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A Study of Dielectric Membrane Breakdown in the *Fucus* Egg

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Summary. Unfertilized eggs and zygotes of the marine brown alga, *Fucus serratus,* have been subjected to single external electric field pulses of 1 to 1760 μsec duration (τ_p) and 50 to 400 V field strength ($U_p cm^{-1}$). During exposure, the difference in electric potential across the plasmalemma (V_m) was recorded intracellularly from single eggs, and the efflux of $^{86}Rb^+(K^+)$ from the cytoplasm was measured on egg populations. A given single pulse instantaneously depolarizes the plasmalemma by a few (i.e., 6) millivolts and releases a certain fraction (i.e., 5%) of the cytoplasmic $^{86}Rb^+(K^+)$. The dependence of these responses upon U_p and τ_p is fully consistent with the assumption that the membrane undergoes a localized reversible dielectric breakdown and reseals within <3 sec. The data are treated in terms of the electro-mechanical model for a compressible membrane by H.G.L. Coster and U. Zimmermann (1975, *J. Membrane Biol.* **22**:73) and verify this model on a nonvacuolated plant cell. A threshold V_m for membrane breakdown (V_c) of 0.58 and 0.51 V is estimated for the turgorless unfertilized eggs and the turgescent (4.8 bar) zygotes, respectively. Using these values for V_c, and a reasonable value of the membrane's elastic modulus (i.e., $Y_m \approx 10^6$ Nm^{-2}), possible sites of membrane breakdown are dicussed in terms of membrane thickness and relative permittivity.

In the past years evidence has accumulated that the cell membrane is a compressible structure. Experiments are guided especially by an electro-mechanical model advanced by Zimmermann, Coster, and coworkers [6, 21–24]. It postulates that there exists a dimensional equilibrium of the membrane

$$P_T + P_e + P_m = 0 \tag{1}$$

where the compressive forces due to the turgor pressure, P_T, and the electric membrane potential difference, P_e, are balanced by a mechanical restoring force, P_m, arising from the strained elastic membrane components. The physiological significance of this model is that it provides a plausible mechanism for the ability of a plant cell to sense and regulate its turgor, i.e., the difference in hydrostatic pressure across its outer

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cell membrane (plasmalemma) [7]. The following set of equations has been developed to treat dielectric membrane breakdown experiments in terms of the electro-mechanical model [7, 22, 24].

The electric compressive force per unit area of membrane P_e is related to the membrane potential V_m and thickness of the stressed membrane δ by

$$P_e = \varepsilon_r \, \varepsilon_o \, V_m^2 / 2\delta^2 \tag{2}$$

where ε_o is the electric permittivity of free space and ε_r the relative permittivity (dielectric constant) of the membrane material at the breakdown site. The restoring force P_m is given by

$$P_m = Y_m \cdot \ln (\delta/\delta_o) \tag{3}$$

where Y_m is the membrane's elastic modulus defined as the rate at which the membrane thickness δ decreases with the imposed compressive forces. δ_o is the thickness of the unstressed membrane when V_m, hence P_e, is zero.

At a given constant turgor pressure Eqs. (1)–(3) yield that in equilibrium, $P_e = -P_m$, and hence

$$\varepsilon_r \cdot \varepsilon_o \cdot V_m^2 / 2\delta^2 = -Y_m \cdot \ln (\delta/\delta_o). \tag{4}$$

Evidently, when δ becomes sufficiently small with increasing membrane compression, P_e will increase more rapidly with $1/\delta^2$ than P_m with the logarithmic term in Eq. (4). Explicitly, the instability condition is met when a critical δ value is reached where

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

The critical V_m for electro-mechanical membrane breakdown, V_c, may be obtained from Eqs. (2)–(5):

$$V_c = 0.606 \cdot \delta_o \cdot [Y_m \cdot (\varepsilon_r \cdot \varepsilon_o)^{-1}]^{\frac{1}{2}} \quad \text{at } P_T = 0. \tag{6}$$

It should be kept in mind that Y_m is taken to be independent of δ (ideal elastic material), and secondly, that ε_r does not take into account changes in the permittivity of a given membrane site normal to the plane of the membrane. At present, V_c is the only experimentally accessible parameter of Eq. (6). An estimate of Y_m is available from the dependence of V_c upon P_T [22].

Substantial evidence supporting and specifying the electro-mechanical model has been obtained from the marine coenocytic alga, *Valonia utricularis* [7, 21–23]. However, V_c measurements on the vacuolated *Valonia*

