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The Mechanism of Electrical Breakdown in the Membranes of *Valonia utricularis*

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Summary. The dielectric breakdown in the membranes of cells of *Valonia utricularis* was investigated using intracellular electrodes and 500-μsec current pulses. Electrical breakdown, which occurs when the membrane potential reaches a well-defined critical value, is not associated with global damage to the cell or its membranes (the membrane reseals in <5 sec). It was thus possible to investigate the effect of temperature on dielectric breakdown in single cells. It was found that the critical potential for breakdown was strongly dependent on temperature, decreasing from ~ 1000 mV at 4 °C to ~ 640 mV at 30 °C. The decrease in the breakdown potential with increasing temperature and the very short rise-time of the breakdown current (~ 1 μsec) suggests that the Wien field dissociation does not play a major role in the breakdown process. It is shown that the nonlinear $I-V$ characteristics observed at different temperatures can be accurately accounted for with no adjustable parameters, by considerations of the mechanical compression of the membrane due to stresses induced by the electric field. Electrical breakdown on this scheme results from an electromechanical instability in the membrane. On this basis the present results indicate that the elastic modulus of the region of the membrane where breakdown occurs, decreases by a factor of 2 with increasing temperature from 4 to 30 °C. On the assumption of a thickness of 4.0 nm and a dielectric constant of 5, the elastic modulus is estimated to have a value of $5 \times 10^6 \text{Nm}^{-2}$ at 20 °C.

The influence of intense electric fields on the permeability and electrical conductance properties of cell membranes has been the subject of a number of experimental and theoretical studies [11, 17, 21].

Often the possibility of breakdown of the dielectric properties of the cell membrane at sufficiently high field strengths has been overlooked. It is well known that at sufficiently high membrane potential differences and hence field strengths, artificial bimolecular lipid membranes will rupture [7, 8, 20, 22]. Dielectric breakdown in thin oil layers and in nonconducting liquids in the bulk phase have been studied in considerable detail (e.g. *see* Ref. [18]).

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When dielectric breakdown occurs in a cell membrane it might be expected that this is associated with a dramatic increase in the membrane conductance, together with, perhaps, a release of some cellular material. Further, it would be expected that on removal of the electric field the restoration of the insulating properties of the membrane only occurs after the completion of a resealing (regeneration) process and is therefore not immediate.

The above effects have been observed in experiments with erythrocytes, *Escherichia coli* B and *Ochromonas malhamensis* in Coulter Counters and aqueous discharge chambers using external electrodes [23–27] and in *Valonia utricularis* using intracellular electrodes [5, 6].

It could be shown in the experiments with *V. utricularis* that dielectric breakdown did not lead to global damage of the cells and was not the result of localized heating in the membrane. In the experiments with erythrocytes it was demonstrated that dielectric breakdown was followed by a release of hemoglobin. The membranes of these resealed, a process which was also observed more directly in the experiments with intracellular electrodes using *V. utricularis*.

Apart from being a possible interesting new tool for membrane research, dielectric breakdown of cell membranes provides also a new technique for the preparation of very uniform erythrocyte ghosts [26] and for the injection of foreign materials into microscopic cells.

In this communication we examine possible mechanisms for the breakdown based on the Wien field dissociation effect and electro-mechanical forces. The strong dependence of the membrane potential difference for dielectric breakdown in *V. utricularis* on the temperature, reported here, is an important factor in these considerations.

Materials and Methods

The cells of *Valonia utricularis* used in the present experiments were grown in the laboratory in Mediterranean sea water. Usually ovoid cells 3–5 mm by 2–3 mm were used.

The electrophysiological apparatus and methods used have been described in detail elsewhere [5]. Briefly, the cells were mounted in an Ag/AgCl grid which served to mechanically fix the cell and also as the external current electrode. Current pulses (0.2–1.0 msec duration) were injected into the cell via a platinum/iridium microwire introduced into the cell through a 5 μ tip diameter micropipette previously manipulated into the cell. The membrane p.d. was measured with intra- and extracellular micropipettes filled with 2 N KCl and a high impedance electrometer amplifier with capacitance neutralization.

The current pulses and the potential responses were displayed on a storage oscilloscope.

The plexiglass cell holder and the flowing sea water bathing the cell were temperature controlled, the temperature being monitored with a thermistor mounted approximately 2 mm from the cell. In the experiments the temperature was continuously cycled at $\frac{1}{2}$ to 1 hour intervals, between room temperature (22 °C) and various other temperatures at which measurements were desired.

At each temperature the breakdown p.d. was determined by injecting a series of (usually 0.5 msec) current pulses of increasing magnitude into the cell. Once breakdown had occurred two or more additional current pulses, again of increasing magnitude (hereafter referred to as supracritical pulses) were injected to verify the value of the breakdown p.d.

Since the cell wall of this species is very tough and elastic and the cytoplasmic layer is very thin, it is not possible to insert microelectrodes into the cytoplasm. All measurements of p.d. therefore were made in the vacuole and hence refer to the total barrier, plasmalemma-cytoplasm-tonoplast, but for simplicity will be simply referred to as the membrane p.d.

