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Ionic Relations and the Regulation of Turgor Pressure in the Marine Alga, *Valonia macrophysa*

David F. Hastings* and John Gutknecht**

Department of Physiology & Pharmacology,
Duke University Medical Center, Durham, North Carolina 27710, and
Duke University Marine Laboratory, Beaufort, North Carolina 28516

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Summary. Ionic composition and turgor pressure in the giant celled marine alga, *Valonia macrophysa*, were measured at environmental salinities ranging from $15^0/_{00}$ to $60^0/_{00}$ (11–44 atm). The steady-state turgor pressure, which is normally about 1.5 atm, changes only 2.5 atm in response to a 25 atm change in seawater osmotic pressure. Thus, turgor regulation is 90% effective. The salts important in turgor regulation are KCl and NaCl. During turgor regulation changes in intracellular KCl concentration account for 85% of the change in sap osmolality, and changes in NaCl account for the remaining 15%. Potassium is actively transported into the vacuole, whereas chloride appears to be passively transported as the counter ion. Thus, potassium transport, which we have shown previously to be sensitive to the turgor pressure, accounts for most of the turgor regulation at all salinities.

Turgor pressure in plant cells is required for normal growth and cell wall extension (Green, 1962), and it also maintains the form and mechanical rigidity of the cell (Kramer, 1969). In many marine and brackish water algae turgor pressure protects the cells from sudden changes in the osmolality of the environment (Biebl, 1962). Algae growing in the intertidal zone may allow turgor to fluctuate widely in response to short-term osmotic stress. These algae, which often have thick cell walls and high turgor pressures, can tolerate 0.1 to 3.0 times the osmolality of standard seawater (Biebl, 1962). In contrast, sublittoral algae living at or below mean low tide exhibit a narrower tolerance to short-term osmotic stress, *i.e.*, 0.4–1.8 times standard seawater osmolality (Gessner & Schramm, 1971).

The genus *Valonia* is generally considered to be stenohaline, based on

* *Present address:* Institute of Physiology, University of Aarhus, DK-8000, Aarhus, Denmark.

** *Reprint requests:* Duke University Marine Laboratory, Beaufort, N.C. 28516.

its sublittoral habitat as well as on the known intolerance of this alga to acute changes in environmental osmolality (Taylor, 1960; Gessner, 1967, 1969). The stenohaline classification implies that the alga has little or no ability to osmoregulate. However, Zimmermann and Steudle (1974) recently found that *Valonia utricularis*, a Mediterranean species, can withstand sudden reductions of up to 13 atm in external osmolality and that within about 10 hr the alga regains its original turgor pressure of about 1.5 atm. This work suggests that the osmoregulatory capacity of *Valonia* may be greater than previously suspected.

Our study answers several questions pertaining to salinity tolerance and turgor regulation in *Valonia*. What is the long-term salinity tolerance of *Valonia*? Does *Valonia* regulate turgor pressure over this salinity range? What salts are important in the regulation process? These questions must be answered before we can accept the suggestion of Gutknecht (1968) that the pressure-sensitive potassium transport system in *Valonia* is part of a turgor regulatory mechanism. In this paper we will show that *Valonia macrophysa* is capable of regulating its turgor pressure over a wide range of salinities. The important salts in turgor regulation are KCl and NaCl, and KCl accounts for 85% of the turgor regulation at all salinities within the growth range.

Materials and Methods

Culture

The zoospores from a single *Valonia macrophysa* cell were collected from the vacuole prior to their release into seawater (Pringsheim, 1946). The uncontaminated zoospores were cultured as a unialgal clone in enriched seawater for 4 months. The cultures were illuminated by reflected skylight at about 10 W/m^2 and maintained at 22–25 °C. The cells remained roughly spherical and reached an average diameter of 3 mm. The culture medium was sterile enriched seawater which was changed at 2 week intervals (James, 1969).

The seawater was prepared by raising the osmolality of local off-shore seawater from 1000 mosmol/kg ($33^0/_{00}$) to 1120 mosmol/kg ($37^0/_{00}$) by titrating with a freeze-concentrate of seawater ($100^0/_{00}$) (Barnes, 1954). The seawater was enriched with ALGA-GRO^R (Carolina Biological Supply, Burlington, N.C.), which is a mixture of nitrate, phosphates, micronutrients and vitamins. The enriched seawater was autoclaved at 2 atm pressure for 15 min. To prevent the formation of precipitates in this seawater medium, the pressure was reduced slowly over a 20-min period.