coenocyte are subjected to a major uncertainty, because the peripheral cytoplasmic layer is bounded by two membranes, the plasmalemma facing the cell wall and the tonoplast facing the vacuole. Therefore, membrane potentials can be measured only across both membranes [22]. Consequently, we deemed it useful to apply the dielectric breakdown approach to a nonvacuolated plant cell, namely, to the egg of the marine brown alga, *Fucus serratus*. This and some other fucacean eggs have been studied for decades with respect to cell morphogenesis [11, 14]. The present study focuses upon three developmental stages of the *Fucus serratus* egg, the unfertilized egg (*I*), the 1–3 hr old zygote (*II*), and the 5–8 hr old zygote (*III*). With respect to the present study, e.g., to Eq. (1), these stages might be characterized as follows [3, 19, 20]: In stages *I* and *II*, the egg still lacks a cell wall and hence a turgor ($P_T = 0$); the plasmalemma displays a V_m of -20 to -35 mV and no K^+/Na^+ selectivity. In stage *III*, V_m is about -55 to -75 mV, and the membrane has developed toward a K^+ electrode; a net uptake of mainly KCl has increased the internal osmotic pressure in the now walled zygote by about 0.2 osmol [1], creating a turgor of 4.8 bar. Since most data presented below concern the stages *I* and *III*, the terms "eggs" and "zygotes" will refer to these stages unless it is specified otherwise.

In this study, dielectric membrane breakdown will be diagnosed by intracellular recording of V_m and by following up during efflux experiments the release of $^{86}Rb^+(K^+)$ by *Fucus serratus* egg populations subjected to a single or trains of externally applied electric fields of variable amplitude and duration.

Materials and Methods

Obtaining Fucus Eggs

Fertile fronds of the seaweed *Fucus serratus* were collected at Helgoland/Nordsee. Eggs were obtained, fertilized, and cultured as described earlier [2]. The mean egg radius r was 34 μm. The experimental medium was artificial seawater of pH 7.9 of the following composition (in mM): 495 NaCl, 27.6 $MgSO_4 \times 7\,H_2O$, 24.9 $MgCl_2 \times 2\,H_2O$, 10 $CaCl_2 \times 2\,H_2O$, 9.7 KCl, 2 $NaHCO_3$, 10 Tris/HCl. The K^+ concentration was varied by isoosmolar changes of KCl and NaCl. The temperature was 15 °C, except for the membrane potential measurements which were carried out at 21–23 °C.

The Pulse Discharge Chamber

In order to subject populations of *Fucus* eggs to external electric fields, the electrolytic pulse discharge of Zimmermann *et al.* [23] has been used. Fig. 1A, B shows the chamber

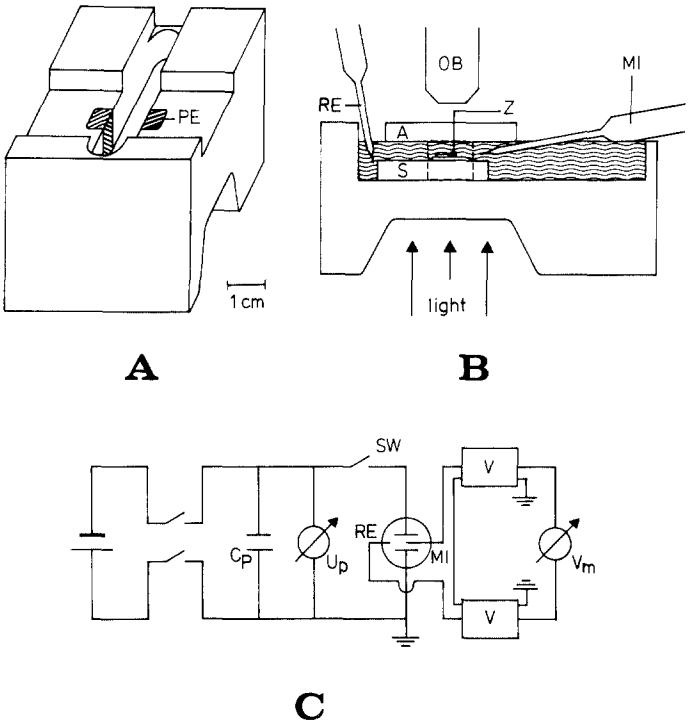

Fig. 1. Apparatus (*A, B*) and circuit diagram (*C*) for the application of electrical pulses to, and simultaneous recording of the membrane potential from *Fucus* eggs. *See* text for description

used for the membrane potential measurements: In a plexiglass chamber, two 1-cm distant Pt-black-coated brass electrodes (PE) provide an electric field, E_o (V cm^{-1}) $\equiv U_p$(V), which is taken to be homogeneous at the site of the eggs (*Z*). The electric pulses (U_p) were generated by discharging through a switch (*SW*) a variable capacitor (C_p) which is recharged from a power supply (Fig. 1*C*). C_p discharges according to $\tau_p = C_p \cdot R_p$, where R_p is the ohmic resistance of the seawater column between the electrodes. From the resistivity of seawater, $\rho_a = 26\,\Omega$cm, and the dimensions of the seawater column, it follows that $R_p = 35\,\Omega$cm. The discharge time τ_p, after which U_p has dropped to $U_p/e = 0.37\,U_p$, is taken as pulse duration in this paper. E_o is received by the egg membrane only if τ_p is longer than $r \cdot c_m\,(\rho_a + 0.5\,\rho_c)$, with c_m membrane capacitance per cm^2 and ρ_c cytoplasmic resistivity in Ωcm [17]; that is, using pertinent values for the *Fucus* egg [13, 20], if $\tau_p > 0.7\,\mu$sec. In the present study τ_p ranged from 0.9 to 1760 µsec (*see* Fig. 4). The pertinent relationship, i.e., the Laplace-equation, is introduced below (Eq. (9)].

