SODIUM AND POTASSIUM TRANSPORT IN THE MARINE ALGA *CHAETOMORPHA DARWINII*

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Summary

Chaetomorpha darwinii is a marine alga with large coenocytic cells. The cell sap contains about 540 mM potassium, 25 mM sodium, and 600 mM chloride, and the vacuole is 10 mV positive to the sea water. The potassium selectivity is due to an active inward pump and an outward sodium pump at the plasmalemma. The fluxes of potassium at the plasmalemma and tonoplast were about 100 and 150 pmoles/cm²/sec, and the fluxes of sodium at these membranes were about 100 and 4 pmoles/cm²/sec, respectively. The potential differences at these boundaries were −35 mV and +45 mV. The cytoplasmic phase contained about 18 μ-equiv/g of potassium and 0·5–1·0 μ-equiv/g of sodium.

Dinitrophenol reduced the flux of potassium at the plasmalemma, and the content of the cytoplasm fell to about 0·5 μ-equiv/g, but it did not induce a net flux from the vacuole. Sodium influx was not affected by dinitrophenol, but the content of the cytoplasm rose from 0·5 to 7 μ-equiv/g, due to inhibition of the sodium efflux.

There were some anomalies in the results, i.e. the high potassium content of the cytoplasm, the high potential difference at the tonoplast, and the lack of any effect of dinitrophenol on the net fluxes. These problems are considered to be due to the organization of the cytoplasm.

I. Introduction

Chaetomorpha darwinii (Hooker) Kuetzing‡ is a marine member of the Cladophorales and grows epiphytically on other marine plants in the sublittoral zone. It is found as filaments up to 20 cm long, consisting of large barrel-shaped to ovoid coenocytic cells, each about 2–4 mm in diameter and 1–2 times as long as broad. The appearance of the strands is shown in Figure 1 and the structure of the coenocytes in Plate 1. There is a large, central vacuole in each cell surrounded by a thin layer of non-flowing cytoplasm. The strands grow by cell enlargement and they increase in cell number by a process of constrictive division in which the cell wall grows into the vacuole, cutting the cell in two. Consequently, there need be no difference in ion content between newly formed and mature cells, as in plants where the proportion of cytoplasm to vacuole changes during cell growth.

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‡ Referred to simply as *Chaetomorpha* in this paper.

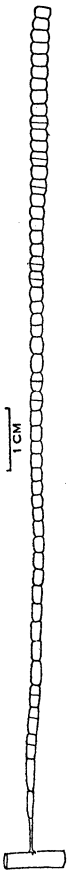

Fig. 1

The fine structure of the cytoplasm is shown in Plate 1. About half the volume of the cytoplasm is taken up by the chloroplasts which are basically disk-shaped to spherical, but are closely packed to form a layer about 2–3 μ thick in the cytoplasm. Between the chloroplast layer and cell wall there is some cytoplasm, but the amount is small and variable. The nuclei are regularly distributed over the cytoplasm, between the chloroplasts and the vacuole, and are about 5–6 μ apart. Between the nuclei is a region containing mitochondria and cell membrane structures, some of which have a golgi-like appearance and are associated with vesicles; these structures are similar to those found in *Ulva lactuca* (West, unpublished data). The cytoplasm near the vacuole contains several large vacuole-like vesicles about 2 μ in diameter. At some stages of development the cytoplasm contains zooids, which are released through pores in the cell wall following disorganization of the cytoplasm.

The volume of cytoplasm is difficult to estimate, but from the dimensions of cytoplasm in electron micrographs it appears to be about 1–2% of the cell volume. The surface area exposed to solution varies with cell shape, and is intermediate between that of a chain of spheres and a cylinder, i.e. about 2·5/radius (cm^2/g). A flux of 1 μ-equiv/g/hr was equal to approximately 15 pmoles/cm^2/sec.

Studies of ion balance in members of the Charales have shown that a satisfactory model for ion transport in these cells is one in which the free space (cell walls), cytoplasm, and the vacuoles are treated as three phases in series. The plasmalemma and tonoplast membranes are the controlling boundaries in this system, and have been suggested as sites of active transport mechanisms responsible for maintaining both potassium and sodium balance and the high salt concentration in the vacuoles. This paper describes experiments in which ion balance and the suitability of this model in *Chaetomorpha* were investigated, and compares the behaviour of *Chaetomorpha* with members of the Charales found in brackish water.

II. Materials and Methods

(*a*) Chaetomorpha *Strands*

Strands of *Chaetomorpha* were collected from the sea and kept in the laboratory at 15°C in aerated sea water. Plants treated in this way remained turgid and maintained high K/Na selectivity for at least 6 weeks, but were used within 6 days of collection for the experiments described in this paper (with the exception of tests of potassium and sodium levels over a longer period). Occasionally zooids were released from experimental material, but it was found that this took place within 24 hr of collection, and cells kept for longer than 24 hr were unlikely to breakdown and become sporangia.