Acclimation

Due to its small surface to volume ratio, *Valonia* can be expected to adjust slowly to any osmotic changes in the environment. Thus, to test the ability of *Valonia* to adapt to a range of salinities, care must be taken to change the salinity gradually, keeping the osmotic stress within the physiological limits of the cell.

Eight groups of weighed cells (1 g/group) were placed in separate culture dishes containing 250 ml of enriched seawater. At 12-hr intervals enough sterile distilled water or sterile $100^0/_{00}$ seawater was added to each dish to produce a 1 atm change in turgor pressure. The addition schedule was continued until each culture dish reached its designated final salinity, *i.e.*, 15, 20, 25, 30, 36.5, 42, 50 and $60^0/_{00}$, as measured by refractometry. Upon reaching the final salinity each culture was replenished with 250 ml of fresh seawater of the proper salinity once a week for 2 weeks. At the end of the 2-week acclimation period the ionic composition of the cells was assumed to have reached a steady state.

Analytical

The growth rate of the cells was calculated from the change in the blotted wet weight during the acclimation period. The daily percent growth (*GR*), was calculated from the equations:

$$k = \ln(wt_1/wt_0)/t \tag{1}$$

$$GR = 100\,k \tag{2}$$

where k is the exponential time constant, t is the growing period in days, and wt_0 and wt_1 are the initial and final wet weights of the cells, respectively.

The vacuolar potentials of acclimated cells were measured by means of ground glass perfusion pipets (100 μm tip diameter) inserted into the vacuole. The seawater bath and the vacuole were electrically coupled to a Keithley electrometer via agar-salt bridges and calomel electrodes (Gutknecht, 1968).

The intracellular fluid was extracted from 1-g samples, containing approximately 60 cells each, by twice freezing the blotted cells and then removing the cell walls and cytoplasmic fragments by centrifugation. Prior to blotting, the cells were exposed to 10 mM lithium in seawater. The Li^+ was intended to serve as a cationic marker of the extracellular space in the cell wall. However, Li^+ proved to be a very poor marker because it binds to cellulose microfibrils (Raelofson, 1959). An independent calculation of the extracellular volume for spherical cultured cells suggests that the extracellular volume is less than 2% of the total cell volume. This estimate assumes a 10 μm aqueous layer in the cell wall and a 3.0 mm cell diameter.

The osmolalities of seawater and cell sap were measured by freezing point depression (Advanced Osmometer, Model 65-31). The precision of the technique, based on replicate measurements of seawater, was ± 2 mosmol/kg (SD). The precision of the technique was independent of concentration over a range of 500–1800 mosmol/kg.

Turgor pressure was estimated from the difference between the osmolalities of seawater and cell sap, using the van't Hoff approximation, *i.e.*, $\Delta\Pi = RT(\Delta$ osmolality). The estimation of the turgor pressure (ΔP) from the osmotic pressure difference ($\Delta\Pi$) assumes that the cell is in osmotic equilibrium, which is not strictly true in a growing cell. However, in our slowly growing (approx. 5%/day) *Valonia* cells the systematic overestimation of ΔP caused by assuming $\Delta P = \Delta\Pi$ is smaller than the random error in our estimation of ΔP (see Table 2). Thus, we have not corrected our estimates of turgor pressure for the effects of cell growth. In two other species of *Valonia* (*ventricosa* and *utricularis*) the osmotic pressure difference was found to equal, within experimental error, the turgor pressure, which was measured directly (Villegas, 1967; Steudle and Zimmermann, 1971).

Sodium, potassium, lithium, calcium, barium and magnesium concentrations were measured by atomic absorption using 4 mM CsCl as the ionization quenching agent (Sanui & Pace, 1968). Chloride-bromide concentration was measured by amperometric titration with silver ions (Buchler-Cotlove chloridometer). Nitrate concentration was measured by the nitrate reductase method of Lowe and Hamilton (1967). Sulfate was measured by the method of barium precipitation (Varian Techtron, 1972).

