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The electrical breakdown of cell and lipid membranes: the similarity of phenomenologies

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The current responses of human erythrocyte and L-cell membranes being subject to rectangular voltage pulses of 150–700 mV amplitude and $5 \cdot 10^{-3}$–10 s duration were recorded by means of the patch-clamp method. The behaviour of planar lipid bilayer membranes of oxidized cholesterol and UO_2^{2+}-modified bilayers of azolectin in a high electric field was investigated for comparison. The gradual growth in the conductance (reversible electrical breakdown) was found for both the cell membranes and lipid bilayers of the compositions studied, with the application of voltage pulses of sufficient duration, to be completed by its drastic enhancement (irreversible breakdown). The time interval preceding the irreversible breakdown and the rate of increase in conductance during the reversible breakdown are determined by the amplitude of the voltage applied. The recovery of the initial properties of the membrane following the reversible breakdown consists of the two stages, the latter substantially differing by their characteristic times. The first very rapid stage ($\tau \ll 1$ ms) reflects the lowering of the conductance of small pores with decreasing voltage across the membrane. The diminishing of the number and mean radii of the pores resulting in their complete disappearance occurs only at the second stage of membrane healing, which lasts several seconds or even minutes. The phenomenological similarity of the cell and lipid membrane breakdown indicates that pores developed during the electrical breakdown of biological membranes arise in their lipid matrices. The structure and the properties of the pores are discussed.

Introduction

The application of a strong electric field to the cell membranes results in a considerable increase in their permeability and conductance. If the amplitude and duration of the electrical treatment are not too high then, after the removal of the field, the membrane returns from this condition of high conductance to its initial state. This phenomenon was referred to as a reversible electrical breakdown [1,2]. In recent years there has been much interest in promising applications of breakdown such as electrofusion [3] and cell loading by various physiologically active compounds for the production of biologically active capsules [4] or the transformation of cells by introduction of foreign genetic material [5]. Furthermore, because cell membranes, as a rule, exist in sufficiently strong electric fields, one can suppose the electrical

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breakdown to play some part in the function of membranes under normal physiological conditions, as well as in some pathological processes [6-8].

The basis of this phenomenon was determined, by means of various experimental methods on the study of the membrane electrical breakdown mechanism, to be the development of pores induced by an electric field. However, it is not known until now where in the membrane these pores arise: in the lipid matrix [9], in the integral protein [10] or in the protein and lipid molecules contact region [3].

From this standpoint special attention should be paid to the comparison of various phenomenological approaches to the electrical breakdown (such a comparison became possible when the reversible breakdown, characteristic for cell membranes, was found to exist also for lipid bilayer membranes of certain compositions [11,12]). The study of a high electric field effect on the membrane conductance carried out by the charge relaxation [11,12] and the voltage-clamp [14-17] methods showed that, apart from the similarity of the results obtained on the biological and model membranes, there exists a unique but essentially fundamental difference among them. Namely, more than several seconds or minutes are necessary to heal the pores developed by the breakdown of cell membranes [13,18], whereas, according to Ref. 19, the same time for planar lipid bilayers is only some microseconds.

In the present paper the electrical breakdown of erythrocyte and L-cell membranes by means of the patch-clamp method [20] is studied and the results obtained are compared with those characteristic for the breakdown of lipid bilayers. Special attention is given to the comparative study of biological and lipid membrane healing processes following the breakdown.

Materials and Methods

Recording of cell membrane electrical breakdown. We have investigated the breakdown of human erythrocyte membranes, as well as those of L-cells (mice fibroblasts, Clone 929). The red blood cells were washed with Hanks' solution and then were treated for 5 min by pronase E (Serva, 0.5 mg/ml) at 37°C. Such a procedure partly removed the glycocalix from the erythrocyte surface, essentially facilitating the giga-seal formation.

The L-cells were grown in glass flasks on the 199 medium with the addition of 10% bovine serum for 48 h. The cells were removed by trypsinization (3–5 min, 37°C) and then washed with Hanks' solution. A small amount of the cells (10–20 cells) was transferred in a little Petri dish, which was placed on the stage of an inverted microscope. Round 10–20 μm cells not attached to the glass surface were taken for the measurements.

The solution inside the pipette contained 104 mM of KCl, 2 mM of $MgCl_2$, 5 mM of Hepes-KOH (pH 7.3). The intracellular solution had the following composition: 140 mM of NaCl, 3 mM of KCl, 1 mM of $CaCl_2$, 2 mM of $MgCl_2$, 5 mM of Hepes-NaOH (pH 7.3). The experiments were carried out at a room temperature (19-22°C) or at 37° C.

The pipettes were made from thn-wall pirex capillary tubes in two stages (as described in Ref. 20). For the experiments with erythrocytes use was made of 10–15 MO resistance pipettes, whereas for those with L-cells this resistance was 2–5 MO.

Typically our measurements were carried out in whole cell recording mode. This configuration (in the case of L-cells) was achieved by pulse of suction, and membrane disruption was accompanied with capacitive current increase (as a response to a 10 ms voltage pulses of 10 mV). For the erythrocytes, the membrane part drown in the pipette was often disrupted spontaneously 1–3 min past formation of a giga-seal.

The measuring circuit consisted of a PAR-175 (Princeton Applied Research) generator, an operational current amplifier used in the 'virtual ground' mode (the current-to-voltage conversion coefficient was 10^8), a Krohn-Hite 3342 filter, and a C8-13 storage oscillograph. The electrical breakdown was recorded by the current response to the rectangular voltage pulses, the system permitting to obtain reliable data on the membrane resistance within the limits of 100 MΩ to 1 GΩ. The order of magnitude of the upper limit is close to the leakage resistance between the membrane and pipette, the mean value of the latter being 5–10 GΩ. On the other hand, a membrane resis-

tance drop below 100 MΩ leads to the clamp error of up to 10%, thus limiting the lower boundary of reliable resistance measurements.

Experiments with bilayer lipid membranes. The bilayers were formed of the oxidized cholesterol (5 mg per ml of decane) and of azolectin (40 mg per ml of decane) by usual technique on 1 mm opening of a two-chamber Teflon cell. Oxidized cholesterol was obtained by oxidation of cholesterol (Calbiochem) in decane solution (5 mg per ml) with a persistent air bubble through during 90 min at 150 °C. The bilayers formed from the resultant mixture were stable enough and displayed reversible electrical breakdown [12]. The measurements were carried out at 30 °C in 1 M KCl. To obtain the reversible electrical breakdown, the membranes from azolectin were modified by UO_2^{2+} ions [16,17]. Previously [12], the comparative study of the behaviour of oxidized cholesterol bilayers and UO_2^{2+}-modified membranes in a high electric field was carried out. Similar qualitative regularities of the reversible electrical breakdown were found to exist in membranes of both compositions. Note that, although the general picture and all the investigated regularities of development of the reversible breakdown on all bilayers are qualitatively reproduced, quantitatively the measurement results vary markedly from membrane to membrane. Therefore, subsequently in all cases the oscillograms of reversible electrical breakdown, given in one figure, refer to the same membrane.

The membrane conductivity measurements during the breakdown and after it were made in voltage-clamp mode. When a train of voltage pulses was applied to membranes, the intervals between pulses were no less than 1 min. For rapid recording of appreciable changes in conductance during the breakdown, in some experiments we used the charge relaxation method, which was employed for the first time in Ref. 11 for the study of lipid membrane breakdown. A simple idea underlies such experiments. A short pulse is applied to the membrane by the generator, which charges the membrane up to some voltage. After the pulse, the discharge of membrane capacitance, C_m, owing to diod being present in the scheme, occurs practically completely through the padding resistor, $R_p = 10$ kΩ, coupled parallel with the membrane. This padding resistor accelerates the discharge of the membrane and, thus, decreases the duration of a strong electric field action on the membrane. Note, that the input resistance of the oscillograph, also coupled parallel with the membrane, far exceeds R_p and does not affect the discharge. The discharge oscillograms, presenting the time-dependence of the voltage applied to the membrane, are exponential with the time constant, $\tau = R_p \cdot C_m$, because usually $R_m \gg R_p$. When a sufficiently high voltage is applied to the membrane, the electrical breakdown occurs, the membrane resistance drops drastically ($R'_m < R_p$) loading to the substantial decrease in the discharge time, $\tau' = R'_m \cdot C_m$. Thus, after the breakdown, the membrane resistance, R'_m, can be easily estimated by the kinetics of voltage relaxation.

The experimental set-up permitting one to carry out the relaxation measurements, as well as to record the current at the fixed voltage applied to the membrane, consists of the following three generators. The first one (Hewlett-Packard 214B) gives very short high-voltage pulses (from 10 ns). The second one (PAR-175) is used for application of test voltage pulses to the bilayer. Finally, the third synchronizing generator (PSI A-100) provides the necessary pause between the voltage pulses of the former two generators connected to the cell through diodes (D 219A). The experimental set-up also includes two oscillographs (C-8-13). The voltage across the membrane is measured by the first oscillograph (this enables one to determine the bilayer conductance at the state of breakdown). The second one records the voltage drop on the resistance, R = 300 Ω, permitting one to measure the current through the membrane.

Results

1. The electrical breakdown of cell membranes

For all the cells investigated the current–voltage characteristics measured in the whole cell recording configuration are practically linear in the range from −130 to +130 mV range; the initial resistance of the L-cells is 0.5–1 GΩ and that of erythrocytes constitutes 2–10 GΩ.

Fig. 1 presents a typical current oscillogram characteristic for the case, when a rectangular voltage pulse of 300 mV amplitude and 100 ms

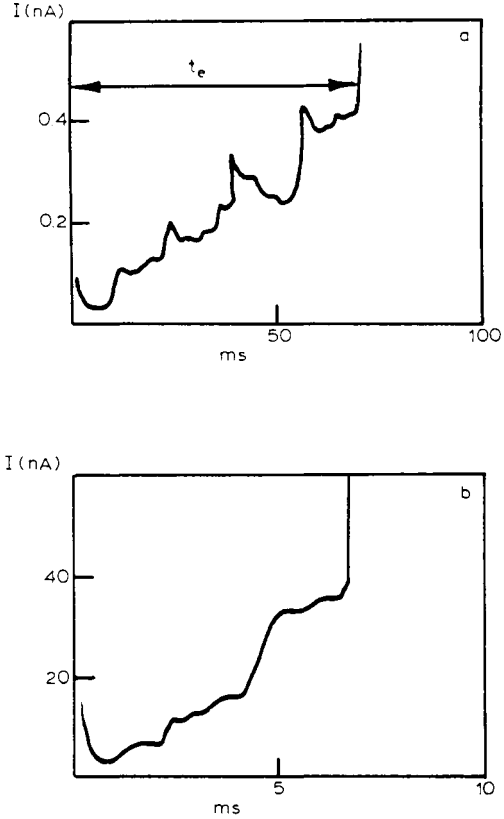

Fig. 1. The breakdown current at the stepwise voltage application. (a) The erythrocyte membrane, $U = 300$ mV. (b) The planar bilayer of the oxidized cholesterol membrane, $U = 200$ mV. The drastic current jump in both the cases corresponds to the irreversible membrane rupture. t_1 is the membrane lifetime.

duration is applied to a membrane. The gradual build-up in the conductance can be seen in this oscillogram with simultaneous appreciable fluctuations, as well as the subsequent rapid current jump. If the voltage applied to the membrane is removed before the current jump, then the membrane, as a rule, returns to its initial state of low conductance. If the interval between the pulses is sufficiently long, then such a reversible breakdown occurring on the same cell can be observed repeatedly. The abrupt jump in the conductance corresponds to the irreversible membrane rupture accompanied by the resistance drop up to 20–100 MΩ. Later on, no recovery of membrane initial properties is observed during 60 min pause at 37 °C. It is substantial that both for the erythrocyte and L-cell membranes qualitatively similar current responses are observed in the whole region investigated (from 10 s and 150 mV to 5 ms and 700 mV, respectively).

At the voltage given, the interval needed for irreversible membrane rupture (in Fig. 1a it is marked in t_1) is a random quantity sufficiently wide spread with respect to its mean value. The mean value, $\bar{t}_1$, in turn, strongly depends upon the voltage applied (Fig. 2a). Every point on the graph is averaged over 8–10 runs. As erythrocytes are sufficiently homogeneous cell population, the simple stochastic treatment (averaging), taking no account of the difference in cell dimensions, was assumed by us to be possible. It is seen in Fig. 2a that when the voltage increases by 100 mV the mean membrane lifetime decreases by nearly one order of magnitude.

