

Direct Demonstration of Dielectric Breakdown in the Membranes of *Valonia utricularis*

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It is shown that if the membrane potential of cells of *Valonia utricularis* was increased rapidly by applying $\sim 500 \mu\text{s}$ current pulses, dielectric breakdown of the membrane occurred when the potential reached a value of $\sim 0.85 \text{ V}$.

The breakdown phenomenon observed was not associated with global damage to the cell or its membrane. The process could be repeated after a short resealing time ($\sim 10 \text{ s}$), many times on a single cell.

The rapidity of the breakdown process ($\sim 1 \mu\text{s}$) rules out the possibility that dielectric breakdown occurs by a mechanism similar to that of punch-through, which involves changes in ionic profiles.

Dielectric breakdown of the membranes of bacteria (*Escherichia coli* B.), erythrocytes and algal cells (*Ochromonas malhamensis*) has been observed in experiments with hydrodynamic focussing Coulter Counters and aqueous electrical discharge chambers^{1–3}. In these experiments breakdown in the cells resulted when the potential difference (p.d.) applied to electrodes placed in the electrolyte media in which the cells were suspended exceeded a critical level. Solution of the Laplace equation for the distribution of the electrical potential in such cell suspensions showed^{2,3}, that breakdown occurred when the p.d. across the membranes was between 1.4 and 1.6 V for erythrocytes, 1.1–1.3 V for bacteria and approximately 2.8 V for *O. malhamensis*.

The dielectric breakdown phenomenon offers a possible new tool for the investigation of membrane substructure and the influence of chemical agents and physical parameters on the structure.

This communication is concerned with direct measurements of the electrical breakdown, using intracellular electrodes, in the giant cells of *Valonia utricularis*.

Experimental

A schematic diagram of the experimental set-up used is shown in Fig. 1.

In the experiments current pulses were passed through the cell membranes *via* an intracellular

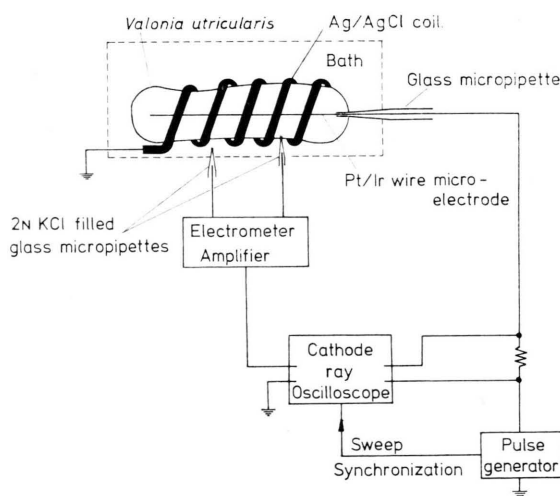


Fig. 1. A schematic diagram of the experimental set-up used in the dielectric breakdown experiments. For explanation see text.

platinum/iridium electrode and a AgCl coated silver wire coil surrounding the cell in the external solution (Mediterranean sea water) bathing the cell. The intracellular current electrode consisted of a Pt/Ir wire which was electro-etched in a KCN solution from an original diameter of $17 \mu\text{m}$ to a tip of $1 \mu\text{m}$ and a shank of $\sim 5 \mu\text{m}$. This wire electrode was inserted longitudinally through a $\sim 5-7 \mu\text{m}$ tip glass micropipette, previously inserted into the cell. The membrane potential was measured with the aid of two 2 N KCl filled glass micropipettes (tip

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diameter $\sim 1 \mu\text{m}$), one inserted into the cell, one immediately outside it. Contact with the electrolyte in these microelectrodes was made with AgCl coated silver wires and these were connected to the input of an electrometer amplifier (input impedance $10^{11} \Omega$), with capacitance neutralization (a modified "Grass" model P 16).

The cells used were generally 3–5 mm long and 2–3 mm in diameter. The experiments were done at 20°C .

Current pulses of 0.1 to 1 ms duration were injected into the cell from a pulse generator and the current was monitored by measuring the potential drop across a 100Ω resistance in series with the pulse generator.

Both the membrane p.d. and the current were displayed on a dual beam storage oscilloscope ("Tektronix" 7623 Oscilloscope).

Results and Discussion

Fig. 2 shows a typical oscilloscope tracing of pulsed current of increasing magnitude (lower trace) and the corresponding membrane potentials (upper trace). The membrane resistance for sub-

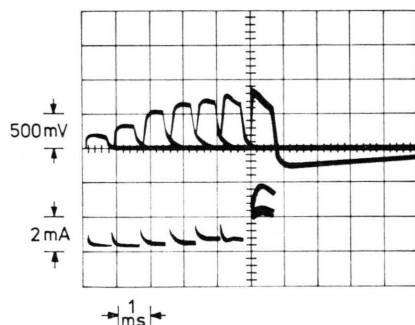


Fig. 2. An oscilloscope tracing of a series of current pulses of increasing magnitude (lower traces) and the corresponding membrane p.d. (upper traces) (intracellular potential positive with respect to the external sea water). The time between each successive current pulse was about 5 s. They were horizontally displaced by adjustment of the oscilloscope. Note the discontinuous (with p.d.), large and rapid increase in the current at a p.d. of $\sim 0.85 \text{ V}$. The last trace for the p.d. is actually a superposition of 4 traces, each corresponding to a larger current as shown in the corresponding lower traces.

critical current pulses for this cell was $\sim 460 \Omega \text{ cm}^2$. Note that at a critical potential of $\sim 0.85 \text{ V}$ the current increased dramatically. There was thus a very clear cut indication of a discontinuous decrease (with potential) of the electrical resistance.