Results

The ionic compositions of cultured cells and wild cells are compared in Table 1. Chemical assay of the vacuolar sap shows that the only major difference between the ionic composition of cultured cells and freshly collected cells is the 45 mM nitrate concentration in cultured cells. All the cells are high in K^+ and low in Na^+, and the cultured cells exhibit the same high variability in Na^+ and K^+ concentrations as wild cells (Steward & Martin, 1937). The SO_4^{2-} concentration in the cultured cells is less than 1 mM, which is close to the amount reported by Osterhout (1924–25), and the Ca^{2+} and Mg^{2+} concentrations are also low. Thus, cultured cells are very similar to the wild cells with regard to their ionic composition, and they offer the advantages of controlled development and an epiphyte-free cell wall.

Fig. 1 shows the growth rates of cultured cells acclimated to salinities ranging from 15 to 60‰. Although the cells remained alive at all salinities, the near zero growth rates at the extremes of 15, 20, and 60‰ suggest physiological dysfunction at these salinities. Thus, although the 10-day viability range of this species is at least 15‰ to 60‰, the growth range is only about 25‰ to 50‰.

Table 2 shows the turgor pressures calculated from the osmolality difference at each salinity in the growth range, *i.e.*, 25‰ to 50‰ or 0.78 to 1.51 osmol/kg. In Fig. 2 these turgor pressures are plotted against the

Table 1. Turgor pressures and ionic composition of *Valonia macrophysa* from several locations and conditions[a]

	Wild cells freshly collected		Wild cells maintained for 2 mo	Zoospores cultured 4 mo
	Florida 5/15/73	Puerto Rico 1/14/74	Florida 8/16/72	Florida 1/14/74
Turgor (atm)	0.79 ±0.1 (6)	1.0 ±0.1 (10)	1.61 ±0.05 (7)	1.71 ±0.03 (4)
K^+ (mM)	483 ±12 (6)	569 ±8 (14)	485 ±12 (7)	431 ±13 (4)
Na^+ (mM)	130 ±15 (6)	69 ±6 (14)	137 ±12 (7)	195 ±20 (4)
Mg^{2+} (mM)	–	–	4.7 ±0.9 (7)	1.1 ±0.6 (3)
Ca^{2+} (mM)	–	–	2.2 ±0.2 (7)	1.0 ±0.1 (3)
Cl^- (mM)	622 ±5 (4)	606 ±5 (10)	633 ±4 (7)	600 ±5 (4)
NO_3^- (mM)	–	0.8 ±0.3 (6)	–	45 ±2 (4)
SO_4^{2-} (mM)	–	–	–	0.3 ±0.2 (3)
pH	5.9 ±0.1 (5)	–	5.8 ±0.1 (5)	5.9 ±0.1 (3)

[a] This table compares cells grown from zoospores in unialgal cultures to cells collected from Florida and Puerto Rico. Results are expressed as the mean ±SE (number of measurements).

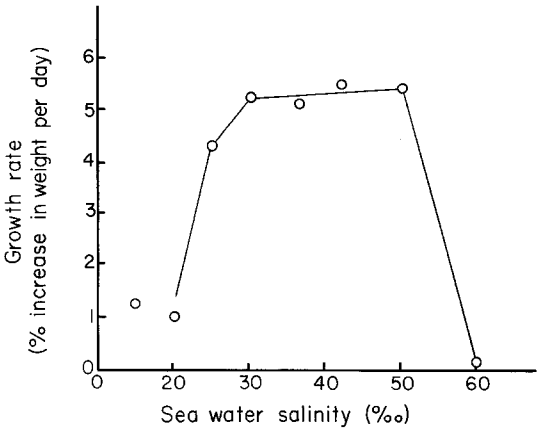

Fig. 1. Growth rates of *Valonia macrophysa* at different environmental salinities. Although the cells remained alive at all salinities, significant expansion growth occurred only within the range, 25‰–50‰

Table 2. Turgor pressures and osmolalities of cell sap in *Valonia macrophysa* adapted to different salinities[a]

Osmolality or turgor	Seawater salinity				
	12‰	30‰	36.4‰	42‰	50‰
Turgor (atm) at $T=22$ °C	3.2 ±0.07 (4)	2.5 ±0.08 (4)	1.7 ±0.05 (5)	1.6 ±0.09 (4)	1.16 ±0.04 (6)
Seawater (Π_{sw}) (mosmol/kg)	767±2	903±2	1120±1.4	1307±2	1512±2
Cell sap (Π_{cs}) (mosmol/kg)	898±2.2 (3)	1007±2.6 (3)	1191±1.4 (4)	1374±3.3 (3)	1560±0.9 (3)
K^+Na^+ salts of sap (Π_{KNa}) (mosmol/kg)	833±5 (3)	978±23 (3)	1122±10 (4)	1302±12 (3)	1487±17 (3)
Residual osmolality ($\Pi_{cs}-\Pi_{KNa}$) (mosmol/kg)	65±6 (3)	29±23 (3)	69±10 (4)	72±12 (3)	73±17 (3)

