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Electrical Properties of *Valonia Ventricosa*

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Summary. The cytoplasmic electrical potential and membrane resistance of mature cells of *Valonia ventricosa* have been measured by inserting a microelectrode concentric with another electrode into the vacuole of the cell. The cytoplasmic region was investigated by advancing the microelectrode into the cell wall from the vacuolar side.

The results revealed a unique region where the vacuolar electric potential and membrane resistance changed in a simultaneous single step to values close to zero. The measured potential always remained positive immediately after the step.

At no time was a highly negative potential region encountered. Further penetration of the microelectrode revealed a low resistance negative potential region of -12.6 ± 1.1 mV associated with the cell wall. Experiments were also carried out on aplanospores of *V. ventricosa* to compare mature and immature cells. The chemical composition of the vacuolar and protoplasmic phases of mature cells was determined. The results agreed with previous results except that the Cl^- ion content of the protoplasm was significantly higher at 381 ± 20 mmoles/liter (H_2O). It was concluded that mature cells of *Valonia* are significantly different from immature cells in that no highly negative potential cytoplasmic region was found in mature cells.

It was considered that the measured step change in electric potential and membrane resistance occurred at the plasmalemma and that the tonoplast was a region of very low resistance. The implications of these findings in terms of models of ion transport into *Valonia* are discussed.

The electrical properties of giant algal cells have been subject to intensive investigation since the early part of this century. A detailed review of the work is given by Hope and Walker (1975) and Raven (1975). The marine algae, *Valonia*, provided one of the classic subjects for study of bioelectric potentials in large plant cells, and it has been studied recently by Gutknecht (1966, 1967, 1968), Zimmermann and Steudle (1974) and Asai and Kishimoto (1975).

The accepted electrical model of *Valonia* is that it has a positive vacuolar potential of 10 to 20 mV relative to seawater. It is also accepted that the cytoplasm of *Valonia* is highly negative relative to the external solution with -71 mV across the plasmalemma. It may be deduced

from these values that an electrical potential difference of some -81 to -91 mV must exist across the tonoplast, with the cytoplasmic side being negative. It is interesting to note that this model rests to a large extent on a determination of cytoplasmic potential (Gutknecht, 1966) using only very young highly cytoplasmic *Valonia* cells. The assumption that the data obtained from very young cells respresents the situation in mature cells requires investigation, as it is known that membrane characteristics change during development or growth (Takahashi & Miyazaki, 1971; Zimmermann, Steudle & Lelkes, 1976).

The resistance of the protoplasm of *Valonia ventricosa* perfused and bathed with artificial sap has been reported as $9.4 \pm 1.4\,k\Omega\ cm^2$ (Gutknecht, 1967). Zimmermann and Steudle (1974) and Zimmermann *et al.* (1976) have presented evidence on *Valonia utricularis*. They state that the membrane resistance is normally .3 to $1\,k\Omega\,cm^2$ at low pressures but that this increases in response to high pressures and reaches a maximum value of $1.5\,k\Omega\ cm^2$ at about 2×10^5 Pa. The low pressure resistance was not found to vary with cell volume but the absolute value of the maximum resistance at high pressure was found to increase with decreasing volume.

The models of turgor pressure regulation of ion transport in *Valonia* (Hastings & Gutknecht, 1974; Zimmermann *et al.*, 1976) have in part been based on the assumption of a highly negative cytoplasm and the ion concentration in the cytoplasm measured by Gutknecht (1966). It is interesting to note that no confirmation of Gutknecht's results on the ion concentration in the cytoplasm have previously been reported.

The present experiments were designed to measure the cytoplasmic electrical potential and membrane resistance of mature cells of *V. ventricosa* and to compare the results obtained with those from aplanospores of the same cells. We show evidence that the electrical characteristics of mature *Valonia* cells may be very different to those previously accepted.

Materials and Methods

Culture Conditions

Cells of *Valonia ventricosa* J. Agardh were obtained from Heron Island, Queensland, Australia, and were maintained in the laboratory in a tank of natural sea water. (Concentration of the major ions in mmoles/liter Na 530, K 11.5, Cl 612). The seawater was aerated continuously and illuminated at about 400 lux for 8–12 hr per day by daylight and fluorescent lighting. The temperature was maintained at 21 ± 2 °C. Aplanopores were produced from mature cells when required by cutting open a cell of 5 to 20 mm diameter and

allowing the cut cell to remain in a bowl of seawater for 4 to 12 hr. Mature cells for the experiments were selected from the collection on the basis of regularity of shape, comparative freedom from encrustations and lack of irregular clumping of the chloroplasts which sometimes preceded the spontaneous formation of aplanospores.

Chemical Measurements

Sap was collected by withdrawing colorless samples with a hypodermic syringe from freshly collected cells. Protoplasm was collected by cutting a large cell, which had been rinsed in Tris sulphate buffer, and allowing the sap to drain out. The remaining cell wall and protoplasm were gently washed for 30 sec in Tris sulphate buffer, pH 7.7, which was isosmotic to the cell sap (1.2 osmols). The protoplasm was rapidly removed from the cell wall and collected into a small previously weighed tube, care being taken to avoid any contamination. The resulting protoplasm and buffer mixture was centrifuged in a hematocrit centrifuge for ten min at $12{,}000 \times g$. The protoplasm was collected and weighed to give mg net weight per sample. The protoplasm was then combined with supernatant buffer and diluted to 10 ml with 1% HNO_3 and allowed to extract for forty-eight hours. The water content of the protoplasm was determined by measuring the dry weight of a number of separate samples.

Sodium and potassium ion concentrations were determined using an EEL flame photometer. Chloride ion concentrations were determined by potentiometric titration (Radiometer). The osmotic pressure of cell sap and the tris buffer was measured with an Advanced Osmometer. The method used to determine protoplasmic ion concentrations was similar to that used by Gutknecht (1966).

Electrical Measurements with Aplanospores

The electrical potential difference (*PD*) between the cytoplasm of aplanospores and seawater was measured by the insertion of a single microelectrode in a manner similar to that of Gutknecht (1966). Microelectrodes of tip diameter 0.5 to 2 μm were prepared from clean lengths of Pyrex glass tubing and filled with 3M KCl solution: they had resistances of 2–20 MΩ and tip potentials of < 5 mV in seawater. In each experiment, an aplanospore of 50–200 μm diameter was mounted in seawater on a slide on the stage of a Zeiss microscope, and positioned with the aid of two fine glass rods mounted on a Zeiss sliding block micromanipulator. The microelectrode was mounted on a second micromanipulator, and was connected to a calomel half-cell by a 3M KCl agar bridge. A second bridge connecting the seawater to another calomel half-cell formed the reference electrode. The potential difference was measured with a Keithley 603 electrometer amplifier and recorded. The microelectrode was inserted into the aplanospore using observation through a $\times 40$ objective; satisfactory insertions were those in which, on removal of the electrode, the tip potential had changed by less than 2 mV and the tip was not visibly damaged. In some experiments, cells were plasmolysed by addition of sucrose to the medium, and the microelectrode tip was forced into the space between the wall and the protoplast. Deplasmolysis was then caused by gently flushing fresh seawater past the cell, and the *PD* was recorded as the stationary microelectrode pierced the cytoplasm.

Microelectrode Measurements in Mature Cells

The plasmalemma *PD* in mature cells of *Valonia* is not amenable to measurement by the insertion of a microelectrode through the cell wall, because the wall is tough and