Membrane Potential Measurements

The cytoplasmic potential V_m was measured by the formerly developed technique of recording from single *Fucus* eggs [3]. The discharge chamber was mounted on a microscope stage (Laborlux II, Leitz). Figure 1*B* shows the Ag-AgCl type reference electrode (*RE*), and the microelectrode (*MI*) which was geared horizontally by a micromanipulator (Leitz).

The eggs (*Z*) were impaled on a supporting cover glass resting on a mobile plexiglas slide (*S*). Microscopical observation (*OB*) was possible through a plexiglas lid (*A*). The circuit for recording V_m by two amplifiers (*V*) is included in Fig. 1*C*. The voltage was displayed on a storage oscilloscope (Tektronix) and a chart recorder.

Tracer Flux Experiments

For pulse application during radio-tracer elution from *Fucus* egg populations, a chamber similar to that in Fig. 1*A* was developed. This flux chamber contained elongated electrodes (4 × 1 cm) and was shaped so that a laminar flow of seawater washed the eggs during elution. (Laminarity of flow was checked by microscopic observation of the distribution of neutral-red added to the flowing medium.) While the 5–8 hr old zygotes reliably adhere to a glass slide, unfertilized and freshly fertilized eggs had to be spread out on a nylon mesh of 15 μm width which provided sufficient flow resistance to the eggs. The nylon mesh also prevented the flowing medium from carrying eggs into the efflux vials. After each experiment the number of eggs was determined microscopically. The average population size was in the range from 100 to 1000 eggs. Radioactivity was assayed using a scintillator (Unisolve, Zinsser) by means of a Packard model 3385 counter. For further details of the employed methods, *see* ref. [9].

Results

Egg Development

One immediate test for the reversibility of the dielectric membrane breakdown comes from evidence that electric pulses sufficient to change V_m and ion fluxes do not impede the regular development of the zygote into the morphologically polarized germling. Figure 2 illustrates that this morphogenetic process, indicated by the percentage of developing

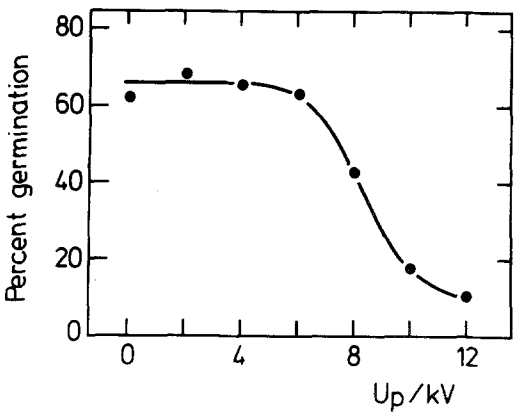

Fig. 2. Percentage of germination of populations of, in total 3100, 3-hr old zygotes of *Fucus serratus* subjected to a 36-μsec pulse of different amplitude, U_p. The electric field strength $E = U_p \text{cm}^{-1}$

cells of a given population is not affected by pulse amplitudes of up to about 6 kV, i.e., by a field strength of 6 kV cm^{-1}. This limiting value exceeds the U_p values used during this study of membrane breakdown by more than one order of magnitude.

Change of Membrane Potential

Figure 3 shows a typical recording of V_m on a *Fucus* zygote which has been subjected to single pulses ($\tau_p = 70$ μsec) of increasing amplitude. When $U_p > 150$ V, V_m suddenly decreases by a few millivolts and recovers within 20–30 sec. The oscilloscope reveals that this depolarization is established no later than 0.1 sec after the pulse. When U_p is stepped up, the triggered ΔV_m from a given egg usually appears at a certain value of U_p and does not increase further with U_p. The critical U_p value, U_c, is reproducible within ± 50 V on a given cell and ranges between 150 and 250 V for $\tau_p = 70$ μsec. Nevertheless, leakage artifacts from the microelectrode-cell system cannot be ruled out completely. For instance, V_m is occasionally two-phased indicating that leakage might occur during the first seconds after the pulse. In this case the slope at $t = 12$ sec was extrapolated to $t = 0$ of pulse application. The ΔV_m value thus obtained was comparable to the ΔV_m values from experiments such as in Fig. 3.