Theoretical Considerations

Some mechanisms which might be involved in the electrical breakdown have been discussed previously [6]. Of these, punch-through and localized thermal damage to the membrane were eliminated and avalanche ion production considered unlikely as possible explanations for the dielectric breakdown effect observed.

We now investigate the effect of electro-mechanical forces and the Wien field dissociation on the I–V characteristics.

Electro-Mechanical Forces

The presence of an electric field in the membrane sets up stresses which lead to a mechanical compression of the membrane. This important effect has recently been analyzed and discussed in relation to the dimensional stability of artificial bimolecular lipid membranes [7]. Thus, the electric compressive force P_e per unit area of membrane, or layer within a membrane, is given by

$$P_e = -\frac{d}{d\delta}\int_0^{\delta}\frac{1}{2}\varepsilon\varepsilon_0 E^2\,dx, \tag{1}$$

where δ is the thickness of the membrane (or layer), E is the electric field strength, ε is the dielectric constant or relative electric permittivity, and ε_0 is the electric permittivity of free space.

If the field within the membrane, or a layer, is independent of the position x, then Eq. (1) reduces to

$$P_e = +\frac{\varepsilon\varepsilon_0 V^2}{2\delta^2} \tag{2}$$

where V is the total p.d. appearing across the layer or membrane.

The compression of the membrane creates elastic strain forces. Following Crowley [7], if we assume that the membrane is an ideal (linear) elastic material, the mechanical restoring force P_m per unit area is given by

$$P_m = Y \int_{\delta_0}^{\delta} \frac{dx}{x} = Y \ln \frac{\delta}{\delta_0}. \tag{3}$$

Here Y is the elastic, compressive, modulus of the membrane, defined as the rate, expressed per unit membrane thickness, at which the thickness decreases with pressure. δ_0 is the original, unstrained, thickness (i.e. at zero p.d.).

For dimensional equilibrium we must have

$$P_e + P_m = 0$$

and hence

$$\frac{\varepsilon \varepsilon_0 V^2}{2\delta^2} = -Y \ln \left(\frac{\delta}{\delta_0}\right). \tag{4}$$

It is at once apparent from Eqs. (2) and (3) that for sufficiently large compressions, that is small values of δ, the electric compressive force will increase more rapidly with decreasing δ than the elastic restoring force. This occurs when

$$\frac{\partial P_e}{\partial \delta} = -\frac{\partial P_m}{\partial \delta}. \tag{5}$$

At this point a catastrophic collapse of the material will occur. For large compressions the membrane is not likely to have linear elastic properties and Eq. (3) is then no longer valid. In the absence of information on the variation, if any, of Y with δ, we proceed by assuming, as a first approximation, that the elastic modulus, Y, remains constant. From Eqs. (2)–(5) the critical p.d., V_c for electro-mechanical collapse [1] is then given by:

$$V_c^2 = \frac{0.3679\, Y \cdot \delta_0^2}{\varepsilon \varepsilon_0}. \tag{6}$$

The movement of ions, which is involved in the typical steady-state non-linear electrical characteristics of cell membranes, have time constants of

1 The first suggestion that electrical breakdown in cell membranes might be due to electro-mechanical forces came from the concordance of the breakdown p.d. calculated from Eq. (6), using physiologically reasonable values of Y, ε and δ_0, and the membrane p.d. at breakdown in hydrodynamic focussing Coulter Counters, calculated from the potential distribution with the external electrodes [25].

the order of 0.01 to 1.0 sec [1, 3][2]. For sufficiently rapidly varying p.d.'s such as those relevant to the present experiments, the membrane behaves simply as a slightly lossy dielectric material [3, 4]. In this case, for membranes of macroscopic dimensions, the resistance measured with very short current pulses is then proportional to the membrane thickness. For sufficiently thin membranes, however, this need not necessarily be so since the profiles of the electrostatic self-energies of the ions in the membrane, due to the electrical image forces, may then not attain steady values. A detailed analysis of this problem has been given by Neumcke and Läuger [12]. Their results show that the ion energy, and hence concentration profiles, reach steady values in ~ 1 nm. Since the maximum reduction in thickness expected (at breakdown) is $\sim 39\%$, we will proceed on the assumption that the resistance, R, per unit area can be expressed as,

$$R = R_0 \frac{\delta}{\delta_0}$$

and hence the current density J is

$$J = \frac{V}{R_0} \cdot \frac{\delta_0}{\delta}. \tag{7}$$

Here R_0 is the resistance when the membrane, or layer within the membrane, has a thickness δ_0, that is, in the unstrained condition when $V = 0$.

It follows [from Eq. (4)] that the I–V characteristics will be nonlinear since the ratio δ/δ_0 is dependent on V. The current as a function of V depends on the ratio Y/ε for the membrane (or membrane layer).

