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Turgor Regulation in *Valonia macrophysa* Following Acute Osmotic Shock

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Summary. The marine alga *Valonia macrophysa* an inhabitant of shallow subtropical waters, is subjected to sudden dilutions of external seawater during rain showers. This study describes the mechanisms involved in turgor pressure regulation following acute hyposmotic shock. Turgor regulation is 88% effective and complete within 4 hr following hyposmotic shocks of up to −10 bar. Loss of vacuolar K^+, Na^+ and Cl^- accounts for the decrease in vacuolar osmotic pressure associated with turgor regulation. A novel mechanism of turgor regulation is exhibited by *Valonia macrophysa* given hyposmotic shocks greater than about −4 bar. Such an osmotic shock causes cell wall tension to increase above a critical value of about 6×10^5 dyne/cm, whereupon the protoplasm ruptures and the cell wall stretches irreversibly at a localized site. The protoplasm rupture is suggested by (1) a large abrupt increase in K^+ efflux (as measured by $^{86}Rb^+$), (2) a rapid decrease in turgor pressure as measured with a pressure probe, and (3) sudden depolarization of the vacuole potential. Evidence for an increase in cell wall permeability includes efflux from the vacuole of dextran (mol wt 70,000), which normally has a very low cell wall permeability, and scanning electron micrographs which show a trabeculated scar area in the cell wall. This mechanism of turgor regulation is physiologically important because 98% of the cells regained normal growth rate and turgor following acute osmotic shock.

Key words Osmoregulation · turgor regulation · ion transport · marine algae · *Valonia*

Introduction

Marine algae exposed to changes in external salinity experience fluctuations in turgor pressure, i.e., the difference in hydrostatic pressure between the inside and outside of a walled cell. Turgor pressure is important in maintaining the growth rate and structure of plant cells. Although many marine and euryhaline algae regulate turgor, the mechanisms involved in turgor regulation are not well understood. For recent reviews *see* Cram (1976), Zimmermann (1977, 1978), Gutknecht, Hastings and Bisson (1978, Kauss (1979) and Kirst and Bisson (1979).

Turgor regulation involves the detection of a change in turgor pressure followed by the modulation of transport properties to cause changes in the concentrations of intracellular solutes until turgor is restored to the desired level. In *Valonia*, a giant celled marine alga, the mechanisms of hyposmotic and hyperosmotic regulation are basically different. During hyperosmotic stress, the active uptake of K^+ is stimulated, causing KCl and water to enter the cell until turgor is restored (Gutknecht, 1968; Hastings & Gutknecht, 1974, 1976; Steudle, Zimmermann & Lelkes 1977). However, during hyposmotic stress, when turgor is high, the pump rate remains low and K^+ permeability increases, allowing a controlled loss of KCl (Steudle et al., 1977).

Zimmermann and Steudle (1974) found that *Valonia utricularis* can regulate turgor following acute hyposmotic or hyperosmotic shocks. Using cultured cells of *Valonia macrophysa* we have confirmed their observations. Furthermore, we have observed a novel mechanism of turgor regulation following acute hyposmotic shocks of greater than −4 bar. This mechanism involves an irreversible localized stretching of the cell wall, a transient rupture of the underlying protoplasm, and rapid loss of cell sap by a pressure-driven bulk flow. This mechanism provides a physiologically important way of rapidly reducing the turgor pressure during acute hyposmotic stress. A preliminary account of this work has been published (Guggino & Gutknecht, 1980).

Materials and Methods

Culture

Cultures of *Valonia macrophysa* were illuminated by fluorescent light at about 15 W/m² on a 12:12 hr light/dark cycle and maintained at 20–25° C. The culture medium was sterile enriched seawater, usually 37‰ salinity (1125 mosmol/kg), which was changed at three-week intervals. The seawater was prepared by raising the osmolality of natural offshore seawater (approx. 1000 mosmol/kg)

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with a freeze concentrate of seawater. The medium was enriched with 20 ml/liter Alga-Gro (Carolina Biological Supply, Burlington, N.C.). Dilute seawater was made by adding distilled water. Cells used in these experiments were 3–8 mm in diameter and nearly ellipsoidal in shape.

Ion Analysis

Known volumes of sap from single cells were drawn into an oil-filled syringe (Gilmont Instruments, Great Neck, N.Y.) attached to a glass pipette which was pulled on a vertical piette puller and bevelled to a 30 μm tip. Pipettes were calibrated by weighing small amounts of water ejected from the pipette. For Na^+ and K^+ analysis, 5 μl of sap was diluted with appropriate volumes of 4 mM CsCl (an ionization quenching agent). Na^+ and K^+ were measured by atomic absorption spectrophotometry. Cl^- was measured by amperometric titration with a Buchler-Cotlove chloridometer.

Electrical Measurements

Microelectrodes containing microfilament glass (A-M Systems, Toledo, Ohio) were pulled on a vertical pipette puller. The microelectrodes were filled with filtered 2.5 M KCl then placed in a Ag/AgCl microelectrode holder (W–P Instruments, New Haven, Conn.). The electrodes had resistances of 1–5 mΩ and tip potentials of less than ±5 mV. Broken tip electrodes were used as reference electrodes. Vacuolar potentials were measured with a Keithley electrometer (Keithley Instruments, Cleveland, Ohio).

$^{86}Rb^+$ Efflux Measurements

$^{86}Rb^+$ (New England Nuclear, Cambridge, Mass.) was used as a K^+ tracer. Rb^+ is an acceptable substitute for K^+ if the transport pathway does not have a high specificity for K^+ (Rhoads, Woo & Epstein, 1977). As an added precaution the specific activity of $^{86}Rb^+$ was kept high and the concentration of Rb^+ relative to K^+ was kept low in order to avoid inhibition of K^+ movement by Rb^+ (Luttge & Higinbotham, 1979).