[a] The osmolalities of seawater in line 2 are the means of two readings on the same sample, and the error is expressed as the SD. The remaining data is quoted in the form: mean ±SE (number of measurements). For a description of lines 4 and 5 see text.

osmolality of the seawater. If the osmolality of the sap were constant, then a plot of the osmolality difference *vs.* the osmolality of seawater would have a slope of −1.0 (dashed line in Fig. 2). However, a linear regression through the data points gives a slope of -0.106 ± 0.017(5)(SE). This represents a 90% regulation of the turgor pressure. The solid line is extrapolated through one point on either side of the growth range, which

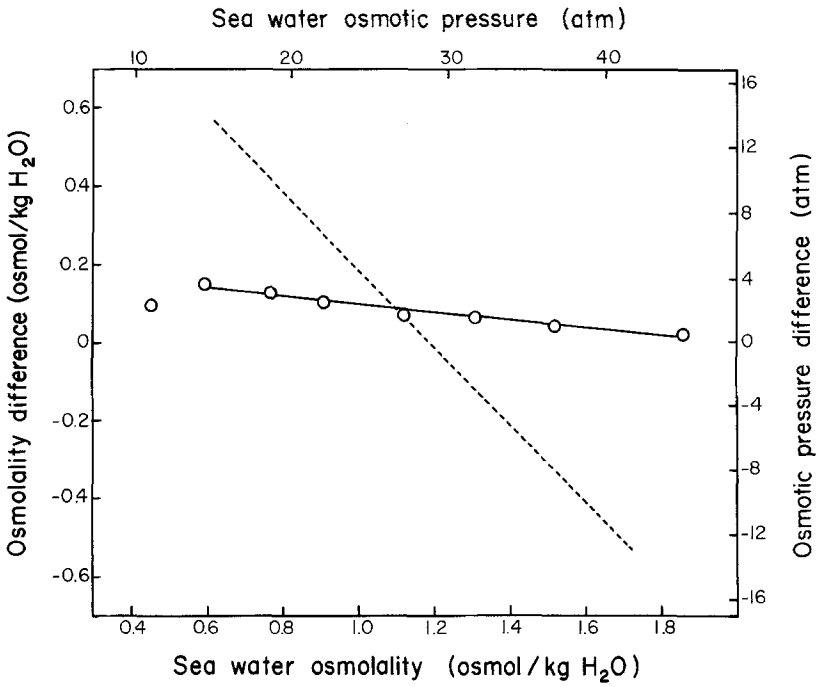

Fig. 2. Turgor pressures in *Valonia macrophysa* at different salinities. The dashed line is the theoretical osmolality difference which would exist if the sap composition remained constant, *i.e.*, if there were no turgor regulation (slope $= -1.0$). The solid line is the linear regression of the observed osmolality difference against the osmolality of seawater. The five points within the growth range (0.77–1.51 osmol/kg) give a regression slope of -0.106 ± 0.017 (5), which represents a 90% regulation of turgor pressure

suggests that turgor regulation may occur over a salinity range which is greater than that of the growth range.

To maintain a constant turgor pressure over a wide range of salinities, the composition of the cell sap must change, and these changes are shown in Table 3. Over the growth range of 25 to 50‰ the sap KCl concentration increases 300 mM, whereas the NaCl concentration increases only 64 mM. At all salinities between 20 and 60‰ the NO_3^- concentration is about 49 mM. Over the growth range from 25 to 50‰ electrical neutrality is maintained by the four ions assayed, *i.e.*, K^+, Na^+, Cl^- and NO_3^-. However, at 15‰ and 20‰ an unidentified cation becomes a significant component of the sap.