(*b*) *Isotopes*

Radioactive solutions were prepared by adding stock tracer to sea water, and compensating for changes in concentration of the major ions. ^{24}Na and ^{42}K were obtained as irradiated carbonates (spectroscopically pure) from the Australian

Atomic Energy Commission (A.A.E.C.), Lucas Heights, N.S.W., and were converted to chlorides. Irradiation was restricted to 24 hr to reduce contamination with long-lived isotopes. ^{22}Na was obtained from the United Kingdom Atomic Energy Authority through the A.A.E.C.

(c) *Measurements*

Isotope measurements were made with a Geiger–Müller liquid counting system but, in addition, uptake of ^{42}K was sometimes measured by direct counting of a short strand of cells under an end-window Geiger–Müller tube. This method was not satisfactory for other isotopes.

Sodium and potassium concentrations were measured with an EEL flame-photometer; chlorine by potentiometric titration against $AgNO_3$; and calcium or magnesium by titration with EDTA using eriochrome black as indicator for total divalent cation, and murexide as indicator for calcium (Ulrich and Johnson 1959).

Potentials were measured with a Vibron electrometer and microelectrodes filled with 2M KCl solution.

(d) *Determination of Fluxes*

Fluxes of potassium and sodium across the plasmalemma and tonoplast were estimated by a method basically the same as that used by MacRobbie and Dainty (1958) and by Pitman (1963). Strands of *Chaetomorpha* about 3 cm long (7–10 cells) were put into labelled sea water and after 12 hr transferred to non-radioactive samples of sea water. The isotope diffusing out of the tissue during this period into unlabelled solution was measured and used to determine the fluxes.

The time course of isotope exchange had three obvious stages. Firstly, there was a rapid exchange between free space and solution, with time for half-exchange of about 16 sec. Secondly, there was a phase in which isotope exchange was dominated by loss of isotope from the cytoplasm, and finally one in which the rate of loss of isotope was proportional to the amount of isotope in the vacuole.

In describing ion transport the following terms have been used:

Q_c = amount of potassium or sodium in the cytoplasmic phase.

Q_v = amount of potassium or sodium in the vacuole.

ϕ_1 = fluxes between free space and cytoplasmic phase (net flux = 0).

ϕ_2 = fluxes between cytoplasmic phase and vacuole (net flux = 0).

The free space was at a specific activity little different from that in the solution, and effectively zero during elution. On this assumption, it can be predicted from the three-phase model that exchange of the isotope in the non-free space is determined by the expression

$$Y = Ae^{k_1 t} + Be^{k_2 t}, \tag{1}$$

where Y = amount of isotope at time t during elution, and A, B, k_1, and k_2, are constants† determined by ϕ_1, ϕ_2, Q_c, and Q_v. These constants can be estimated

† k_1 and k_2 are both negative.

by plotting log Y versus t and log (dY/dt) versus t, and from them ϕ_1, ϕ_2, Q_c, and Q_v can be estimated (see Appendix).

This method assumes that (1) the fluxes ϕ_1, ϕ_2 do not change during the experiment and that net flux is zero; (2) the three phases are arranged in a series. The first assumption is reasonable and supported by measurements of isotope uptake and of potassium or sodium content. There is no unequivocal evidence for the second assumption, though it is appropriate to the cellular organization. This problem will be discussed again later in this paper. Tracer is measured in the units "μ-equivalent*", which is simply counting rate divided by specific activity. Tracer uptake or efflux is called *apparent* uptake or efflux to distinguish it from the estimated fluxes.

TABLE 1

CONCENTRATIONS OF K^+, Na^+, AND Cl^- IONS IN WHOLE CELLS OF *CHAETOMORPHA*, IN THE CYTOPLASM, IN THE VACUOLES, AND IN SEA WATER

Ion	Concentration (μ-equiv/ml) in:				Equilibrium Concentration* (μ-equiv/ml)
	Sea Water	Whole Cells	Cytoplasm	Vacuole	
K^+	13	535±5	541±3	540	9
Na^+	500	66±2	25±3	25	340
Cl^-	523	575±6	601±18	—	770
K/Na ratio	0·026		17–25	21	

* Assuming potential to be 10 mV positive to sea water.

III. RESULTS

(a) *General Ion Distribution and Potential Measurements*

In Table 1 estimates of concentrations of K^+, Na^+, and Cl^- ions in whole strands of *Chaetomorpha*, in the cell sap, in the vacuoles, and in sea water are given. The amount of sodium in whole cells is higher than that in the vacuoles, due to the free-space content which, of course, contains potassium and sodium at the proportions of sea water instead of the vacuoles. From measurements of isotope exchange the free space had a time for half-exchange of about 16 sec and contained 1 μ-equiv/g potassium and 40 μ-equiv/g sodium. This amount of sodium accounts for the observed discrepancy between whole cell and vacuolar estimates.