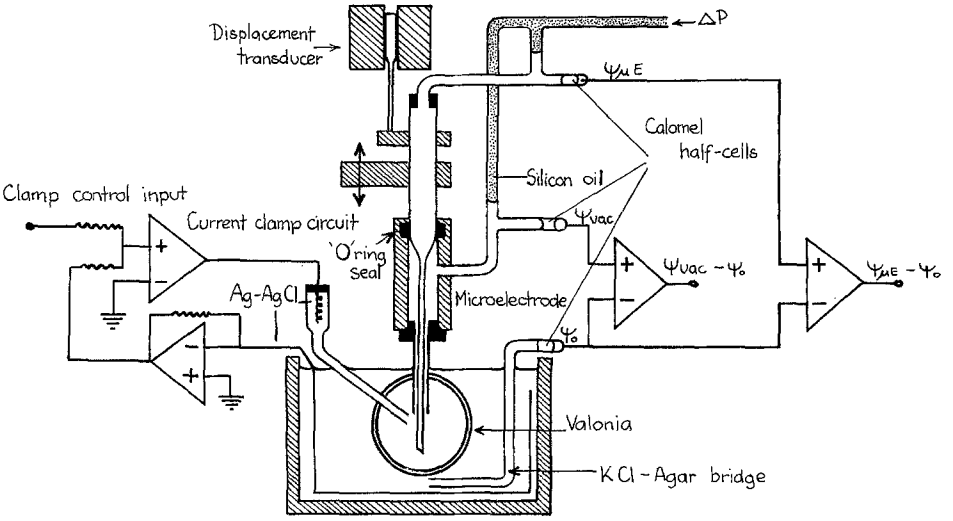

Fig. 1. Assembly for measuring the profile of electrical potential and resistance between the vacuole and outside solution using *Valonia ventricosa*. ψ_{vac} and ψ_O are the potentials of the vacuole and outside solution. $\psi_{\mu E}$ is the potential of the microelectrode. The resistance was determined by means of the current clamp circuit. ΔP is the pressure applied from a mercury manometer. For further explanations *see text*

the cytoplasmic layer thin. Insertion of a microelectrode was achieved using the arrangement shown in Fig. 1. A cell of 10 to 30 mm diameter was mounted in a bath of slowly flowing seawater containing both a current electrode of Ag sheet coated with AgCl and a separate calomel half-cell reference electrode. Two slightly tapered and sharp glass pipettes were inserted through the wall and protoplast into the vacuole, in a manner similar to that of Gutknecht (1967). The smaller pipette (tip diameter 0.5 mm) was connected to an enclosed Ag/AgCl current electrode, and its tip was positioned close to the center of the vacuole. The large pipette had a tip diameter of 0.8 mm, and was connected both to a calomel half-cell and a mercury manometer which controlled the hydrostatic pressure difference between the inside and outside of the cell (zero to 10^5 Pa). Both pipettes were inserted using simple micromanipulators, and were completely filled with artificial sap (7 volume seawater +93 vols. .67M KCl) prior to insertion. The vacuolar potential relative to seawater was measured through the larger pipette using an electrometer amplifier. The resistance of the protoplast could be obtained by the response of this *PD* to current applied through the smaller pipette using a current clamp circuit. Normally, a single cycle of a triangular waveform (period 30s) was applied at 3 min intervals; between cycles the current was zero, as was the long term average current.

To measure the cytoplasmic potential, a glass microelectrode of tip diameter 0.5–2 μm and filled with 3M KCl was arranged to pass concentrically through the larger pipette. The electrodes used had resistances of 2–20 mΩ and tip potentials of <5 mV in seawater. The microelectrode position was controlled by the fine feed of the micromanipulator to which the large pipette was attached. The microelectrode tip could be moved from just within the lumen of the large pipette, across the vacuole, into the cytoplasm and through the cell wall from the vacuolar side. The shank of the microelectrode passed through a rubber 'O' ring in a perspex chamber to which the large pipette was attached. This seal allowed hydrostatic pressure to be applied to the vacuole while allowing the

microelectrode to be advanced. Movement of the microelectrode did not disturb the position of either large pipette, and did not cause significant changes in the applied pressure since the diameter of the microelectrode shank was much less than that of the mercury manometer.