Cells were placed in enriched seawater containing 16–20 μCi/ml of $^{86}Rb^+$ for at least two weeks, which was sufficient time to achieve isotopic equilibrium. Cells were then removed from the radioactive medium, quickly blotted, weighed, and placed under a projector to measure cell perimeter. The surface area was calculated using the method of Hastings (1975). A cell was placed in a 1.5 ml chamber containing a stirring bar below a perforated platform. The chamber was perfused continuously with seawater at a rate of about 1.5 ml/min. In those experiments performed with the micropressure probe a larger chamber volume of 7 ml was used and the perfusion rate was 3.5 ml/min.

Samples were collected at alternate minutes during the control period and then every minute following a hyposmotic shock. At the end of an experiment the cell was homogenized and a cell diluent counted. Total efflux counts and total cell counts were added to obtain total initial cell counts (Q_i^*) for each time interval. Samples were counted in a low background planchet counter or a liquid scintillation counter.

The rate coefficient for K^+ efflux was calculated assuming a two-compartment system, i.e., vacuole and seawater. Tracer efflux is described by the equation,

$$\frac{dQ_i^*}{dt} = \frac{J_{io} A Q_i^*}{V C_i} \tag{1}$$

where Q_i^* is the amount of tracer in the cell (cpm), J_{io} is the one-way efflux (mol/cm^2 sec), t is time (sec), C_i is the vacuolar K^+ concentration (mol/cm^3), V is the cell volume (cm^3), and A is the surface area (cm^2). The rate coefficient ($J_{io} A/VC_i$) was calculated for each 1-min interval, assuming that changes in C_i and Q_i^* were negligible during each interval. Then the rate coefficient was plotted as a function of time following changes in external osmolality (e.g., Fig. 3). It was assumed that the protoplasmic compartment did not contribute significantly to total efflux because the protoplasm contains <1% of the total cell K^+.

Turgor Pressure and Osmolality

The osmolality of 7 μl samples of seawater or vacuolar sap was measured on a vapor pressure osmometer (Wescor Inc., Logan, Utah). Sap osmolality minus seawater osmolality ($\Delta\pi$) was used in some experiments as an estimate of turgor. The osmolality difference was converted to turgor pressure by the relation $\Delta P = R\,T\,\Delta\pi$, where R is the gas constant and T is the temperature.

Turgor pressure was also measured directly with a miniature pressure probe similar to that described by Zimmermann and Steudle (1974). A pressure transducer (model CQS 140-50, Kulite Semiconductor Ltd., Ridgefield, N.J.) was built into a Plexiglas reservoir which was filled with silicone oil and attached to a micropipette (tip diameter 50–100 μm). An ultraprecision micrometer (Gilmont Instruments) was also attached to the reservoir in order to add or remove small fluid volumes to the vacuole via the micropipette. Typically a cell was punctured, then allowed to return to steady turgor pressure by passive water inflow. Occasionally, the turgor was adjusted by adding a small volume of fluid to the vacuole. Efflux experiments were conducted only with cells in which Rb^+ efflux was the same before and after recovery from pressure probe insertion.

^{14}C-Dextran Efflux

Cells weighing from 23–45 mg were injected with ^{14}C-dextran, mol wt 70,000 (New England Nuclear, Boston, Mass.). A 15-μm beveled micropipette was attached to a microsyringe filled with oil. A solution of 0.6 M KCl colored with dextran blue 2000 (Pharmacia, Piscataway, N.J.) and ^{14}C-dextran (0.1 μCi/μl) was drawn into the pipette. A cell was punctured and 2 μl of the solution were injected into the vacuole. The blue dye was added in order to visualize the injection process. Test cells were allowed to heal from one to two weeks in individual containers filled with enriched seawater maintained under normal growth conditions. After one week those cells which showed good healing of the injection site and which showed no isotope leak into the seawater bath were chosen for experiments. Some control cells were injected with 0.6 M KCl, and after one week $\Delta\pi$ was measured. No significant difference in turgor was found between KCl injected and control cultured algae. Therefore the dextran-injected algae were assumed to recover normal turgor after the injection procedure. ^{14}C-dextran efflux was measured by the same method as ^{86}Rb efflux.

Permeability of the Cell Wall

Tracer flux across *Valonia macrophysa* cell walls was measured in a miniature Ussing chamber designed by Zadunaisky and Degnan (1976). A large cell was cut in half, the green protoplasmic layer was removed and the cell wall was placed over the aperture of an X-ray film partition which had been previously covered with a very thin layer of stopcock grease. The cell wall was covered with a similar piece of X-ray film and then inspected under a microscope to make sure the wall extended at least 1 mm beyond the edge of the aperture. The exposed surface area of the cell wall was 0.07 cm^2. The volume of seawater in each compartment was 2 ml.

The following tracers were used for cell wall permeability measurements: ^{14}C-dextran, ^{3}H-inulin, ^{14}C-mannitol, and ^{14}C-urea. At $t=0$, 5–20 μl of radioactive solution were added to one side.

Fifty μl aliquots were removed from both sides during experiments and counted by liquid scintillation. The cpm's collected on the cold side were divided by the surface area of exposed wall and the time of collection to give the tracer flux (J^*) in cpm/cm^2 sec. Permeability coefficients (cm/sec) were calculated by $P=J^*/\Delta C^*$, where ΔC^* is the difference in concentration of tracer on the left and right sides at the given experimental times. Flux measurements were terminated before the tracer concentration in the cold side reached 2% of that in the hot side.