To find out whether the observed changes in sap K^+ and Na^+ salt concentrations can account for the observed changes in sap osmolality, we measured the osmolalities of artificial sap solutions for each salinity over the growth range of 25 to 50‰. The artificial sap solutions contained

Table 3. Ionic composition of the cell sap of *Valonia macrophysa* adapted to different salinities[a]

Seawater salinity (‰)	Ion concentration (mM)				Electrical neutrality ([cations] − [anions]) (meq/liter)
	K^+	Na^+	Cl^-	NO_3^-	
15 (2)	103±2	108±5	272±2	29±0.7	−90±10
20 (2)	152±7	175±3	352±4	53±0.8	−78±15
25 (3)	257±12	244±12	430±3	49±0.8	22±26
30 (3)	293±10	251±11	507±13	55±0.4	−18±20
36.4 (4)	422±13	201±20	600±5	45±1.6	−23±14
42 (3)	534±11	285±12	697±7	51±0.6	50±43
50 (3)	557±16	308±16	808±10	47±0.0	10±27
60 (2)	629±5	466±36	1002±4	45±0.5	47±45

[a] The numbers in parentheses are the numbers of samples assayed at each salinity, and the errors are the standard errors of the means. Each sample contained the pooled sap from about 60 cells.

Na^+ and K^+ at the respective assayed concentrations and Cl^- and NO_3^- at the same ratios as those observed in the cell saps at each salinity. If K^+ and Na^+ salts account for all of the changes in sap osmolality, then the difference between the osmolality of the test solutions and the observed osmolality of the cell saps will be constant. Table 2 compares the observed osmolality of the cell saps (line 3) with the osmolality of the K^+—Na^+ salt solutions (line 4). The rather large standard errors in line 4 reflect the large uncertainty in the anion concentrations (see Table 3) which were used to define the K^+—Na^+ test solutions at each salinity. The fifth line in Table 2 shows that the difference between the osmolalities of the two solutions is not correlated with salinity. The regression slope equals 0.039±0.033(5) with 3 degrees of freedom. The slope is not significantly different from zero ($P > 0.3$). Thus, the residual osmolality difference can contribute at most only 4% to the osmoregulation of the cell, and the changes in sap osmolality must be due to changes in the KCl and NaCl concentrations of the sap.

Fig. 3 evaluates the separate roles of KCl and NaCl salts in turgor regulation. Using a method adapted from Millero (1974), we calculated the osmotic contribution of each salt from the formula, $\Pi^i = 2k\,C_+$, where C_+ is the molar concentration of cations. First, the osmolar coefficient, k, of the vacuolar "salt," $KNaClNO_3$, was measured for each mixed salt solution. Because the osmotic coefficients of KCl, KNO_3, NaCl and $NaNO_3$ are very similar at the ionic strength of *Valonia* sap, we assumed the osmolar coefficient of the mixed salt, $KNaClNO_3$, to be a

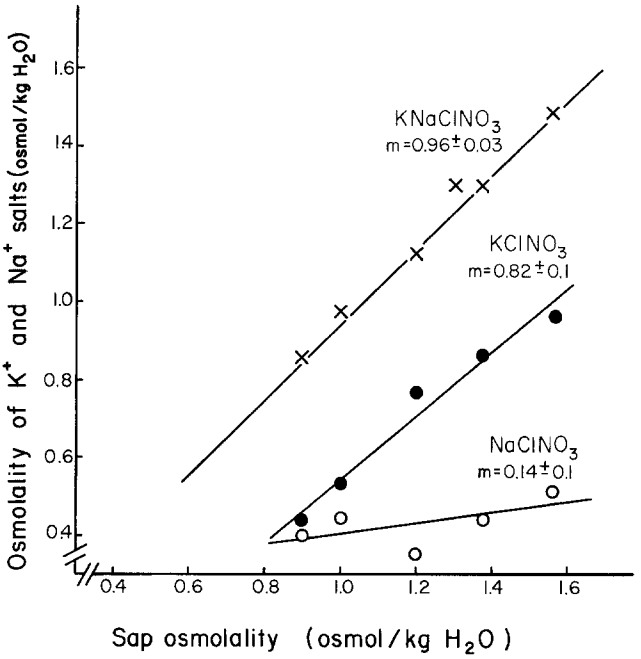

Fig. 3. Osmolality of artificial sap solutions plotted against the observed osmolality of the sap (upper curve). The artificial sap contains Na^+ and K^+ at the same concentrations as measured in the sap, plus Cl^- and NO_3^- at the same ratio as measured in the sap. The lower two curves show the calculated contributions of Na^+ and K^+ salts to the observed osmolality of the sap