In Fig. 4 average values of ΔV_m from developing *Fucus* zygotes have been plotted as a function of the pulse amplitude for pulse durations from 0.9 to 1760 μsec. A significant ΔV_m is observed, independently of U_p, only for $\tau_p > 9$ μsec. The magnitude of ΔV_m, on the other hand,

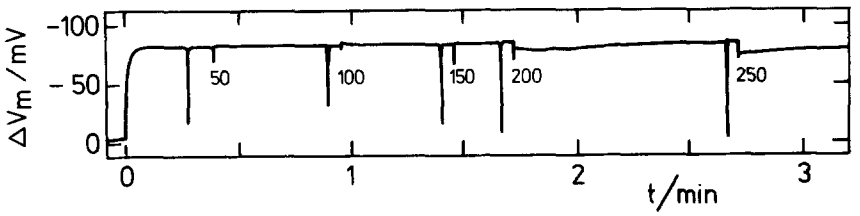

Fig. 3. Recording of the membrane potential, V_m, of a 10-hr old zygote of *Fucus serratus* subjected to 70-μsec pulses of different amplitudes, U_p, indicated by the numbers (in volts). The time of pulse application is indicated by the small vertical stroke above the numbers. The conspicuous large stroke preceding the response is an artifact from recharging the pulse capacitor (C_p in Fig. 1 *C*). The microelectrode was inserted at $t = 0$

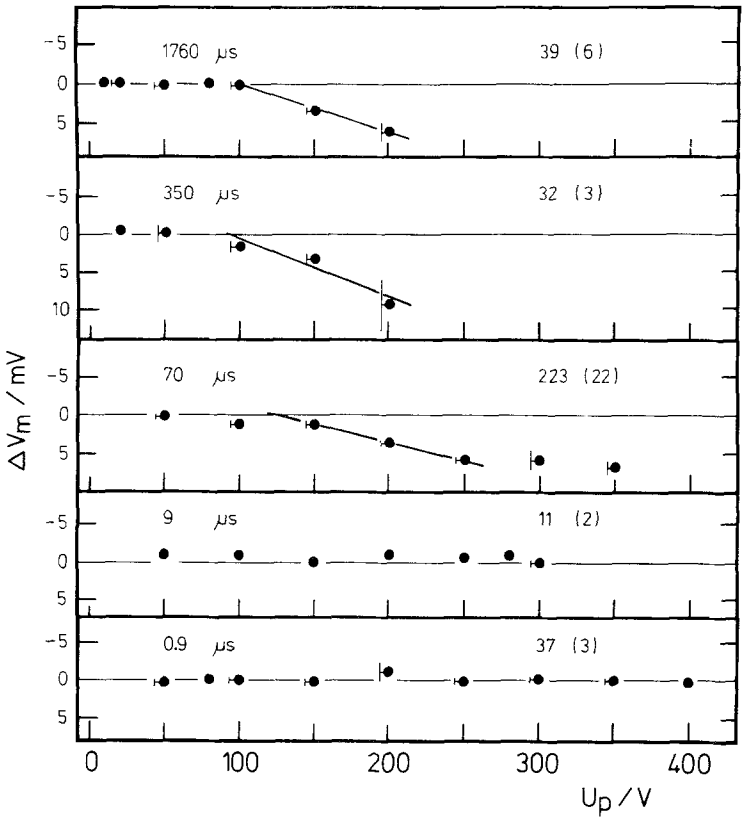

Fig. 4. Change of membrane potential, $\Delta V_m \pm$ SEM of zygotes of *Fucus serratus* as a function of the pulse amplitude, U_p, for five different durations given by the left-hand figures (in µsec). The right-hand figures give the total number of pulses (cells) for each case

averages 6 mV and is independent of τ_p. Another, more important feature is that U_c, i.e., the critical pulse amplitude just sufficient to elicit a ΔV_m, decreases from 150 to 100 V when τ_p is increased from 70 to 350 µsec. No further decrease of U_c is observed for $\tau_p = 1760$ µsec.

Since the applied electric field is superimposed upon V_m, a change of V_m through the K^+ concentration of the seawater should affect U_c; an increase of K^+ from 10 to 100 mM, for instance, depolarizes the plasmalemma of *Fucus serratus* zygotes (5–8 hr old) from -65 to -35 mV [3] and hence should increase the threshold pulse amplitude, U_c (*see* Eq. (9) below). Figure 5 is basically consistent with this presumption. A numerical evaluation will be postponed until the *Discussion*.

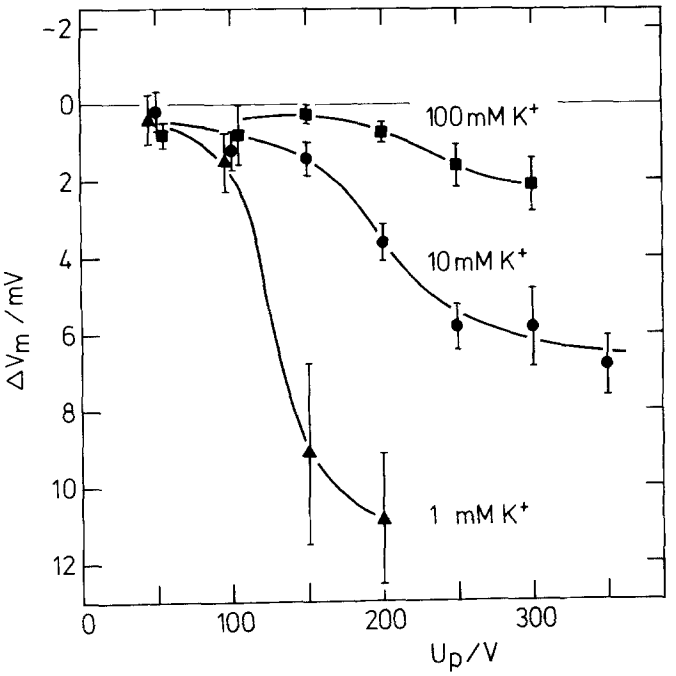

Fig. 5. Change of membrane potential, ΔV_m, of zygotes of *Fucus serratus* as a function of the pulse amplitude, U_p, in the presence of 1, 10, or 100 mM K^+ in the seawater. Pulse duration was 70 μsec, $\Delta V_m \pm$ SEM from 323 pulses on 31 cells

