=0$). Y_m represents the so-called elastic compressive modulus transverse to the membrane plane. Y_m is a very complex parameter, a more precise definition of it being given in the literature [34].

The introduction of Eqs. (5) and (6) in Eq. (3) yields for electromechanical equilibrium:

$$P+\frac{\varepsilon_m \cdot \varepsilon_0}{2d^2} V_m^2 + Y_m \ln \frac{d}{d_0} = 0. \tag{7}$$

It can readily be shown that at maximum voltage breakdown occurs. The breakdown voltage derived from Eq. (7) is given by [11]:

$$V_c^2 = \frac{Y_m \cdot d_0^2}{e \cdot \varepsilon_m \cdot \varepsilon_0} \quad \text{at} \quad P=0. \tag{8}$$

This equation can be used for the calculation of Y_m when certain assumptions are made for the dielectric constant and the membrane thickness, d_0. Young's modulus Y_m for lipid bilayer membranes has been calculated according to theoretical considerations [16] and from exact measurements of the voltage-dependent capacity of solvent-free membranes [1]. In addition, Y_m has been estimated from the breakdown voltage [34] (Eq. (8)) and from the dependence of V_c on turgor pressure [35]. The Y_m-values were calculated for cell membranes and lipid bilayer membranes to be of the order of 5×10^6 N/m^2 [34, 35] and 1.4×10^8 N/m^2 (1), respectively. The Y_m-value of the bilayer membranes corresponds to a breakdown voltage of 5.6 V ($\varepsilon_m = 2.1$). The electro-mechanical model (Eq. (7)) predicts that mechanical and electrical forces are coupled within the membrane. There is some evidence in the literature [34] which confirms this conclusion and supports the interpretation of the breakdown in terms of the electro-mechanical model.

However, it has to be noted that the membrane has to be compressed by 39% to induce the electrical breakdown of the cell membrane. This value seems to be unlikely, particularly considering the breakdown of the lipid bilayer membrane. Some other processes have therefore to be considered if the membrane is compressed only to a certain degree, say 10 to 20%, which then finally leads to the breakdown of the cell membrane or the bilayer membrane.

Thinning of the membrane by electric compressive forces may lead to the situation that the energy of the ions in the electric field reach the Born energy which is required to inject ions into an oil phase of low dielectric constant [22]. The high electrical field across the membranes when the breakdown voltage range is reached results in a high energy of the single ions moving through the membranes. The energy for a

monovalent ion is calculated to be 1 eV (corresponding to 9.6 $\times 10^4$ J/mol) and is on the order of the Born energy, which is given by equation:

$$w = \frac{z^2 e_0^2}{8 \Pi \varepsilon_0 r} \left(\frac{1}{\varepsilon_m} - \frac{1}{\varepsilon_w} \right) \tag{9}$$

with $e_0 = 1.6 \times 10^{-19}$ A sec, an ion radius r of 2×10^{-10} m and $\varepsilon_m = 2$ and $\varepsilon_w = 80$ as dielectric constants of the membrane and of the aqueous phase, respectively, w has a value of 1.7×10^5 J/mol (valency $z = 1$). Equation (9) has to be modified if the oil phase, i.e., the lipid bilayer, is very thin [22]:

$$w = \frac{z^2 e_0^2}{8 \pi \varepsilon_0 r} \left(\frac{1}{\varepsilon_m} - \frac{1}{\varepsilon_w} \right) - \frac{z^2 e_0^2}{4 \pi \varepsilon_0 \varepsilon_m d_0} \ln \left(\frac{2 \varepsilon_w}{\varepsilon_w + \varepsilon_m} \right). \tag{10}$$

