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SODIUM, POTASSIUM, AND CHLORIDE TRANSPORT AND MEMBRANE POTENTIALS IN VALONIA VENTRICOSA

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In 1891 Meyer discovered that coenocytic cells of the marine alga *Valonia* have a high potassium and low sodium content. *Valonia* and other giant-celled algae subsequently became popular organisms for studies of ion permeability and transport, mainly because large quantities of vacuolar sap could be collected without contamination by protoplasm or extracellular fluid. From these early studies came Osterhout's theories on the nature of protoplasmic surfaces and his models of membrane transport by carrier molecules (Osterhout, 1931, 1940).

Electrical studies on *Valonia,* which were conducted with the permeability and transport studies, were initiated by Osterhout *et al.* (1924, 1927) and pursued later by Blinks (1940, 1955). *Valonia* was, in fact, the first cell in which a steady electric potential between internal and external solutions, *i.e.,* sap and sea water, was demonstrated. Osterhout's discovery that the vacuole of *Valonia* is electrically positive to external sea water did not seem unusual, since few data from other cells were available for comparison. The positive vacuole potential appears now, however, to be restricted to *Valonia* and its close relatives (see Blinks, 1949).

Later work on ion transport in *Valonia* suggested a relation between net K^+ uptake and external phosphate concentration (Mullins, 1949) and the existence of separate mechanisms for Na^+ and K^+ transport (Scott and Hayward, 1955). Another marine coenocyte, *Halicystis ovalis,* was shown to extrude Na^+ at the plasmalemma and actively absorb Cl^- at the tonoplast (Blount and Levedahl, 1960). Similar transports of Na^+ and Cl^- were found in *Nitellopsis,* a brackish-water characean (MacRobbie and Dainty, 1958a). Additional work on marine algae is summarized by Gutknecht (1965a).

In *Valonia* the relations between ion concentrations and fluxes and the electric potentials have not been studied. Therefore it is not known with certainty which ions are actively transported nor at which membrane, plasmalemma or tonoplast, the transport takes place. My objective is to answer these questions by using electrochemical methods similar to those of Dainty, MacRobbie and others working on the fresh-water Characeae (see reviews by Dainty, 1962, 1964).

I will show that the ionic and electrical properties of *Valonia ventricosa* differ in several respects from those of other plant cells. The high salt content of the vacuole is produced by an inward transport of Na^+ and especially K^+ at the tonoplast, whereas Cl^- uptake into the vacuole appears to be a passive process. The electric potential of the cytoplasm is highly negative to the vacuole, which contrasts with the small or zero potential across the tonoplast of other cells. Like most other plant and animal cells, *Valonia* actively extrudes Na^+ at the plasmalemma, and its cytoplasm is electrically negative to the external sea water.

Methods

Culture methods

The *Valonia* cells used in this study were either 1–3 cm. diameter, shipped by air from Puerto Rico, or 0.3–0.8 cm. diameter, grown from aplanospores in the laboratory. Similar results were obtained with both sizes. The experimental and culture medium was an enriched sea water similar to that described by Keck (1964). Briefly, the medium consisted of natural sea water plus nitrate, phosphate, soil extract, and enough artificial sea salts (Utility Chemical Co., Paterson, N. J.) to raise the salinity to about 37‰—similar to the water from which the cells were collected.

Cultures were started with young cells, called aplanospores, which formed spontaneously from the protoplasm of mature coenocytes (see Steward, 1939). Soon after spore formation, the outer wall of the coenocyte was cleaned with alcohol to remove epiphytes. The wall was then pierced with a syringe, and a suspension of spores was withdrawn and transferred to the sea-water medium. Cultures started in this way were free from contaminating algae, which was important since other species usually grow faster than *Valonia.*

The cells were grown in large fingerbowls, and the medium was changed at about two-week intervals. The temperature was 25 ± 1° C., and illumination was 3000–4000 lux, 8–12 hr./day (natural daylight plus fluorescent lighting). Some of the bowls contained marble chips, as prescribed by Steward (1939); within several weeks many cells became attached to the marble substrate by rhizoid processes. This attachment did not appear necessary for normal growth and development, however, for in three months both attached and unattached cells grew to a diameter of about 0.5 cm. and showed similar ionic and electrical properties.

Chemical measurements

Sap from large cells was obtained by piercing the cell surface with a syringe and withdrawing about 200 μl. Sap from small cells was collected by washing the cell for one minute in 0.9 *M* sucrose, blotting with absorbent tissue, puncturing with a sharp needle, and gently squeezing. The sap was uncontaminated by protoplasm, as indicated by the absence of chloroplasts in the sample.

Protoplasm was collected by cutting a large coenocyte in half and allowing the sap to drain out. The wall and adhering protoplasm were washed gently for 20–25 seconds in a large volume of Tris-sulfate buffer, pH 7.7–7.8, approximately isosmotic to sap (1.14 molal). The protoplasm, along with some buffer, was gently scraped from the wall with a scalpel, taken up in a capillary tube, and centrifuged in a hematocrit centrifuge for 10 minutes at about 12,000 *g*. The protoplasm fraction was forced out of the tube and weighed. The protoplasm from several large cells was pooled to give 20–40 mg. wet weight per sample. The protoplasm was combined with the supernatant buffer, diluted to 5 ml. with 0.1 N HNO_3, and allowed to extract for several days.

The amount of water in the protoplasm was measured in separate samples by drying overnight at 100–110° C. Na^+, K^+, and Cl^- were measured with a Beckman DU flame spectrophotometer and an Aminco-Cotlove chloride titrator. The osmotic pressure of sea water, sap, and Tris was measured with a Fiske osmometer.

This method of collecting protoplasm was not quantitative, for there was always some loss during washing and scraping. The amounts agreed approximately, however, with those predicted from Doyle's (1940) measurements of protoplasm volume and density. No attempt was made to separate the cytoplasm from the chloroplasts, which comprise 60–70% of the protoplasm volume in *V. ventricosa* (Doyle, 1935, 1940).

Electrical measurements

Electric potentials of the vacuole and cytoplasm were measured with microcapillary electrodes and a high-impedance voltmeter (Keithley Model 600A). The electrical circuit was similar to that described by Gutknecht (1965a). Micropipets were drawn from 1-mm. Pyrex tubing and filled with 0.6 *M* KCl by boiling under reduced pressure. Tip diameters ranged from <1 μ to about 10 μ. The larger tips were necessary to pierce the tough outer wall of old cells. Electrode tip potentials in either sea water or artificial sap were <2 mv.

To measure the vacuole potential, a microelectrode was forced through the cell wall and underlying protoplasm (5–10 μ thick) until the tip protruded about 1 mm. into the vacuole. The puncture was made with the aid of a micromanipulator and dissecting microscope. The potential difference between vacuole and sea water was initially low, then rose gradually to a maximum and remained steady (± 2 mv) for many hours. The cells were illuminated during both the electrical and flux measurements (3000–4000 lux), which was important because the vacuole potential in *V. ventricosa* is sensitive to light (Marsh, 1939).

The electric potential of the cytoplasm was measured in aplanospores 5–15 hours old and 100–300 μ in diameter. These young cells were largely protoplasm rather than vacuole, and their thin wall offered little resistance to microelectrode penetration. The cells were held by gentle suction, similar to the method of Eckert (1965). When the electrode tip entered the cytoplasm, the potential rose rapidly to a maximum, then usually decreased slowly after 1–2 minutes. This decrease was due possibly to a cytoplasmic sealing process like that in *Nitella* (Walker, 1955). Potentials that were steady (± 2 mv) for 1–2 minutes were recorded.

Aplanospores of fairly uniform age were obtained by cutting a large cell in half and holding it in sea water for several hours. During this time the protoplasm changed from a continuous layer into a large number of multinucleate spores, 20–300 μ in diameter (see Doyle, 1940). Alternatively, a small coenocyte was centrifuged at 1000 *g* for 10 minutes and the protoplasm, concentrated at one end of the cell, was allowed to stand for several hours in sea water. Spores formed by the latter method were usually larger but gave similar results.

Flux measurements

Ion fluxes between vacuole and sea water were measured in spherical cells of *Valonia,* 5–6 mm. in diameter and 60–110 mg. in weight. The small rhizoidal cells and hapteron cells on the surface of the main vesicle were removed with forceps. Unidirectional fluxes were measured with radioisotopes, Na^{22}, K^{42}, and Cl^{36}. Conventional detectors were used (Baird-Atomic Model 510 and Nuclear Chicago Model C-115). Tracer specific activities were high enough that the concentrations

of stable ions in the radioactive media were increased less than 1%. Net fluxes were measured by the weight increase of a cell during an influx experiment (1–3 days). This was possible because the intracellular ion concentrations were constant and the average rate of growth was about 1 mg./day. Efflux was calculated from the unidirectional influx minus the net influx. Efflux of Na^+ and K^+ was also measured directly from the rate of isotope efflux into a large non-labeled environment. Both methods gave similar results, but the former method was preferred because it provided simultaneous estimates of both influx and efflux in a single cell.

To measure the influx from sea water to vacuole, a weighed cell was placed in 10 ml. of labeled medium under constant illumination. Cells were removed after 20–50 hours, washed for one minute in non-active medium, blotted, weighed, measured, and pierced with a sharp needle. The sap was squeezed out, taken up in a calibrated pipet, and transferred to a planchet for counting. The initial influx was calculated by the equation

$$M_i = \frac{C_i^* C_o V}{t C_o^* A}$$

where M_i is the influx in M/cm^2. sec., C_i^* is the concentration of labeled ions in the sap after time t, C_o is the external ion concentration, C_o^* is the external concentration of labeled ions, V is the volume, and A is the surface area of the spherical cells. The specific activity of the sap at the end of the influx period was less than 10% of the specific activity outside; therefore the efflux of labeled ions during the influx measurement was neglected.

The net flux was calculated by the equation

$$M_{net} = \frac{\Delta W C_i}{A t}$$

where M_{net} is the net influx in M/cm^2. sec., ΔW is the weight increase during time t, and C_i is the internal ion concentration.

Efflux was estimated either by subtracting the net flux from the unidirectional influx, or from direct measurements of the rate of isotope efflux into a large non-labeled environment by the equation

$$M_o = \frac{2.3 C_i V}{t A} \log \frac{C_{i0,}^*}{C_i^*}$$

where M_o is the efflux in M/cm^2. sec., and $C_{i,o}^*$ is the initial concentration of labeled ions inside.

These equations were derived from standard flux equations which treat the cells as a single compartment (see, for example, Sheppard, 1962). This was justified because the cells used for flux measurements were >99% vacuole.

Results

Ion concentrations in sea water, protoplasm, and sap

Concentrations of Na^+, K^+, and Cl^- in sea water, protoplasm, and vacuolar sap are shown in Table I. Sap was analyzed from large Puerto Rican cells and from

smaller cells grown in the laboratory. Protoplasm was collected only from the large coenocytes, since the small cells contained only about 0.5 μl. protoplasm per cell. Protoplasmic ion concentrations are expressed in terms of protoplasm water, which was 58.6 ± 1.0% (10) of the wet weight. Results are presented in the sequence: mean, standard error, and (in parentheses) number of measurements.

Potassium was highly concentrated in the protoplasm (434 m*M*) and especially in the vacuole (625 m*M*). Sodium was largely excluded from both protoplasm (40 m*M*) and sap (44 m*M*). The Cl^- concentration was relatively low in the protoplasm (138 m*M*), but was higher in the sap (643 m*M*) than in the external sea water. These concentrations for the sap of *V. ventricosa* are slightly higher

TABLE I

*Concentrations of Na^+, K^+, and Cl^- in sea water and protoplasm and sap of Valonia ventricosa**

Ion	Sea water (*mM*/liter)	Protoplasm (*mM*/liter water)	Sap (*mM*/liter)
Na^+	508 ± 5 (13)	40 ± 2 (8)	44 ± 1 (18)
K^+	12.1 ± 0.2 (10)	434 ± 9 (8)	625 ± 5 (18)
Cl^-	596 ± 2 (9)	138 ± 4 (8)	643 ± 2 (10)

* Mean, SE, and number of measurements.

than those of Cooper and Blinks (1928) and are similar to those of Steward and Martin (1937).

Electric potentials across plasmalemma and tonoplast

The potential difference between cytoplasm and sea water was −71 ± 1 mv (20). The measurements were made on cells 5–10 hours old, which were mainly protoplasm surrounded by a thin cell wall. Vacuoles in cells of this age were small and not clearly defined (see Doyle, 1935). When the electrode was forced toward the center of these cells, the potential sometimes reversed sharply from negative to positive (+6 to +14 mv), which suggested entry into a vacuole. Cells 20–30 hours old resembled mature *Valonia* in having a large vacuole surrounded by a thin layer of protoplasm. In cells of this age I was sometimes able to measure a cytoplasmic potential of −70 ± 5 mv (6), whereas the vacuole potential was +12 ± 1 mv (12).

The potential difference between vacuole and sea water in older cells (3–4 months) was +17 ± 1 mv (16), vacuole positive. These cells were illuminated continuously for 2–3 days prior to the potential measurements to simulate conditions under which ion fluxes were measured. This value agrees with the early work of Blinks (1929), who used much larger, and presumably much older, cells of *V. ventricosa.*

The vacuole potential is the algebraic sum of the potentials across plasmalemma and tonoplast. Thus, subtracting the plasmalemma potential in young cells (−71 mv) from the vacuole potential in mature cells (+17 mv) gives a tonoplast potential of about −88 mv, cytoplasm negative to vacuole. This value should be confirmed by direct measurements on large cells. I have not accomplished this,

however, mainly because the cell wall is as thick as the underlying protoplasm (5–12 μ; Doyle, 1940) and cannot be penetrated easily by a glass capillary of 1 μ diameter.

These results support qualitatively the early work of Umrath (1938), who found that the potential difference between cytoplasm and sea water in *V. macrophysa* was about −20 mv. Umrath measured cytoplasmic potentials in slowly deplasmolyzing cells by allowing the protoplasm to be pierced by a stationary microelectrode inside the cell wall. His microelectrodes were filled with a too dilute solution of KCl (0.1 *M*), however, for which he was criticized by Briggs, Hope and Robertson (1961, p. 109). So, although his method was not entirely satisfactory, he concluded correctly that the cytoplasm of *Valonia* is more negative toward the vacuole than toward the external sea water.

Ion fluxes

Ion fluxes between sea water and vacuole of *V. ventricosa* are shown in Table II. Unidirectional fluxes ($\mu\mu M$/cm^2. sec.) were 3.3–3.6 for Na^+, 86–89 for K^+, and 11–18 for Cl^-. Influxes were somewhat larger than effluxes, because

TABLE II

Fluxes of Na^+, K^+, and Cl^- between sea water and vacuole *, **

Ion	Influx	Efflux	Net flux
Na^+	3.64 ± 0.32 (20)	3.32 ± 0.33 (20)	0.32 ± 0.03 (35)
K^+	88.7 ± 3.0 (18)	86.2 ± 3.0 (18)	5.6 ± 0.6 (33)
Cl^-	18.5 ± 1.4 (30)	10.9 ± 1.1 (30)	7.6 ± 0.5 (30)

* Mean, SE, and number of measurements.
** Units are $\mu\mu M$/cm^2. sec.

the cells were growing at a rate of 0.5–1.5%/day and the intracellular concentrations were constant. The net fluxes, estimated from the increase in weight and average concentration of ions in the sap, were about 0.3 for Na^+, 5.6 for K^+, and 7.6 for Cl^-.

The efflux of Na^+ and K^+ from *V. ventricosa* was also measured directly from the rates of Na^{22} and K^{42} efflux into a large non-labeled environment. The cells were labeled for only a short time (1–5 hours), so that the specific activity of the protoplasm would be higher than that of the sap at the start of the efflux measurement. Some typical results are shown in Figure 1. A rapid loss of both Na^{22} and K^{42} from the wall and extracellular space was complete in about 0.5 minute. The remaining Na^{22} exchanged at a nearly uniform rate and probably represents exchange of vacuolar Na^+. The rate of K^{42} exchange was non-uniform during the first hour but was constant thereafter, presumably representing exchange of vacuolar K^+. Effluxes of Na^+ and K^+ from vacuole to sea water were estimated from the slopes of the linear parts of the curves, K^{42} B and Na^{22}, as described under Methods. The efflux of Na^+, measured between 0.3 and 65 hours, was 3.8 ± 0.3 (8) $\mu\mu M$/cm^2. sec. The efflux of K^+, measured between 1 and 26 hours, was 89 ± 9 (5) $\mu\mu M$/cm^2. sec. These values agree with those obtained by the more convenient method of subtracting the net influx from the unidirectional influx (Table II).