For pulses of sufficient magnitude to take the membrane to the breakdown p.d., rapid (one per

second) repetition of such pulses resulted in a further increase in current without an increase in the membrane p.d., that is, the resistance decreased further. This is evident both from the oscilloscope tracing in Fig. 2 as well as the cumulative results for the p.d. as a function of (pulsed) current obtained for this cell (shown in Fig. 3).

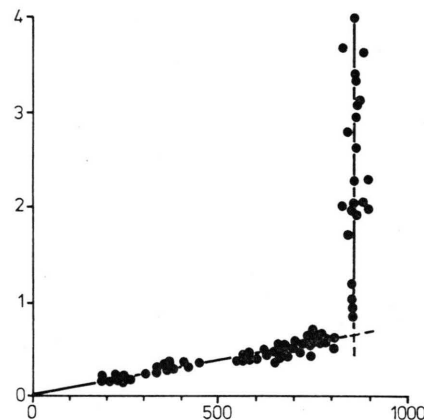


Fig. 3. The cumulative data of pulsed current as a function of membrane potential (inside with respect to outside) for 13 runs (such as that shown in Fig. 2) on a single cell. The sharp break in the I–V relation at $\sim 0.85 \text{ V}$ was observed in all cells examined. Below the electrical breakdown p.d. the I–V relation appeared quite linear; the membrane resistance for this cell being $\sim 1300 \Omega$ ($\equiv 460 \Omega \text{ cm}^2$ for this cell). 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.

An important feature of the breakdown phenomenon observed was that for a given cell, after a delay of the order of approximately 10 s, the process could be repeated (several times) with quantitatively identical results.

However, when the time between supracritical current pulses was very short, less than 10 s, the cells deteriorated rapidly. The membrane resistance then decreased to $30 \Omega \text{ cm}^2$ and no further electrical breakdown could be detected even at very large membrane p.d.'s and the membrane I–V characteristics became quite linear — see Fig. 4. However, often these cells still appeared healthy.

Following electrical breakdown, the membrane p.d., which usually had a value of +3 to +10 mV, often decreased by several millivolts, but recovered over a period of 10–20 min. If the cells were not subjected to further electrical breakdown during this recovery period, the electrical breakdown pro-

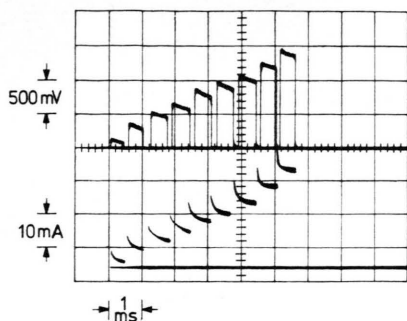


Fig. 4. An oscilloscope tracing of pulsed current, of increasing magnitude (lower traces), as a function of the membrane p.d. (upper traces) for a dead cell at the end of a 5 hour experiment. The cell now had a much lower membrane resistance (note the different current scale from that in Fig. 2). No electrical breakdown was observed.

cess could be repeated more often over a long period of time without deterioration of the cells.

The value of the critical breakdown potential observed in the present experiments was of similar magnitude to the calculated membrane potentials generated at dielectric breakdown of red blood cells, bacteria and algal cells using a Coulter Counter with hydrodynamic focussing. In the latter experiments mechanical effects caused by the hydrodynamic focussing forces, heating effects or punch-through effects⁴ could be definitely excluded.

The breakdown phenomenon here described is again qualitatively quite different from the punch-through effect observed in other algal cells^{5,6}. The punch-through effect is associated with ionic currents and changes in ion profiles in the membrane⁷.

Such processes have time constants of the order of 0.01 to 1 s. The latter is also evident from the fact that while at low frequencies (≤ 100 Hz) the membrane capacitance of cells, for example, 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 and the latter is then frequency independent⁸. In contrast, it is evident from Fig. 2 that the rise time for the breakdown current was of the order of $1 \mu\text{s}$ which rules out the possibility of ionic punch-through.

The possibility that breakdown occurred as a result of heating can also be ruled out by the following argument. There is a clear cut, discontinuous, increase in the current at the dielectric breakdown potential. However, the subcritical currents ($\sim 200 \mu\text{A}/\text{cm}^2$) could be of the same order of magnitude as the supracritical currents. The initial heating effects at breakdown are therefore also of the same order of magnitude as at such subcritical currents and the latter did not lead to damaging heating effects, irrespective of the pulse length used.

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¹ U. Zimmermann, J. Schultz, and G. Pilwat, *Biophys. J.* **13**, 1005 [1973].

² U. Zimmermann, G. Pilwat, and F. Riemann, *Membrane Transport of Plants* (U. Zimmermann and J. Dainty, eds.), p. 146, Springer, Heidelberg 1974.

³ U. Zimmermann, G. Pilwat, and F. Riemann, *Biophys. J.* **14**, 881 [1974].

⁴ U. Zimmermann, G. Pilwat, and F. Riemann, *Z. Naturforsch.* **29 c**, 304 [1974].

⁵ H. G. L. Coster, *Biophys. J.* **5**, 669 [1965].

⁶ H. G. L. Coster, *Aust. J. Biol. Sci.* **22**, 365 [1969].

⁷ H. G. L. Coster and A. B. Hope, *Aust. J. Biol. Sci.* **21**, 243 [1968].

⁸ H. G. L. Coster and J. R. Smith, *Membrane Transport in Plants* (U. Zimmermann and J. Dainty, eds.), p. 154, Springer, Heidelberg 1974.