Release of $^{86}Rb^+$ (K^+)

A reversible dielectric membrane breakdown may be indicated by a transient release of intracellular ions driven out by an appropriate electrochemical driving force, and by the turgor if a bulk flow is assumed to pass the breakdown site [15]. The conventional efflux analysis [18] seems appropriate to detect a sudden release of intracellular radioactivity. The existence of only one intracellular $^{42}K^+$ flux compartment in *Pelvetia fastigiata* [16] is confirmed by our $^{86}Rb^+(K^+)$ efflux experiments on eggs and zygotes of *Fucus serratus*. During wash-out the cellular radioactivity, Q_c^*, declined according to

$$Q_c^* = I_c \cdot \exp(-k_c t) \tag{7}$$

where I_c is Q_c^* $(t=0)$ and k_c the rate constant. The wash-out kinetics, including the undisturbed periods of pulse experiments (Fig. 6), returned mean k_c values of $(2.8 \pm 1.0) \times 10^{-5}$ sec^{-1} and $(6.1 \pm 1.1) \times 10^{-5}$ sec^{-1}

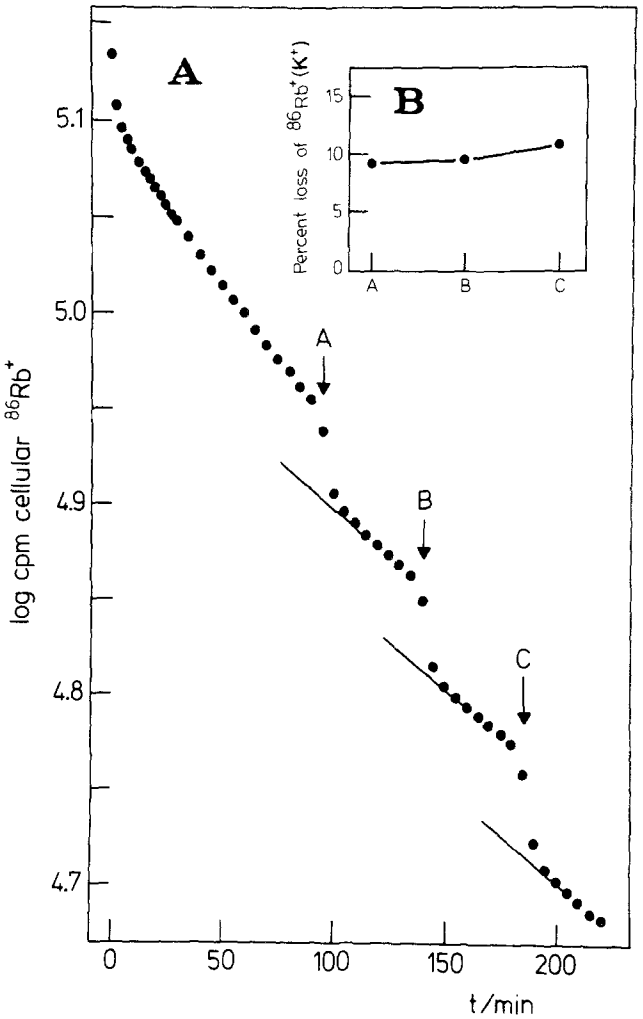

Fig. 6. (*A*): Efflux of $^{86}Rb^{+}(K^{+})$ from 600 zygotes of *Fucus serratus*. The zygotes were loaded from 1–6 hr, and tracer wash-out was started 6 hr after fertilization. At the times *A*, *B*, and *C* a 42-μsec pulse of 350 V was administered. (*B*): Loss of $^{86}Rb^{+}$ (K^{+}) after each pulse as a fraction of the radioactivity present in the cells just before the pulse

for the eggs and, respectively, zygotes. Plasmalemma fluxes, ϕ_{co} (mol $m^{-2}sec^{-1}$), may be obtained from

$$\phi_{co} = k_c \cdot Q_c \tag{8}$$

where Q_c (mol m^{-2}) is calculated from the total cellular K^{+}, Q_T (mM = mol m^{-3}), times $r/3$ (m), i.e., the volume-to-surface-ratio of the *Fucus* egg. Q_T values of 180 and 300 mM K^{+} have been assayed on the corre-

sponding developmental stages of *Pelvetia fastigiata* [1]. Then, using these Q_T values, ϕ_{co}^{K+} values of 57 and 207 nmol m^{-2} sec^{-1} follow from Eq. (8). The respective values from *Pelvetia* are 94 and 300 nmol m^{-2} sec^{-1} [1].