Preliminary calculations suggest that the exchange of intracellular K^{42} proceeds at two exponential rates. The rapidly exchanging fraction (K^{42} A) was obtained by subtracting the extrapolated portion of the slow fraction (K^{42} B) from the total activity. The faster rate probably represents exchange of protoplasmic K^+ rather

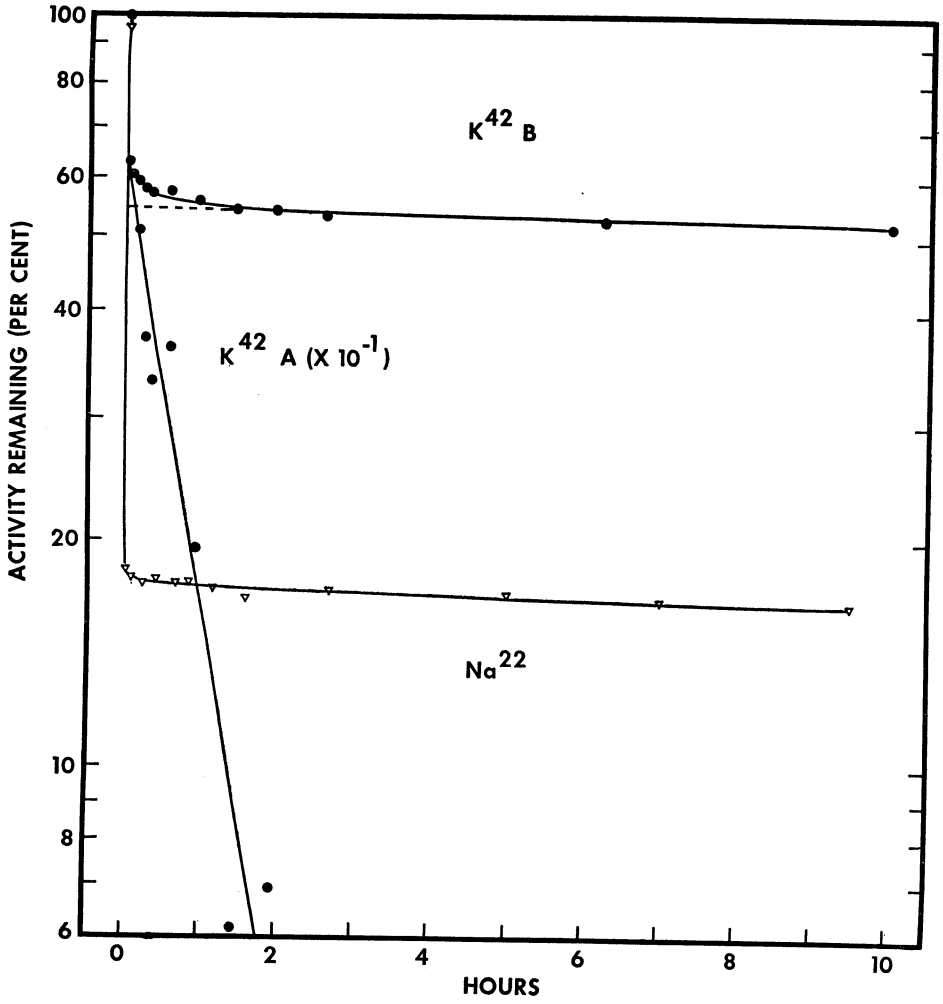

FIGURE 1. Efflux of Na^{22} and K^{42} from cells of *V. ventricosa,* previously labeled for 5 hours (Na^{22}) or one hour (K^{42}) in the light. Rapidly exchanging fraction of intracellular K^{42} (K^{42} A) was obtained by subtracting the extrapolated portion of the slow fraction (K^{42} B) from the total activity. Each point represents a measurement of 11–30 cells.

than cell-wall K^+, based on the rate of exchange and the fact that an analogous compartment for Na^+ was not observed. The fast fraction had an apparent half-time of about 0.5 hour and a rate coefficient of about 1.3 hr.$^{-1}$. Efflux of K^+ from protoplasm to sea water was estimated by multiplying the rate coefficient by the K^+

concentration of the protoplasm (about 13×10^{-8} M/cm^2.), which gave a value of about 50 $\mu\mu M/cm^2$. sec.

The K^+ flux from protoplasm to sea water, the rate coefficient, and the apparent size of the protoplasm compartment are all underestimated, however, by the graphical analysis of the washing-out curve (Fig. 1). The error is caused by the series arrangement of protoplasm and vacuole, the relatively small size of the protoplasm compartment, and the similarity between the fluxes across plasmalemma and tonoplast (see Solomon, 1960; Huxley, 1960). First, the protoplasm loses radioactivity to both vacuole and sea water during the washing-out process. Second, the specific activity of the protoplasm after several hours in a K^{42} medium is probably not equal to the specific activity of the medium, but is at a quasi-steady level below that of the medium.

Discussion

The occurrence and direction of any active transport processes in *V. ventricosa* can be determined from a knowledge of the ion concentrations and electric potentials under steady-state conditions. Active transport is indicated by any large differences between the electrochemical potentials of an ion in sea water, cytoplasm, or sap. The difference in electrochemical potential can be estimated by comparing the measured membrane potential with the Nernst equilibrium potential, *i.e.,* the potential at which the ion would be in passive flux equilibrium between the two phases.

$$E_j = \frac{2.3RT}{zF} \log \frac{C_1}{C_2}$$

E_j is the equilibrium potential, C_1 and C_2 are the ion concentrations on either side of the membrane, z is the algebraic valency, and R, T, and F have their usual meanings. In the discussion which follows, the sign of the potential will always be that of the cytoplasm. The sea water and vacuole are taken as ground for the potentials across the plasmalemma and tonoplast, respectively.

