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

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Dielectric Breakdown, Cell Membrane, *Valonia utricularis*

It is shown that if the membrane potential of cells of *Valonia utricularis* was increased rapidly by applying ~500 μs current pulses, dielectric breakdown of the membrane occured when the potential reached a value of ~0.85 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 (~10 s), many times on a single cell.

The rapidity of the breakdown process (~1 μs) 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 occured 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 platinum/iridium electrode and a AgCl coated silver wire coil surrounding the cell in the external solution (Mediterenean 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 μm to a tip of 1 μm and a shank of ~5 μm. This wire electrode was inserted longitudinally through a ~5 – 7 μm tip glass micropipette, previously inserted into the cell. The membrane potential was measured with the aid of two 2 NKCl filled glass micropipettes (tip

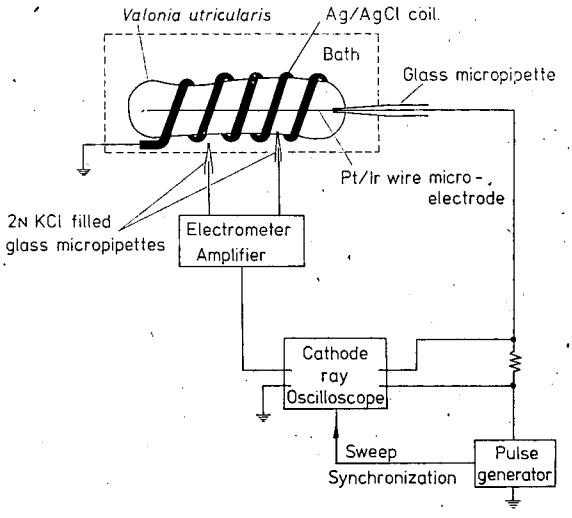

Fig. 1. A schematic diagram of the experimental set-up used in the dielectric breakdown experiments. For explanation see text.

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diameter $\sim 1\,\mu$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 $100\,\Omega$ 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-

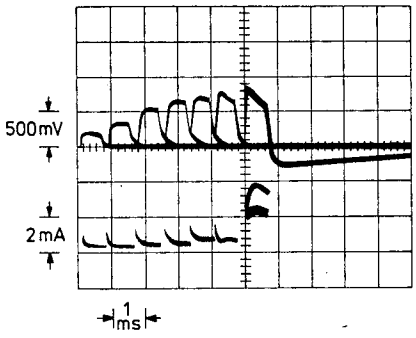

Fig. 2. An oscilloscope tracing of a series of current pulses of increasing magnitude (lower traces) and the corresponding membrane p.d. (upper traces) (intracellular potential positive with respect to the external sea water). The time between each successive current pulse was about 5 s. They were horizontally displaced by adjustment of the oscilloscope. Note the discontinuous (with p.d.), large and rapid increase in the current at a p.d. of ~0.85 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 $\sim 460\,\Omega$ cm^2. Note that at a critical potential of ~ 0.85 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) repitition 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).

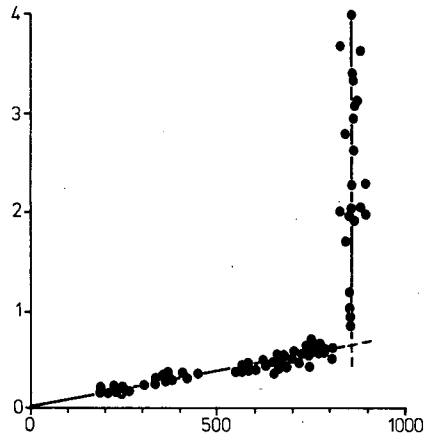

Fig. 3. The cumulative data of pulsed current as a function of membrane potential (inside with respect to outside) for 13 runs (such as that shown in Fig. 2) on a single cell. The sharp break in the I–V relation at ~0.85 V was observed in all cells examined. Below the electrical breakdown p.d. the I–V relation appeared quite linear; the membrane resistance for this cell being $\sim 1300\,\Omega$ ($\equiv 460\,\Omega$ 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 then 10 s, the cells deteriorated rapidly. The membrane resistance then decreased to $30\,\Omega$ 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-

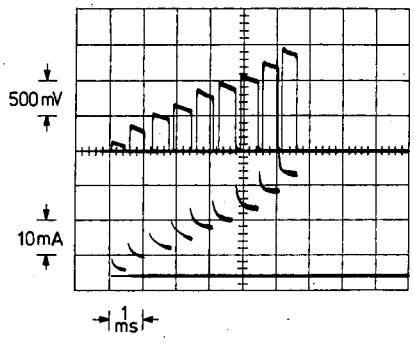

Fig. 4. An oscilloscope tracing of pulsed current, of increasing magnitude (lower traces), as a function of the membrane p.d. (upper traces) for a dead cell at the end of a 5 hour experiment. The cell now had a much lower membrane resistance (note the different current scale from that in Fig. 2). No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

The breakdown phenomenon here described is again quilitatively 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 membrane [7]. 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 ($\lesssim$100 Hz) the membrane capacitance of cells, for example, the alga *Chara corallina*, is strongly dependent on frequency, at high frequencies ($>$200 Hz) the contribution of diffusion polarization to the membrane capacitance is negligible and the latter is then frequency independent [8]. In contrast, it is evident from Fig. 2 that the rise time for the breakdown current was of the order of 1 μs which rules out the possibility of ionic punch-through.