Since the surface area of the cell wall was small, edge leakage may contribute to the lowest permeability, i.e., dextran. In order to estimate edge leakage the flux of dextran across an impermeable barrier of plastic Saran Wrap® was measured. A single or double layer of Saran Wrap was cut to the same size as the experimental cell walls in order to duplicate the amount of wall overlap around the edge of the aperture. A permeability for Saran Wrap was then measured. There was no difference between single or double layers of Saran Wrap so all leakage must have occurred at the edges. The dextran permeability of Saran Wrap was about 1% of the permeability of the cell wall. Thus, edge leakage was assumed to make a negligible contribution to the total permeability.

Scanning Electron Microscopy

A modification of Bell's fixation technique was followed (Bell, Barnes & Anderson, 1969). Algae were fixed for 1.5 h in ice-cold 6% glutaraldehyde containing 0.2 M phosphate buffer, pH 7.7 (1070 mosmol/kg). Cells were rinsed four times in cold phosphate buffer or distilled water and then post-fixed in 1% osmium in 0.2 M phosphate buffer plus sufficient KCl to increase the osmolality to 1050 mosmol/kg. Cells tended to lose volume and wrinkle unless a distilled water wash was given after the fixation steps. After an hour of osmium fixation, cells were washed four times with distilled water, resuspended in distilled water and dehydration was begun. Ethanol was added dropwise to the cell solution until 100% ethanol concentration was reached after 24 hr. Cells were stored in cold 100% ethanol for 3 days and then critical point dried. The chamber was flushed five times with CO_2 to remove all ethanol before drying. Cells were sputter coated with gold palladium and viewed with an ETEC Autoscan model UI scanning electron microscope at 10 kV power.

Statistics

All values are quoted as mean ± standard error (number of cells) unless otherwise stated. Values were considered to be significantly different if a Student's t-test showed $p<0.01$.

Results

Turgor Pressure and Ionic Regulation

Steady-state osmolality difference ($\Delta\pi=\pi^i-\pi^o$) was used as an estimate of steady-state turgor pressure. Cells taken from normal seawater were placed directly into various hyperosmotic and hyposmotic salinities and allowed to establish a new steady state. Cells given hyperosmotic shocks of +240 mosmol/kg required several days to regain steady-state turgor. Cells given hyposmotic shocks of −240 mosmol/kg regained normal turgor within about 4 hr (Fig. 1). A plot of steady-state turgor versus external osmotic pressure ranging from 700 to 1600 mosmol/kg (17.0 to 38.6 bar) gave a linear regression slope of -0.123 ± 0.005 (177 cells). This means that turgor regulation is about 88% effective, i.e., turgor decreases 0.12 bar for each 1-bar increase in external osmotic pressure. These results are similar to those of Hastings and Gutknecht (1976), who adapted cells slowly by means of small stepwise changes in salinity.

To find out whether turgor pressure is related to cell volume or cell wall tension we measured steady-state turgor as a function of cell size. Figure 2 shows that turgor is independent of cell size over a range of 3.0 to 8.6 mm diameter (volumes ranging from about 14 to 320 μl). The approximate tension (T) in the cell wall (assuming spherical shape) is given

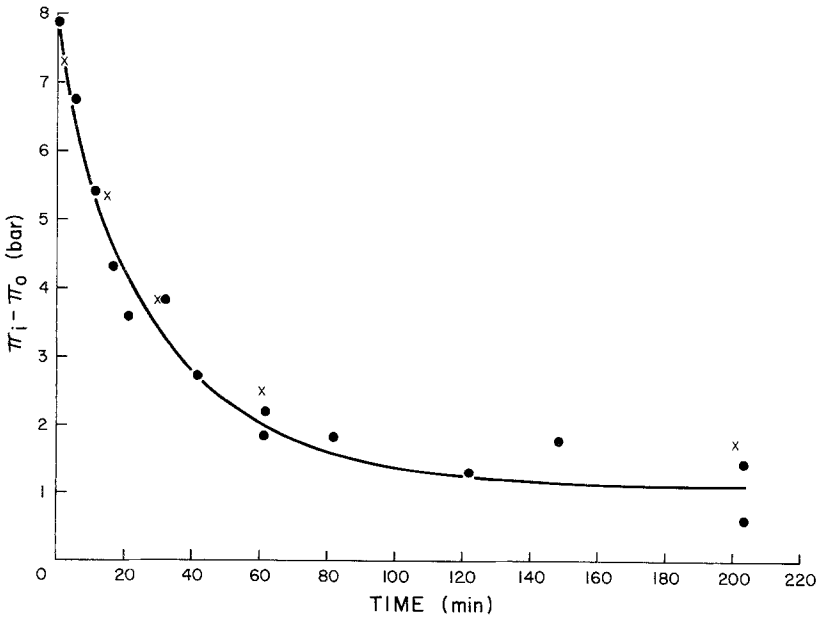

Fig. 1. Decrease in sap osmolality minus seawater osmolality ($\Delta\pi$) after a hyposmotic shock of −242 mosmol/kg. Closed circles indicate osmolality differences measured directly from seawater and sap samples. X indicates the combined sum of sap Na^+, K^+ and Cl^- times the osmotic coefficient of 0.89 (*see* Table 1). Each point represents 1 cell

by the law of Laplace, i.e., $T=\Delta P\ r/2$, where ΔP is the turgor and r is the radius. Since the cells used in this experiment had an average turgor of 1.9 bar (1.9×10^6 dyne/cm^2), we estimate that cell wall tension ranged from about 1.4×10^5 dyne/cm in the smallest cells to about 4.1×10^5 dyne/cm in the largest cells. Thus, turgor is independent of volume over a 23-fold range and independent of cell wall tension over a three-fold range.