Possible active transports of Na^+ and K^+ in *V. ventricosa* may be tested by using the Nernst equation, because these ions are in an approximately steady-state (Table II). Equilibrium potentials for Na^+ and K^+ across plasmalemma and tonoplast are shown in Table III. Also shown are the differences, ΔE, between the measured potential and the equilibrium potential for each ion. The magnitude of the ΔE indicates the relative driving force acting on the ion. The sign of the ΔE, along with the valency of the ion, indicates the direction of that driving force. The large values for ΔE_{Na} at the plasmalemma (−136 mv), and for both ΔE_{Na} and ΔE_K at the tonoplast (−90 and −97 mv) indicate active transport of these ions. An active efflux of Na^+ from protoplasm to sea water and active transports of both Na^+ and K^+ from protoplasm to vacuole are indicated. An active influx of K^+ at the plasmalemma is likely, although the ΔE_K at this membrane is relatively small (+21 mv).

Where marked deviations from flux equilibrium occur, as with Cl^- (Table II), the flux-ratio equation of Ussing (1949) and Teorell (1949) may be used to test

TABLE III

*Equilibrium potentials for Na^+ and K^+ across plasmalemma and tonoplast**

Ion	Plasmalemma		Tonoplast	
	E_j**	$\Delta E = E_{obs} - E_j$	E_j**	$\Delta E = E_{obs} - E_j$
Na^+	+65	−136	+2	−90
K^+	−92	+21	+9	−97

* Calculated from data in Table I.

** $E_j = \frac{-2.3RT}{zF} \log \frac{C_i}{C_o}$ millivolts.

for passive, independent, ion movement.

$$\frac{M_i}{M_o} = \frac{C_o}{C_i \exp(zFE/RT)}$$

M_i/M_o is the predicted flux ratio (influx/efflux), E is the measured vacuole potential, and the other symbols have the same meanings as before. Close agreement between predicted and measured flux ratios is strong evidence for passive ion movement. Discrepancies suggest, but do not prove, active transport, since such passive processes as exchange diffusion and single-file diffusion can cause deviations from the predicted flux ratios (see Ussing, 1960, p. 49).

Measured and predicted flux ratios for Na^+, K^+, and Cl^- between sea water and vacuole are shown in Table IV. The predicted values were calculated from the average vacuole potential of +17 mv and the sap and sea-water concentrations shown in Table I. The large differences between the measured and predicted values for both Na^+ and K^+ support the earlier indications of active efflux of Na^+ and active influx of K^+. On the other hand, the close agreement between the predicted and measured flux ratios for Cl^- (1.70 and 1.79) indicates that Cl^- uptake into the vacuole of growing cells is a passive process.

The absence of active transport of Cl^- from sea water to vacuole, however, does

TABLE IV

Measured and predicted flux ratios for Na^+, K^+, and Cl^- between sea water and vacuole

Ion	Influx/Efflux, M_i/M_o	
	Measured*	Predicted**
Na^+	1.10	5.78
K^+	1.03	0.01
Cl^-	1.70	1.79

* Calculated from data in Table II.

** $\frac{M_i}{M_o} = \frac{C_o}{C_i \exp(zFE/RT)}$.

not preclude the possibility of some active uptake of Cl^- into the protoplasm at both membranes. The predicted flux ratio at the plasmalemma (M_{oc}/M_{co}) is about 0.3, and the predicted flux ratio at the tonoplast (M_{cv}/M_{vc}) is about 6. The actual flux ratios are not known, but the measured value of 1.7 between sea water and vacuole (M_{ov}/M_{vo}) sets an upper limit on the flux ratios. A lower limit of about 1 is set by the experimental conditions, *i.e,* growing cells which maintain a constant Cl^- concentration inside. Thus, the actual flux ratios at plasmalemma and tonoplast must be 4–6 times higher and lower, respectively, than the predicted flux ratios. This suggests either that there is active uptake of Cl^- into the protoplasm at both membranes or that the estimated Cl^- concentration in the protoplasm is too high. The latter possibility is more likely, because either contamination by sap or the presence of minute sap-filled vesicles in the protoplasm (see Buvat, 1963; Sutcliffe, 1962) could raise the estimated Cl^- concentrations above the equilibrium level. Two other possible sources of error are the ion selectivity of the chloroplasts and any changes in the cytoplasmic potential with age.

Valonia ventricosa differs from other plant cells by not showing active uptake of Cl^- into the vacuole. Active Cl^- absorption occurs, for example, in the marine algae *Rhodymenia, Halicystis,* and *Gracilaria* (MacRobbie and Dainty, 1958b; Blount and Levedahl, 1960; Gutknecht, 1965a). In giant characean cells, as well as in roots, storage tissues, and leaves of higher plants, an inward Cl^- or anion pump is essential for maintaining the high salt content of the vacuole (see Dainty, 1964). In *V. ventricosa,* however, Cl^- appears to follow passively while Na^+, and especially K^+, are pumped into the vacuole. Thus, the hypertonicity of the cell interior, which is essential for the growth and expansion of all walled cells (see Rothstein, 1964), is primarily due to an inward transport of cations rather than anions. In this respect *Valonia* resembles yeast and bacteria more than other algae and higher plants.

Active transport of one or more ions from cytoplasm to vacuole is necessary for osmotic reasons, summarized recently by Tosteson (1964). Otherwise, unless the tonoplast were pressure-resistant, the protoplasm would be osmotically unstable toward the sap. For this and other reasons the inward Cl^- pump in both *Halicystis* and *Nitellopsis* was sited at the tonoplast (Blount and Levedahl, 1960; MacRobbie and Dainty, 1958a). In *Nitella* it appears that Na^+ is pumped in at the tonoplast, whereas K^+ and Cl^- are in equilibrium between cytoplasm and sap (Spanswick and Williams, 1964). In *V. ventricosa,* however, it is primarily K^+ which is pumped from cytoplasm to sap.