The possibility that breakdown occured 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 μA/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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Irrtümer beim Maschineschreiben und ihr Hinweis auf Systemeigenschaften zentraler Entscheidungsmechanismen

Errors in Type-writing and Their Indications for Special Characters of the Data Processing Controlling the Typing Process

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Errors in Writing, Neural Data Processing, Short Term Memory, Motor Programming, Response Time

Twenty students having a comparable typing skill and speed (about 150 strokes per min) performed series of typewritings with contents well familiar to them. These writings were examined for errors (error = deviation from a given program of stroke sequences).

The errors could not be caused by ingnorance of writing or spelling of the words. Two categories of errors ("exchanges" = "Verwechslungen" and "omissions" = "Auslassungen") were explained to result from special "faults" in the data processing controlling the typing process (Fig. 3). The occurrence of these types of errors dependend on interactions of the following factors:

1. Preferences of succession which (in general) related the first letter of an actual letter-combination with the second one (also) in contrary succession ($i \rightleftharpoons e$) or with other letters, not occurring in the combination ($n \rightarrow d$; $n \rightarrow g$) (Tab. I).
2. Tendencies of the particular letters of an actual sequence of letters (= word) to occur already before and/or after the right sequential position (sequential interval of this effect $\leqq 4$ strokes; temporat interval: about 1 sec; Figs 1, 2).
3. Correspondences in particular characteristics of special letters (vowel-vowel-exchange, etc.).
4. The notice to type as quickly as possible.

Interactions of these factors resulted in erroneous "anticipations" and "postpositions" of letters.

Die Analyse von Abfolgen motorischer Muster (Verhaltensmuster) kann Hinweise liefern auf die Arbeitsweise der zentralen Mechanismen, die diese Abfolgen steuern [1, 2]. Die erfolgreiche Durchführung derartiger Analysen setzt die Vergleichbarkeit der Muster voraus und erfordert dabei deren verläßliche Quantifizierbarkeit. Beide Forderungen lassen sich gut erfüllen: Zum einen für Verhaltensmuster, deren Parameter sich akustisch messen lassen (Lautäußerungen verschiedener Art) [3–5], zum anderen für Muster aus hand- und maschinengeschriebenen Zeichen, wie sie vor allem unter der Frage nach typischen „Musterfehlern" bereits mehrfach untersucht wurden [6–10].

An der Übertragung von beliebigen, aber sinnvollen und verständlichen Buchstaben- und Wortfolgen in einen maschinengeschriebenen Text sind notwendig eine Vielzahl von Prozessen beteiligt. In diese gehen die Systemeigenschaften der schreibenden Person und der Maschine mit ein. Einige der Systemeigenschaften bedingen das Vorkommen charakteristischer Irrtümer. Nachstehend beschriebene Untersuchungen sind ein erster Versuch, von bestimmten Irrtümern (hier auch „Tippfehler" genannt) auf Eigenschaften jener Systeme zurückzuschließen, die Grundlage der zentralen Erzeugung und Durchsetzung von „Tippkommandos" sind.

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Methodik

Aus einer größeren Zahl von Personen wurden 20 nach Schreibtechnik (Finger-Buchstabenlage) und Tippgeschwindigkeit (ca. 150 ± 50 Anschläge pro min) vergleichbare Versuchspersonen ausgewählt. Diese schrieben dann auf der gleichen Maschine und jeweils mehrfach einen 35-zeiligen Text, der aufgrund von Voruntersuchungen zusammengestellt war. Die Versuchspersonen hatten die Anweisung, möglichst schnell zu tippen. Sie benötigten für die 35 Zeilen 5 bis 15 min. Ihre Zeitunterschiede ergaben sich durch Pausen, die sie zwischen einigen Sätzen und Absätzen machten. Die Rohauswertung der Texte sowie zusätzlich anderer Maschinenmanuskripte der Versuchspersonen hatte I. Hülswitt übernommen.

Zur Bewertung der Fehler und ihrer Abhängigkeiten wurde für jeden einzelnen Fehlertyp eine gesonderte Matrix für Beobachtungswerte aufgestellt. Und zwar nach dem Muster: