J. Membrane Biol. 72, 75–84 (1983)

The Journal of
Membrane Biology

Relationship between Cell Turgor Pressure, Electrical Membrane Potential, and Chloride Efflux in *Acetabularia mediterranea*

Stephan Wendler, Ulrich Zimmermann and Friedrich-Wilhelm Bentrup*

Arbeitsgruppe Membranforschung am Institut für Medizin, Kernforschungsanlage Jülich, Postfach 1913, D-5170 Jülich, West Germany and *Institut für Allgemeine Botanik und Pflanzenphysiologie der Justus-Liebig-Universität, Senckenbergstrasse 17–21, D-6300 Giessen, West Germany

Summary. Turgor pressure P, electrical membrane potential V_m and release of $^{36}Cl^-$ have been measured on individual cells of the marine green alga *Acetabularia mediterranea*. In contrast to other marine and pond water algal cells a continuous turgor pressure increase is observed in many cells of *A. mediterranea* (0.8 to 5×10^{-4} bar sec^{-1}), even though the osmolarity of the external seawater is kept constant. Above a critical pressure value of 2.26 ± 0.22 bar ($n = 25$, $T = 18$ and 23 °C), the turgor pressure exhibits continuous decreases and increases in a range of 0.22 ± 0.13 bar ($n = 23$). At lower temperatures (6 to 10 °C) the threshold for pressure regulation is shifted towards higher values. Pressure regulations in the critical range are accompanied by bursts of ^{36}Cl-efflux and in most cases by action potentials, but not every action potential is associated with a decrease in turgor pressure. The frequency of spontaneous action potentials increases with rising cell turgor. The amount of ^{36}Cl-released, i.e. about 4% of the cellular Cl^- content (H. Mummert, Ph.D. Thesis, University of Tübingen, Germany, 1979), compares well with the observed reduction in turgor pressure after the burst. The kinetics of turgor reduction, however, are not compatible with a release of chloride by vesicular transport as suggested by H. Mummert and D. Gradmann (*In*: Plant Membrane Transport: Current Conceptual Issues. Elsevier/North-Holland, Amsterdam, 1980). It is concluded that the steady turgor increase is generated by a net influx consisting largely of KCl due to the electrogenic chloride import pump and the strong inward driving force for K^+ whereas the subsequent occurring regulations of turgor pressure can be understood on the basis of a passive chloride channel in the plasmalemma which is controlled by turgor.

Key words turgor pressure · action potential · chloride efflux · intrinsic regulations · *Acetabularia mediterranea*

Introduction

Giant algal cells are ideal objects for the study of cell-membrane properties and transport mechanisms involved in the regulation of internal ion composition and turgor pressure [21–23, 28, 29]. In the fresh-water alga *Chara corallina*, the action potential is reported to be accompanied by a change in turgor pressure [1, 2]. Furthermore, in this species, action potentials can be triggered by applied changes in turgor pressure [24].

In the study reported here, we applied the pressure-probe technique to another giant alga, the marine green alga *Acetabularia mediterranea*. This alga exhibits various temporary electrical and ionic events, namely metabolic action potentials and large electrically silent outbursts of chloride [9; the relevant literature is cited in this review]. Nuccitelli and Jaffe [17] and later Gradmann and Mummert [9] speculated that the chloride outbursts could be involved in adjustment of turgor pressure. In order to test this hypothesis and reveal possible causal relationships between turgor pressure and the temporary ionic events in question, the turgor pressure P, the electrical membrane potential V_m and the release of ^{36}Cl-were recorded continuously on individual cells of *A. mediterranea*. In most experiments, two of these parameters were measured simultaneously. In addition, sudden changes in turgor pressure were brought about by the pressure probe, and the corresponding changes in pressure and membrane potential recorded.

Materials and Methods

Acetabularia mediterranea were kept in a 12-h light/dark regime (3000 lux, crypton 100 W lamp or Osram Fluora) at 20 °C. Sometimes, a suboptimal illumination was used to reduce cap formation, while elongation growth continued [19]. Erdschreiber medium was used for cultivation. The total osmolality was 1050 mosmol/kg. The majority of cells used had not yet formed a cap (cell volume 0.4 to 4.3 µl and 12.3 µl). Measurements were performed in artificial seawater (ASW) (for composition, *see* [13]).

Changes in the external osmolarity were brought about by the addition of the appropriate amounts of distilled water, distilled water plus 10 mM/liter KCl and 10 mM/liter $CaCl_2$, or ASW containing sucrose or NaCl.

The turgor pressure was measured and manipulated by means of both the nonminiaturized and the miniaturized pressure probe [28, 29]. The volumetric elastic modulus of the cell wall ε, and the hydraulic conductivity of the membrane Lp were determined with the aid of the pressure probe; the tech-

nique is described elsewhere [28]. The volumetric elastic modulus ε for *A. mediterranea* is found to range from 14 to 45 bar for a turgor pressure of 2 bar. This value is comparable to the range of values measured for cells of *Halicystis parvula* [11, 25], the range recorded in other giant algal cells being in the order of several 100 bar [21, 22]. ε exhibits a linear dependence on turgor pressure in *A. mediterranea* over the whole observable pressure range ($\varepsilon = a \cdot P + b$; $a = 12.3 \pm 3.1$; $b = 7.4 \pm 2.9$ bar; independent measurements on 8 cells). A volume dependence of ε was not found.

The values for the hydraulic conductivity as determined from the half-times of turgor relaxations lie in a range of 0.1 to 1.5×10^{-6} cm bar^{-1} sec^{-1}. Values for exosmotic measurements were found to be slightly higher than for endosmotic experiments. The membrane potential was recorded with different electrode systems, depending on temperature. At room temperature (18 and 23 °C) conventional symmetrical Ag/AgCl electrodes were used; they were made pressure-tight in order to prevent a large drop in cell turgor following the insertion of the electrode. For this purpose, a silver wire was introduced into a glass tube about 2 cm long (internal diameter 1 mm) and glued into the end of the tube. The tube was subsequently filled with 3 M KCl solution containing agar, and the silver wire chloridized. A 2- to 3-cm long capillary tube (internal diameter about 0.5 mm, external diameter 1 mm) was filled with 3 M KCl, then introduced into the tube containing the silver wire and sealed in with a mixture of beeswax and colophony.