The energy requirement for K^+ transport in *V. ventricosa* is of interest because of the rapid influx of K^+ (89 $\mu\mu M/cm^2$. sec.) and the large electrochemical potential gradient against which K^+ is pumped (about 2500 cal./M). The cells in which K^+ fluxes were measured contained about 5×10^{-4} g. protoplasm cm^2. The energy requirement is thus about 1.6 cal./g. protoplasm per hour, which is rather high and suggests that part of the K^+ fluxes may be due to a mediated, but not active, process, such as exchange diffusion (see Ussing, 1960).

The large potential across the tonoplast is another unusual feature of *V. ventricosa.* The marine alga *Halicystis ovalis* and root hair cells of *Avena* show no potential across the tonoplast (Blount and Levedahl, 1960; Etherton and Higinbotham, 1960). Fresh-water Characeae show either no potential or very small

potential (Walker, 1955; Kishimoto, 1959; Nagai and Kishimoto, 1964; Findlay and Hope, 1964; Spanswick and Williams, 1964).

At the plasmalemma, both the large negative potential and the active extrusion of Na^+ are features common to many cells. Two other marine algae, *Halicystis* and *Porphyra,* show cytoplasmic potentials of −80 and −42 mv, respectively (Blount and Levedahl, 1960; R. W. Eppley, personal communication). In fresh-water algae and higher plants the plasmalemma potentials are usually somewhat larger (Walker, 1955; Umrath, 1956; Findlay and Hope, 1964; Spanswick and Williams, 1964). Finally, the outward transport of Na^+, possibly coupled with an inward transport of K^+, occurs at the outer plasma membrane of many plant and animal cells (see Dainty, 1962, 1964).

The suitability of *Valonia* for salt-transport studies is questioned by Steward

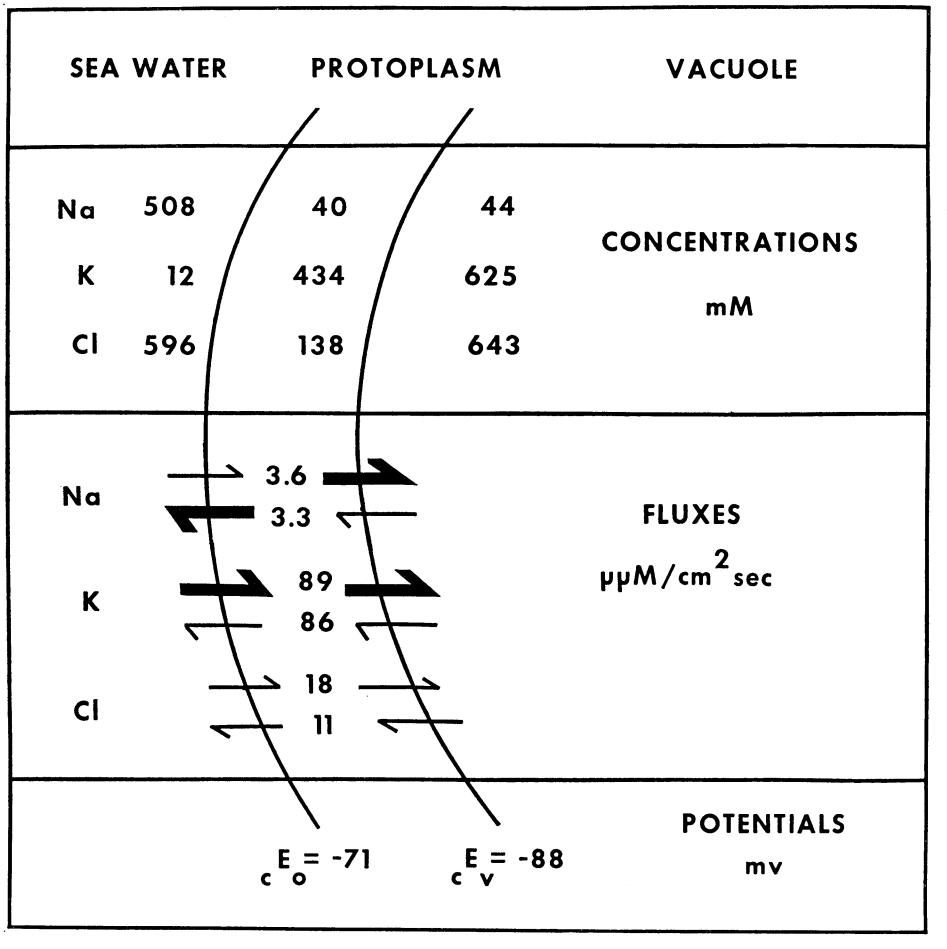

FIGURE 2. Ionic relations in *V. ventricosa.* Provisional scheme showing concentrations, fluxes, and potentials. Large arrows indicate active transport.

and Sutcliffe (1959, p. 289), because the sap is not subject to rapid changes in composition, and because the cells grow slowly and only from aplanospores which send rhizoidal processes into the substratum, *e.g.*, marble chips. They suggest that the rhizoidal processes, as well as the small hapteron cells on the surface of the main vesicle, may be the functional absorbing organs. My results indicate that neither the rhizoidal cells, nor the substratum, nor the hapteron cells are necessary for fairly rapid growth and rapid ion movements. The growth rate of 0.5–1.5% per day in fairly large cells (60–110 mg.) is comparable with that in some other marine algae under fairly similar conditions (Gutknecht, 1965b), and the fluxes of ions across the surface of the main vesicle are more rapid than in most other plant and animal cells. That the sap is not subject to rapid changes in composition is probably due mainly to the large volume:surface ratio.