Equation (10) states that the Born energy decreases when the membrane becomes thinner. To calculate the Born's energy according to Eq. (10), it is suitable to substitute the unknown membrane thickness d_0 by the membrane capacity C_m, which can be determined in bilayer experiments:

$$w = \frac{z^2 e_0^2}{8 \pi \varepsilon_0 r} \left(\frac{1}{\varepsilon_m} - \frac{1}{\varepsilon_w} \right) - \frac{z^2 e_0^2 C_m}{4 \pi (\varepsilon_0 \varepsilon_m)^2} \ln \left(\frac{2 \varepsilon_w}{\varepsilon_w + \varepsilon_m} \right). \tag{11}$$

With $C_m = 555$ nF/cm^2 and $\varepsilon_m = 2$, the Born's energy is calculated to be 1.5×10^5 J/mol (univalent ions). Assuming a value of $\varepsilon_m = 3$, the energy decreases to 1.0×10^5 J/mol. This value is very close to the energy which a monovalent cation or anion receives by the electric field across the membrane. It should be noted that the assumption is made that the membrane is homogeneous with a dielectric constant which is independent of surface coordinates. Cell membranes and solvent containing bilayers are, on the other hand, inhomogeneous, and it is quite plausible that the heterogeneity leads to a further reduction of the energy required for injecting ions into the lipid-bilayer phase. It can also be readily shown that a compression of the membrane by about 10 to 20% in response to the electric field leads to a value of the Born's energy identical with that value of the energy of the ions in the electric field. The consideration of the Born's energy is able to explain the difference in the breakdown voltages observed for 1:1 and 2:2 salts. According to Eq. (9), the energy needed to inject a single ion into the membrane is four times higher for a divalent ion in comparison with a monovalent ion ($w = 4.2 \times 10^5$ J/mol). In the case of the movement of a divalent ion through the membrane in presence of a potential difference of 1 V, the energy is 2 eV

(corresponding to 1.9×10^5 J/mol). Thus, about 2 V are needed to inject a divalent ion into the membrane. In the case of $MgSO_4$, V_c has a value of 1.7 V. Although the increase of value of the breakdown voltage in the presence of $MgSO_4$ can be interpreted in terms of the Born's energy, it is also possible to relate this effect to the very low activity coefficient of bivalent salts. The activity coefficient of a 0.5-M $MgSO_4$ solution is 0.068, whereas the activity coefficient for a 1-M KCl solution is 0.61.

An open problem is the experimental finding that the lipid bilayer membranes remain in the high conductance state during the voltage relaxation after breakdown has occurred. The original low conductance state of the membrane is reached in all experiments after a couple of microseconds. It seems quite possible that local heating in the membrane when high currents passing the membrane in the breakdown areas cause thermal-induced structural changes in the membrane. If this is true, it has to be assumed that the time constant of the reversal of the thermal-induced structural changes is of the order of μsec, that means that hysteresis effects may occur. Such effects seem to be very likely, especially if it is considered that the ions injected into the membrane during breakdown may alter conformation and arrangements of molecules within the membrane.

Electrical breakdown experiments on cell membranes have also led to the conclusion [12, 40] that local heating may play an important role after the primary process of electrical breakdown. This secondary process should be not confused with the electric field effects resulting in breakdown. There are some theoretical and experimental evidences [12] that thermal effects can be excluded as a primary reason for the observed breakdown phenomenon.

On the other hand, if the high conductance is partly dependent on the field imparting sufficient energy to an ion for it to jump the potential energy barrier due to the difference in image force between water and lipid, the conductance should decrease, on removal of the field, with a time constant similar to that of the discharging process. Further, the current voltage relation for the membrane will not necessarily be linear, even when the concentrations in the electrolyte phases of either side are equal, and the shape of the $V-I$ relation may be dependent on the value of the concentration.

In summary, the experimental finding that lipid bilayers exhibit an electrical breakdown phenomenon may open an elegant way to study the mechanism of this electrical phenomenon in more detail than is currently possible with cell membranes.

The authors wish to thank Dr. P. Läuger for many helpful discussions. This work has financially been supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereiche 138 (R.B.) and 160 (F.B. und U.Z.)).

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