At low temperatures (6 to 10 °C) the presence of a voltage electrode, used in addition to the pressure probe, caused leaks since either the resealing properties of the membrane were apparently diminished at low temperatures or, due to the fixed positions of the inserted pressure probe and voltage electrode, a small contraction of the cell during cooling caused leaks. Therefore, membrane potential measurements at low temperatures were carried out with a chloridized silver wire inserted into the capillary of the pressure probe itself.

After the cells had been impaled by the pressure probe, a small amount of cell sap was sucked up with the aid of the metal rod in the apparatus, so that the tip of the chloridized silver wire was surrounded by cell sap, and the voltage of the silver wire was equal to the potential in the cell interior, i.e. to the vacuolar potential. The reference electrode was also a chloridized silver wire.

In order to measure the changes in the chloride flux during turgor pressure regulation, *Acetabularia* cells were loaded with ^{36}Cl-(100 µCi in 4 ml ASW, incubation time >100 hr). After the adsorbed radioactive isotope had been washed from the cell walls, the loaded cells were fixed in a channel and washed with "cold" seawater. Seawater was pumped into the channel at a velocity of about 1.2 ml (30 drops) per min, and was then collected in scintillation vials which were changed every 30 sec (sometimes every 60 sec). The amount of ^{36}Cl-washed out in this process was determined in a Tricarb liquid scintillation counter (Packard). At the same time, the cell turgor was recorded with the pressure probe. The cell turgor could be raised as desired by diluting the ASW with distilled water.

Results

In contrast to other giant algal cells and cells of higher plant tissues, the cell turgor of *A. mediterranea* only rarely remained constant over any length of time. Instead, a continuous increase in pressure of the order of 0.8 to 5×10^{-4} bar/sec ($T = 18$ and 23 °C) was observed in many cells,

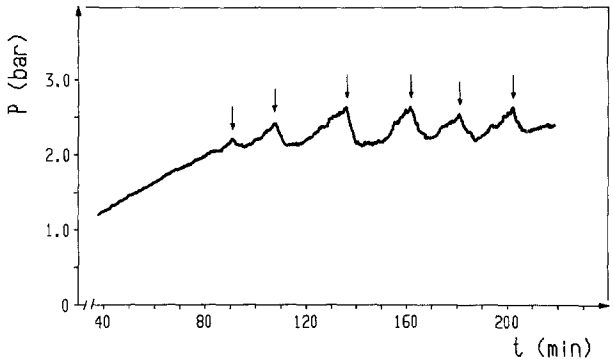

Fig. 1. Turgor pressure of *Acetabularia mediterranea*. At constant external osmolarity (seawater) the turgor pressure of this cell slowly increases intrinsically and is regulated between 2.2 and 2.6 bar at intervals from 16 to 28 min. The onsets of turgor regulation are denoted by arrows. The small disturbances also seen result from manual adjustment of the oil/sap boundary in the microcapillary and therefore are not part of a regulation step

even though the external osmotic pressure (seawater) remained constant. When the cell turgor reached a value between 2 and 2.8 bar, the cells began to regulate turgor pressure. The gradient of the pressure curve dP/dt changed spontaneously and took on a smaller, often even a negative value. Sometimes this type of pressure regulation was repeated until the turgor pressure had stabilized below the threshold value or in the critical pressure range. The threshold value which triggers this regulatory phenomenon was found to be 2.26 ± 0.22 bar (independent measurements on 25 cells). The average range of this apparent turgor regulation was 0.22 ± 0.13 bar (23 cells). A typical example of such a series of pressure regulations in *A. mediterranea* is shown in Fig. 1. An intrinsic increase in pressure is observed for the first 50 min ($t = 90$ min) and prior to each turgor regulation. In this particular cell the interval between the subsequent decreases of the turgor pressure is from 16 to 28 min.

Similar turgor pressure responses were observed when the cell turgor was raised by adding distilled water to the external seawater (*see*, e.g., Figs. 5 and 6).

In a second set of experiments turgor pressure and membrane potential were monitored simultaneously.

At room temperature, most cells had a resting potential of -179 ± 15 mV (independent measurements on 33 cells). The resting potential did not appear to be pressure-dependent, but varied randomly by about ± 5 mV in any given algal cell. The values for the resting potential quoted here were slightly more negative than the values reported in the literature (-169 ± 13 mV) [6]. Spon-

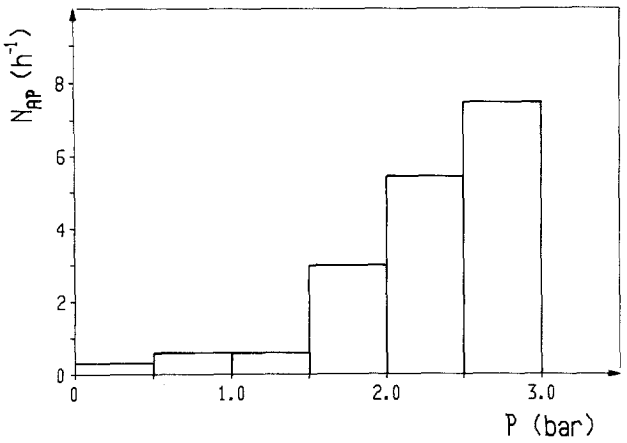

Fig. 2. Number of action potentials per hour N_{AP} in dependence on turgor pressure P plotted in 0.5-bar intervals (average values from independent measurements on five cells). Note that the values for P less than 1.5 bar might be underestimated, as the cells were subjected to this pressure range never longer than 45 min

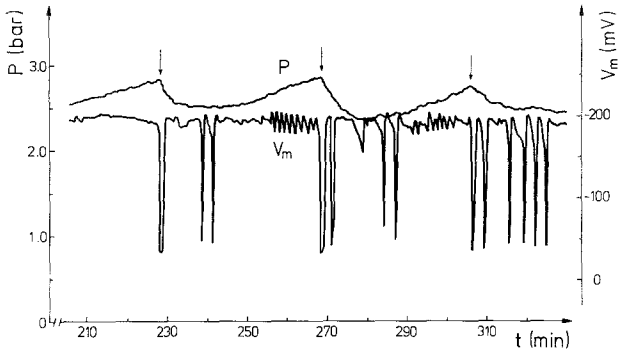

Fig. 3. Turgor pressure P and membrane potential V_m of a cell of *Acetabularia mediterranea* at constant osmolarity of the seawater. Turgor regulations and action potentials coincide, but not every action potential is accompanied by a turgor regulation. The onset of turgor regulation is denoted by an arrow

taneous action potentials were observed over the entire pressure range; small depolarizations of the membrane potential were also common. The frequency of spontaneous action potentials increased with rising cell turgor. Figure 2 shows the number of action potentials per hour as a function of cell turgor [*cf.* 13,16]. The values represent average values obtained from independent measurements on five cells. Note, that the frequency of action potentials for pressures below 1.5 bar might be underestimated, as the cells were not kept at these pressures for more than 45 min. Pressure regulations in the critical turgor range were in most cases accompanied by action potentials, but not every action potential was associated with a decrease in turgor pressure. Occasionally, pressure regulations were observed without a concomitant depolarization of the membrane. There was thus no strict correlation between the two phenomena. Figure 3 shows as a function of time the changes in pressure and the membrane potential in a cell in which each pressure change during the regulation phase was accompanied by an action potential. It is evident from Fig. 3 that further spontaneous action potentials occur between two pressure regulations which do not lead to any measurable changes in turgor pressure.