Some examples of the theoretical I–V characteristics for various values of the parameters are shown in Fig. 1. The broken part of the curve represents the rapid increase in current due to the collapse of the membrane when the critical p.d. is reached.

An independent measure of the parameters Y/ε and δ_0 can be experimentally determined from the ciritical p.d. for breakdown [Eq. (6)]. Thus, combining Eqs. (4) and (6) yields,

$$\left(\frac{\delta}{\delta_0}\right)^2 \ln\left(\frac{\delta}{\delta_0}\right) = -0.1839 \frac{V^2}{V_c^2}. \tag{8}$$

2 This is borne out, for example, by the fact that while at very low frequencies ($\lesssim 100$ Hz) the membrane capacitance of the giant cells of the alga *Chara corallina* is strongly dependent on frequency; at high frequencies (>200 Hz) the contribution of diffusion polarization to the membrane capacitance is negligible [3]. At these high frequencies the membrane capacitance is independent of frequency and the presence of membrane inhomogeneity cannot be detected. The latter is also true in artificial lipid bilayer membranes [4].

On the other hand in the presence of gramicidin A, jump rates for K^+ and Na^+ of the order of 10^8–10^9 sec^{-1} can occur in artificial lipid bilayers [10].

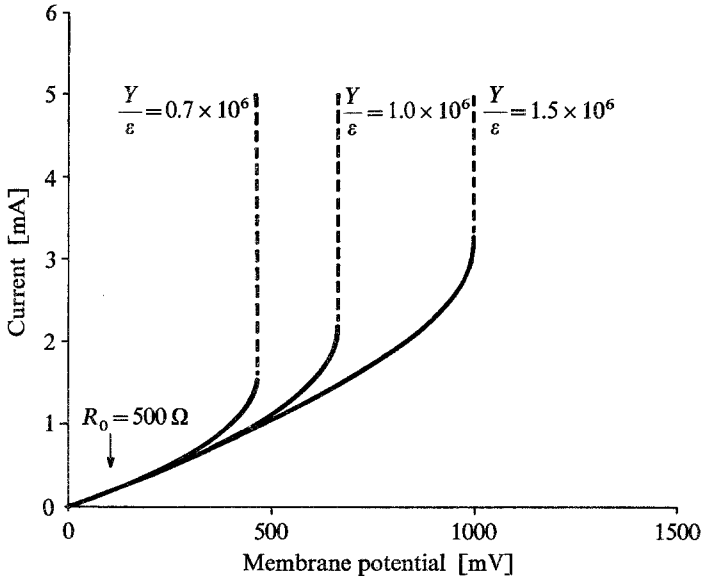

Fig. 1. The theoretical $I-V$ characteristics calculated from Eqs. (7) and (4) for three values of the ratio of the elastic modulus to the dielectric constant (Y/ε). The membrane thickness δ_0 was taken as 4.0 nm and a value to 500 Ω was used for the membrane resistance, R_0, for small currents. At a critical membrane potential, given by Eq. (6), the membrane collapses, which then leads to electrical breakdown. This is indicated by the dashed lines

The I–V characteristics are therefore in practice completely determined by two experimentally accessible parameters, R_0 and V_c.

The foregoing treatment applies to a homogeneous membrane or one in which both the resistance and the high frequency reactive (dielectric) impedance is very much larger than any other layer which is in juxtaposition to it[3].

Otherwise when the membrane contains more than one layer the treatment would still be valid provided the ratio V/δ is the same for the whole membrane and the layer of interest within it[4].

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3 This indeed is, to a good approximation, true for the hydrocarbon region of lipid bilayers. From low frequency impedance dispersion studies [4] it has been shown that while the hydrocarbon region has a capacitance of ~ 0.5 μF/cm^2 and a resistance of $\sim 10^6$ Ω cm^2, the polar head regions each have a capacitance of ~ 30 μF/cm^2 and a resistance of ~ 500 Ω cm^2.

4 In the cell membranes of *Chara corallina* for instance the reactive impedances of the elements in a three-layer electrical equivalent model (corresponding to the three layers seen in electron-micrographs) are approximately equal [2, 3], suggesting that the corresponding values for V/δ for high frequency signals are also approximately equal. The d-c resistance of one layer, however, is 500× larger than the other two.

Field Dissociation (Wien) Effect

In the foregoing analysis it was assumed that the resistance per unit area of the membrane was field dependent only through the dependence of membrane thickness on the field strength. However, the intrinsic conductivity (that is for a unit volume element) depends on the concentration and degree of ionization of the ion pairs in the membrane. At high field strengths, such as those of interest in dielectric breakdown, the dissociation constant and hence specific conductivity will be a function of field strength. Here, in particular, we wish to examine briefly the effect of temperature on the increase in conductivity due to field dissociation.