When during tracer wash-out a pulse sufficient to elicit a change of V_m is administered, a sudden increase of the rate of tracer loss occurs, as indicated by the inflection of the wash-out curve after the pulses

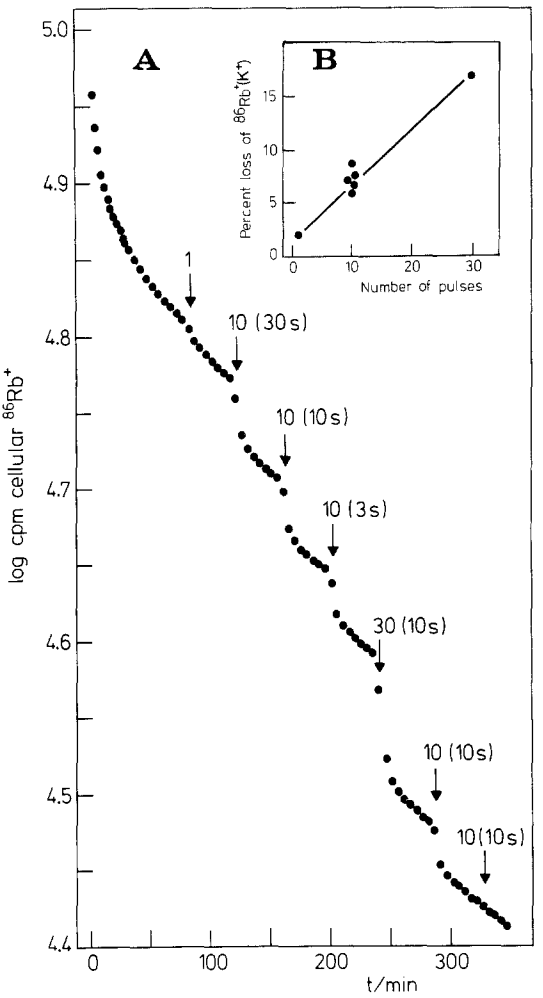

Fig. 7. (*A*): Efflux of $^{86}Rb^+(K^+)$ from 2100 unfertilized eggs of *Fucus serratus*. Tracer loading time was 18 hr. The arrows indicate seven periods of pulse application. Pulse duration was 42 μsec, the amplitude 350 V, except for the last period of 80 V. At each application, the pair of numbers denote the number of pulses and the interval (in seconds) between them, respectively. (*B*): Fractional loss of $^{86}Rb^+(K^+)$ *vs.* the pulse number calculated from the efflux kinetics of *A*

A, *B* and *C* in Fig. 6*A*. The extra loss of $^{86}Rb^+/K^+$ may be estimated from the 5-min samples after the pulse, which are above the extrapolated line of the steady-state rate k_c reestablished after the pulse. The sluggish return of the kinetics back to k_c is due to the low rate of exchange of the efflux chamber; this was checked by blank wash-out experiments. Thus the kinetics of the pulse-triggered ion release remains concealed. The relative loss of $^{86}Rb^+(K^+)$ per pulse is given by the ratio of released $^{86}Rb^+$ per cellular $^{86}Rb^+$ before the pulse. It is about 10% for each of the three pulses in Fig. 6*A*, as shown by Fig. 6*B*.

Another, more elaborate test for the assumption that the pulse-triggered ion release reflects a reversible and reproducibly sized dielectric