The vacuole of the cells was found to be about 10 mV positive to sea water. This value has been confirmed by Dr. N. A. Walker (unpublished data) who also used electrodes of different shapes to show that the cytoplasm was 75 mV negative to sea water, making the vacuole about 85 mV positive to the cytoplasm. By allowing electrodes to be sealed with cytoplasm we have found the cytoplasm to be about 30 mV negative to sea water, i.e. somewhat lower than Walker's values:

Site of Electrode	Potential (mV)	No. of Determinations
Vacuole	$+10 \pm 0{\cdot}5$	35
Cytoplasm	$-32 \pm 3{\cdot}1$	16

The concentrations of sodium, potassium, and chloride that would be in equilibrium with sea water for a potential difference of +10 mV are 335, 9, and 780 m-equiv/l, respectively. Ion balance at flux equilibrium therefore requires an inward potassium pump and an outward sodium pump, but the values do not give any clear demonstration of a chloride pump as the ratio of concentrations in sap and sea water is equivalent to only $+3{\cdot}5$ mV.

A high level of discrimination between potassium and sodium was found in samples collected at all times of the year (Fig. 2), and this level was maintained by cells kept in aerated sea water in the laboratory for at least 6 weeks after collection. There was some variability between cells in a strand, but no systematic difference between young and older cells, possibly due to the mode of formation of new coenocytes.

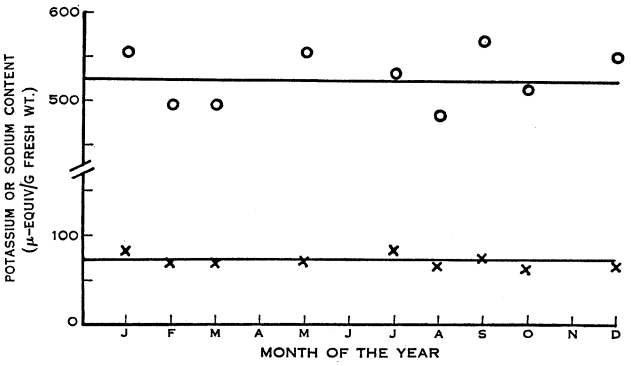

Fig. 2.—Potassium (○) and sodium (×) in strands of *Chaetomorpha* at different times of the year.

The K/Na ratio in the cells was not altered by transferring tissue from the light to darkness, nor did this reduction in photosynthesis immediately alter the apparent potassium uptake, i.e. the cation ion-transport mechanism was not critically dependent on photosynthesis, as in *Ulva* (Scott and Hayward 1953). Potassium uptake by cells in nitrogen was unaffected if the tissue was in the light, but in the dark there was a rapid loss of potassium and uptake of sodium (Fig. 3). Inhibitors of metabolism such as dinitrophenol (DNP) or cyanide could be used to inhibit potassium uptake and were reversible for short exposures. Uptake of potassium isotope had a Q_{10} of $3{\cdot}1$ in the range 10–15–20°C, the apparent uptakes being $1{\cdot}8$, $3{\cdot}3$, $5{\cdot}9$ μ-equiv*/g/hr, respectively. The K/Na discrimination in *Chaetomorpha* thus appears to be metabolically controlled, as in other plant cells. The cardiac glycoside oubain was found to have no effect on potassium uptake.

(*b*) *Sodium Fluxes*

The uptake of tracer sodium (apparent sodium uptake) was only 3–4 pmoles*/cm²/sec (0·2 μ-equiv*/g/hr), and after 13 hr in labelled sea water the amount of tracer in the vacuole and cytoplasmic phase was a small proportion of the total in the tissue, due to the large free space content (40 μ-equiv/g). However, it was possible to make a good separation between free space and cytoplasmic phase as the half-time for free-space exchange (18 sec) was short compared with that of the cytoplasmic phase (6 min). The distinction between cytoplasmic phase and vacuole was also clear and the graphs of log Y versus t and log ($\mathrm{d}Y/\mathrm{d}t$) versus t both rapidly became straight lines of the same slope (k_2). In all, determination of k_1, k_2, A, and B was most satisfactory.