For electrolytes in organic solvents the association constant for the electrolyte is independent of the field strength. The dissociation constant increases rapidly with field strength in very strong fields ($\sim 10^7$ V/m); the equilibrium constant being given by [13, 14]:

$$K = K_0 \left(1 + \frac{1}{2}\beta + \frac{\beta^2}{2!\,3!} + \frac{(\beta)^3}{3!\,4!} + \cdots\right) \tag{9}$$

where

$$\beta = e^3 |E| / \varepsilon \varepsilon_0 k^2 T^2.$$

Here $|E|$ is the absolute value of the field strength, K_0 the equilibrium constant at zero field, e the electronic charge, k the Boltzmann constant, T the absolute temperature, and ε, ε_0 are as defined before.

The effect of field dissociation on the conductivity of lipid bilayers has recently been analyzed by Neumcke, Walz and Läuger [13]. Considerations of simultaneous diffusion and membrane-solution boundary effects must be taken into account and these present some mathematical difficulties. The effect on the membrane conductivity depends in a complex way on the ratio of the rate of diffusion and field dissociation relaxation time and the relative resistances of the membrane interior and the surface barriers. The results of Neumcke, Walz and Läuger show, however, that for membrane p.d.'s of only 100 mV the membrane conductance can increase fivefold and rises very steeply with increasing field strength.

From Eq. (9) it would also follow[5] that the relative increase in the conductivity with increasing field strength should decrease with increasing temperature. If the field dissociation is primarily responsible for the breakdown of the membrane, it would be expected therefore that the onset of the

5 For some materials the dielectric constant ε decreases with temperature. However, over the temperature range of interest this effect is very small and is swamped by the rapid decrease in the value of the factor $1/T^2$ as T increases.

rapid increase in conductivity would occur at higher p.d.'s as the temperature is increased, a feature also evident in the theoretical I–V curves for lipid bilayers presented by Neumcke *et al.* [13].

Results

To elucidate the potential responses, lightly longer than usual pulses (0.7–1.0 msec) were sometimes used. A typical set of results so obtained is shown in Fig. 2. For the first two pulses, traces *A* and *B*, the current was subcritical, that is, did not take the membrane to the breakdown potential. For such subcritical currents the membrane had a resistance of $\sim 1400\ \Omega$ ($= \sim 580\ \Omega\ \text{cm}^2$ for this cell).

Despite the fact that the increments in the membrane p.d. in each of the traces *A*, *B* and *C* were about equal, the increment in current in trace *C* was

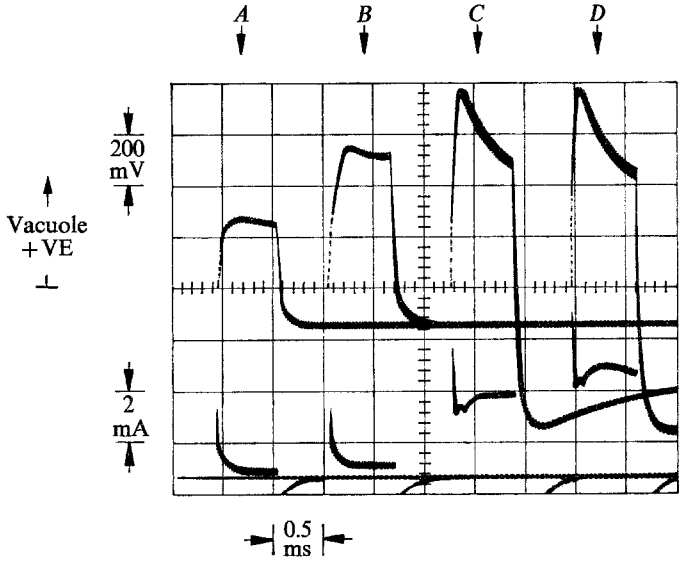

Fig. 2. The potential response, vacuole (positive) with respect to the external solution (upper traces) to 700-μsec current pulses (lower traces) for a cell of *V. utricularis*. In the first two sets of traces (*A* and *B*) the pulses were subcritical, that is, did not take the membrane p.d. to the breakdown value. Pulses *C* and *D* were supracritical (i.e. electrical breakdown occurred). Note that on breakdown the current increased dramatically (*cf.* currents in traces *A* and *B* with that in *C*). For supracritical pulses a further increase in the current did not lead to a further increase in the membrane p.d. as is evident from trace *D*. For supracritical pulses (*C* and *D*) moreover, once breakdown had occurred, the membrane p.d. during the current pulse dropped 300–400 mV below the peak, critical breakdown value. The current, however, remained substantially constant. To obtain the complete pulsed $I-V$ characteristics, a sequence of 10 to 15, 500-μsec current pulses of increasing magnitude were injected into the cell *(see text)*

many times higher than that for traces *A* and *B*. This result was characteristic of the breakdown phenomenon; at the breakdown p.d. the current increased very dramatically. For a supracritical pulse any further increase in the current did not lead to a measurable increase in the membrane p.d., as is evident from trace *D* in Fig. 2.