To answer the question whether action potentials are elicited by changes in turgor pressure or by the absolute turgor pressure itself, pressure pulses of different amplitude, duration and sign were injected into the cells with the aid of the pressure probe. The absolute turgor pressure was first decreased to values between 0.5 and 2 bar by increasing the osmolarity of the external medium, in order to prevent the critical turgor range from being exceeded by large positive pressure pulses which would then activate the regulatory mechanism of the cell. In contrast with analogous experiments on *Chara corallina* [24], the amplitudes of the pressure pulses were therefore limited to 0.2 to 1.0 bar. The duration of the pulses was varied between 8 and 180 sec, and at the same time, the pressure probe was used to maintain constant pressure. Neither negative (turgor pressure decrease) nor positive pressure pulses (turgor pressure increase) had a measurable influence on the membrane potential. Positive pressure pulses only elicited action potentials when their duration exceeded about 30 sec and when the cell turgor exceeded the critical threshold value during the pulse, i.e. when the amplitude of the pressure pulse was chosen to be sufficiently high. The action potential is thus not elicited by the pressure pulse as such but by the absolute value of the turgor.

In order to ascertain whether the turgor-pressure regulation depends on the magnitude of the membrane potential, both turgor pressure and potential were simultaneously recorded at 6 to 10 °C. Initially, the potential maintained a value corresponding to the resting potential at room temperature, but it could then usually be raised to a value between −60 and −40 mV by turning off the direct illumination of the cell [halogen-ellypsoid-reflector lamp (Philips) with light tube, filtered for the infrared, at a distance of about 2 cm from the cell]. This observation has been interpreted in terms of the electrogenic Cl^- pump which at temperatures below 10 °C ceases to pump Cl^- into the cell [6]. In Fig. 4 the external medium was cooled down to 7 °C and the potential stayed in the range between −75 and −40 mV as long as

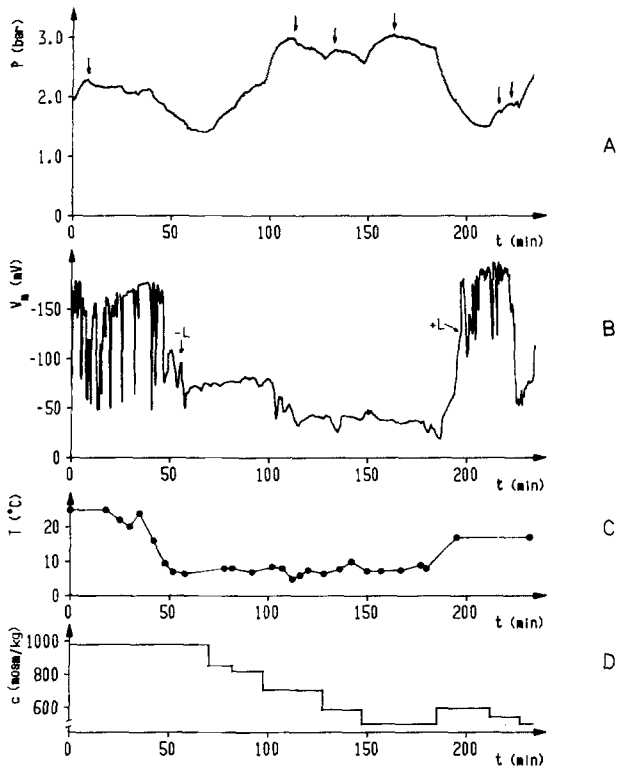

Fig. 4. Simultaneous recording of turgor pressure P (*A*) and membrane potential V_m (*B*) of a cell of *Acetabularia mediterranea* as well as of the temperature of the bathing solution T (*C*) and its osmolality c (*D*). Note, that the turgor regulation at low temperature occurs at a higher turgor pressure. Turgor pressure regulations at constant system parameters (temperature, osmolarity) are denoted by arrows. The pressure decrease (*A*), observed 50 min after insertion of the capillary into the cell, results from the temperature decrease. The turgor pressure was increased by decreasing the external osmolarity to cause turgor pressure regulations. The pressure decrease at $t=$ 180 min is due to the relatively small increase of the external osmolarity brought about by the addition of ASW. At the same time, the temperature is raised to 17 °C. The membrane potential shows spontaneous action potentials at room temperature and values between −40 and −70 mV at a temperature less than 10 °C. Switching off the direct illumination of the cell ($-L$) stabilizes the less negative membrane potential, while illumination at $t=195$ min ($+L$) leads to a rapid recovery of the resting potential

the temperature was lower than 10 °C. Avoiding direct illumination (Fig. 4*B*, -*L*) resulted in a stabilization of the membrane potential at less negative values. The cell turgor was then raised by adding distilled water to the external medium. Pressure regulation was initiated at a slightly higher turgor level than it would at room temperature, but it nevertheless proceeded in spite of the inactivated Cl^- pump and the low membrane potential. For the following reasons, there can be no doubt that a change of the internal osmolality must have occurred, when the external osmolality was reduced from about 1 osmol/kg to about 0.5 osmol/kg. The change in external osmolarity corresponds to a change in osmotic pressure of $\Delta\pi \simeq 12.5$ bar. Assuming values of $\varepsilon = 25$ bar and $\pi_o = 27$ bar for the volumetric elastic modulus and the internal osmotic pressure of the cell, the turgor pressure should have increased by $\varepsilon \cdot \Delta\pi/(\varepsilon + \pi_o) \simeq 6$ bar. A final turgor of about 8 bar was therefore expected, but was found to be less than 3 bar. Therefore, the internal osmolarity must have changed dramatically. The recovery to normal behavior of the cell in Fig. 4 was tested by warming it to room temperature. The resting potential returned to its normal level of −180 mV, and the pressure regulations were also seen to be normal. The results of Fig. 4 were repeated in experiments on two more cells.