good estimate of the osmolar coefficient of the K^+ and Na^+ salts in the sap. Using the measured osmotic coefficients of $KNaClNO_3$ and the measured ion concentrations in *Valonia* sap, we calculated the osmolalities of $KClNO_3$ and $NaClNO_3$ and plotted these values against the observed osmolality of the sap (lower two curves in Fig. 3). Linear regression of the calculated osmolality of $KClNO_3$ against the total osmolality of the vacuolar sap yields a slope of 0.82 ± 0.1 (df = 3). Since the NO_3^- concentration is constant, 82% of the change in sap osmotic pressure is due to changes in the KCl concentration. Of the residual 18%, 14% can be attributed to changes in the NaCl concentration which has a slope of 0.14 ± 0.1 (df = 3). The Na^+ slope differs from zero with a probability of only 0.3. Thus, intracellular Na^+ concentration is not clearly related to salinity within the growth range. The combined osmolality of K^+ plus Na^+ salts has a regression slope of 0.96 ± 0.03, which is not significantly different from 1.0 (upper curve in Fig. 3). Thus, KCl accounts for about 85% of the observed osmotic regulation, *i.e.*, 100 (0.82/0.96), and NaCl accounts for the remaining 15%, *i.e.*, 100 (0.14/0.96).

Table 4. Effects of salinity and metabolic inhibitors on the vacuolar potential (V_{vo}) and equilibrium potentials (E_j) of the major intracellular ions in *Valonia macrophysa*[a]

E_j or V_{vo}	Salinity		
	15‰	36.4‰	60‰
E_K (mV)	−82	−96	−93
E_{Na} (mV)	15	22	13
E_{Cl} (mV)	0.8	0.4	−0.4
E_{NO_3} (mV)	121	116	133
V_{vo} (mV)	8	7	6
V_{vo} in presence of CN^- (1 mM) or azide (1 mM) (mV)	1.0	1.0	1.5

[a] Equilibrium potentials between sap and seawater were calculated by means of the Nernst equation, *i.e.*,

$$E_j = \frac{59}{z_j} \log \frac{C_j^0}{C_j^i} \text{ mV at } 24\,^\circ\text{C}.$$

Some additional information on the ionic relations of *V. macrophysa* at different salinities is given in Table 4, which shows that the vacuolar potential (V_{vo}) and the equilibrium potentials (E_j) for the major ions remain approximately constant over a wide range of salinities. Active uptake of K^+ and NO_3^- is suggested by the fact that these ions are far out of electrochemical equilibrium at all salinities ($V_{vo} - E_K \simeq 97$ mV, and $V_{vo} - E_{NO_3} \simeq -116$ mV). E_{Na} and E_{Cl} are both close enough to V_{vo} that conclusions regarding the possible active transport of these ions requires additional information, some of which will be discussed below. The metabolic inhibitors, sodium cyanide (1 mM) or sodium azide (1 mM), reduce V_{vo} within 2 min from its normal value of about 7 mV to about 1 mV, which is almost identical to E_{Cl} (Table 4).

Discussion

Regulation in a physiological system requires the output of the system to modify a controlled process in a manner which will shift the output toward the set point. In the case of turgor regulation in *Valonia*, the output is the turgor pressure, the controlled process is the rate of salt transport, and the set point is the normal turgor pressure. The difference between the actual turgor and the set point is defined as the error signal, *i.e.*, error signal = turgor − set point. The error signal may modify transport activity either by changing the physical driving force for ion movements or by

affecting the transport process directly, *i.e.*, by changing either the pumping rate or the ionic permeability.

In *Valonia* three ions may be important in turgor regulation, *i.e.*, K^+, Na^+ and Cl^-. Of these three, K^+ is the most directly involved in turgor regulation. The net transport of K^+ into the vacuole is stimulated by a decrease in turgor pressure. The stimulation of K^+ uptake is not due to an increase in external K^+ concentration, a substantial decrease in cell volume, a negative shift in V_{vo}, or a coupling to osmotic movements of water (Gutknecht, 1968; Cram, 1973; Hastings & Gutknecht, 1974; Zimmermann & Steudle, 1974). Furthermore, the influx of K^+ is clearly an active transport process, based on (1) a large net influx under short-circuit conditions, (2) large deviations between observed and predicted flux ratios, (3) inhibition of net K^+ influx by cyanide and azide and (4) maintenance of a vacuolar K^+ concentration which is about 100 mV out of electrochemical equilibrium at all salinities (Aikman & Dainty, 1966; Gutknecht, 1966, 1967; Hastings & Gutknecht, 1974; Table 4, and *unpublished data*). Thus, in stimulating net K^+ uptake the error signal is acting primarily on the active transport process rather than on the electrochemical driving force or the potassium permeability.