The microelectrode was connected to a third calomel half-cell and the *PD* between this and the bath reference electrode was measured with a further electrometer amplifier and recorded. The resistance of the microelectrode was checked during experiments by briefly applying a small current (10^{-9} A) between the inputs of this electrometer and noting the change in *PD*. After each experiment the microelectrode was removed and the tip diameter again measured. Experiments in which there was a greater than 20% change in the resistance of the microelectrode tip between the beginning and end of the experiment were discarded, as were experiments in which the electrode tip broke after passing into the wall.

To overcome any error due to slipping of the microelectrode shank in the manipulator clamp, the position of the shank was independently monitored with a linear displacement transducer (Hewlett-Packard 24DCDT-050, sensitivity 4 mV μm^{-1}). The transducer was mounted on a separate stand from the micromanipulator, and the sliding core of the transducer rested freely on a collar attached to the microelectrode shank. The transducer output was recorded together with the two potential differences; pertubations in these *PD*'s in response to the applied current cycles gave the total protoplast resistance and the resistance between the tip of the microelectrode and the bathing seawater. The current-voltage linearity of both of these resistances was checked using a dual *X-Y* display on a storage oscilloscope.

Cells were illuminated at about 500 lux and the temperature of the bathing solution was maintained at 21 ± 2 °C. throughout the experiments.

Results

Ion Concentration in the Compartments of Valonia and the Bathing Medium

Concentrations of Na^+, K^+ and Cl^- in the seawater bathing the cells, protoplasm and vacuolar sap are shown in Table 1. The protoplasmic concentration expressed in terms of protoplasm water refers to the entire protoplasm as no attempt was made to remove the chloroplasts that were present.

Table 1. Concentrations of Na^+, K^+ and Cl^- in seawater, protoplasm and sap of *Valonia ventricosa*

Ion	Seawater mmoles/liter	Protoplasm mmoles/liter (H_2O)	Sap mmoles/liter
Na^+	530 ±4 (9)	37±5 (6)	35±2 (34)
K^+	11.5±1.1 (9)	454±2 (6)	603±7 (34)
Cl^-	612 ±5 (9)	381±20 (6)	672±8 (34)

Results are quoted as mean ± SE (number of measurements).

The density of the cytoplasm was found to be 1.15 which was a value similar to that reported by Doyle (1940). The protoplasm water was determined as 57% of the wet weight, which is a result similar to that found by Gutknecht (1966). The results obtained for the ion concentrations are similar to those reported by Gutknecht (1966) except for a significantly higher concentration of Cl^- in the protoplasm.

The Electric Potential of the Cytoplasm of Aplanospores

Two different approaches to measuring the cytoplasmic potential of aplanospores were tried. In one case the microelectrode was inserted directly through the cell wall into the cytoplasm. In the other case the cell was plasmolyzed, the electrode inserted through the wall, and the cell deplasmolyzed so that the cytoplasm was impaled by the electrode tip. The results of these experiments are shown in Table 2.

It was found immediately upon penetration by the electrode that a peak negative potential was recorded. This potential usually decreased to a slightly negative plateau value within one minute of initial penetration. No significant differences were found between the two techniques that were employed. The peak values obtained were significantly lower than those reported by Gutknecht (1966) but of similar magnitude to those of Umrath (1938). The peak potentials were more rapidly transient than those reported by Gutknecht (1966) but the plateau potential was stable for periods of up to 15 min. The results support the finding that the cytoplasm of aplanospores has a negative electric potential with respect to the outside bathing medium but the value appears to be lower than previously accepted, being in the possible range of -31.1 to -3.6 mV. It is also worth noting that in these experiments it was found that the cell wall of the aplanospores had a significantly negative potential with respect to the bathing solution. The value of the potential obtained was -11.8 ± 2.6 (12) mV.

Table 2. The electric potential of the cytoplasm of aplanospores of *Valonia ventricosa*

	Peak potential (mV)	Plateau potential (mV)
Nonplasmolyzed cells	-31.1 ± 2.7 (22)	-3.6 ± 1.0 (22)
Plasmolyzed cells	-20.8 ± 7.2 (5)	-4.9 ± 1.0 (5)

Results are quoted as mean ± SE (number of determinations).