Simultaneous measurements of chloride release and the turgor pressure in the critical pressure range (Fig. 5) revealed a clear correlation between the beginning of a turgor pressure regulation and an increased chloride efflux. The increase in the Cl^- efflux could be demonstrated over two measurement intervals, each lasting 30 sec. Measurements on 6 cells with a total of 22 pressure regulations showed that the Cl^- burst and pressure regulation always coincide.

During the Cl^- burst, the Cl^- efflux was 2 to 6 times higher than the steady-state efflux level. There is a large scattering of the values for the steady-state Cl^- efflux given in the literature, spanning a range from 2 to 20 μmol m^{-2} sec^{-1} [7, 9, 10, 14, 19]. Assuming a steady-state efflux of 10 μmol m^{-2} sec^{-1}, the Cl^- efflux reached values of up to 60 μmol m^{-2} sec^{-1} during the bursts, in agreement with Mummert and Gradmann [14]. Assuming that the time required for Cl^- extrusion is less than the interval of measurement (30 sec), the flux could be even higher.

Figures 5 and 6 show analogous experiments on two cells where the turgor pressure was raised to the critical pressure range by adding distilled water to the seawater. Obviously, the regulatory mechanism underlying the periodic changes in turgor pressure is sufficient not only to stop the turgor from rising any further but also to reduce it. In Fig. 5 the $^{36}Cl^-$ release and in Fig. 6 the membrane potential are recorded together with the cell turgor. It is evident that the pressure regulations of the cells are accompanied by both action potentials and Cl^- bursts. The latter probably are the primary mechanism of turgor regulation in the critical pressure range. This is further supported by the finding that Cl^- bursts only occur for a period of about 30 to 60 sec and are then succeeded by a turgor relaxation lasting a total of 5 to 10 min (Fig. 5).

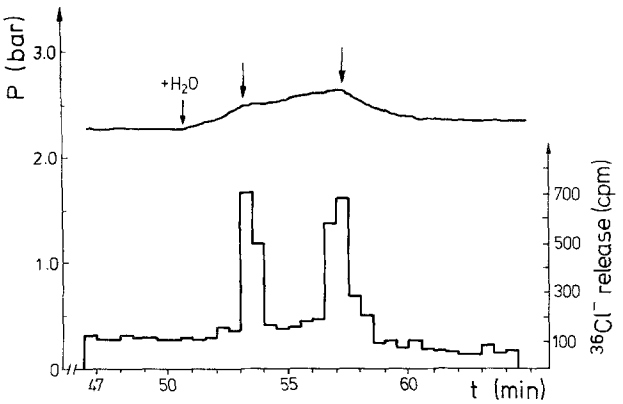

Fig. 5. Relationship between turgor pressure and Cl^- efflux bursts of a cell of *Acetabularia mediterranea* in response to a change in the external osmolarity. The turgor is increased by adding water to the bathing solution. The subsequent turgor regulations (arrows) are accompanied by Cl^- bursts, i.e. an increase of the Cl^- efflux by a factor of nearly 6 for a period of 60 sec. The first regulation results in a change of the rate of turgor pressure increase, the second regulation in a reduction of the turgor pressure (for further explanation, *see* text)

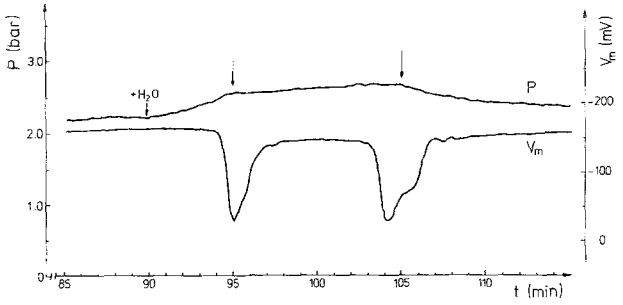

Fig. 6. Changes in turgor pressure P and membrane potential V_m of a cell of *Acetabularia mediterranea* in response to a change in the external osmolarity. The turgor pressure is raised by adding water to the bathing solution. The first regulation results in a change of the rate of turgor pressure increase, the second regulation in a reduction of turgor pressure. Each of the subsequent turgor regulations (arrows) is accompanied by an action potential

Discussion

Like other algal cells, *Acetabularia* has to create sufficient cell turgor for growth and at the same time must avoid bursting, i.e. it has to regulate the osmotic pressure difference between the cell interior and the outside medium, seawater. Another problem arises from the unusually low membrane potential of *A. mediterranea*, $V_m = -180$ mV, which is about 90 mV more negative than the K^+ equilibrium potential E_K. No other marine plant or animal cell is known to have a membrane potential which is significantly lower than E_K. In *A. mediterranea*, V_m is presumably generated by an electrogenic chloride import pump (ATPase). This would account for the intracellular accumulation of chloride and, by electrical coupling, of K^+ [6, 8, 15]. This unique property of *Acetabularia* seems to involve a complex array of the following temporary events:

The action potential occurs spontaneously and may be triggered electrically or by "light-off" signals. It is conventional insofar as the underlying ionic events are voltage-controlled temporary effluxes of Cl^- and K^+ of about 10 μmol m^{-2} sec^{-1} each [9]. The phenomenon is linked to the energy metabolism of the cell, and as a rule, the spontaneous frequency of the action potential is sufficient for the cell to periodically reset its intracellular K^+ concentration which would otherwise rise because of the large K^+ driving force, $V_m - E_K$ [13]. This regulation of the intracellular K^+ relies upon the fact that during the peak of the action potential, V_m undershoots E_K, and the K^+ conductance rises dramatically so that there is a large K^+ efflux during this short period of about 30 sec.

Gradmann et al. [10] have shown that spontaneous action potentials and Cl^- bursts may occur side by side. While the turgor pressure was not controlled in their experiments, the present study reveals that turgor pressure regulations are associated with the ionic and electrical events. In fact, the absolute value of the turgor pressure seems to be of primary importance for the regulation of transport across the membrane [17].