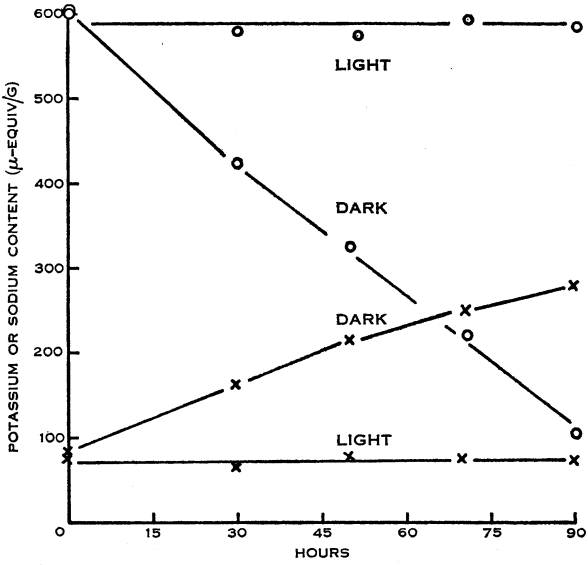

Fig. 3.—Potassium (○) and sodium (×) content of *Chaetomorpha* cells in an atmosphere of nitrogen and exposed to the light and the dark.

Estimates of fluxes (ϕ_1, ϕ_2) and sodium content of the cytoplasmic phase are given in the following tabulation (the values therein are means of six separate determinations):

ϕ_1 (pmoles/cm²/sec)	ϕ_2 (pmoles/cm²/sec)	Sodium Content (μ-equiv/g)	Apparent Uptake (pmoles*/cm²/sec)
110±12	3·7±0·4	1·15±0·20	3·6

The low apparent uptake is clearly due to the flux across the tonoplast, but this low flux is a result of the low sodium concentration in the cytoplasmic phase. There is good reason to believe that the inward movement of sodium (ϕ_1) is passive, so the permeability of the outer membrane, P_1, can be defined by

$$\phi_1 = -P_1 \cdot \frac{zEF}{RT} \cdot \frac{C}{1-\exp(zEF/RT)},$$

where C is the external concentration and E the potential of the inside relative to outside, say -35 mV. The permeability of the inner membrane for sodium can be defined by a similar expression using vacuolar concentration and the potential between vacuole and cytoplasm, i.e. -45 mV. In this case P_1 is $1{\cdot}2\times10^{-7}$ cm/sec and P_2 is $0{\cdot}7\times10^{-7}$ cm/sec. The average concentration of sodium in the cytoplasmic phase would be between 60 and 120 μ-equiv/ml if the volume were 1–2%.

(c) *Potassium Fluxes*

The apparent uptake of potassium was much larger than that of sodium, the usual value being about 80–120 pmoles/cm²/sec, but values up to 240 and as low as 40 pmoles/cm²/sec have been measured. The determination of the fluxes of potassium was much less satisfactory than of sodium. The graphs of log Y versus t and log ($\mathrm{d}Y/\mathrm{d}t$) versus t could be treated as for sodium to yield values of "k_1", "k_2", "A", and "B", but the subsequent calculation of ϕ_1, ϕ_2, and Q_c leads to some ridiculously high values of Q_c. For example, if Q_c were 90 μ-equiv/g (Table 2) then

TABLE 2

POTASSIUM FLUXES, APPARENT UPTAKE, AND CONTENT OF THE CYTOPLASMIC PHASE

No. of Replicates	ϕ_1 (pmoles/cm²/sec)	ϕ_2 (pmoles/cm²/sec)	Q_c (μ-equiv/g)	Apparent Uptake (pmoles/cm²/sec)
5	210 ± 35	950 ± 100	98 ± 8	175
3	135 ± 4	920 ± 100	32 ± 3	120
3	135 ± 6	350 ± 30	23 ± 2	100
3	$65\pm0{\cdot}3$	100 ± 4	$17{\cdot}7\pm0{\cdot}3$	40

the concentration in the cytoplasm would have been 4500 μ-equiv/ml and the concentration in the vacuole some 20% less than the average of the cell as a whole. This difference in concentration was never found in direct measurements of the sap.

The primary data from such an experiment are shown in Figure 4 plotted as log Y versus t (curve A) and log ($\mathrm{d}Y/\mathrm{d}t$) versus t (curve B). The overestimation of Q_c is mainly due to the persistent curve in the latter, which is a more sensitive estimate than curve A of the rate constant k_1.

The equation for isotope loss from the tissue given above includes the assumption that

$$\text{isotopic flux} = \text{flux}\times\text{specific activity in bulk of phase.}$$

When fluxes are large compared with diffusion in the phase the specific activity at the surface could be very different from that in the bulk of the phase and the simple equation no longer applicable, particularly if diffusivity of potassium is low in the bulk of the phase (cf. Plate 1). This difference is suggested as an explanation for

the very large values of Q_c when apparent uptake was high. In these conditions observed isotope diffusion out of the tissue would be smaller than expected, and the time for exchange of the cytoplasmic phase would be increased, making k_1 an underestimate. In this case the most reliable values are the lowest ones, i.e. about 17–20