Electric Potential and Resistance Profile between the Vacuole and Bathing Medium in Mature Cells of Valonia

The resting potential and resistance of the mature *Valonia* cells reached a steady value in 30 to 40 min after initial insertion of the electrodes. Initially the tips of both voltage sensing electrodes were positioned at approximately the center of the vacuole and similar values of potential and resistance were recorded from each of them. The inner microelectrode was then advanced slowly across the vacuole towards the cytoplasmic layer and the cell wall. The potential and resistance were monitored continuously by both electrodes. As the microelectrode neared the estimated region of the cytoplasm and cell wall it was advanced in 5 μm steps until a position was reached where the measured potential and resistance changed suddenly. The values of potential and resistance as measured by the stationary electrode remained unchanged. The microelectrode was then withdrawn a distance of 50 μm and the region reinvestigated in much greater detail. The values of potential and resistance recorded by the microelectrode after this withdrawl were not significantly different from those being measured by the stationary electrode. Table 3 shows the values that were obtained from nine experiments with the vacuole held at a pressure of 7×10^3 Pa. In each experiment an initial unique region was encountered where the potential and resistance changed suddenly upon a 1 μm movement of the microelectrode. The potential depolarized to a small but still positive value while the resistance became close to zero. The results from different experiments have been compared by normalizing them so that the position where the initial maximum change of potential and resistance occurred is considered to be the zero distance. Positive distances are on the vacuolar side of the zero position. The potential and resistance values measured by the microelectrode are compared with the values of the same parameters as measured by the stationary electrode in the vacuole in each experiment. It can be seen from these ratios that a single well defined region exists where a significant change in potential and resistance occurs.

In every case the potential and resistance changed in a single step and at no time was a large negative potential region encountered. Further insertion of the microelectrode revealed a region of slightly negative potential with a still low resistance to the bathing solution. This was considered to be the potential of the cell wall (V_{wall}). When the microelectrode was inserted still further a region was found where the potential and resistance remained steady and zero. It was considered that this

Table 3. Measurements of electrical potential and resistance relative

V_{vac} (mV)	R_{vac} (kΩcm^2)	V_1 (mV)	V_2 (mV)	V_3 (mV)	R_1 (kΩcm^2)	R_2 (kΩcm^2)	R_3 (kΩcm^2)
7.6 ±5.5	1.4 ±0.7	7.4 ±5.4	7.5 ±5.4	1.3 ±0.2	1.3 ±0.6	1.3 ±0.7	0.06 ±0.1

[a] All experiments were performed with the pressure of the vacuole held at 7×10^3 Pa. V_{vac} and V_1, V_2 and V_3 are the potentials measured at the center of the vacuole and at 50 μm, 2 μm and −2 μm with respect to the position corresponding to the maximum initial change of voltage. R_{vac} and R_1, R_2 and R_3 are the corresponding measurements

region indicated that the microelectrode had broken through the cell wall into the bathing solution. At all times the stationary electrode continued to record steady values for the vacuolar potential and protoplast resistance.

Fig. 2 shows a composite diagram of the way in which the potential and resistance profile between the vacuole and the bathing solution varied. The vacuolar values of potential and resistance as measured by the stationary electrode have been normalized to 10 mV and 1 kΩ cm^2, respectively. The measurements of potential and resistance measured by the microelectrode at 50 μm, 2 μm and − 2 μm relative to the previously defined zero have been adjusted accordingly. The standard deviation of each average value is shown on the diagram. The negative potential value shown to the left of the zero position is an average maximum potential which has not been normalized to the vacuolar potential. The distance at which the maximum occurs is also an average value (-12.5 ± 3 (6) μm).

The experiments were repeated on cells with their vacuolar pressure held at 10^5 Pa. Some difficulty was encountered in performing the experiments under the increased pressure condition but though the value of the resistance was markedly increased, as expected, the general shape of the potential and resistance profile was not significantly different from that obtained in the low pressure experiments. Again no region of significantly high negative potential was encountered in passing from the vacuole to the bathing medium and the change in potential and resistance occurred in a unique single step.