Chloride appears to be passively transported as the counter ion to K^+. This statement is based on (1) agreement between the observed and predicted Cl^- flux ratios when active K^+ transport is occurring in normally growing cells and (2) absence of a net Cl^- flux under short-circuit conditions in internally perfused cells (Gutknecht, 1966; and *unpublished data*). There are two reports of active Cl^- uptake into the vacuole of *Valonia*, but at least one of these is erroneous. First, in *V. ventricosa* our reported net Cl^- influx under short-circuit conditions was an artifact due to the presence of ^{40}K, a naturally occurring radioisotope, in the flux samples (Gutknecht, 1967, and *unpublished data*). Aikman and Dainty (1966) also reported an active Cl^- influx based on a Cl^- flux ratio of 1.0 in *V. ventricosa* and a vacuole potential of -20 mV, which is more negative than E_{Cl} ($+1$ mV). However, V_{vo} is rarely negative for any species of *Valonia*. A negative V_{vo} may occur when cells are excreting salts in response to hyperturgor stress (Steudle & Zimmermann, 1974) or in response to a permeant weak acid in the external seawater (Blinks, 1955). If we assume that the normal V_{vo} in *V. ventricosa* is $+17$ mV (Blinks, 1929; Gutknecht, 1966; Blei, 1967), then only passive Cl^- transport into the vacuole is indicated under normal conditions.

The conformity of Cl^- transport to predicted passive behavior occurs both at the set point and when turgor $= 0$ atm. When net K^+ uptake is

stimulated by the error signal, net Cl^- uptake is stimulated also. Since the net Cl^- flux is equal to the Cl^- conductance (g_{Cl}) times the electrochemical driving force ($V_{vo} - E_{Cl}$), the error signal might stimulate Cl^- uptake by increasing either g_{Cl}, $V_{vo} - E_{Cl}$, or both. Our preliminary results suggest that both g_{Cl} and V_{vo} are increased by the error signal. Total membrane (protoplasm) conductance definitely increases with the error signal (Steudle & Zimmermann, 1974; Hastings & Gutknecht, 1974).

Sodium makes a small (15 %) and highly variable (± 10 %) contribution to turgor regulation (Fig. 3). Our data do not clearly establish that Na^+ transport is an important part of the turgor regulation system. E_{Na} is more positive than V_{v0} (Table 4), which suggests either that Na^+ is normally pumped out of the vacuole or that the growth rate exceeds the passive Na^+ influx. The first explanation is likely to be the correct one. Gutknecht (1966) has suggested that two Na^+ pumps are required. One pump at the plasmalemma pumps Na^+ out of the cell, and a second pump at the tonoplast pumps Na^+ into the vacuole. This "back to back" arrangement of Na^+ pumps is required to explain the low Na^+ concentration in the cytoplasm, which is maintained in spite of a -70 mV cytoplasmic potential (Gutknecht, 1966; Davis, *personal communication*). The Na^+ transport system appears to be arranged symmetrically around the cytoplasmic layer, which suggests that the Na^+ transport system is more important in regulating the volume and composition of the cytoplasmic layer than in regulating the turgor pressure of the cell.

In conclusion, *V. macrophysa* regulates its turgor pressure by regulating the KCl concentration in the vacuole. These findings confirm the suggestion of Gutknecht (1968) that the pressure sensitive K^+ pump in *Valonia* is part of a turgor regulating system. Additional support for this hypothesis was obtained recently in *V. utricularis* (Zimmermann & Steudle, 1974). Evidence for turgor regulation in some other marine algae was obtained by Kesseler (1964, 1965) and Bisson and Gutknecht (1975). Recently a pressure sensitive Cl^- pump was found in the giant celled marine alga, *Halicystis parvula* (Graves, 1974). Thus, pressure-sensitive ion transport may be a common component of turgor regulating systems in marine algae. For recent reviews on ionic and osmotic regulation in algae *see* Hope and Walker (1975), MacRobbie (1975), Cram (1976) and Raven (1976).

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