Electrically neutral Cl^- bursts with fluxes of up to 60 μmol m^{-2} sec^{-1} [12] are shown in the present study to occur mainly in the pressure range between 2 and 2.8 bar. They are strictly correlated with the changes in turgor and less strictly with the occurrence of action potentials. Occasionally pressure regulations without the occurrence of an action potential have been observed. Experiments carried out at temperatures of 6 to 10 °C in which turgor regulation was maintained even though the membrane potential rose to −40 to −60 mV, have demonstrated that action potentials are not necessary for the initiation of turgor regulation nor in turn, for electrically neutral extrusion of Cl^- together with its counter-ion. Hence we must be careful to distinguish between these two apparently independent processes.

Beside the turgor pressure regulations (*see below*), in many measurements of the cell turgor, a slow and steady turgor-pressure increase has been observed corresponding to a rate of change in turgor pressure of 0.8 to 5×10^{-4} bar sec^{-1}. A plausible reason for this would be an imbalance between the influx and efflux of solutes. If the

Table 1. Calculation of the solute flux J_s during intrinsic pressure increase (dP/dt = rate of change of turgor pressure)

Cell no.	Volume/area $\times 10^3$ (cm)	$(dP/dt) \times 10^4$ (bar sec^{-1})	J_s ($\mu mol\ m^{-2}\ sec^{-1}$)
1	8.3	1.0	0.34
2	8.5	2.2	0.77
		2.0	0.70
3	8.4	1.0	0.34
4	7.7	1.0	0.32
5	8.4	1.4	0.48
		0.8	0.28
6	7.2	3.1	0.92
7	7.1	1.1	0.32
		1.2	0.35
		1.2	0.35
8	8.5	5.0	1.74
		4.7	1.64
		1.1	0.38
9	8.9	0.8	0.29

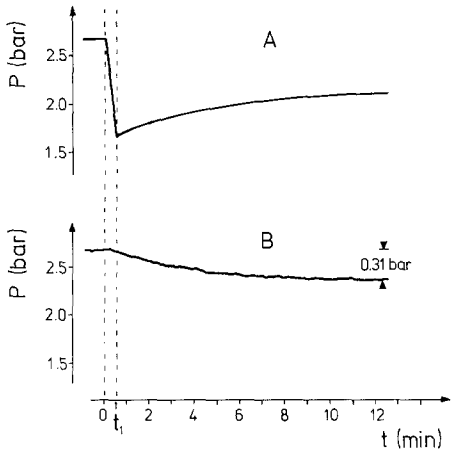

Fig. 7. The hypothetical change of turgor due to an isosmotic vesicular transport (*A*) is compared to a typical turgor regulation observed experimentally (*B*). A Cl^- extrusion of 4% of the cellular contents is assumed during the time interval from $t=0$ to $t=t_1$ (30 sec, dashed lines). For further explanations, *see* text

efflux of ions is smaller than the influx, a net inward flow is created which causes a steady increase in the internal osmolarity. This in turn induces a flow of water and a corresponding increase in cell turgor. If we assume that the cell is in water flow equilibrium at any given time during the intrinsic pressure increase, we can estimate the corresponding net ion flow J_S from the pressure change dP/dt (*see* Eq. (A3) in Appendix A). These data are listed in Table 1. For a mean pressure change $dP/dt = (1.8 \pm 1.4) \times 10^{-4}$ bar sec^{-1}, J_S is $(0.6 \pm 0.5)\,\mu mol\ m^{-2}\ sec^{-1}$.

The unusually high membrane potential of *Acetabularia* seems to be the ultimate reason for the net ion uptake, essentially of K^+ and Cl^-, and hence necessitates a mechanism to prevent a continuous increase in cell turgor. We suggest that the observed changes in turgor-pressure in the range between 2 and 2.8 bar, which are clearly correlated with an increased Cl^- efflux lasting 30 to 60 sec, reflect this regulatory mechanism. The hypothesis that turgor pressure triggers regulation is supported by the following observation. Turgor relaxations with exosmotic water flow end at a lower pressure than expected, if the pressure step inducing the relaxation exceeds the critical pressure range. It is assumed that in these cases a Cl^- efflux burst occurs during the relaxation which increases the driving force for water flow. Therefore the final pressure is smaller than calculated from the externally applied pressure step and the cell's water flow parameters.

What is the transport mechanism responsible for the large Cl^- efflux bursts? Mummert and Gradmann [14] have suggested that Cl^- could be extruded by way of vesicle transport which mainly sequesters NaCl and KCl from the vacuole to the outside by some direct route bypassing the cytoplasmic compartment. However, as the following considerations will show, the concept of vesicular transport is incompatible with the turgor pressure regulation observed in this study.

If we assume that vesicular transport does exist, the vesicular contents must be isosmotic with the surrounding cytoplasm since a vesicle has no rigid structure which could sustain the hydrostatic pressure resulting from an osmotic pressure difference across the membrane. Since the amount of Cl^- released during a single burst constitutes up to 4% of the total Cl^- content of the vacuole [12], isosmolar vesicular extrusion of about 4% of vacuolar fluid would cause a cell volume reduction of 4%. With the aid of the Philip equation [18], $\varepsilon = V \cdot (dP/dV)$, and a typical value of $\varepsilon = 25$ bar measured on *A. mediterranea* it can be estimated that a 4% reduction in the volume ($\Delta V/V = 0.04$) would cause the cell turgor to drop by 1.0 bar, i.e. about 40% of the turgor pressure, during the burst (30 sec). This is in contradiction with our results (*see* Fig. 7). Figure 7 compares the time course of the turgor pressure for hypothetical transport by isosmolar vesicles (*A*) and a representative turgor regulation curve observed experimentally here (*B*). The cell, the turgor regulation of which is shown in Fig. 7*B*, had an ε value of 41 bar at a turgor pressure of 2.6 bar, a volume of 2.4 µl and a surface area of 34 mm^2. From these data Lp can be calcu-

lated from the half-time of the relaxation after the onset of the turgor regulation ($T_{1/2}=160$ sec) [28]. This results in an Lp value of 0.47×10^{-6} cm bar^{-1} sec^{-1}, which fits well in the range of Lp values measured by induced turgor pressure relaxations (*see* Materials and Methods). The hypothetical curve in Fig. 7*A* was calculated for a relatively low ε value of 25 bar to give the worst case of estimation. A higher ε value would result in an even greater pressure drop during the burst. After the drop, turgor pressure has changed dramatically, but as the loss of cell volume was isosmolar, the internal osmolarity is unchanged. Therefore the pressure slowly increases, the dynamics of the relaxation being controlled by the hydraulic conductivity of the membrane and the volumetric elastic modulus of the cell wall ($Lp=0.47\times10^{-6}$ cm bar^{-1} sec^{-1} and $\varepsilon=25$ bar were assumed).