A further characteristic feature of the results shown in Fig. 2 is that for supracritical pulses (traces *C* and *D*) the current showed some instabilities. More significantly, the membrane p.d. following breakdown decreased by some 300–400 mV during the remainder of the current pulse but this was not associated with a decrease in the current. For subcritical pulses (traces *A* and *B*), on the other hand, the membrane p.d. was substantially constant during the current pulse. For these pulses the current showed initial spikes due to, presumably, the membrane capacitance.

To determine the breakdown p.d. usually a sequence of 10 to 15, 0.5-msec current pulses of increasing magnitude were used. In the experiments only positive current pulses were used, that is, current pulses which make the intracellular potential more positive with respect to the external sea water. Electrical breakdown could also be demonstrated with negative current pulses but these were avoided in the present experiments because of gas evolution at the electrodes which then occurs and becomes a problem when many, long sequences of current pulses are used in experiments which must of necessity last for many hours in order to obtain the temperature dependence of the breakdown p.d.

Electrical breakdown did not lead to global damage to the cells or to the membrane and the process could be repeated many times with identical results provided that the time between supracritical pulses was more than 10 sec. A complete set of *I–V* characteristics was reproducible if a time of 10–20 min was allowed between two measurement sequences as discussed in an earlier paper [6].

I–V characteristics were obtained in this way at various temperatures with a given cell. Examples of the *I–V* characteristics at three temperatures for a particular cell are shown in Fig. 3. In this particular case the results presented are the cumulative data (at these temperatures) of several runs, obtained over a period of ~ 5 hr. Note particularly the very large membrane potentials involved; these *I–V* curves are quite unlike the usual characteristics obtained with long application of currents (e.g. *see* Ref. [1]). The curves shown are numerical solutions of Eqs. (7) and (8) using the values of R_0 and V_c corresponding to the data at these temperatures. It is apparent that the theoretical *I–V* characteristics predicted from the electro-mechanical effects can very adequately account for the experimental data. Note the

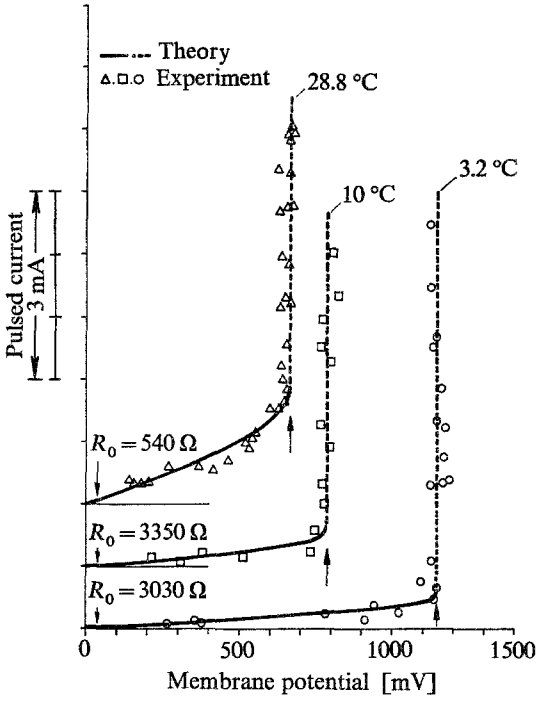

Fig. 3. The theoretical (curves) and experimental (points) $I-V$ characteristics for a cell of *V. utricularis* at three different temperatures. The vacuolar potential plotted here is positive with respect to the external solution. The results at each temperature are the cumulative data of several runs at these temperatures obtained over a time interval of about 5 hr. *Note:* The membrane potentials involved are very large, and these experimental $I-V$ characteristics, though perhaps similar in form, should not be confused with the usual characteristics obtained with long applications of currents. The theoretical $I-V$ curves were determined numerically from Eqs. (7) and (8). The only parameters in this calculation are the resistance R_0 (at $V \sim 0$) and the critical breakdown p.d., V_c. The values of these quantities were contained in the experimental data and are indicated on the curves. The dashed portion of the curves represents the situation, as in Fig. 1, when electrical breakdown has occurred

very sharp rise in the I–V characteristics, particularly at the low temperatures. The measured *slope* resistance beyond breakdown is virtually zero.

The breakdown p.d.'s determined from I–V curves at various temperatures for one cell are given in Fig. 4. The scatter in the results shown appears greater than that evident from the I–V curves in Fig. 3. It should be noted, however, that measurements in any one temperature range were not made sequentially; the temperature was cycled between 22 °C and the various other temperatures. The scatter in the results for the breakdown p.d. at different temperatures appeared to be due to long-term (~ 10 hr) changes which take place in the membrane after repeated electrical breakdown.