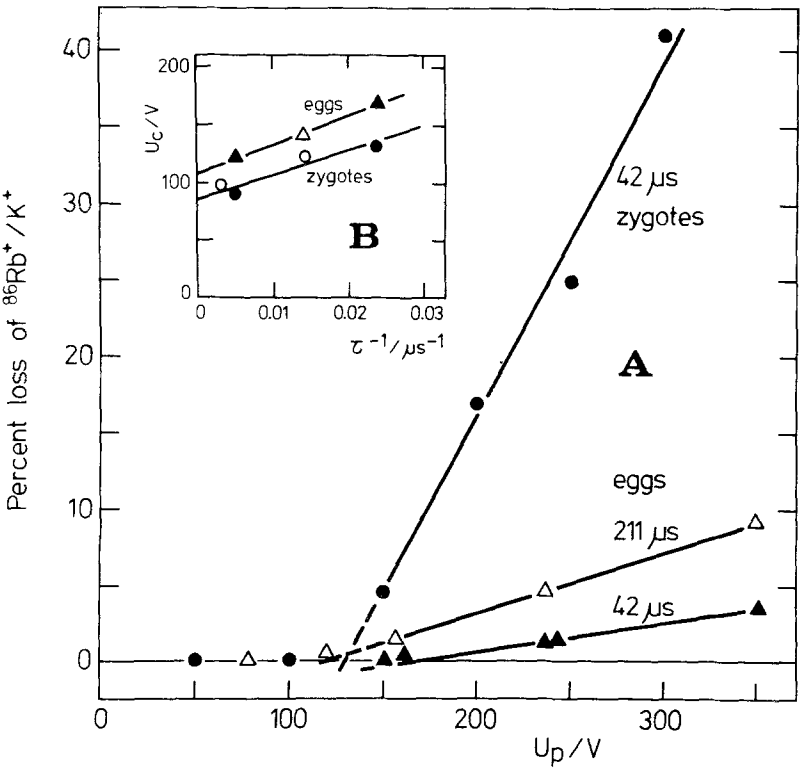

Fig. 8. (*A*): Fractional loss of $^{86}Rb^+(K^+)$ from 1300 zygotes and, respectively, 2700 unfertilized eggs of *Fucus serratus* as a function of the amplitude of a series of ten pulses each. Pulse duration τ_p was 42 µsec (zygotes), and 42 or 211 µsec (eggs), as indicated. (*B*): Critical pulse amplitude U_c as a function of the reciprocal τ_p for eggs and zygotes, respectively. The U_c values have been obtained by extrapolation to zero loss in Fig. 8*A* which holds 3 out of the 4 data given in Fig. 8*B*, and to zero ΔV_m from zygotes (Fig. 4) and eggs. The $^{86}Rb^+(K^+)$ loss and ΔV_m data in Fig. 8*B* have been plotted as filled and open symbols, respectively

membrane breakdown is illustrated by Fig. 7. This plot concerns unfertilized eggs which have been subjected to 1, 10, or 30 pulses with intervals between subsequent pulses of 3, 10, and 30 sec (Fig. 7*A*). The inset graph (Fig. 7*B*) shows the relative loss of $^{86}Rb^+(K^+)$ being proportional to the number of pulses, irrespective of the chosen intervals. Apparently, the membrane returns to its normal state within <3 sec after the pulse.

In order to assess the expected role of the membrane potential in the breakdown process, the pulse duration and amplitude was varied. The relative loss of $^{86}Rb^+(K^+)$ triggered by a series of ten pulses has been abstracted from wash-out kinetics like those in Figs. 6 and 7. The results have been plotted as a function of the pulse duration in Fig. 8*A*. The loss by the turgorless unfertilized eggs is obviously smaller than by already turgescent zygotes. It increases when τ_p is increased from 42 to 211 μsec (in Fig. 8*A* this is plotted for the unfertilized eggs only). Extrapolation to zero loss of $^{86}Rb^+(K^+)$ yields U_c values between 100 and 180 V. Figure 8*B* will be considered in the following paragraph.

The Critical Membrane Potential

A critical, threshold pulse amplitude, U_c, may be obtained by extrapolating to zero the ΔV_m and $^{86}Rb^+(K^+)$ loss data in Figs. 4 and 8. Figure 8*B* shows, then, that U_c depends upon τ_p. This is plausible when τ_p is not large compared with the membrane charging time, because a short pulse will already decay before it has been able to charge the membrane capacitance completely. Hence U_c increases as τ_p decreases. Outside the scale of, but consistent with Fig. 8*B* is the result of Fig. 4 that $U_c \gtrsim 300$ V for $\tau_p = 9$ μsec. From the hyperbolic relationship $U_c = a/\tau_p + b$ of Fig. 8*B* follows that $U_c(\tau_p \to \infty) = b = 107$ V holds for eggs, and $b = 87$ V for zygotes. (This hyperbolic function has been long known to describe the likewise τ_p-dependent depolarization to a threshold V_m required for excitable membranes to fire an action potential.) Although the difference between 107 and 87 V, due to the few data, may not be significant statistically, we tentatively calculate the critical membrane potential, V_c for both cases separately. The change in V_m of a spherical cell of radius r generated by an external electric field E_o is given by the well-known Laplace equation (*cf.* [13, 17, 23])

$$\Delta V_m = 1.5 \cdot r \cdot E_o. \tag{9}$$

Then, assuming a linear superposition of E_o and V_m, at breakdown threshold

$$V_c = V_m + 1.5 \cdot r \cdot U_c. \tag{10}$$

(Note that, according to Fig. 1, $E_o = U_p$ when E_o is given in V cm^{-1}).

Using the pertinent V_m values of *Fucus serratus* [3, 19, 20], $V_c = -(33+546) = -579$ mV for eggs, and $-(65+444) = -509$ mV for zygotes.

Discussion

What is the evidence that the observed changes in membrane potential and $^{86}Rb^+$ (K^+) exchange indeed are caused by those short-lived localized episodes of electro-mechanical instability of the plasmalemma predicted by the electro-mechanical model [6, 21–24]?

1) The amplitude of the applied electric field pulses is by orders of magnitude below the 8 kV cm^{-1} which is necessary to impede cell development (Fig. 2). We mention, on the other hand, that steady electric fields of only 0.1 to 1 V cm^{-1} are sensed by such cells, because they effectively orient the morphological polarity when applied during cell development [2, 6, 13].