The sap osmolality is accounted for by the major vacuolar ions, K^+, Na^+ and Cl^-. Table 1 and Fig. 1 show that the loss of these ions accounts for the decrease in π^i after a hyposmotic shock of -242 mosmol/kg. The amount of each ion lost is roughly proportional to its vacuolar concentration, although there is considerable variability in the data collected from osmotically shocked cells. At steady state, a combined total of about 188 mM of K^+, Na^+ and Cl^- are lost, causing π^i to decrease by about 179 mosmol/kg. Thus, steady-state turgor increases by 33 mosmol/kg when external salinity decreases by 242 mosmol/kg (Table 1).

Most cells subjected to the largest decreases in salinity, i.e., -550 mosmol/kg, ruptured irreversibly and died; the few surviving cells turned pale in color and showed no turgor pressure. Cells given hyposmotic shocks between -150 and -425 msomol/kg exhibited a novel mechanism of turgor regulation which involves a rapid volume efflux. The remainder of the Results section describes in detail the turgor regulation process in cells given hyposmotic shocks of about -240 mosmol/kg (-6 bar).

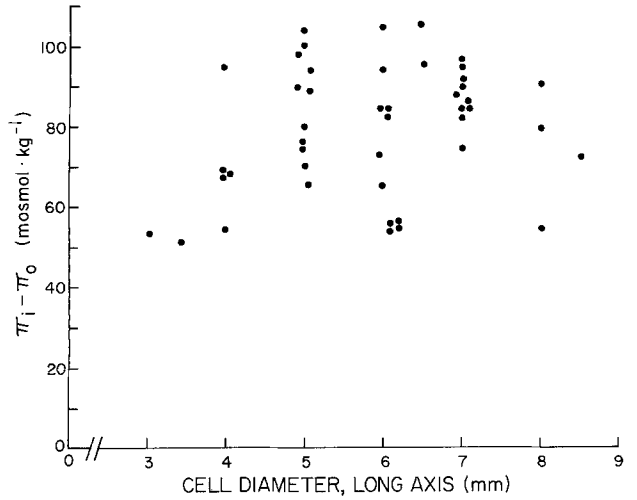

Fig. 2. Relation between steady-state turgor pressure and cell size in normal seawater (1130 mosmol/kg). Each point represents a sincle cell correlation coefficient = 0.21

$^{86}Rb^+$ Efflux, Turgor Pressure and Vacuole Potential

The $^{86}Rb^+$ efflux from control cells in 1125 mosmol/kg seawater had a rate coefficient of $(5.5\pm0.01)\times10^{-6}$ sec^{-1} (11). After a -250 mosmol/kg decrease in external osmolality the rate coefficient increased within 5 min to $(7\pm2)\times10^{-5}$ sec^{-1} (6). This elevated efflux continued for 2–3 hr, except when interrupted by large bursts of $^{86}Rb^+$ efflux with a rate coefficient of at least $(2.7\pm0.1)\times10^{-4}$ sec^{-1} (10) (Fig. 3) The large abrupt increase in $^{86}Rb^+$ efflux occurred only once in most cells but occurred 2–3 times in some cells. Estimation of the volume lost during these bursts in efflux was made by assuming that the volume lost is proportional to the tracer lost in excess of the normal elevated efflux. A calculated volume of about 3.5% of the total vacuolar volume is lost with each burst in $^{86}Rb^+$ efflux.

Direct measurement of turgor pressure by means of the pressure probe allowed us to study the relation

Table 1. Ion concentrations and osmolalities of vacuole sap of *Valonia macrophysa* given a -242 mosmol/kg (-6 bar) hyposmotic shock

	Vacuole sap concentration (mM)				Vacuole sap osmolality (mosmol/kg)		
	K^+	Na^+	Cl^-	Σions	π^i calculated[a]	π^i measured	$\pi^i-\pi^o$ measured
Control (38‰, 1174 mosmol/kg)	537 ± 7(7)[b]	137 ± 2(9)	707 ± 3(5)	1381	1229	1219	45 ± 3(4)
Test (31‰, 932 mosmol/kg)							
10 min	520 ± 17 (4)	125 ± 6(3)	647 ± 14(4)	1292	1150		
30 min	507 ± 12 (3)	107 ± 12(3)	615 ± 34(3)	1229	1081		
Steady state	483 ± 9(11)	116 ± 7(6)	594 ± 5(8)	1193	1050	1010	78 ± 5(4)

[a] A solution of 538 mM KCl and 138 mM NaCl has an osmotic coefficient of 0.89. A solution of 481 mM KCl and 113 mM NaCl has an osmotic coefficient of 0.88. The calculated value for π^i is the Σ ions multiplied by the osmotic coefficient.

[b] Number in parentheses means number of cells.

between $^{86}Rb^+$ efflux and turgor. Figure 4 shows a typical experiment. After a hyposmotic shock, turgor rises rapidly for 5–10 min. Sudden decreases in turgor occur within 10–30 min after hyposmotic shock (Figs. 4 and 5). These decreases in turgor are always associated with large increases in $^{86}Rb^+$ efflux. From Laplace's law we estimate the critical tension at which a burst of $^{96}Rb^+$ efflux occurs to be about 6×10^5 dyne/cm.

In *Valonia macrophysa* the vacuole potential (V_{vo}) is slightly positive under normal conditions (Hastings & Gutknecht, 1976). We found that after hyposmotic shock V_{vo} becomes -40 ± 7 mV (11), except when interrupted by brief periods when the potential shifts toward zero. Such depolarizations are associated with sudden decreases in turgor (Fig. 5) and bursts of $^{86}Rb^+$ efflux (*data not shown*).