If we tentatively neglect the concept of isosmolarity between vesicles and cytoplasm and vacuole, respectively, we can estimate how much chloride ion must be concentrated in the vesicles in order to bring about the observed Cl^- bursts without an appreciable drop in pressure and, therefore, to comply with the experimental curve of Fig. 7*B*. Assuming an elastic modulus of $\varepsilon=25$ bar, a change in turgor pressure of 25 mbar (which would not lead to a measurable pressure drop and which corresponds to a relative volume change $\Delta V/V$ of 0.1%) and furthermore assuming a burst lasting 30 sec and an ionic flux of 60 μmol m^{-2} sec^{-1}, a cell of normal size ($V=2$ μl, $A=25$ mm^2) would lose about 45 nmol of Cl^- ions. With a vesicle volume of $\Delta V=2$ nl (0.1% of the total cell volume) the required Cl^- concentration would thus be 22.5 moles/liter which is an unrealistically high concentration.

Thus, it is unlikely that vesicular transport is responsible for the observed turgor regulation in *Acetabularia*. Turgor regulation apparently is accomplished by a change in the internal osmolarity due to salt extrusion (Cl^- and counter-ion) via a reversible change in the membrane permeability, the molecular mechanism being unknown. This is equivalent to saying that we have a reduced reflexion coefficient and/or an increased permeability (as defined in thermodynamics of irreversible processes). In line with the electro-mechanical model [3, 4, 21, 22, 23], it is conceivable that local changes in the intrinsic electric field in response to turgor pressure lead to local breakdown and, in turn, to the creation of some transient pathways (channels, pores) [26]. This interpretation would explain that a higher turgor pressure is required to induce Cl^- bursts at lower temperatures. In terms of the electro-mechanical model higher turgor pressures are required for compression of the membrane and, in consequence, for transformation of the pressure signal into electric field and ion transport changes at low temperatures due to the increase of the elastic compressive modulus perpendicular to the membrane plane.

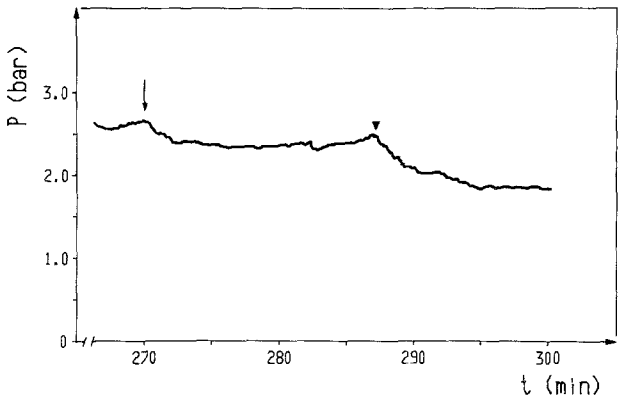

Fig. 8. Turgor pressure of *Acetabularia mediterranea* 270 min after insertion of the pressure probe. The first decrease of pressure (at $t=270$ min, ↓) is a typical autonomous pressure regulation (decrease of internal osmolarity with subsequent water flow directed outwards). At $t=287$ min the external solution was replaced by a solution with higher osmolarity (ASW + sucrose, ▾), which resulted in an imposed decrease of turgor pressure

Phenomenologically, if an osmotic gradient is set up across the membrane of a cell as a result of the rapid loss of Cl^- ions, a water flow directed outwards and a concomitant decline in turgor pressure will occur (*cf.* Figs. 5 and 6, second arrow). Alternatively, if an osmotic gradient already exists driving an inwardly directed water flow and thus causing a pressure increase in the cell, a Cl^- efflux burst will weaken the gradient and likewise cause a clearly detectable diminution in the rate of turgor pressure increase (*cf.* Figs. 5 and 6, first arrow).

On the basis of these considerations the decline in pressure during turgor regulation is related to a transport of water which is elicited by an osmotic gradient between the cell and the external medium, created by Cl^- bursts. This idea is confirmed by the similarity of the regulation process to an experiment, in which an osmotic gradient is imposed by changing the external osmolarity.

Figure 8 shows a turgor relaxation caused by a change of the internal osmolarity, due to Cl^- burst (arrow), followed by a relaxation caused by an osmolarity change of the external solution (triangle). Both turgor relaxations resemble each other, although they are caused by different events. The relaxation processes are both controlled by the hydraulic conductivity of the membrane and

Table 2. Calculation of the solute flux J_s during Cl^- bursts, assuming a constant flow for a 30-sec period

Cell no.	Volume (µl)	Area (mm^2)	ε (bar)	$\pi_o = P_o + \pi_e$ (bar)	ΔP (bar)	$\Delta \pi_i$ (bar)	J_s (µmol m^{-2} sec^{-1})
6	0.6	8.3	24	25	0.38	0.76	76
					0.31	0.62	62
7	2.4	34	41	24	0.24	0.38	37
					0.31	0.49	47
					0.13	0.21	20
8	3.6	43	28	27	0.09	0.18	20
					0.24	0.47	54
					0.34	0.66	76
10	3.4	41	14	27	0.07	0.20	23
					0.18	0.52	59
				21	0.34	0.83	94
11	0.9	13	29	27	0.27	0.52	49
					0.37	0.71	67
					0.09	0.17	16
					0.34	0.65	61
					0.49	0.93	88
					0.31	0.59	56
12	2.6	26	40	27	0.13	0.22	30
					0.16	0.27	37
					0.12	0.20	27

ε = volumetric elastic modulus; π_o = internal osmotic pressure; P_o = turgor pressure prior to Cl^- bursts; π_e = external osmotic pressure; ΔP = pressure decrease after Cl^- burst; $\Delta \pi_i$ = calculated change of the internal osmotic pressure

the volumetric elastic modulus of the cell wall. Evaluating the hydraulic conductivity from the half-times of both relaxations, *Lp* results to be 1.2×10^{-6} cm bar^{-1} sec^{-1} and 0.9×10^{-6} cm bar^{-1} sec^{-1}, respectively.