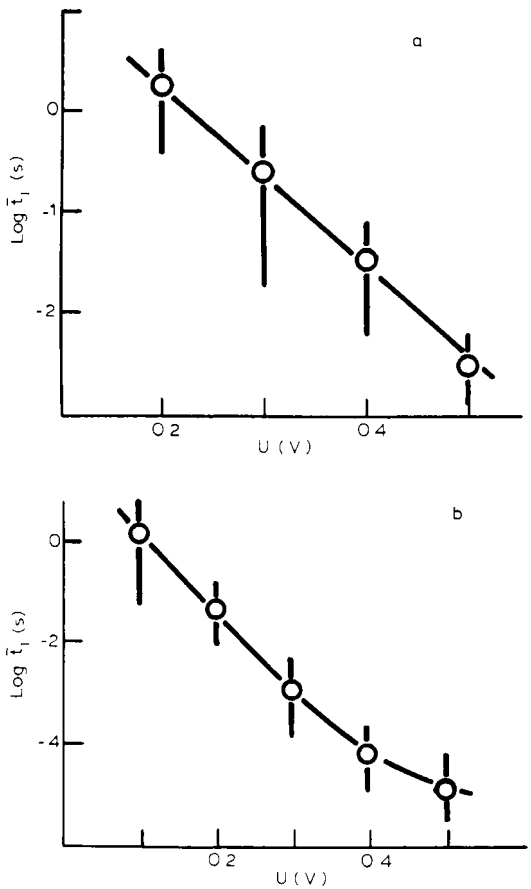

Fig. 2. Dependence of the mean membrane lifetime on the voltage. (a) For erythrocyte membranes. (b) For bilayers formed from the oxidized cholesterol in 1 M KCl. Each point corresponds to the mean value with respect to 10–20 membranes.

Consider now the reversible stage of the electrical breakdown. The L-cell turned out to be more suitable subjects of observation than the erythrocytes, because these membranes can reversibly bear higher currents. This fact is likely to be related to that the area of L-cell membranes is sufficiently larger than that of erythrocytes.

A series of oscillograms obtained with the application to the L-cell membrane the rectangular voltage pulses of different amplitudes is given in Fig. 3a. The rate of conductance growth and the current level reached at the end of the pulse is seen to depend substantially on the voltage applied.

Sometimes, when the breakdown pulses of sufficiently high amplitude and duration (>600 mV, 10 ms) are many times applied to the membrane (with short pauses), the breakdown current becomes higher and hlgher with each subsequent pulse. The membrane loses its ability to reseal and its resistance remains unchanged (50–200 MΩ). If, however, the amplitudes of breakdown pulses are not too high (300–400 mV), the opposite effect occurs, i.e., the application of similar pulses to the membrane causes the current to lower noticeably with each following pulse (the membrane 'gets accustomed' to the high electric field action see also Ref. 3). In spite of the above-mentioned, one is able to choose the cells, for which the reversible breakdown oscillograms are quite reproducible when similar pulses separated by sufficiently long pauses (> 1 min) are applied to the membrane. In addition, the shape and the value of the current responses are practically independent on the polarity of breakdown pulses. In the experiments under consideration, we used the hyperpolarizing pulses (inside negative) and the zero voltage was supported across the membrane during the pauses.

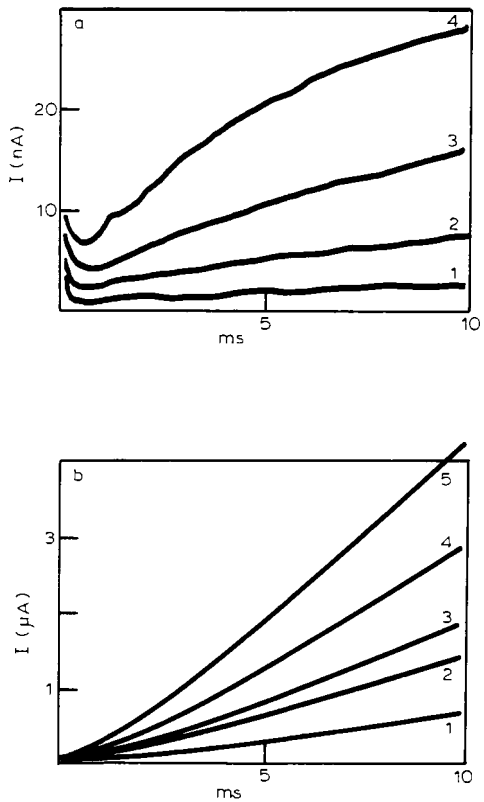

Fig. 3. The reversible breakdown current with the application to the membrane of rectangular voltage pulses of 10 ms duration. (a) Current oscillograms obtained for L-cell membrane; the pulse amplitudes: 200 mV (1), 300 mV (2). 400 mV (3), and 500 mV (4). (b) Current oscillograms for UO_2^{2+} modified azolectin membrane; the pulse amplitudes: 700 mV (1). 720 mV (2), 730 mV (3), 740 mV (4), and 750 mV (5); 0.1 M KCI.

In principle, the oscillograms presented in Figs. 1a and 3a could be accounted for by the electrical rupture of the giga-seal between the membrane and pipette, i.e., by the leakage growth, whereas the conductance and permeability of the membrane proper remain unchanged. The appreciable enhancement of the cell membrane water penetrability is shown, however, to occur after the electrical breakdown. The experiments were carried out as follows. After the formation of a high-ohmic contact between the L-cell membrane and pipette, the solution outside the cell was diluted by water as 1 : 3. (In this run the membrane area pulled into the pipette was not damaged, and the cell membrane integrity was not affected (the cell-attached configuration)). Ten minutes after the dilution, the diameters of both the cell under study and the cell at the bottom of the dish increased, their morphological integrity and clear boundary remained unchanged. Then the breakdown pulse was applied to the membrane under investigation (700 mV, 10 ms). The rapid lysis of the cell followed the pulse, i.e., after 2 min the cell enlarged 2–3-times becoming practically transparent with poorly distinguishable boundary. At the same time, the cells, which were not affected by the field, remained

almost the same (their dimensions only slightly increased). The rapid osmotic lysis of the cell following the field application can be easily accounted for by the growth in the membrane water permeability due to the electrical breakdown. Thus, for the systems used by us, the application of an electric field, indeed, results in the electrical breakdown of the cell membrane.