The maximum turgor following a -6 bar hyposmotic shock was about 3 bar, substantially less than the maximum $\Delta\pi$ of about 7 bar. At least two factors contribute to this difference. First, the sudden drop in turgor usually occurs before the cells reach osmotic equilibrium (Figs. 4 and 5). Second, K^+ permeability increases markedly at high turgor (Figs. 3 and 4) (Steudle et al. 1977). Thus, the cells begin to lose salt with the onset of hyposmotic stress before the critical cell wall tension is reached. From our value of the Rb^+ efflux rate coefficient (7×10^{-5} sec^{-1}) we estimate that this increased K^+ leakage could re-

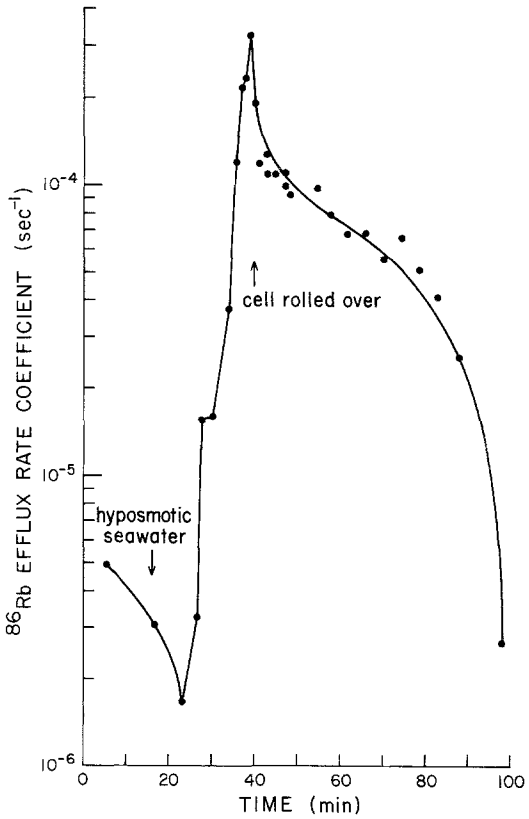

Fig. 3. Time course of $^{86}Rb^+$ efflux following hyposmotic shock. The rate coefficient increases after hyposmotic shock and remains elevated as long as turgor is above normal. At $t=40$ min the cell rolled over and a burst of tracer efflux occurred

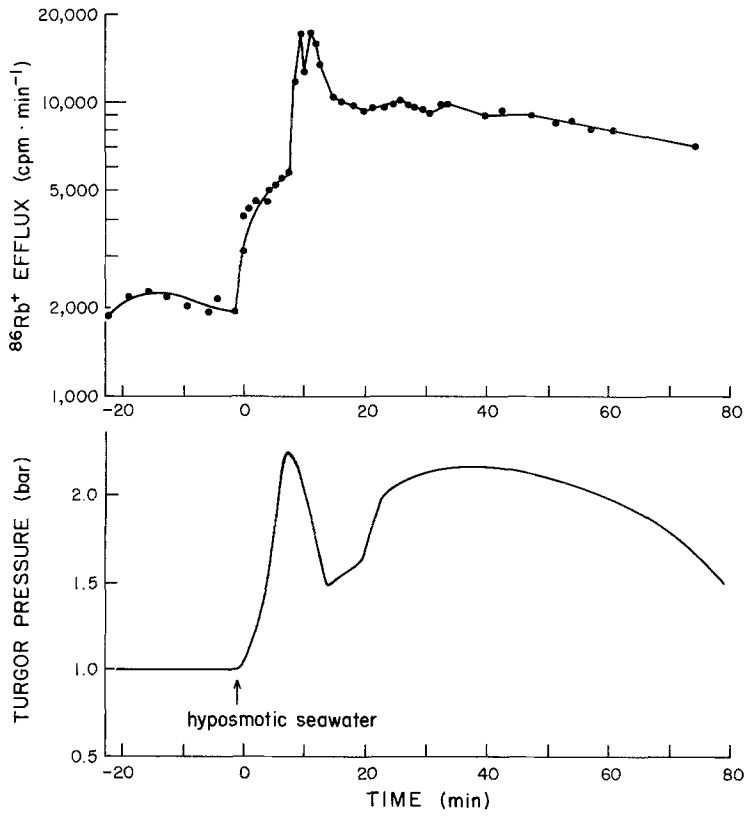

Fig. 4. Simultaneous measurements of $^{86}Rb^+$ efflux and turgor pressure following hyposmotic shock. $^{86}Rb^+$ efflux increases as turgor begins to rise. A large burst in $^{86}Rb^+$ efflux corresponds to a time of decreased turgor

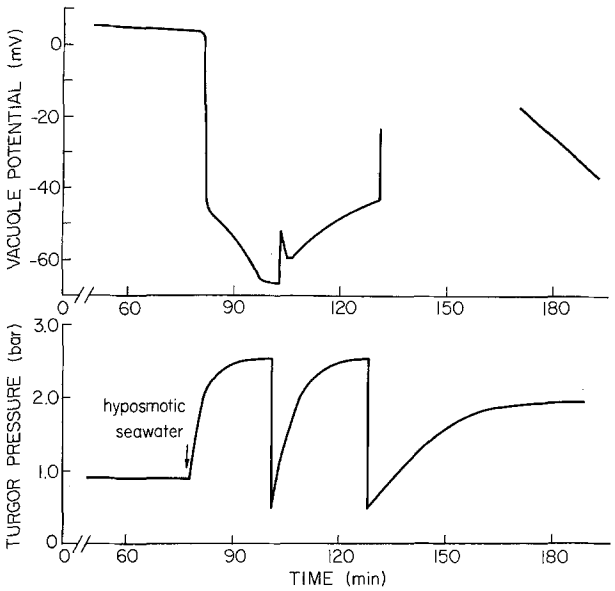

Fig. 5. Simultaneous recording of vacuole potential and turgor pressure following hyposmotic shock (−6 bar). Pressure probe was inserted at $t=0$, and hyposmotic seawater was applied at $t=76$ min. Technical difficulties interrupted the latter part of the vacuole potential recording