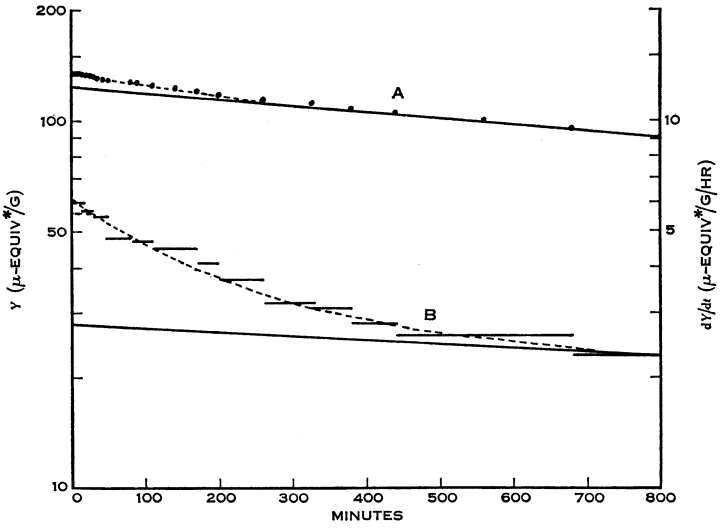

Fig. 4.—*A*, log Y versus t; *B*, log (dY/dt) versus t.

μ-equiv/g, but even these may be too large. Taking the cytoplasmic volume as 3% these estimates give reasonable potassium concentrations in the cytoplasm of 500–600 μ-equiv/ml. Fluxes in this particular experiment were at the low end of the range ($\phi_1 = 60$, $\phi_2 = 100$), and it must be recognized that higher values are found.

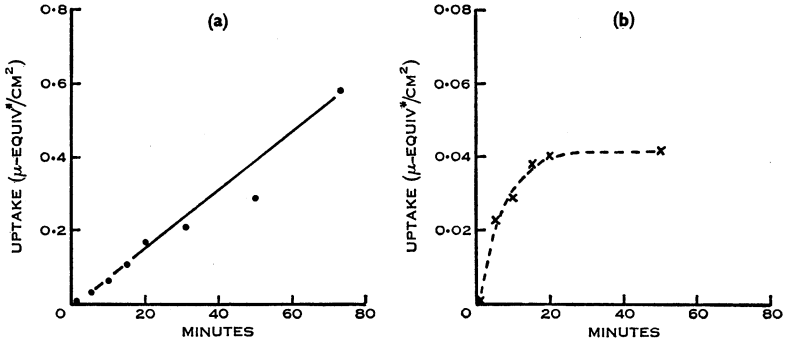

Fig. 5.—Uptake of potassium isotope to (*a*) cell vacuoles and (*b*) cytoplasm.

As apparent uptake is equal to $\phi_1 \cdot \phi_2/(\phi_1+\phi_2)$, both ϕ_1 and ϕ_2 must be larger than the observed apparent uptake, and in some cases over 165 pmoles/cm²/sec.

Other evidence to support the suggestion that k_1 can be underestimated comes from measurements of potassium tracer uptake to the cell vacuole, using micro-

pipettes to sample the vacuoles directly. From comparison of the vacuolar and whole cell uptakes it is possible to estimate the tracer uptake to the cytoplasm. Figure 5 shows the time course of uptake to the vacuoles (observed) and to the cytoplasm (estimated).

Uptake to the cytoplasm was small, and half complete in about 5 min; this time for half-exchange was very much shorter than that observed in experiments as shown in Figure 4, which also gives correspondingly higher values of k_1. The time course of uptake, showing no "shoulder", is what would be expected if ϕ_1 were much smaller than ϕ_2, and the specific activity of the cytoplasm closer to that of the vacuole than of the solution. Due to the rapid exchange of the cytoplasm there was no detectable lag in the rate of uptake to the vacuole as the cytoplasmic specific

TABLE 3

CONTENT OF THE CYTOPLASMIC PHASE, VACUOLAR CONCENTRATIONS, AND FLUXES OF POTASSIUM AND SODIUM IN SOLUTIONS CONTAINING 10^{-4}M DINITROPHENOL (DNP)

Solution	ϕ_1 (pmoles/cm²/sec)	ϕ_2 (pmoles/cm²/sec)	Q_c (μ-equiv/g)	Vacuolar Concentration (m-equiv/l)	
				Potassium	Sodium
		Potassium			
Control	65±0·3	100±4	17·7±0·3	520	45
+DNP (replicate 1)	19	13	0·9	490	50
+DNP (replicate 2)	11	3·0	0·2	524	28
		Sodium			
Control 1	110±12	3·8±0·4	1·15±0·20	500	28
+DNP	90	5·0	6·7	560	20
Control 2	40	2·6	0·40	385	95
+DNP	58	62	15·0	320	150

activity increased. The low specific activity of the cytoplasm would partly account for the small uptake to the cytoplasm (0·9 μ-equiv*/g), but it is still small compared with the apparent contents estimated experimentally. The lowest values found were about 2·5 μ-equiv*/g (equivalent to 17·7 μ-equiv/g for Q_c from Table 3).