Because of the similarity of the two processes, which is illustrated in Fig. 8, the mathematics used to describe osmotic experiments is equally well applicable [5, 27]. In favorable cases (i.e., assuming water equilibrium prior to the Cl^- burst) it is possible to determine the change in the internal osmolarity with Eq. (B6) (*see* Appendix B). The results of the calculations are shown in Table 2. The values for the flow of ions J_s, corrected for surface area, during the burst are obtained on the assumption that the burst consists of a constant flow over the period of 30 sec. The ion flows (16 to 94 µmol m^{-2} sec^{-1}) calculated in this way are in the same order of magnitude as those derived from tracer measurements (60 µmol m^{-2} sec^{-1}) [14].

Finally we would like to point out that no other pressure changes than the pressure regulations described above were observed during spontaneous action potentials generated in cells of *A. mediterranea*. On the other hand, Barry [1, 2] has reported that action potentials in *Chara australis* are associated with water flow directed outwards and a corresponding decrease in turgor pressure. The apparent discrepancy with the results reported here might be explicable in terms of the different values for the volumetric elastic modulus of the cell wall and of the hydraulic conductivity of the membrane as well as of different thermodynamic coupling coefficients and different diffusion coefficients for KCl in the cell wall of *A. mediterranea*. Further investigations should make clear, if phenomena like ion water coupling and enhancement of salt concentration in the cell wall contribute to the pressure decrease during a Cl^- burst.

Conclusion

We relate the observed oscillatory (and apparently regulatory) changes in the turgor pressure of *Acetabularia mediterranea* above a threshold value, which are unprecedented in the literature, to the other unique feature of this alga, the particularly negative membrane potential. To our knowledge no other marine alga or marine organism sustains a membrane potential much more negative, i.e. by about 90 mV, than the K^+ equilibrium potential.

Obviously both active electrogenic chloride import which is responsible for this unusually negative membrane potential of about −170 to −180 mV [6, 20] and passive net K^+ influx tend to steadily increase the cell turgor; the observed bursts of net passive chloride efflux, accompanied by a cation as yet unidentified will then periodically reduce the turgor.

The action potentials are not strictly associated with this hypothetical mode of turgor regulation. They seem to provide an independent mechanism of reducing the cellular K^+ concentration as has been suggested by Mummert and Gradmann [13].

The dependence of the chloride efflux bursts upon the absolute turgor pressure strongly indicates that this event is essential to turgor regulation as assumed by Nuccitelli and Jaffe [17]. We conclude, therefore, that the plasmalemma of *A. mediterranea* is able to sense the turgor pressure and to link it to the transport of Cl^-. Indeed, the data are consistent with the idea that the chloride permeability, i.e. the passive chloride pathway, is turgor-controlled. Export of chloride salts by vesicles as discussed by Mummert and Gradmann [14] can be excluded to provide the turgor regulation observed in the present study.

We thank Dr. K.-H. Büchner for valuable discussion and kind suggestions. We are also obliged to A. Kühnle and H. Jäckel for their assistance in cultering *Acetabularia*. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (Zi 99/8) to U.Z.

References

1. Barry, P.H. 1970*a*. Volume flows and pressure changes during an action potential in cells of *Chara australis*. I. Experimental results. *J. Membrane Biol.* **3**:313–334
2. Barry, P.H. 1970*b*. Volume flows and pressure changes during an action potential in cells of *Chara australis*. II. Theoretical considerations. *J. Membrane Biol.* **3**:335–371
3. Coster, H.G.L., Steudle, E., Zimmermann, U. 1977. Turgor pressure sensing in plant cell membranes. *Plant Physiol.* **58**:636–543
4. Coster, H.G.L., Zimmermann, U. 1976. Transduction of turgor pressure by cell membrane compression. *Z. Naturforsch.* **31c**:461–463
5. Dainty, J. 1963. Water relations of plant cells. *Adv. Bot. Res.* **1**:279–326
6. Gradmann, D. 1970. Einfluss von Licht, Temperatur und Aussenmedium auf das elektrische Verhalten von *Acetabularia crenulata*. *Planta* **93**:323–353
7. Gradmann, D. 1975. Analog circuit of the *Acetabularia* membrane. *J. Membrane Biol.* **25**:183–208
8. Gradmann, D. 1976. "Metabolic" action potentials in *Acetabularia*. *J. Membrane Biol.* **29**:23–45
9. Gradmann, D., Mummert, H. 1980. Plant action potentials. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas and J. Dainty, editors. pp. 333–344. Elsevier/North-Holland, Amsterdam
10. Gradmann, D., Wagner, G., Gläsel, R.M. 1973. Chloride efflux during light-triggered action potentials in *Acetabularia mediterranea*. *Biochim. Biophys. Acta* **323**:151–155
11. Graves, J., Gutknecht, J. 1976. Ion transport studies and determination of the cell wall elasticity in the marine alga *Halicystis parvula*. *J. Gen. Physiol.* **67**:579–597
12. Mummert, H. 1979. Transportmechanismen für K^+, Na^+ und Cl^- in stationären und dynamischen Zuständen bei *Acetabularia*. Ph.D. Thesis, Universität Tübingen, Germany
13. Mummert, H., Gradmann, D. 1976. Voltage dependent potassium fluxes and the significance of action potentials in *Acetabularia*. *Biochim. Biophys. Acta* **443**:443–450
14. Mummert, H., Gradmann, D. 1980. Multi channel Cl^- pathways in *Acetabularia*. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas and J. Dainty, editors. pp. 439–440. Elsevier/North-Holland, Amsterdam
15. Mummert, H., Hansen, U.-P., Gradmann, D. 1981. Current-voltage curve of electrogenic Cl^- pump predicts voltage-dependent Cl^- efflux in *Acetabularia*. *J. Membrane Biol.* **62**:139–148
16. Novák, B., Bentrup, F.W. 1972. An electrophysiological study of regeneration in *Acetabularia mediterranea*. *Planta* **108**:227–244
17. Nuccitelli, R., Jaffe, L.F. 1976. Current pulses involving chloride and potassium efflux relieve excess pressure in *Pelvetia* embryos. *Planta* **131**:315–320
18. Philip, J.R. 1958. The osmotic cell, solute diffusibility, and the plant water economy. *Plant Physiol.* **33**:264–271
19. Saddler, H.D.W. 1970. The ionic relations of *Acetabularia mediterranea*. *J. Exp. Bot.* **21**:345–359
20. Saddler, H.D.W. 1971. Spontaneous and induced changes in the membrane potential and resistance of *Acetabularia mediterranea*. *J. Membrane Biol.* **5**:250–260
21. Zimmermann, U. 1977. Cell turgor regulation and pressure mediated transport processes. *In:* Integration of Activity in the Higher Plant. D. Jennings, editor. pp. 117–154. Cambridge University Press, Cambridge
22. Zimmermann, U. 1978. Physics of turgor- and osmoregulation. *Annu. Rev. Plant Physiol.* **29**:121–148
23. Zimmermann, U. 1980. Pressure mediated osmoregulatory processes and pressure sensing mechanism. *In:* Animals and Environmental Fitness. R. Gilles, editor. pp. 441–459. Pergamon Press, Oxford and New York
24. Zimmermann, U., Beckers, F. 1978. Generation of action potentials in *Chara corallina* by turgor pressure changes. *Planta* **138**:173–179
25. Zimmermann, U., Hüsken, D. 1980. Turgor pressure and cell volume relaxation in *Halicystis parvula*. *J. Membrane Biol.* **56**:55–64
26. Zimmermann, U., Scheurich, P., Pilwat, G., Benz, R. 1981. Cells with manipulated functions: New perspectives for cell biology, medicine, and technology. *Angew. Chem.* **20**:325–344; *Int. edition* **93**:332–351
27. Zimmermann, U., Steudle, E. 1974. The pressure dependence of the hydraulic conductivity, the membrane resistance and membrane potential during turgor pressure regulation in *Valonia utricularis*. *J. Membrane Biol.* **16**:331–352
28. Zimmermann, U., Steudle, E. 1978. Physical aspects of water relations of plant cells. *Adv. Bot. Res.* **6**:45–117
29. Zimmermann, U., Steudle, E. 1980. Fundamental water relations parameters. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas and J. Dainty, editors. pp. 113–127. Elsevier/North-Holland, Amsterdam