2. The breakdown of lipid membrane

Consider now the main phenomenological features of the electrical breakdown of lipid bilayer membranes. The detailed study of the behaviour of lipid bilayers of various compositions in a high electric field was carried out already [11,12,15].

Fig. 1b shows the current oscillogram obtained when 200 mV voltage pulse of 20 ms duration was applied to an oxidized cholesterol membrane. The oscillogram clearly presents the transition from the stage of reversible conductance increase to that of irreversible membrane rupture (an abrupt current jump in the oscillogram). The lifetime, t_1 (the time from the onset of the voltage pulse to the beginning of bilayer rupture) is a random quantity with a rather wide distribution with respect to the mean value. The mean lifetime of the membranes rapidly drops with increasing voltage (Fig. 2b). The kinetics of the reversible stage of breakdown is substantially influenced by the voltage applied. Fig. 3b presents the data on the dependence of the rate of reversible breakdown current increase upon the voltage for UO_2^{2+}-modified azolectin membranes.

The data presented in Figs. 1b, 2b, 3b permit one to compare the experimental results obtained for lipid membranes with those of cell membranes (Figs. 1a, 2a, 3a).

3. The resealing of membranes after an electrical breakdown

Of special interest is the problem of the characteristic times of resealing of lipid and cell membrane after a reversible electrical breakdown. Fig. 4a presents the current response obtained for L-cell membrane being subject to the two-step voltage pulses. The comparison of the current level reached at the end of the first step with that at the second step onset (after a transition process related to the membrane capacitance) shows the

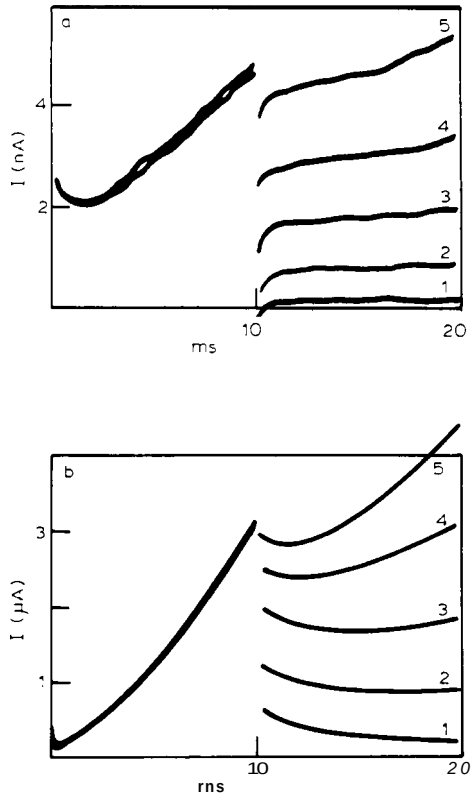

Fig. 4. Changes in membrane conductance with the stepwise voltage decrease. (a) The reversible breakdown current with the application to the L-cell membrane of two successive voltage pulses (without a pause). The first pulse amplitude is fixed and constitutes 550 mV; the second pulse amplitudes are: 100 mV (1), 200 mV (2), 300 mV (3), 400 mV (4) and 500 mV (5). (b) The data on similar experiments corresponding to the UO_2^{2+}-modified planar bilayer membrane. The first pulse amplitude is 720 mV; those of the second pulse are (mV): 500 (1); 600 (2); 650 (3); 670 (4); and 680 (5).

rapid decrease in bilayer conductance to occur along with the voltage drop at the end of the first step. The less the second step amplitude is, the more noticeably this effect is developed.

To make a comparison, in Fig. 4b a similar current oscillogram, obtained by the application of a two-step voltage pulse to an UO_2^{2+}-modified bilayer, is given. We can see the very rapid ($< 5\ \mu s$ [17]) decrease in membrane conductance to occur also in this case, at the moment of voltage drop. Obtained in the course of such experiments is the dependence of bilayer conductance at the state of reversible breakdown as a function of voltage on

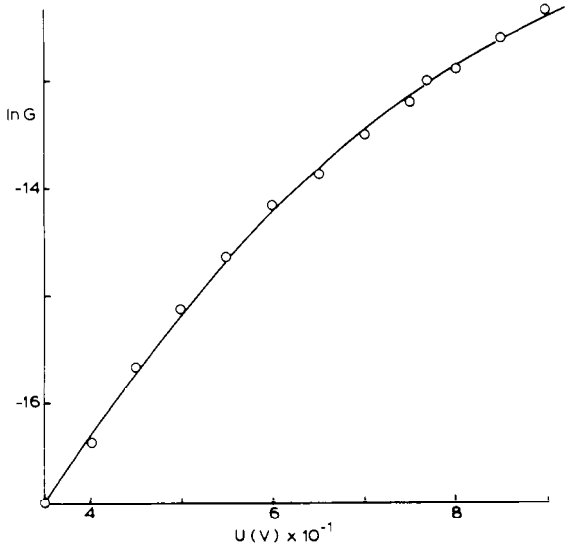

Fig. 5. Dependence of the UO_2^{2+}-modified membrane conductivity on the voltage across the membrane at the state of reversible breakdown. The experimental points are obtained for the current at the onset of the second voltage pulse, the amplitudes of the latter being plotted as abscissa. The amplitudes and duration of the first pulse in all cases constitute 770 mV and 10 ms. The solid line is the theoretical curve plotted in accordance with Eqn. 1 (see text).

the second step of the pulse (Fig. 5).

Important information on the times of recovering following the breakdown gave the experiments, in which the membrane was subject to similar voltage pulses separated by different pauses. The results of such experiments for the L-cell membrane are shown in Fig. 6. To check the conductance between the pulses, a relatively low voltage (100 mV) was applied to the membrane. As seen in Fig. 6a, when the pause is small (5 ms) the current oscillogram, corresponding to the second pulse, looks as it were the continuation of that referring to the first pulse. Note, that in this run, along with the decrease in voltage at the end of the first pulse, the membrane conductance appreciably diminishes, just as in the experiment presented in Fig. 4a. When the interval between the pulses grows, the current response to the second pulse resembles more and more that in the course of the first pulse (Figs. 6b, c). When the pause is 0.5-1 s the current oscillograms, obtained with the application to the membrane of both the pulses, practically coincide.