(*d*) *Effect of Dinitrophenol on Potassium and Sodium Transport*

As the pH of sea water (about 8) was much higher than that at which DNP is effective, solutions containing 10^{-4}M DNP and buffered to pH 6·2 with sodium acetate/acetic acid were used. *Chaetomorpha* has a small pH tolerance and collapses at about pH 5·5, leaving only a small pH range in which DNP is active but the cells unaffected by low pH. DNP inhibited both the uptake of potassium isotope and the loss of isotope from labelled tissue to non-radioactive solutions. This inhibition was reversible for short exposures to DNP (Fig. 6). In spite of inhibition

of potassium transport, the amount of potassium and sodium in the tissue was unchanged for up to 40 hr, or in some experiments even longer (Fig. 7). Subsequently

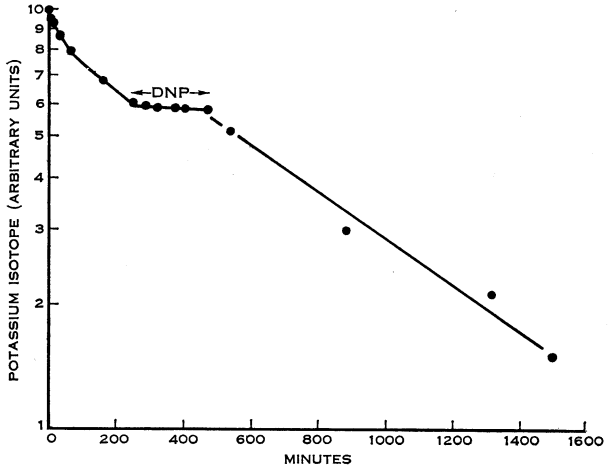

Fig. 6.—Effect of dinitrophenol (DNP) on the loss of potassium isotope from tissue to unlabelled sea water.

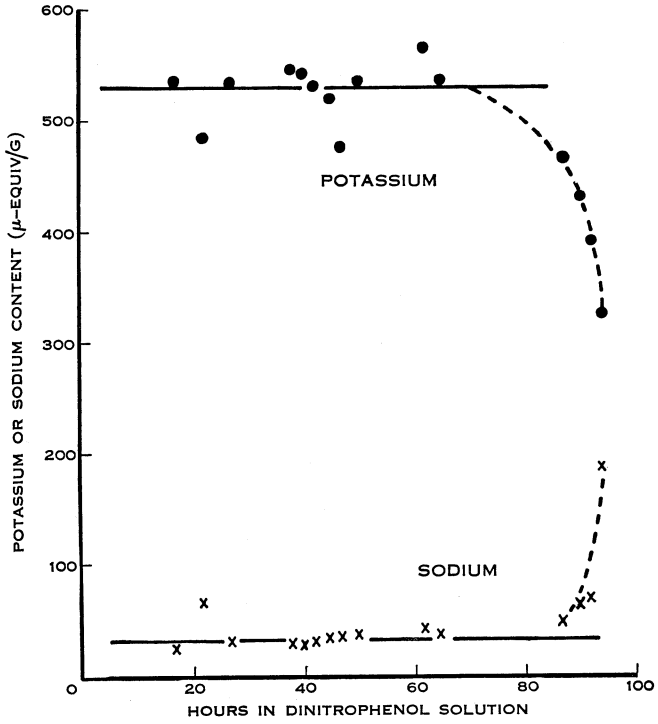

Fig. 7.—Potassium and sodium content of tissue in dinitrophenol solution.

there was a rapid loss of potassium and uptake of sodium, which was irreversible. There was no change in the overall potential when DNP was added to the solution.

During the period of flux inhibition there was no detectable net transport of potassium and sodium, so it was possible to use the method described above to estimate fluxes. Table 3 summarizes these results. As a result of DNP action at the outer boundary the potassium flux (ϕ_1) was inhibited and the content of the cytoplasmic phase changed from high potassium–low sodium to low potassium–high sodium. The effect on fluxes into and out of the vacuole of this changeover in K/Na ratio in the cytoplasmic phase was unexpected. Not only was potassium influx reduced, but so was potassium efflux. Similarly, uptake of sodium did not increase commensurately with the change in sodium content, though in the second example when evidently some net uptake of sodium has occurred, the flux of sodium (62 pmoles/cm^2/sec) is within the range expected for an increase in cytoplasmic phase content.