2) The effective pulse duration seems to be sufficiently long compared with the membrane charging time, so that the applied electric field pulses last long enough to change V_m. Larger τ_p values, i.e., $\tau_p > 1$ msec, have been avoided in the present study (except for one experiment in Fig. 4), because they will substantially change the ionic profile within the membrane. This may lead to a membrane punch-through which is observed, for instance, in *Chara* [5] and *Valonia* [21]. In the fucacean *Pelvetia,* 5-msec pulses cause a drastic conductance increase, resembling punch-through, when the plasmalemma is hyperpolarized beyond -280 mV [19]. This figure is clearly below the above given V_c values. Similarly, in *Valonia utricularis* the V_c value measured across plasmalemma and tonoplast is about 0.8 V, whereas 20-sec pulses will not polarize these membranes beyond about ± 0.1 V [21].

3) The percentage of $^{86}Rb^+$ (K^+) released by a given pulse is fairly reproducible (Fig. 6), and a linear function of the pulse number, but independent of the interval between two consecutive pulses (Fig. 7). Obviously, the membrane reseals in a time < 3 sec. A resealing time < 5 sec has been observed on *Valonia* [7]. Figure 8 shows that the percentage of released $^{86}Rb^+$ (K^+) is also a linear function of the pulse amplitude.

However, a given single pulse releases about 10–14% of the intracellular $^{86}Rb^+(K^+)$ from zygotes, but only 1–6% from eggs. Apparently, the turgor pressure of the zygotes enhances the ionic release which presumably is a bulk flow. On the other hand, recently fertilized eggs show a significantly smaller loss than unfertilized eggs (not shown), although both stages do not differ in turgor pressure ($P_T = 0$) and membrane potential (*see* the introduction).

A loss of 14% causes a drop of the K^+ diffusion potential of about 4–5 mV. This drop is matched by the average ΔV_m in Figs. 3–5. ΔV_m and $^{86}Rb^+(K^+)$ loss data are also comparable with respect to the extrapolated U_c values (Fig. 8*B*). The difference in temperature, 15 *vs.* 22 °C, would decrease U_c from the ΔV_m data only by about 10% according to the data on *Valonia* [7], hence would stay within the statistical margin of the present results.

4) It is clear from Eq. (10) that the membrane breakdown potential V_c will be reached at a lower (higher) pulse amplitude U_c when V_m is raised (lowered). The result of Figure 5, therefore, appears plausible; numerically, however, this reasoning is not supported: since the mean K^+ dependent shift of V_m does not exceed 30–40 mV [3], the above estimated V_c value of -509 mV would be changed only insignificantly. The observed K^+ dependent shift of V_c, however, seems substantially larger: Despite obvious uncertainties in obtaining unambiguous U_c values, the K^+ dependent shift of U_c, i.e., of U_p ($V_m = 0$) is roughly 30%, as extrapolated from the steepest slope of the curves in Fig. 5. (A similarly large shift of U_c results, if one chooses the U_p for half-maximal ΔV_m as the measure of U_c.) At present, we cannot exclude that V_c is affected by the K^+ concentration of the seawater other than through V_m, hence by a mechanism outside the electro-mechanical model in its current form.

It is instructive to compare the V_c values of 0.51 and 0.58 V with the pertinent value of *Valonia utricularis*. In this vacuolated cell the membrane breakdown voltage across both plasmalemma and tonoplast averages 0.96 V [22]. Therefore, by analogy with *Fucus*, the V_c value of the individual membrane would be near 0.5 V, if both *Valonia* membranes are comparable in electrical resistance. On the other hand, the likewise vacuolated brackish-water alga *Ochromonas malhamensis* and red blood cells exhibit V_c values of 2.8 and 1.1 V, respectively [15, 24]. Roughly, then, cell membranes appear to feature V_c values from 0.5 to 1.5 V. Interestingly, a similar range of V_c values has been reported for lipid bilayer membranes [4].

The V_c values of *Fucus* may be plugged into Eq. (6), so that appropriate combinations of the elastic modulus Y_m, the relative permittivity ε_r, and the effective thickness δ of the plasmalemma may be considered. A value of Y_m of 10–20 bar ($1–2 \times 10^6$ N m^{-2}) would cover the two limiting cases where Eq.(6) is applicable; that is, dielectric breakdown might concern either a lipid bilayer region of $\varepsilon_r = 2–3$ and $\delta = 2.4–4$ nm [8, 12], and/or an integral protein (lipoprotein) of $\varepsilon_r \approx 5–10$ and $\delta = 7–9$ nm [10, 17]. In any case, the assumed Y_m value would cover both V_c values. On the other hand, it is not clear whether Y_m remains constant during egg development, because the virtual drop of V_c from 0.58 to 0.51 V is greater than follows from the concomitant increase of P_T from zero to 4.8 bar on the grounds of the electro-mechanical model; *cf.* [21].

We conclude that the plasmalemma of the nonvacuolated *Fucus* egg, which is an essential component of the morphogenetic machinery during early egg development [2, 11], may be subjected to reversible dielectric breakdown. The membrane breakdown voltage and the putative range of the elastic modulus are consistent with pertinent figures from vacuolated plant cells [6]. At present, no conclusion is offered concerning developmental changes of the electromechanical membrane properties.

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