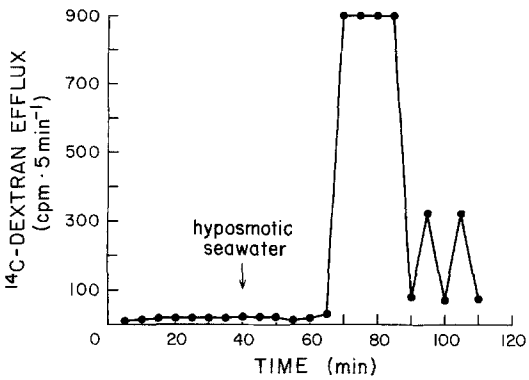

Fig. 6. ^{14}C-dextran efflux from the vacuole of *Valonia* following −5 bar hyposmotic shock. At $t=65$ min the cell rolled over and a scar site was visible

duce the vacuolar KCl concentration by 40–50 mM (about 2 bar) in 20 min.

To predict the time course of the rise in turgor following hyposmotic shock requires a knowledge of the hydraulic conductivity (L_p) and the volumetric elastic modulus (ε) (Dainty, 1963). Although L_p and ε are not known in *Valonia macrophysa*, Fig. 5 shows that the half-time for attaining a quasisteady state was 5–10 min. A similar rate of osmotic adjustment was observed in *Valonia utricularis*, which is similar in many respects to *Valonia macrophysa* (Steudle & Zimmermann, 1971; Zimmermann, 1977). In *V. utricularis*, $L_p \simeq 1 \times 10^{-6}$ cm/sec bar and ε ranges from 40 to 400 bar, depending upon both the turgor pressure and the cell volume. The slow rise in turgor in both these giant-celled algae is probably due primarily to the small surface-to-volume ratio rather than to exceptionally small values of L_p and ε. Calculations of the type outlined by Dainty (1963) suggest that osmotic gradients in the unstirred layers play only a minor role in controlling the rate of change in turgor following hyposmotic shock.

Cell Wall Permeability

Fluxes of various nonelectrolytes through isolated cell walls were measured in order to estimate the normal permeability of the wall. Dextran (mol wt 70,000) has a low cell wall permeability of $(4.7 \pm 0.05) \times 10^{-6}$ cm/sec (2). The permeability to inulin (mol wt 5,000) is $(2.3 \pm 0.9) \times 10^{-5}$ cm/sec (2), the mannitol permeability (mol wt 182) is $(2.8 \pm 0.4) \times 10^{-4}$ cm/sec (2), and the urea permeability (mol wt 60) is 4.0×10^{-4} cm/sec (1).

Mannitol and urea are probably unstirred layer limited as judged by their similar permeabilities despite differing molecular sizes. If we assume that urea diffusion is unstirred layer limited, then the combined thickness (δ) of the wall (approx. 10 μm) and unstirred layer is given by $\delta = D/P$, where D is the aqueous diffusion coefficient (1.18×10^{-5} cm^2/sec) and P is the permeability coefficient. This yields an unstirred layer thickness of about 290 μm. A similar calculation for mannitol ($D = 6.8 \times 10^{-6}$ cm^2/sec) gives an unstirred layer thickness of 243 ± 40 μm.

^{14}C-dextran efflux from the vacuole during hyposmotic stress would indicate a disorganization or rupture of the cell wall, because under normal circumstances the cell wall permeability to dextran is very low. Cells injected with dextran showed no significant efflux in normal seawater ($n=6$) (Fig. 6). Cells given a small (−50 mosmol/kg) hyposmotic shock also showed no efflux of dextran ($n=4$) and the cell walls showed no detectable damage (*data not shown*). Cells given a −217 mosmol/kg hyposmotic shock showed no immediate dextran efflux, but after 20–30 min large abrupt losses of dextran occurred ($n=6$ cells) (Fig. 6). At the same time that dextran loss occurred, the cells rolled over or a rupture site was observed. Cell movement and appearance of a rupture site were shown above to be associated with periods of rapid $^{86}Rb^+$ loss and drops in turgor pressure. Thus, the simultaneous losses of both Rb^+ and dextran suggest that the protoplasm and cell wall rupture together.

Scanning Electron Microscopy

Scanning electron microscopy shows the morphology of the volume efflux site. The trabeculated scar area,