The very low resistance negative region found beyond the position where the initial sudden change in potential and resistance occurs is considered to be a region associated with the wall of the cell. The average value of V_{wall} of -12.6 ± 1.1 (6) mV from the profile experiments com-

to the bathing medium in *Valonia ventricosa* bathed in sea water[a]

X_1	X_2	X_3	Y_1	Y_2	Y_3	V_{wall} (mV)
0.98 ±0.01	0.99 ±0.01	0.24 ±0.05	0.99 ±0.01	0.97 ±0.01	0.04 ±0.01	−12.6 ±1.1(6)

of resistance. X_1, is the ratio of V_1 to V_{vac} for each experiment. X_2 and X_3 are similar ratios with V_2 and V_3. Y_1, Y_2 and Y_3 are the corresponding ratios for the resistance measurements. V_{wall} is the maximum potential measured in the wall. All values are mean ± SD ($n=9$ unless shown otherwise).

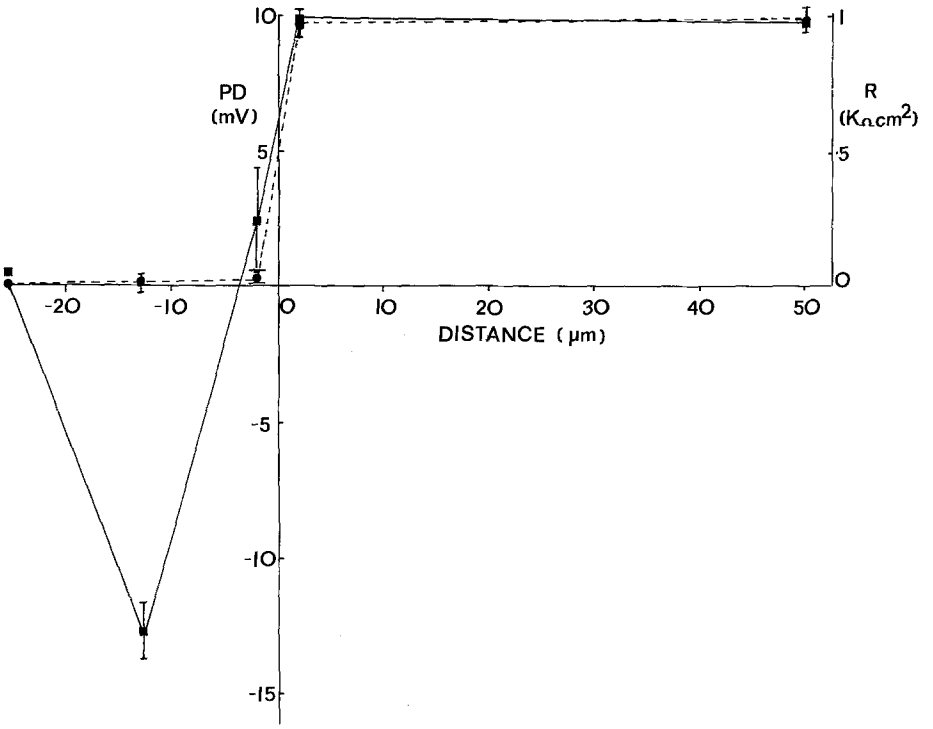

Fig. 2. A composite diagram of the profile of electric potential (■ — ■) and resistance (● ··· ●) in *Valonia* with distance from the vacuole to the outside solution. The vacuolar *PD* and resistance measured by the stationary electrode have been normalized to 10 mV and 1 kΩ cm², respectively. Microelectrode measurements have been adjusted accordingly. The distances have been normalized so that the initial position of maximum change of potential and resistance is considered to be zero. The distances are positive on the vacuolar side of the zero position. The negative potential shown to the left of zero has not been normalized to the vacuolar potential

pares very well with the average value obtained from naked wall material of −16.3±2.5 (13) mV and the value of −11.8±2.6 (12) mV obtained from the wall material of aplanospores. Another indication that this

region lies in the wall is that the apparent distances moved by microelectrode are much larger than any known phase. This could be accounted for if the microelectrode or wall flexes upon penetration of the electrode. A third indication is that the microelectrode tip was often found to break in this region, as indicated by a sudden reduction in electrode tip resistance.