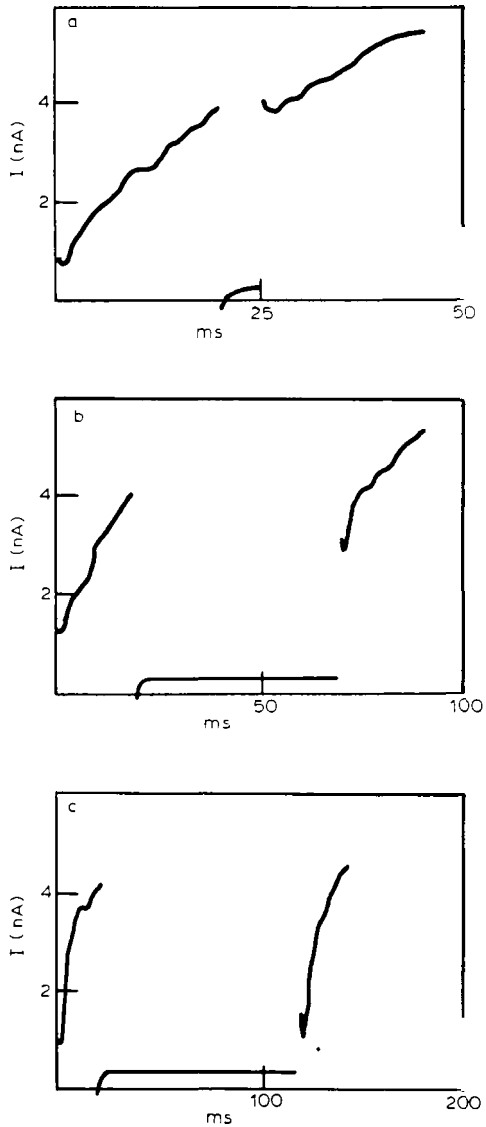

Fig. 6. Current oscillograms of the reversible breakdown of L-cell membranes with the application of two similar voltage pulses ($U = 300$ mV, $t = 20$ ms) separated by different pauses. The voltage amplitude during the pause is 100 mV. The pause durations are: (a) 5 ms, (b) 50 ms, and (c) 100 ms.

Similar experiments were also carried out for lipid membranes (Fig. 7a). For the 4 ms pause the current oscillogram, corresponding to the second pulse of 0.75 V looks as continuation of that referring to the first pulse. In the series shown in Fig. 7b we gradually increased the pause between the first and second pulses in order to obtain the

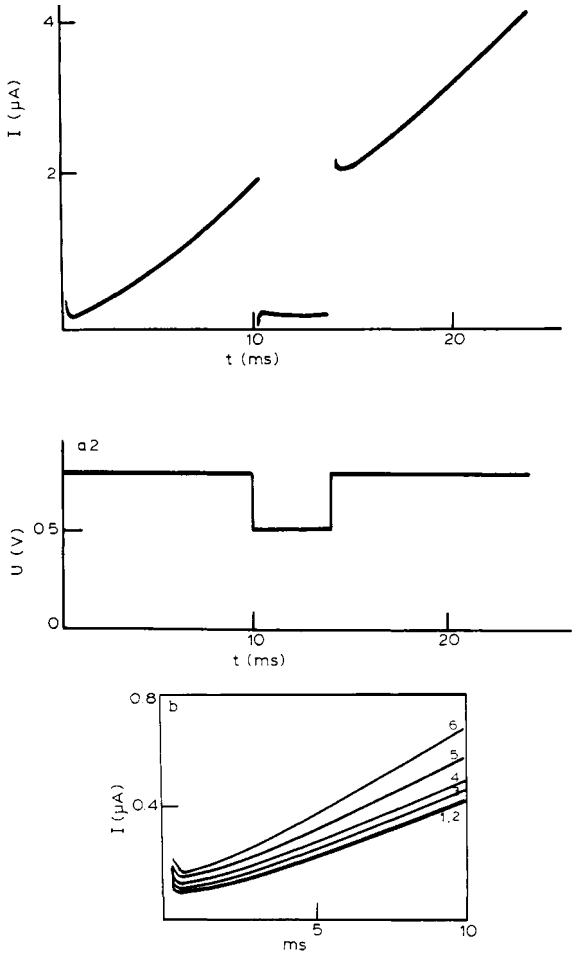

Fig. 7. Current oscillograms of UO_2^{2+}-modified azolectin membrane with the successive application of two identical pulses with a pause between them. (a) The case of two identical pulses (0.75 V, 10 ms) applied on the membrane with a 4 ms pause between them, during which a voltage 0.5 V was maintained across the bilayer. (b) Two identical voltage pulses (0.68 V, 10 ms) separated by different pauses were applied on the membrane. In all the experiments the currents in response to the first pulse are the same. The oscillograms 2–6 are obtained in response to the second pulse applied after a pause of: 30 s (the second curve coincides with the first one), 10 s (3), 2 s **(4).** 0.5 s (5), and 0.1 s (6), respectively.

same current response. As seen in Fig. 7b, the current oscillograms corresponding to the voltage pulses of 0.68 V amplitude and 10 ms duration coincide when the pause between the pulses is 30 s (curves 1 and 2). For UO_2^{2+}-modified bilayer, if

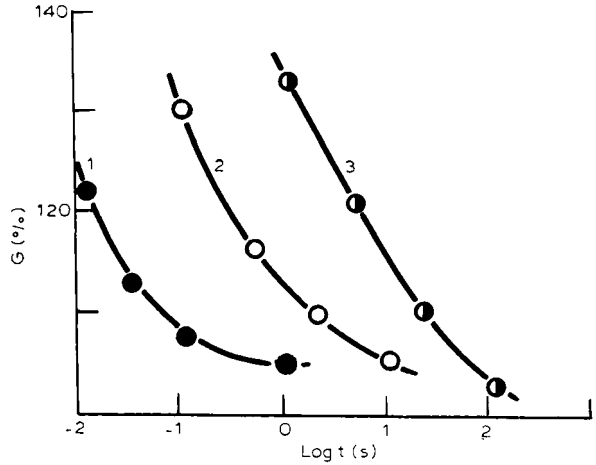

Fig. 8. Lipid bilayer recovery after the reversible breakdown caused by various electrical treatments. The first breakdown voltage pulse was followed, after a certain pause, by the second probe pulse. The amplitudes and durations of both the breakdown and probe pulses were the same and constituted: 0.8 V, 1 ms (1); 0.68 V, 10 ms (2); and 0.59 V, 100 ms (3). The conductance being reached at the end of the probe pulse is presented as ordinate. The conductance at the prepulse end ($G = 9 \cdot 10^{-7}\ \Omega^{-1}$) is taken to be 100%. The duration of the pause separating the prepulse and the probe one is plotted as abscissa.

the pause are shorter, the current oscillogram of the second pulse is higher; the membrane 'remembers' the action of a high electric field.