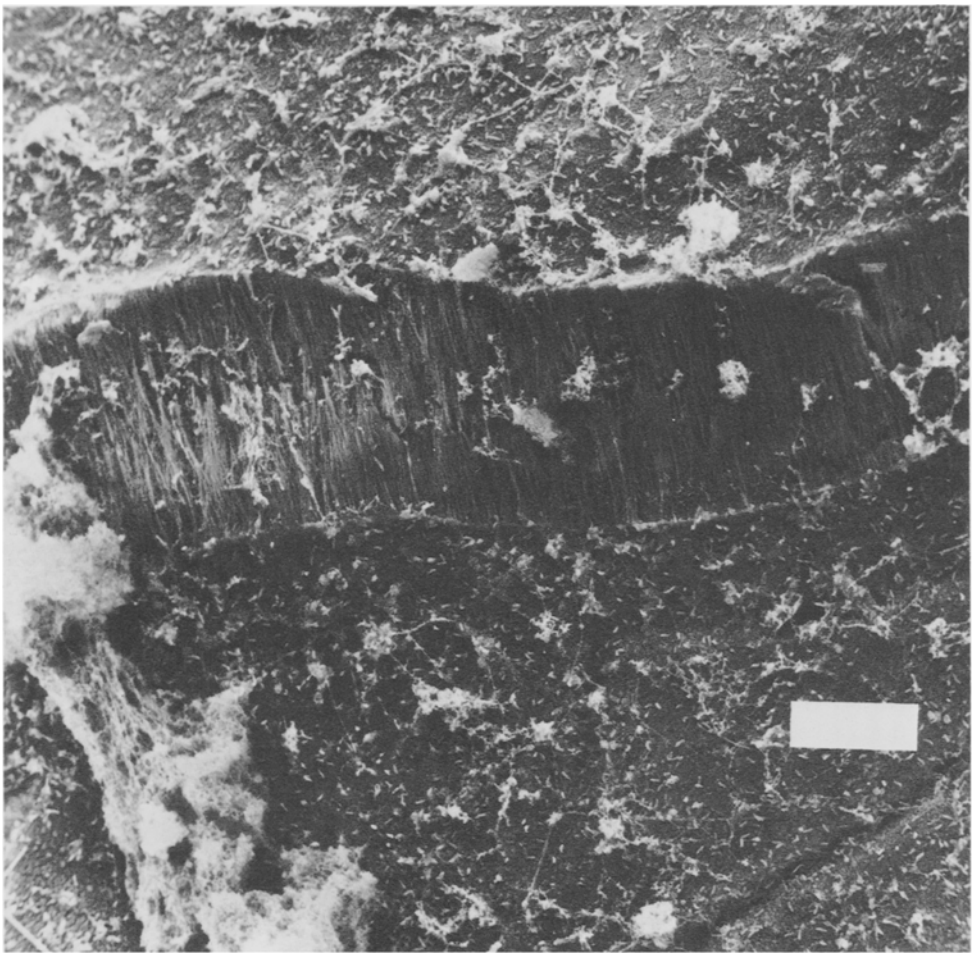

Fig. 7. Scanning electron micrograph showing part of the scar site through which rapid volume efflux occurs following hyposmotic shock. The scar is about 20 μm wide and 500 μm long. Scale marker = 10 μm. Magnification = 500 ×

part of which is shown in Fig. 7, is about 20 μm wide and 500 μm long. The microfibril bundles appear to be stretched apart and loosely packed.

Discussion

Turgor Regulation and Ionic Regulation

Turgor regulation is approx. 88% effective after acute hyposmotic or hyperosmotic shocks (Fig. 1, Table 1). Hastings and Gutknecht (1976) found 90% effective turgor regulation after gradual adaption to hyposmotic and hyperosmotic solutions. Therefore, whether the osmotic shock is applied abruptly or stepwise, *V. macrophysa* can turgor regulate with similar effectiveness. Although *Valonia* has previously been considered a stenohaline species, our data indicate that *Valonia macrophysa* can cope with abruptly changing osmolalities such as those encountered in a shallow water habitat.

Under normal conditions the ionic relations of *Valonia* are dominated by an active uptake of K^+ which results in a very high K^+ vacuolar sap (Table 1). The vacuole potential is normally about +5 mV and the Nernst equilibrium potentials for K^+, Na^+ and Cl^- are: $E_K = -97$ mV, $E_{Na} = +35$ mV and $E_{Cl} = +1$ mV. Thus there is a large outward gradient for K^+, a moderate inward gradient for Na^+, and Cl^- is in approximate electrochemical equilibrium. Decreasing the external salinity by −250 mosmol/kg causes small changes in the equilibrium potentials, but V_{vo} becomes highly negative, i.e., approximately −40 mV. Thus, during hyposmotic stress the outward gradient for K^+ (i.e., V_{vo}–E_K) becomes about +60 mV, the inward gradient for Na^+ becomes about −69 mV, and the outward Cl^- gradient becomes about −49 mV. If net ion efflux during hyposmotic stress occurred by simple electrodiffusion through a nonselective (e.g., dextran permeable) pathway, we would expect the net fluxes to be proportional to

the electrochemical gradients. However, Table 1 shows that during hyposmotic stress K^+, Na^+ and Cl^- are all lost from the vacuole, roughly in proportion to their vacuolar concentrations. This suggests that ions are lost primarily by a bulk flow mechanism.

Under normal conditions the small positive vacuole potential (V_{vo}) in *Valonia* is equal to the algebraic sum of a large negative potential across the plasmalemma and a larger positive potential across the tonoplast (Gutknecht, 1966; Davis, 1981). The plasmalemma potential is dominated by a K^+ diffusion potential, and the tonoplast potential is due partly to electrogenic pumping of K^+ into the vacuole (Gutknecht, 1967; Davis, 1981). The origin of the large negative V_{vo} during hyposmotic stress is uncertain. Zimmermann and Steudle (1974) have suggested that high turgor causes a reversal in the direction of the electrogenic K^+ pump. Alternatively, an inhibition of the K^+ pump and/or changes in the ionic selectivity of the tonoplast could partially depolarize the tonoplast potential and allow V_{vo} to be dominated by the negative plasmalemma potential. During hyposmotic stress the negative vacuolar potential is interrupted by brief periods of depolarization associated with abrupt decreases in turgor (Fig. 5). The simplest explanation for this observation is the development of transient low-resistance pathways through which ion efflux occurs.