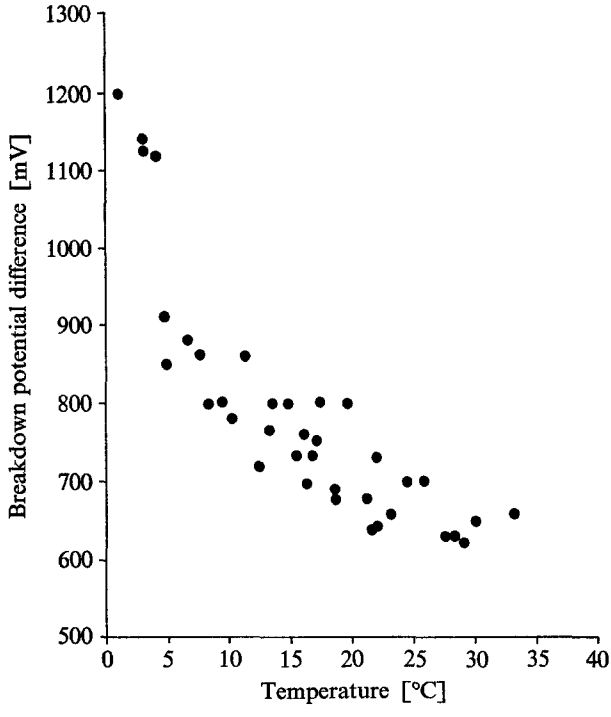

Fig. 4. The breakdown p.d., determined from $I-V$ curves such as those shown in Fig. 3, at various temperatures for the same cell of *V. utricularis*. It should be noted that the measurements at different temperatures were not done sequentially; the temperature was cycled between room temperature and the various other temperatures. This particular experiment (on one cell) was conducted over a period of 36 hr

Such changes were not related, however, to the deterioration of the cell. The latter was always obvious from a rapid lowering of the membrane resistance accompanied by a disappearance of the breakdown phenomenon at which stage the I–V characteristics also became linear (*see* Fig. 5).

Similar results to those shown in Fig. 4 were obtained for all cells examined. The cumulative data for 10 cells for which the breakdown p.d. was obtained for the whole of the temperature range 1–30 °C is given in Fig. 6. It is apparent from both Figs. 4 and 6 that the breakdown p.d. increased rapidly with decreasing temperature.

The membrane resistance, as might be expected, also increased with decreasing temperature as is evident, for instance, from the I–V relations, below the critical breakdown p.d., in Fig. 2. The cumulative data for the resistance for subcritical currents for the same cell (to which Figs. 3 and 4 refer) is shown in Fig. 7. The scatter in the results here is much greater than for the breakdown p.d. for this same cell (Fig. 4).

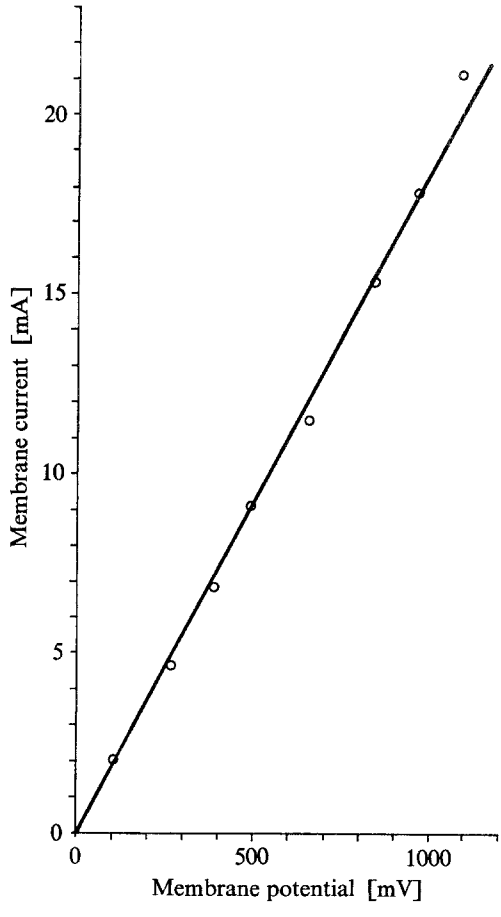

Fig. 5. The pulsed $I-V$ characteristics for a dead cell at the end of an 8-hr experiment; vacuolar p.d.'s (positive) with respect to the external sea water. Note the large current scale used in the plot and the absence of electrical breakdown

Discussion

If the Wien effect plays a significant role in the high field effects reported here, it would be expected that this would be evident in a decrease in the membrane resistance, at high membrane p.d.'s, in excess of that expected from the reduction in membrane thickness alone. It is evident from the results in Fig. 3 that this is not the case. The variation with temperature of the potential for the onset of the rapid increase in conductance with membrane p.d. (Fig. 6) is also contrary to what is expected from the Wien field dissociation effect. Further, the time constants for the field dissociation in typical lipid bilayers was estimated by Neumcke *et al.* [13] to be