It is known that in the case of reversible breakdown, to obtain the same value of membrane conductance, G, the duration of the field effect depends on the voltage amplitude. It turned out that, for the same G value, the longer the first pulse, the greater the time of membrane resealing should be. In the run presented in Fig. 8, the same value of conductance ($9 . 10^{-7}\ \Omega^{-1}$) was attained with the application of pulses of various amplitude and duration. The membrane was successively subject to two similar pulses separated by different pauses. Then the bilayer conductance at the end of the second pulse was defined depending upon the length of the pause. The graphs show the conductance, corresponding to the pause of 1 s and pulses having the duration of 1 ms, for the second pulse to be by 5% greater than for the first one. When the pulse duration grows up to 10 and 100 ms the conductance difference reaches 14% and 34%, respectively.

The characteristic time of resealing of membrane properties rises when the conductance, reached at the end of the first pulse, as well as the pause voltage grow. It is interesting to note that the greater the second pulse amplitude, the longer the time interval lasts, when one is able to observe the consequence of the first pulse.

To study the reversible electrical breakdown caused by very short pulses of high voltage, we employed the charge relaxation method. In this run, the charging pulses of $2 \cdot 10^{-9}$ to $9 \cdot 10^{-9}$ C amplitude and 20 ns duration were applied to the UO_2^{2+}-modified bilayer. Then the kinetics of membrane discharge were followed. An abrupt diminishing in the discharge time constant was observed with the application of $4 \cdot 10^{-9}$ C charge (reflecting a drastic growth in conductance caused by an electrical breakdown). (The greater the pulse amplitude is, the higher the conductance and, correspondingly, the less the time constant are). As a whole, the oscillograms obtained with the application of the charge relaxation method to UO_2^{2+}-modified membranes resemble very much those described in Ref. 11 for the bilayers of oxidized cholesterol. Note, however, that the breakdown voltage of UO_2^{2+}-modified membranes is substantially higher than that of the membranes mentioned (see also Ref. 21).

To estimate the lifetime of defects developed in the membrane during the breakdown when the polarization is very strong and short, and UO_2^{2+}-

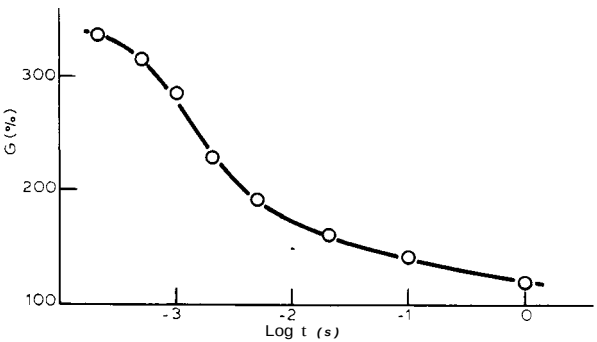

Fig. 9. Dependence of the conductance at the end of the probe pulse (0.8 V, 100 μs) on the duration of the pause separating it from the breakdown charging pulse (20 ns, $6 \cdot 10^{-9}$ C). The conductance of $G = 2.6 \cdot 10^{-6}\ \Omega^{-1}$ reached at the end of the probe pulse (prepulse is absent) is taken to be 100%.

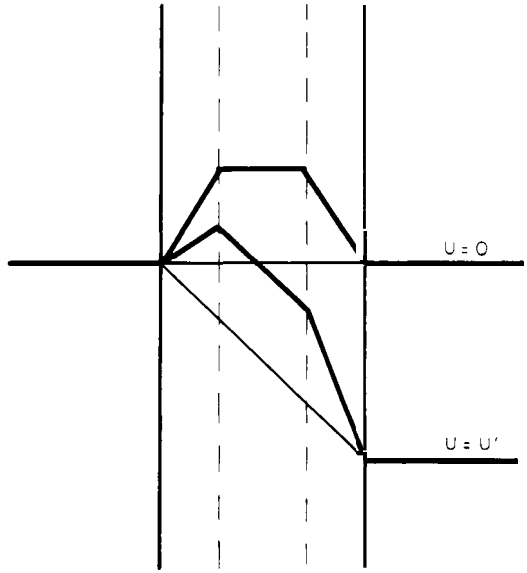

Fig. 10. The energetical profile of the narrow pore in a membrane. The external electric field lowers the barrier and thus enhances the ion transport through the pore (see text).

modified bilayer was subject to a charging pulse of 20 ns duration (the voltage across the membrane at the pulse end was **4.5** V) followed by a rectangular voltage pulse (800 mV, 100 µs) separated from the previous one by a certain pause. At the end of the first pulse the membrane conductance increased up to a value of $\mathbf{5} \cdot 10^{-2}\ \Omega^{-1}$ (due to a breakdown), and after 100 μs the membrane voltage dropped to 1 mV. The second relatively long voltage pulse was applied to the membrane and the current oscillograms were recorded. The oscillograms obtained were compared with those observed in the case, when no first pulse is applied to the membrane. Fig. 9 shows the dependence of the normalized membrane conductance, measured at the end of the second pulse, on the duration of the pause separating the two successive pulses. As seen in Fig. 10, in this case the current response for 1 s (i.e., for a period, which is by nearly eight orders of magnitude greater than that of the very high electric field action) is also higher than in the absence of the first pulse (when the same voltage pulses are applied to the membrane).

Discussion

The similarity of the phenomenologies of electrical breakdown of cell and of lipid membranes

The phenomenology of the electrical breakdown of erythrocyte membranes was sufficiently well studied [22-25]. In these investigations, as a rule, the appreciable growth in the membrane permeability in a high electric field was observed for the two substantially different ranges of electrical actions: (i) at the short but very strong polarizations (1–500 ps, 0.5–1 V) [22,23], and (ii) conversely, at the long (from several seconds to minutes) but relatively low voltages (150–200 mV [24,25]). As shown in this paper, the electrical breakdown, recorded in our case by the growth in conductance of the single cell membrane, can be observed practically in the whole interval separating these two ranges. This fact, as well as the qualitative similarity of the general features and regularities of the breakdown, caused by the application to the membrane of voltage pulses of different amplitude and duration, indicate that the electrical breakdowns, within the wide range of voltages and times of their application, are common in nature.

The comparative study of the electrical breakdown of cellular and bilayer membranes carried out in the present paper is indicative of the phenomenological similarities of these phenomena. The oscillograms of breakdown currents (Fig. 1) and the dependences of t_1 on U (Fig. 2) for the erythrocyte (a) and lipid bilayer (b) membranes are qualitatively similar.

The common regularities of the breakdown of cell and lipid membranes are also observed at the reversible stage of the process. When the voltage across the cellular or artificial membranes is kept sufficiently high a gradual growth in membrane conductance occurs, the rate of the growth being substantially dependent on the voltage (Figs. 3a, b). Note, that the oscillograms of reversible membrane breakdown of squid giant axon given in Ref. 14 also very much resemble the ones of reversible breakdown of lipid bilayer membranes (Fig. 3b; see also Ref. 16).

The study of the kinetics of membrane resealing after the reversible breakdown in the experiments with two successive voltage pulses, applied to the cell and lipid membranes (separated by a pause or without pause), showed nearly the same results (Figs. 4–9). Consider the latter in detail. As seen in Figs. 4, 5, the rapid decrease in membrane conductance occurs when the voltage diminishes. According to the data, obtained by the charge relaxation method, the growth in cell membrane resistance following the reversible breakdown occurs sooner than 10 μs [12]. The conductance developed by the breakdown drops no slower also in the case of lipid bilayers [17,19]. One can suppose this conductance change to be related to the rapid decrease in the number and mean radius of the conducting defects in the membrane, i.e., to the membrane 'healing' after the breakdown [19]. The alternative hypothesis is also possible, viz., the lifetime of the conducting defects resulted from the breakdown (during the first step of the two-step voltage pulse) is substantially longer. As for the rapid non-ohmic current drop during the voltage decreases (Figs. 4, 5), it is the consequence of the voltage dependence of the conductance of defects. The data presented in Figs. 6–9 confirm the latter hypothesis. It is obvious, that when defects disappear and the membrane returns to its initial state the probe voltage pulse of any amplitude will cause the same current response before and after the breakdown. However, as seen in Fig. 6, the application to the membrane of two similar voltage pulses separated by a pause of 5 ms (the latter exceeds the characteristic time of conductance decrease during voltage diminishing by more than three orders of magnitude) results in the current oscillogram due to the second pulse looking as if it is the continuation of the current response to the first pulse. The pause duration growth leads to the current at the second pulse onset approaching that at the beginning of the first pulse and the current responses to the both pulses coincide (Figs. 6 and 7). This means that the membrane has time for returning to its initial state (or to the state close to the initial one). In the case of both the cellular and the lipid membranes the complete reproducibility of oscillograms is usually observed when the pauses between the pulses are longer than 1 s (Figs. 6–9). Thus, these experiments prove the characteristic times of healing of the pores to be in the order of some seconds or even minutes. The recovery times obtained for cell membranes are in accordance with the data of other authors [3,9].

The results presented show the rapid (for several microseconds) and significant decrease in the

membrane conductance at the breakdown state caused by the voltage drop, to be related to the voltage dependence of their conductance rather than to the healing of defects. It is to be emphasized, that this dependence is characteristic both for cellular (Fig. 4a, see also Ref. [26]) and lipid (Figs. 4b, **5)** membranes. The different extent of conductance change after voltage decrease on these figures we attribute to the certain experimental limitations of our patch-clamp measurements. Most probably we did not create and registrate on small area of cell membrane large population of small pores since we could not apply on the membrane sufficiently short and high voltage pulses (the time constant of our system is too high).

In spite of the difference stated, both the general picture and the main regularities of the electrical breakdown of cellular and lipid membranes, discovered by the study of the phenomenon using the voltage-clamp method, turn out to be strikingly alike. In earlier papers [11,13,27] the comparative investigation of reversible electrical breakdown of biological membranes and lipid bilayers has been carried out by means of the charge relaxation method. In this case it was also found that the basic regularities of cellular and lipid membrane electrical breakdowns are very close.

The similarity of the phenomenologies of cell and lipid membrane breakdowns is indicative of the common mechanism of these phenomena. It testifies also that the pores, developed during the electrical breakdown of cell membranes, arise in their lipid matrix. It is noteworthy, however, that for the mediums of low ionic strength the formation of pores in channel proteins was observed also [10].

The structure and properties of the pores developed during the breakdown

A number of mechanisms of the initial stage of the electrical breakdown was proposed [11,28-31], i.e., of the potential-dependent appearance of pores in the lipid membranes. In the present work no such problem should be considered. We shall only dwell on the structure, characteristic dimensions and some other properties of pores, being responsible for an increase in membrane conductance occumng at breakdown.

In principle, the pores in lipid bilayers can be either hydrophobic or hydrophilic [15]. In the first case, the pore walls are made from the hydrocarbon chains of lipids, whereas in the second one the inner surfaces of the pores are covered by their polar heads. The estimations show the hydrophobic pores filled by water to be energetically relatively disadvantageous and, therefore, they should have very short lifetimes. In our experiments, the conducting defects formed in the membrane as a result of a reversible breakdown have relatively long lifetime (> 1 s) and, in all probability, are hydrophilic pores. The same conclusion was made by the authors of Ref. 9 in which the electrical breakdown was shown to increase the rate of lipid transition between the monolayers constituting the erythrocyte membranes.

It is found in the present study that the conductance of the pores, being responsible for the disturbing of the membrane bamer function, depends on the voltage, U, applied (Fig. 5). We shall emphasize that the dependence concerned does reflect the variation in pore conductance.

We assume the non-ohmic behaviour of of membrane in the breakdown state is related to ion's electrostatic interaction with the wall of the pore. Parsegian [33] has shown that the ion energy inside the narrow pore formed in low dielectric membrane is markedly higher than the ion energy in the bulk of electrolyte. Correspondingly, the pore conductance is to be lower than in the bulk, and its value is expected to decrease drastically with the pore radius diminishing. Therefore, ions crossing the membrane through the narrow pore must overcome some energetical barrier. The voltage applied to the membrane reduces this bamer significantly. The pore conductance becomes higher due to this effect with the voltage increases.