IV. Discussion

The magnitudes of the fluxes for potassium and sodium observed in these experiments and the transmembrane potentials observed may be summarized as follows:

	ϕ_1 (pmoles/cm^2/sec)	Potential (mV)	ϕ_2 (pmoles/cm^2/sec)	Potential (mV)
Potassium	70–150	−35	200	+45
Sodium	85–125		4	

It is considered that the behaviour conformed adequately to the three-phase model, particularly as there is some evidence from Figure 5 that the rate of uptake to the vacuole increased from zero, and did not rise immediately the cells were put into the labelled solution. Such behaviour is characteristic of the serial arrangement of the phases in the model.

DNP had the following effects on sodium and potassium in the tissue:

(1) Uptake of potassium isotope was inhibited.

(2) Efflux of potassium isotope was inhibited.

(3) No large efflux of potassium or influx of sodium took place.

(4) The ratio of potassium to sodium in the cytoplasmic phase was much reduced.

The vacuolar concentrations of potassium and sodium and the overall potential of +10 mV require inward potassium transport, and outward sodium transport. From the action of DNP it is clear that pumps of this kind were located at the outer boundary. Thus the influx (ϕ_1) of potassium was much reduced but that of

sodium unaffected. Although the sodium fluxes were little affected, the content of the cytoplasmic phase increased rapidly.

The observed potential across the outer boundary was about −35 mV, and assuming the sodium influx (ϕ_1) was passive the permeability can be estimated [defined as in Section III(*b*)] as $1{\cdot}2\times10^{-7}$ cm/sec. If the concentration of sodium in the cytoplasmic phase were known the *passive* contribution to the efflux (ϕ_1) could be estimated: a reasonable value of the concentration is *c.* 60 m-equiv/l in which case the efflux would be 4·5 pmoles/cm²/sec. This value is most unlikely to be smaller by a factor of more than 2, and shows that the sodium efflux, normally about 100 pmoles/cm²/sec, must be predominantly active. Lack of information about the potassium concentration again makes a calculation of potassium permeability doubtful if based on the efflux (ϕ_1). If the potassium concentration is as low as 600 m-equiv/l, the permeability is about 3×10^{-7} cm/sec,† but this value would be correspondingly reduced if the concentrations were larger. The *passive* potassium influx (ϕ_1) is thus at the most about 7 pmoles/cm²/sec, and again the flux is predominantly active, and it is well within the experimental limits that the active fluxes are of equal magnitude.

If these were the only active fluxes of potassium and sodium in the cell, then the concentrations in vacuole and cytoplasm should be in electrochemical equilibrium, but this would require about 3200 m-equiv/l of potassium and 150 m-equiv/l of sodium in the cytoplasm. While the sodium estimate is not unreasonable (as it is equivalent to 1 μ-equiv/g in about 0·7% of the tissue) the potassium concentration seems physiologically large—though not necessarily impossible. However, it would have to be located in a very small proportion of the cytoplasm if direct measurements of sap concentration are to agree with other measurements of potassium in the whole cell. This high estimate is due to the large positive potential difference found between cytoplasm and vacuole (about 45 mV). This value is much larger than that found for characean cells (Spanswick and Williams 1964), but it seems well established by conventional methods of measurement. If these observations are accepted then a potassium pump into the vacuole from the cytoplasm is necessary. The present observations do not require any active transport of sodium.

The action of DNP also presents some anomalies if active transport of potassium and sodium were only at the plasmalemma. Inhibition of these fluxes should lead to a reduced potassium and increased sodium content of the cytoplasmic phase. Unless very large potential changes took place, there should then be a net potassium efflux and sodium influx to the vacuole. In fact, this was not found until about 40–80 hr after inhibition and the estimated potassium efflux from the vacuole fell from 200 to about 10 pmoles/cm²/sec. The overall potential change in DNP solutions was at the most about 10 mV decrease. It would appear then that DNP is inhibiting a potassium *efflux* from the vacuole—i.e. requiring a pump in the opposite direction

† Taking ϕ_1 as 85 pmoles/cm²/sec.

to that required by electrochemical consideration. Similar arguments can be used to support an active sodium influx to the vacuole.

However, the introduction of systems of pumps tailored to fit a few observations offends principles of simplicity and it may be better to look for the explanation elsewhere. The layer of chloroplasts in the cytoplasm has few discreet channels through it (Plate 1) and effectively forms a separate serial phase. Observations on the chloroplast layer in characean cells shows them to contain large amounts of potassium and sodium, probably maintained by active transport. Electrodes inserted into the cytoplasm might well enter only this layer and record *chloroplast*–solution potential difference, leading to overestimates of the cytoplasmic–vacuole potential difference. Moreover, diffusion of potassium (and sodium) across the cytoplasm to the vacuole is in the pathways between chloroplasts but also *through* the chloroplasts. Hence exchange of potassium in the cytoplasm may be restricted by diffusion through the chloroplasts (leading to overestimate of cytoplasmic content—cf. Fig. 4). The chloroplast layer could also act as a buffer between tonoplast and plasmalemma reducing the effect of DNP. If transport of potassium into the chloroplasts were reduced by DNP action the effect would be to reduce diffusion of tracer potassium across the cytoplasm and prevent large-scale leakage of potassium from the vacuole.