A provisional scheme of the ionic relations in *V. ventricosa* is shown in Figure 2. The important differences between *V. ventricosa* and other vacuolated plant cells are (1) the absence of active transport of Cl^- into the vacuole, (2) the inward transport of both Na^+ and K^+ at the tonoplast, and (3) the large potential across the tonoplast. Two features of *V. ventricosa* that are similar to other vacuolated plant cells, as well as to plant and animal cells in general, are (1) the active efflux of Na^+ and probably active influx of K^+ at the plasmalemma, and (2) the large negative potential of the cytoplasm relative to the external solution. Thus, the main differences between *Valonia* and other plant cells lie at the vacuole membrane. These results may therefore support the view of Steward (1939) and Steward and Sutcliffe (1959), based on morphological evidence, that the vacuole of *Valonia* and its relatives is not homologous to the vacuole of most other plant cells.

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Summary

1. The marine alga *Valonia ventricosa* regulates its ionic content by means of several ion pumps. An active extrusion of Na^+ and probably an active uptake of K^+ occur at the plasmalemma. At the tonoplast there is an inward transport of Na^+ and especially K^+. The evidence for these ion pumps is that Na^+ efflux at the plasmalemma and Na^+ and K^+ influx at the tonoplast occur against large electrochemical gradients under steady-state conditions.

2. Ion distribution and membrane potentials across plasmalemma and tonoplast were measured. The ion concentrations were: Na^+ 508, K^+ 12.1, and Cl^- 596 m*M*/liter sea water; Na^+ 40, K^+ 434, Cl^- 138 m*M*/liter protoplasm water; Na^+ 44, K^+ 625, Cl^- 643 m*M*/liter sap. The resting potential across the plasmalemma was about 71 mv, cytoplasm negative to sea water. The potential across the tonoplast was about 88 mv, cytoplasm negative to sap. The large potential across the tonoplast is a feature not found in other vacuolated plant cells.

3. Ion fluxes between sea water and vacuole were measured in growing cells of *Valonia*. The fluxes were: Na^+ 3.3–3.6, K^+ 86–89, Cl^- 11–18 $\mu\mu M/cm^2$. sec. The ratio of influx:efflux for Na^+ and K^+ was close to unity, whereas the flux ratio for Cl^- was 1.70. The latter value is close to that predicted by the Ussing-Teorell equation for a passively moving ion, which suggests that *Valonia,* unlike other vacuolated plant cells, does not actively transport Cl^- into the vacuole.

LITERATURE CITED

BLINKS, L. R., 1929. Resistance and potential measurements across the protoplasm of *Valonia ventricosa. Carnegie Inst. Wash., Year Book No. 28,* pp. 277–279.

BLINKS, L. R., 1940. The relations of bioelectric phenomena to ionic permeability and to metabolism in large plant cells. *Cold Spring Harbor Symposia Quant. Biol.,* **8**: 204–215.

BLINKS, L. R., 1949. The source of bioelectric potentials in large plant cells. *Proc. Nat. Acad. Sci.,* **35**: 566–575.

BLINKS, L. R., 1955. Some electrical properties of large plant cells, pp. 187–212. *In:* T. Shedlovsky (ed.) Electrochemistry in Biology and Medicine. Wiley, New York.

BLOUNT, R. W., AND B. H. LEVEDAHL, 1960. Active sodium and chloride transport in the single celled marine alga *Halicystis ovalis. Acta Physiol. Scand.,* **49**: 1–9.

BRIGGS, G. E., A. B. HOPE AND R. N. ROBERTSON, 1961. Electrolytes and Plant Cells. Blackwell Scientific Publications, Oxford, 217 pp.

BUVAT, R., 1963. Electron microscopy of plant protoplasm. *Internat. Rev. Cytol.,* **14**: 41–157.

COOPER, W. C., AND L. R. BLINKS, 1928. The cell sap of *Valonia* and *Halicystis. Science,* **68**: 164–165.

DAINTY, J., 1962. Ion transport and electrical potentials in plant cells. *Ann. Rev. Plant Physiol.,* **13**: 379–402.

DAINTY, J., 1964. Physiological aspects of ion transport in plant cells and tissues, pp. 41–52. *In:* J. F. Hoffman (ed.) The Cellular Functions of Membrane Transport. Prentice-Hall, Englewood Cliffs, New Jersey.

DOYLE, W. L., 1935. Cytology of *Valonia. Carnegie Inst. Wash., Papers from Tortugas Lab.,* **29**: 15–21.

DOYLE, W. L., 1940. The structure and composition of *Valonia ventricosa. Carnegie Inst. Wash., Papers from Tortugas Lab.,* **32**: 144–152.

ECKERT, R., 1965. Bioelectric control of bioluminescence in the dinoflagellate *Noctiluca. Science,* **147**: 1140–1145.

ETHERTON, B., AND N. HIGINBOTHAM, 1960. Transmembrane potential measurements of cells of higher plants as related to salt uptake. *Science,* **131**: 409–410.

FINDLAY, G. P., AND A. B. HOPE, 1964. Ionic relations of cells of *Chara australis,* VII. The separate electrical characteristics of the plasmalemma and tonoplast. *Australian J. Biol. Sci.,* **17**: 62–77.

GUTKNECHT, J., 1965a. Ion distribution and transport in the red marine alga, *Gracilaria foliifera. Biol. Bull.,* **129**: 495–510.

GUTKNECHT, J., 1965b. Uptake and retention of Cs^{137} and Zn^{65} by seaweeds. *Limnol. Oceanogr.,* **10**: 58–66.