Discussion

The accepted distribution of electrical potential in *V. ventricosa* is that the vacuole is +17 mV and the cytoplasm −71 mV relative to the outside solution. These values give a calculated potential difference of −88 mV across the tonoplast. With the vacuolar pressure clamped at 7×10^3 Pa the resistance between the vacuole and the outside medium was found to be 1.4 ± 0.7 (9) kΩ cm^2. It would be reasonable to speculate that this resistance might be made up of two distinct components occurring at the plasmalemma and tonoplast respectively, though the relative magnitudes are unclear. A simple picture of the profile of electrical potential and resistance between the vacuole and outside solution might therefore be a two step change in electrical potential, with a significant negative potential region being encountered, accompanied by a two-step change in resistance.

The present results consistently show a single well defined step in both potential and resistance. At no time was a significant negative region encountered except for a slightly negative potential region of very low resistance that appears to be associated with the cell wall. This result is very unexpected in view of the accepted data for mature *Valonia* cells as it implies that the cytoplasm is not at a negative potential, as is the case for all other reported measurements on algal cells.

It was not possible to accurately identify the site of the step change in potential and resistance but as the resistance changed from the initial value to a value close to zero and remained at that value regardless of further penetration by the microelectrode it is reasonable to assume that the side of the step change is the plasmalemma. If this is the case, it implies that the cytoplasm of mature *Valonia* cells has an electrical potential very close to that of the vacuole and that the tonoplast has a very low resistance. It is interesting to note that *Acetabularia mediterranea* and *Halicystis ovalis* show similar properties (Saddler, 1970; Blount & Levedahl, 1960) but the potential of the vacuole is highly negative in each case.

The unexpected nature of the present results means that critical examination of the experimental technique is required. The initial insertion of a sharp tipped electrode with tip diameter of ~ 0.8 mm ensured that the electrode assembly initially broke cleanly through the cell wall and protoplasm. The tip of the large electrode was positioned near the center of the vacuole and the cell sealed around the shaft of the electrode to the extent that the vacuolar pressure could be clamped at 10^5 Pa. The microelectrode was then moved slowly forward into the vacuole and both electrodes recorded similar values of potential and resistance. It seems extremely unlikely that the values recorded were not those representing the vacuolar phase.

The situation at the position where the step change occurred is perhaps less unequivocal. The thickness of the protoplasmic layer by our own observations is of the order of 8 μm thick, which is in agreement with Doyle (1940). The microelectrode was advanced in 1 μm steps in this region. It is conceivable that the tonoplast would flex on contact with the microelectrode but it seems very unlikely that it would distort to the extent that the microelectrode would penetrate the tonoplast and plasmalemma simultaneously without at least revealing a transient highly negative potential, if it existed in the cytoplasm. The experimental finding that the step change was entirely accomplished within a 4 μm movement of the electrode tip in all cases and usually occurred upon a 1 μm movement of the electrode supports the contention that the electrode tip did enter the cytoplasmic region for a significant period. Nevertheless, the possibility that the plasmalemma and tonoplast were penetrated simultaneously cannot be entirely excluded.

If the present findings are accepted then the absence of a highly negative cytoplasmic region in mature *Valonia* cells has interesting implications for the ion transport mechanisms present in the cell. The lack of a significant electrical potential difference across the tonoplast means that *Valonia* is more similar to other algal cells in that respect than has previously been accepted. This can be seen from the data on algal cells presented by Raven (1975).

If the assumption is made that Na^+ and K^+ ions in *Valonia* are in approximately steady state (Gutknecht, 1966), then the possible active transport of Na^+ and K^+ may be tested by using the Nernst equation. This equation was used to calculate the equilibrium potentials, E_{Na} and E_K from the gradients of concentration across the plasmalemma and tonoplast. The calculated values are shown in Table 4 and they are compared with the observed values by calculation of ΔE for each value.

Table 4. Equilibrium potentials for Na^+ and K^+ across the plasmalemma and tonoplast[a]

Ion	Plasmalemma		Tonoplast	
	E_{ion} (mV)[b]	$\Delta E = E_{obs} \psi\chi - E_{ion}$ (mV)[c]	E_{ion} (mV)[b]	$\Delta E = E_{obs} - E_{ion}$ (mV)[c]
Na^+	+68	−60	+1.4	−1.4
K^+	−94	+104	+7.3	−7.3

[a] Concentrations taken from Table 1.
[b] $E_{ion} = (-RT/ZF) \ln (Ci/Co)$ volts.
[c] E_{obs} taken from Table 3 (considered zero at tonoplast).

It can be seen from Table 4 that an active flux of K^+ from the seawater into the cytoplasm and an active flux of Na^+ from the cytoplasm to the seawater are indicated. This is in agreement with Gutknecht (1966), but the evidence for active transport of K^+ is much stronger. The results at the tonoplast, in contrast to those presented by Gutknecht (1966), indicate only very tentative evidence for active transport of Na^+ and K^+ from the cytoplasm into the vacuole. The results do not support the concept of an electrogenic pump at the tonoplast of *Valonia*.

The position with regard to Cl^- is different to that proposed by Gutknecht (1966) as the present results show that the concentration of Cl^- in the cytoplasm is well below that equating to passive distribution between the external solution and the cytoplasm. The results suggest that active transport of Cl^- may occur from the cytoplasm across the tonoplast into the vacuole but that the process is nonelectrogenic. This result is interesting as the same finding is indicated from the short circuit current studies reported by Gutknecht (1967) in which the summed ion fluxes accounted for only about one half of the inward positive current.

The finding that the cytoplasm of mature cells is not highly negative with respect to the outside solution suggests, in the absence of other evidence, that the influx of Cl^- to the cytoplasm may be by passive diffusion down its electrochemical gradient. Gutknecht (1966) suggests that there was a large discrepancy between the probable range of the ratio of Cl^- influx to Cl^- efflux at the plasmalemma ($\sim$1 to 1.7) and the ratio predicted on the basis of a highly negative cytoplasm ($\sim$0.3). This discrepancy would be largely abolished by our findings, since the predicted flux ratio at the plasmalemma would be about 2.2, and Gutknecht's suggestion that Cl^- may be actively transported out of the cyto-

plasm at the plasmalemma, as well as at the tonoplast, would be unnecessary.

Passive influx of Cl^- at the plasmalemma would also not be inconsistent with the results of Asai and Kishimoto (1975) who found that the relative conductance for Cl^- in *Valonia aegagropila* was much larger than that of Na^+ or K^+, as determined from the response of the vacuolar potential to external concentration changes.

The work of Zimmermann *et al.* (1976) suggests that both turgor pressure and the elastic properties of the cell wall may influence ion fluxes into *V. utricularis*. In view of this work it seems reasonable to suggest that the electrical properties of mature *Valonia* cells may be quite unlike that of aplanospores. The results presented by us in this paper suggest that mature cells of *Valonia* are very different from the immature cells in that the cytoplasm is slightly positive and not highly negative. In addition, there appears to be no electrical potential across the tonoplast and no evidence for the active transport of Na^+ and K^+ at that site. It can be argued that the presence of an electrically positive cytoplasm relative to the external solution produces important similarities for *Valonia* with other vacuolar plant cells in terms of the ion transport mechanisms that may be present. The present results appear to be compatible with other experiments on mature *Valonia* cells, and, perhaps, offer a more acceptable model of ion transport in *Valonia* than that proposed by Gutknecht (1966) which requires the cell to exhibit some unusual ion transport sites.

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