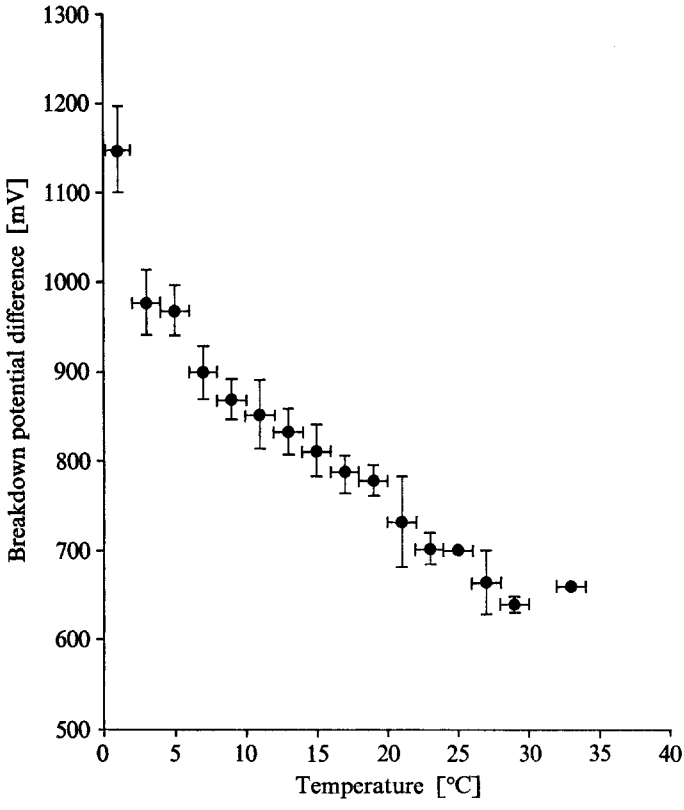

Fig. 6. The cumulative data of the breakdown p.d. as a function of temperature for 10 cells. Only data is included for cells for which the breakdown p.d. was determined over most of the temperature interval shown. The temperature dependence in these experiments was measured in the same manner as indicated in the legend for Fig. 4 *(also see text)*. The data shown is grouped into 2 °C intervals and the vertical bars indicate the standard deviation from the mean

40 μsec or more. Significantly, for dielectric breakdown the rise-times of the currents are very much smaller than this.

On the other hand, the I–V characteristic predicted from considerations of electro-mechanical effects in the membrane are in very good agreement with the experimental data, particularly considering that once the membrane resistance R_0 (at $V \cong 0$) and the critical breakdown voltage V_c are determined, there remain no adjustable parameters.

While the effect of temperature on R_0 can be readily envisaged, for instance through an increase in ion pair dissociation with increasing temperature, its effect on V_c and hence Y/ε remains to be elucidated.

It is of interest also to consider some numerical values of Y/ε calculated, using Eq. (6), from the observed breakdown p.d.'s. For this we need to

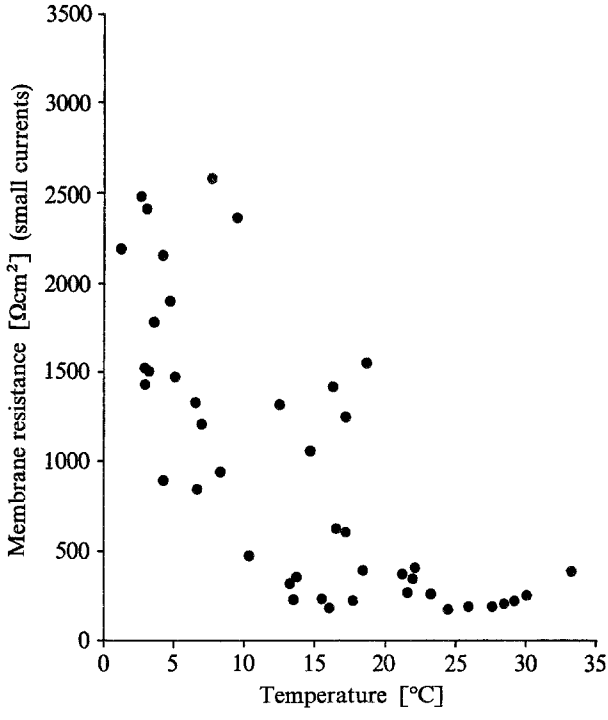

Fig. 7. The membrane resistance R_0 (for very small current pulses) as a function of temperature for the same cell to which Figs. 3 and 4 refer. The scatter in the values of R_0 is relatively large compared with the scatter in the breakdown p.d. but is typical of such measurements of membrane resistance as a function of temperature

know the thickness δ_0 of the membrane, or membrane layer, which breaks down and the fraction of the total p.d. that appears across this layer. No quantitative electron-microscopic or impedance dispersion data are available for the membranes of *Valonia utricularis*. For reasons outlined earlier (*see* Theoretical Considerations) we can take, for purposes of calculation[6], δ_0 to be in the range 3.0–9.0 nm.

For this range of values of δ_0, Y/ε from Eq. (6) is in the range $Y/\varepsilon = 1.5 \times 10^5$ to 1.5×10^6 Nm^{-2} (0.15–1.5×10^7 Dynes/cm^2) at 21 °C. The dielectric constant for the lipoprotein regions in the membrane is likely to be about 5. This yields values of $Y = 0.75$–7.5×10^6 Nm^{-2} (0.75–7.5×10^7 Dynes/cm^2) for the elastic modulus of the membrane material. This range of values

6 If in the region of breakdown the membrane is simply like naked a lipid bilayer the appropriate value of $\delta_0 \sim 4.0$ nm. For reasons outlined earlier, for cell membranes, impedance studies suggest that V_c/δ_0 for the whole membrane will be of the same order as that for the individual layers which might be present (perhaps only in patches). The appropriate value of δ_0 for the present calculations is then ~ 9.0 nm.

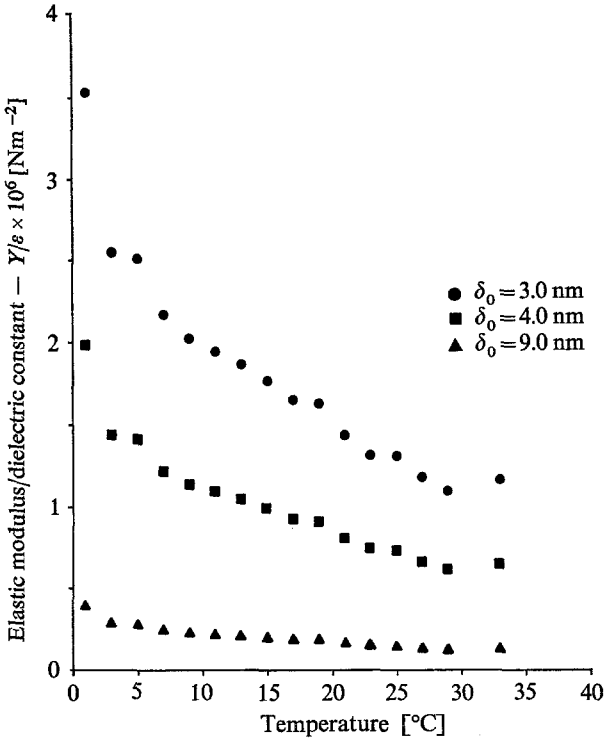

Fig. 8. The value of the ratio of the elastic modulus to the dielectric constant (Y/ε) as a function of temperature, calculated from the experimental data shown in Fig. 6 using Eq. (6) for values of $\delta_0 = 3.0$, 4.0 and 9.0 nm

is indeed similar to the values quoted in the literature for the elastic modulus [7] of the red cell membrane [9, 15] although the values quoted do not strictly refer to the modulus for deformations normal to the plane of the membrane as relevant here. The values deduced from the present study are also close to those reported recently for "solvent-free" lipid bilayer membranes [16].

If the electro-mechanical instability is responsible for the dielectric breakdown, the present results show that the ratio Y/ε for the membrane material (where breakdown occurs) must be strongly dependent on the temperature. This is illustrated in Fig. 8 where the calculated values of Y/ε from the breakdown p.d. data of Fig. 6, for $\delta_0 = 3.0$, 4.0 and 9.0 nm, are plotted as a function of temperature. The decrease in elastic modulus with temperature also immediately suggests that in the region of electrical breakdown, the contribution to the elastic restoring force due to internal energy

7 Indeed the values of the breakdown p.d. for erythrocytes measured in hydrodynamic focussing Coulter-Counters [25] are also consistent with these values of Y.

changes is greater than that due to entropy changes. This is unlike, for instance, the behavior of elastin or elastic polymers such as rubber.

A feasible alternative mechanism for electrical breakdown, not discussed so far, is as follows. In the fluid mosaic model [16, 19] the membrane is envisaged to contain protein units embedded in a lipid bilayer. It is conceivable that some of these protein units normally would not reach completely through the membrane but would do so if the membrane thickness was reduced. The latter occurs as the field strength is increased. Thus, at a particular field strength these units would then form a conducting channel through the membrane.

The actual potential at which this would occur would again depend on the elastic and dielectric constants as discussed previously and also on the hypothetical protein subunit, as well as any hydrophobic and hydrophylic forces at the protein-lipid and protein-water interfaces. As, at best, only order of magnitude estimates of these would be possible, no quantitative calculation of the critical p.d. for breakdown can be made for comparison with the experimental data.

Conclusion

The present study allows us to conclude that:

1. The I–V characteristics of the membranes of cells of *Valonia utricularis*, measured with 500-μsec current pulses are very nonlinear at large membrane potentials.

2. The potential for electrical breakdown in cells of *V. utricularis* is strongly dependent on temperature; decreasing from ~ 1000 mV at 4 °C to ~ 640 mV at 30 °C.

3. Considerations of the compression of the membrane due to stresses set up by the electric field in the membrane can accurately account (with no adjustable parameters) for the experimental I–V curves, once the membrane resistance for very small currents and the critical breakdown potential have been determined.

4. Electrical breakdown can occur as a result of electro-mechanical instabilities in the membrane when a critical potential is reached. On this basis the present results indicate that the elastic modulus, for deformations normal to the plane of the membrane, decreases by a factor of ~ 2 with an increase in temperature from 4 to 30 °C.

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