Changes in the Cell Wall and Protoplasm during Hyposmotic Stress

The dextran permeability of the cell wall is normally about one order of magnitude lower than the inulin permeability and two orders of magnitude lower than urea and mannitol permeabilities. Extrapolating the results of Carpita, Sabularse, Montezinos and Delmer (1979) for molecular diameters and molecular weights of dextran polymers, we estimate the molecular diameter of dextran 70,000 to be about 65Å. Scanning electron micrographs of Hastings (1975) show that the gaps between the cellulose microfibrils normally range from 0 to 50 Å, averaging about 9 Å. Thus, the low cell wall permeability to dextran, the moderate permeability to inulin (diameter ca. 30 Å) and high permeability to urea and mannitol (diameters ca. 4 and 8 Å, respectively) agree qualitatively with morphological observations.

Scanning electron micrographs show that during hyposmotic stress the cell wall "stretches" locally, producing a trabeculated scar area (Fig. 7). However, the wall does not rupture completely because turgor is always >0 (Figs. 4 and 5). Since the cell wall increases in area, both the plasmalemma and tonoplast must either increase in area or rupture. The transient efflux of dextran (Fig. 6) indicates that both the cell wall and underlying protoplasm undergo drastic increases in permeability during hyposmotic stress. Observations with Nomarski optics (Zeiss Photomicroscope II) show that the chloroplasts and protoplasm appear to be retracted away from the scar site. The protoplasm and cell membranes must subsequently reseal, however, because both turgor and V_{vo} recover fully follwing dextran and volume loss from the vacuole.

Our results show that the cell wall stretches in local regions when wall tension exceeds about 6×10^5 dyne/cm. The initial response to high turgor could be a stretching of the wall at weak areas. Scar sites were often observed near the points of attachment of other epiphytic algae or rhizoids (algal rootlets). However, we cannot rule out the possibility that a local rupture of the protoplasm actually preceeds the localized stretching of the wall. If the protoplasm ruptured first, the wall would be exposed to vacuolar sap which has a low pH (5.9) and low $[Ca^{2+}]$ (1–2 mM), compared to seawater which has a pH of 8.0 and 11 mM Ca^{2+} (Hastings & Gutknecht, 1976). Tepfer and Cleland (1979) found that exposure to low pH and low Ca^{2+} causes generalized wall loosening in *Valonia ventricosa*, and a similar phenomenon might conceivably cause localized wall loosening in *Valonia macrophysa*. Further work is needed to elucidate the sequence of events involved in this novel response to hyposmotic stress.

Mechanisms Involved in Turgor Regulation

Previous studies have shown that different mechanisms are involved in hyperosmotic and hyposmotic turgor regulation in *Valonia*. During hyperosmotic stress (low turgor) active K^+ uptake is stimulated, which results in KCl and water influx until turgor is restored to normal (Gutknecht, 1968, Hastings & Gutknecht, 1976). During hyposmotic stress (high turgor) K^+ permeability increases, which results in KCl and water loss (Steudle et al., 1977). These changes in K^+ permeability and active transport are caused by changes in turgor pressure rather than by changes in absolute pressure, ionic gradients, osmotic gradients, or net water movements (Gutknecht, 1968; Hastings & Gutknecht, 1974; Zimmermann & Steudle, 1974; Steudle et al., 1977). Possible changes in Na^+ and Cl^- permeability during osmotic stress have not been investigated.

Our results indicate the existence of a second mechanism of hyposmotic regulation which becomes important when cell wall tension exceeds 6×10^5 dyne/cm. This process is characterized by abrupt increases in $^{86}Rb^+$ and dextran efflux, abrupt decreases in tur-

gor and transient depolarization of the vacuole potential (Figs. 3–6). Our data indicate that vacuolar sap is lost directly to the external seawater. We were first alerted to the existence of this novel mechanism when we observed that cells subjected to acute osmotic shock often rolled over due to the intensity of the pressure-driven volume efflux. The turgor pressure which triggers this mechanism is dependent upon cell volume; i.e., larger cells require smaller turgor pressures in order to attain the critical wall tension which triggers the abrupt increase in permeability. There is also considerable variation in the time course of the volume efflux. In some cells the drop in turgor is rapid, and subsequent recovery begins within 1 min (Figs. 3 and 5). In other cells the decrease and recovery of turgor occur over periods of up to 20 min (Figs. 4 and 6). Since 98% of the cells survive and resume normal growth following acute hyposmotic shock, we believe that this volume efflux mechanism is physiologically important in *Valonia macrophysa*.

Other examples of pressure or volume-sensitive solute loss through nonspecific pathways can be found in bacterial, plant and animal cells. For example, when *E. coli* is subjected to hyposmotic shocks, a rapid loss of sugars, nucleotides and K^+ occurs, and the barrier damage is repaired in less than 2 sec (Epstein & Schultz 1965; Tsapis & Kepes, 1977). *E. coli* also possesses a turgor-sensitive K^+ uptake system which is similar in some respects to that of *Valonia* (Rhoads & Epstein, 1978). The alga, *Platymonas suecica*, loses 50% of its photoassimilated carbon within 10 min after acute hyposmotic shock. Solutes lost include mannitol, amino acids and other unknown solutes (Hellebust, 1976). When human red blood cells are subjected to hyposmotic shock, many 200 Å diameter holes appear in the membrane with subsequent resealing after 10–20 sec (Seeman, 1967). Thus, transient breakdown of the permeability barrier and subsequent repair can occur in various types of cells, and in euryhaline cells this is an important mechanism for surviving acute osmotic stress.

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