HUXLEY, A. F., 1960. Compartmental methods of kinetic analysis, appendix 2, pp. 163–166. *In:* C. L. Comar and F. Bronner (eds.) Mineral Metabolism, Vol. I, Part A. Academic Press, New York.

KECK, K., 1964. Culturing and experimental manipulation of *Acetabularia,* pp. 189–213. *In:* D. M. Prescott (ed.) Methods in Cell Physiology. Academic Press, New York.

KISHIMOTO, U., 1959. Electrical characteristics of *Chara corallina. Ann. Rep. Scient. Works, Fac. Sci., Osaka Univ.,* **7**: 115–146.

MACROBBIE, E. A. C., AND J. DAINTY, 1958a. Ion transport in *Nitellopsis obtusa. J. Gen. Physiol.,* **42**: 335–353.

MACROBBIE, E. A. C., AND J. DAINTY, 1958b. Sodium and potassium distribution and transport in the seaweed *Rhodymenia palmata. Physiol. Plant.,* **11**: 782–801.

MARSH, G., 1939. Effect of light on the inherent E. M. F. of *Valonia ventricosa*. *Carnegie Inst. Wash., Papers from Tortugas Lab.*, **32**: 65–84.

MEYER, A., 1891. Notiz über die Zusammensetzung des Zellsaftes von *Valonia utricularia*., *Ber. deutsch. bot. Ges.*, **9**: 77–79.

MULLINS, L. J., 1949. Potassium accumulation in *Valonia* as a function of phosphate ions. *Pubb. Staz. Zool. Napoli*, **21**: 255–260.

NAGAI, R., AND U. KISHIMOTO, 1964. Cell wall potential in *Nitella*. *Plant Cell Physiol.*, **5**: 21–31.

OSTERHOUT, W. J. V., 1924. On the importance of maintaining certain differences between cell sap and external medium. *J. Gen. Physiol.*, **7**: 561–564.

OSTERHOUT, W. J. V., 1931. Physiological studies of single plant cells. *Biol. Rev.*, **31**: 369–411.

OSTERHOUT, W. J. V., 1940. Some models of protoplasmic surfaces. *Cold Spring Harbor Symposia Quant. Biol.*, **8**: 51–62.

OSTERHOUT, W. J. V., E. B. DAMON AND A. G. JACQUES, 1927. Dissimilarity of inner and outer protoplasmic surfaces in *Valonia*. *J. Gen. Physiol.*, **11**: 193–205.

ROTHSTEIN, A., 1964. Membrane function and physiological activity of microorganisms, pp. 23–39. *In:* J. F. Hoffman (ed.) The Cellular Functions of Membrane Transport. Prentice-Hall, Englewood Cliffs, New Jersey.

SCOTT, G. T., AND H. R. HAYWARD, 1955. Sodium and potassium regulation in *Ulva lactuca* and *Valonia macrophysa*, pp. 35–64. *In:* A. M. Shanes (ed.) Electrolytes in Biological Systems. American Physiological Society, Washington, D. C.

SHEPPARD, C. W., 1962. Basic Principles of the Tracer Method. John Wiley & Sons, New York, 282 pp.

SOLOMON, A. K., 1960. Compartmental methods of kinetic analysis, pp. 119–167. *In:* C. L. Comar and F. Bronner (eds.) Mineral Metabolism, Vol. I, Part A. Academic Press, New York.

SPANSWICK, R. M., AND E. J. WILLIAMS, 1964. Electrical potentials and Na, K, and Cl concentrations in the vacuole and cytoplasm of *Nitella translucens*. *J. Exp. Bot.*, **15**: 193–200.

STEWARD, F. C., 1939. The growth of *Valonia ventricosa* and *Valonia ocellata* in culture. *Carnegie Inst. Wash., Papers from Tortugas Lab.*, **32**: 86–98.

STEWARD, F. C., AND J. C. MARTIN, 1937. The distribution and physiology of *Valonia* at the Dry Tortugas, with special reference to the problem of salt accumulation in plants. *Carnegie Inst. Wash., Papers from Tortugas Lab.*, **31**: 87–170.

STEWARD, F. C., AND J. F. SUTCLIFFE, 1959. Plants in relation to inorganic salts, pp. 253–478. *In:* F. C. Steward (ed.) Plant Physiology, Vol. 2. Academic Press, New York.

SUTCLIFFE, J. F., 1962. Mineral Salts Absorption in Plants. Pergamon, Oxford, 194 pp.

TEORELL, T., 1949. Membrane electrophoresis in relation to bio-electrical polarization effects. *Arch. Sci. Physiol.*, **3**: 205–219.

TOSTESON, D. C., 1964. Regulation of cell volume by sodium and potassium transport, pp. 3–22. *In:* J. F. Hoffman (ed.) The Cellular Functions of Membrane Transport. Prentice-Hall, Englewood Cliffs, New Jersey.

UMRATH, K., 1938. Über das elektrische Potential und über Aktionsströme von *Valonia macrophysa*. *Protoplasma*, **31**: 184–193.

UMRATH, K., 1956. Elektrophysiologische Phänomeme, pp. 747–778. *In:* R. Ruhland (ed.) Handbuch der Pflanzenphysiologie, Vol. 2. Springer, Berlin.

USSING, H. H., 1949. The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.*, **19**: 43–56.

USSING, H. H., 1960. The alkali metal ions in isolated systems and tissues, pp. 1–195. *In:* O. Eichler and A. Farah (eds.) The Alkali Metal Ions in Biology. Springer, Berlin.

WALKER, N. A., 1955. Microelectrode experiments on *Nitella*. *Australian J. Biol. Sci.*, **8**: 476–489.