Received 8 March 1982; revised 9 June 1982

Appendix A

Calculation of the Net Ion Flux into the Cell during an Intrinsic Pressure Increase

If we assume that the cell is in water equilibrium at any time during an intrinsic pressure increase and that the reflexion coefficients for the solutes are unity, the following equation holds:

$$P(t)=\pi_i(t)-\pi_e \tag{A1}$$

with $P(t)$=turgor pressure (bar), $\pi_i(t)$=internal osmotic pressure (bar) and π_e=(constant) external osmotic pressure (bar). The assumption is justified because of the small rate of pressure increase observed in the experiments.

Derivation of Eq. (A1) yields:

$$\frac{dP}{dt}=\frac{d\pi_i(t)}{dt}\simeq\frac{RT}{V_o}\frac{dn}{dt} \tag{A2}$$

where R and T have their usual meanings, V_o(cm^3) is the cell volume and n the number of moles of intracellular solutes. It is assumed that the volume change can be neglected.

From Eq. (A2) the net ion flux into the cell, $J_s=(1/R)\cdot(dn/dt)$, can be calculated to be:

$$J_s=\frac{V_o}{RT\cdot A}\cdot\frac{dP}{dt} \tag{A3}$$

with A=membrane area (cm^2).

All parameters on the right side of Eq. (A3) can be experimentally determined.

Appendix B

Estimation of the Internal Osmolarity Response due to Cl^--Extrusion

From the change of turgor pressure after a Cl^- burst the change of the cell's internal osmotic pressure can be estimated, if the cell was in water equilibrium prior to the burst. An impermeable membrane is assumed ($\sigma=1$). Suppose that the internal osmotic pressure π_o changes to $\pi_o-\Delta\pi_i$ at $t=0$, then the phenomenological equation for the volume flow $J_v=Lp\cdot(P(t)-(\pi_i(t)-\pi_e))$ leads to a differential equation for $P(t)$ with the following substitutions:

$$J_v=-\frac{V_o}{A\varepsilon}\frac{dP}{dt};$$

$$\pi_i(t)=(\pi_o-\Delta\pi_i)\cdot\left(1-\frac{\Delta V(t)}{V_o}\right)\quad\text{for}\quad\frac{\Delta V}{V_o}\ll 1;$$

$$\Delta V(t)=\frac{V_o}{\varepsilon}\cdot(P(t)-P_o).$$

The solution of the differential equation is given by:

$$P(t)=P_o+\frac{\varepsilon\cdot\Delta\pi_i}{\varepsilon+\pi_o-\Delta\pi_i}\cdot(e^{-t/\tau}-1) \tag{B1}$$

with $P(t)$=turgor pressure (bar), P_o=turgor prior to the burst (bar), $\pi_i(t)$=internal osmotic pressure (bar), π_o=internal osmotic pressure prior to the burst (bar), $\Delta\pi_i$=change of internal osmotic pressure by Cl^- extrusion (bar), π_e= external osmotic pressure (bar), ε=volumetric elastic modulus (bar), τ=time constant (sec) of the relaxation given by $V_o/(A\cdot Lp\cdot(\varepsilon+\pi_o-\Delta\pi_i))$, A=membrane area (cm^2), V_o=cell volume (cm^3) and Lp=hydraulic conductivity of the membrane (cm bar^{-1} sec^{-1}).

For $t\to\infty$, the cell reaches a new water equilibrium with a turgor pressure P_∞ given by:

$$P_\infty=P_o-\frac{\varepsilon\cdot\Delta\pi_i}{\varepsilon+\pi_o-\Delta\pi_i}. \tag{B2}$$

Rearrangement of Eq. (B2) yields:

$$\Delta\pi_i=\frac{\varepsilon+P_o+\pi_e}{\varepsilon+\Delta P}\cdot\Delta P \tag{B3}$$

with $\Delta P=P_o-P_\infty$ and $P_o+\pi_e=\pi_o$.

With the assumption that volume flow is negligible and therefore the cell volume is unchanged, Eq. (B4) gives the number of moles of Cl^-, Δn, that have been extruded during the burst:

$$\Delta n\simeq\frac{V_o}{RT}\cdot\Delta\pi_i. \tag{B4}$$

Assuming a constant efflux rate over a period of 30 sec, we can estimate an ion efflux during Cl^- extrusion by Eq. (B5):

$$J_s=\frac{\Delta n}{A\cdot 30}. \tag{B5}$$

Using Eqs. (B3), (B4) and (B5), we get:

$$J_s=\frac{1}{30}\cdot\frac{V_o\cdot(\varepsilon+P_o+\pi_e)}{A\cdot R\cdot T\cdot(\varepsilon+\Delta P)}\cdot\Delta P. \tag{B6}$$

Equation (B6) can be used to estimate the ion flux during a Cl^- burst, because all terms on the right side of the equation can be determined experimentally.