The energetical profile of the pore is shown in Fig. **10.** To simplify the calculations we assume the profile to have a trapezoidal shape, with the sides corresponding to the entrance regions of the pore. According to Ref. **34,** the current-voltage characteristic of the membrane with some non-monotonous profile $W(x)$ is expressed generally by the equation

$$I = \alpha \frac{e^z - 1}{\int_0^h \exp[z\psi(1 - x/h) + W(x)]\,dx} \qquad (1)$$

where $\psi = eU/kT$, e = proton charge, h = membrane thickness, α = factor independent of potential. Energy $W(x)$ is measured in kT units.

In conditions of trapezoidal profile determined as

$$((x) = \begin{cases} Ax/h_1 & 0 < x < h_1 \\ A & h_1 < x < h - h_1 \\ A(h-x)/h_1 h - h_1 < x < h \end{cases}$$

(A = the barrier height when $\psi = 0$, h_1 = the length of entrance region of the pore) the current-voltage relation could be written

$$I = \frac{\alpha}{e^{W_{eff}}(n/W_{eff} + 1/\psi) - n/W_{eff}} \qquad (2)$$

here factor $n = h_1/h$ and $W_{eff} = (A - n\psi)$, the effective barrier height.

Fig. 5 represents the result of computer fitting of the theoretical curve determined by Eqn. 2 to the experimental current-voltage relationship (points). The best coincidence was obtained when $A = 8.7\,kT$ and n = 0.31 i.e. entrance region of the pore constitutes one third of the total membrane thickness. According to Parsegian [33], the barrier height A relates to the pore radius as $r = h_0/A$ (here h_0 is the combination of parameters, which is equal to 5 nm when dielectric constant of the membrane material $\varepsilon_m = 2$). Therefore we can estimate $r \simeq 0.6$ nm.

Note, that when $h = 5$ nm, the entrance part of the pore h, $\approx 2r$. This correlation is quite reasonable because it reflects the boundary effect of ion energy decrease for ions occurring near the pore ends. We suppose that the characteristic distance from the pore end, in which ions inside the pore still 'sense' the presence of an external electrolyte, could be estimated as around r. The computer fitting by only one parameter A, assuming h, $= 2$ r gives the same result as shown in Fig. 10.

It is to be noted that the energetical barrier decreases gradually and then completely disappears with the voltage increase. The corresponding voltage, U^*, depends on the pore radius. Thus, the barrier disappearing condition is

$$eU^* r/kTh = h_0/r \qquad (3)$$

Hence, we obtain the dependence of voltage on the pore radius

$$U^* = kThh_0/er^2 \qquad (4)$$

When $h = 5$ nm, r = 1 nm then

$$U^* = 0.31 \text{ V}.$$

It was earlier stated the mean radius of the pores, developed due to cellular [18,23] and lipid [12] membrane breakdowns, to rise when the duration of the electrical treatment increases. In accordance with the theoretical conceptions discussed, in the voltage range of $U < U^*$ the voltage dependence of conductance grows more drastically than in the case of $U > U^*$. As seen from Eqn 4, U^* sharply diminishes when the pore radius increases, and the range of steep dependence of conductance on the voltage is narrowed. Correspondingly, the nonlinear dependence of conductance on the voltage is predicted in the whole voltage range to reduce with the growth of pore radii.

The data obtained in this study permit one to conclude that the process of membrane conductance relaxation, occurring after the electrical breakdown, includes, as a rule, the two following stages. At the moment of voltage drop a very rapid diminishing in conductance occurs, the latter being related to the voltage dependence of pore conductance. Then a much longer relaxation stage follows, which for the breakdown voltage durations and amplitudes investigated lasts several seconds or even minitus and is concerned with the decreasing in the number of pores and their mean radius. The qualitatively regularities, discovered during the investigation of membrane 'memories' after a breakdown, seem to be quite explicable. It is natural to thmk that the healing time of the pores having great radii and arising at longer electrical treatment [12] should be greater than that in the case of small pores. Exactly this regularity was observed experimentally (Fig. 8). Further, as shown above, the conductance of small pores grows especially strongly with the voltage build-up. Therefore, the less the mean radius of the pore at the given moment is, the stronger the dependence of the pore conductance on voltage, and, in agreement with the experiment to reveal such pores, the greater the probe pulse amplitude should be.

In conclusion, let us consider the problem of the nature of the irreversible growth in membrane

conductance (see above, Figs. 1, 2). The phenomenon of the irreversible disturbance of barrier functions of cell membranes at sufficiently long and strong polarizations is known for many years [18,35,36]. The character of corresponding current oscillograms (Fig. 1) permits one to assume the irreversible electrical breakdown of biological membranes, just as in the case of artificial lipid ones, to be concerned with the appearance of one or a number of relatively large defects. The origin of the irreversible breakdown of planar bilayers is the metastable character of the bilayer-meniscus system. Indeed, when a defect inside a bilayer reaches a certain critical size then, under the action of the surface tension forces, the defect spontaneously develops resulting in the rupture of the whole membrane [15]. Unlike the planar bilayer, the cell membranes are closed and have no menisci (correspondingly, there is no tension). It is to be anticipated that also in this case the appearance of pore in a membrane causes the energy of the system to increase with the pore perimeter [37]. Therefore, in the absence of a field the existence of pores in such a system is disadvantageous. From this point of view, one can suppose the conductance growth, being considered above as an irreversible process, to be, as a matter of fact, the reversible one. However, 1 h incubation at 37°C turns out to be insufficient for pore healing. Indeed, an analysis of literature leaves the reader the impression that, after the pore formation, the characteristic time of recovering of barrier functions of cell membranes may be divided into two intervals. Obviously, until the pores developed during the breakdown are realized in the membrane lipid part, the healing times are in accordance with those of bilayer membranes (several seconds or minutes). However, if with the growth of pores the protein particles are involved in the region of defects, stabilization of the pore edge can presumably occur making difficult the membrane healing.

The present comparative study of the phenomenologies of cell and lipid bilayer membrane electrical breakdowns permit one to suppose that the pores developed with the application of a high electric field to cell membranes arise, in the first place, in the lipid matrices of the membranes. Therefore, the properties of these pores (including the healing times and voltage dependence of conductance) turn out to be close to those of pores in lipid bilayer membranes.

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