The organization of the cytoplasm near the tonoplast also shows the presence of small vacuoles, about 2 μ in diameter, which are remarkably similar to those found in *Chara* (Chambers and Mercer 1964). The cytoplasm around these vacuoles was well fixed in the electron-microscope preparation (Plate 1) and they do not appear to be artefacts. If these vacuoles are forming and discharging into the main vacuole (arrow, Plate 1, Fig. 3), this is another reason why flux estimated from isotope uptake by the present model is an overestimate of flux into the vacuole. They could also explain the action of DNP in appearing to inhibit fluxes into and out of the vacuole.

V. Acknowledgments

The authors are grateful to Professor N. Higinbotham for his advice and help in measuring potentials.

VI. References

Chambers, T. C., and Mercer, F. V. (1964).—Studies on the comparative physiology of *Chara australis*. II. The fine structure of the protoplast. *Aust. J. Biol. Sci.* **17**: 372–87.

MacRobbie, E. A. C., and Dainty, J. (1958).—Ion transport in *Nitellopsis obtusa*. *J. Gen. Physiol.* **42**: 335.

Pitman, M. G. (1963).—The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. *Aust. J. Biol. Sci.* **16**: 647–68.

Scott, G. T., and Hayward, H. R. (1953).—Metabolic factors influencing sodium and potassium distribution in *Ulva lactuca*. *J. Gen. Physiol.* **36**: 659.

Spanswick, R. M., and Williams, E. J. (1964).—Electrical potentials and Na, K, and Cl concentration in the vacuole and cytoplasm of *Nitella translucens*. *J. Exp. Bot.* **15**: 193–200.

Ulrich, A., and Johnson, C. M. (1959).—Analytical methods for use in plant analysis. Bull. Calif. Agric. Exp. Stn. No. 766.

Appendix

The differential equation for isotope uptake to the vacuole is

$$\frac{d^2Y_3}{dt^2}+\frac{dY_3}{dt}\left[\frac{(\phi_1+\phi_2)}{Q_2}+\frac{\phi_2}{Q_3}\right]+\frac{\phi_1\phi_2}{Q_2Q_3}Y_3=\frac{\phi_1\phi_2}{Q_2}S_o.$$

If tissue is in the isotope solution for a time t and then transferred to non-radioactive solution, the decrease in isotope in the tissue with time (t') is

$$Y_2+Y_3=1/(k_2-k_1)\{[\phi_1+k_2(Q_2+Q_3)](1-e^{k_1t})e^{k_1t'}-[\phi_1+k_1(Q_2+Q_3)](1-e^{k_2t})e^{k_2t'}\},$$

where k_1,k_2 are the roots of

$$x^2+\beta x+\gamma=0,$$

and

$$\beta=[(\phi_1+\phi_2)/Q_2+(\phi_2/Q_3)],$$

$$\gamma=\phi_1\phi_2/Q_2Q_3.$$

Hence $k_1k_2=\gamma$, $k_1+k_2=-\beta$, and have the same meaning as in equation (1) in the text. The constants A and B of equation (1) have the following values:

$$A=[\phi_1+k_2(Q_2+Q_3)](1-e^{k_1t})/(k_2-k_1),$$

and

$$B=[\phi_1+k_1(Q_2+Q_3)](1-e^{k_2t})/(k_1-k_2).$$

(Q_2+Q_3) is determined experimentally, so ϕ_1, ϕ_2, Q_2, Q_3, can be estimated from these relationships. When Q_3 is large compared with Q_2, the following approximations can be made:

(1) If S_v is small compared with S_o,

$$B=\phi_2s_2t=\phi_1\phi_2t/(\phi_1+\phi_2),$$

or alternatively

$$Bk_2=-\phi_1\phi_2/(\phi_1+\phi_2)S_v,$$

$$\phi_1\phi_2/(\phi_1+\phi_2)=-k_2(Q_2+Q_3). \qquad \text{(if } Q_2 \ll Q_3\text{)}$$

(2) $$Ak_1=-\phi_1^2(\phi_1+\phi_2).$$

(3) $$k_1=-(\phi_1+\phi_2)/Q_2$$

From these approximations ϕ_1, ϕ_2, and Q_2 can be estimated.

Explanation of Plate 1

Sections of the cytoplasm of *Chaetomorpha darwinii* fixed in potassium permanganate (1·5% for 45 min) and mounted in Araldite. *C*, chloroplast; *CV*, cytoplasmic vacuoles; *N*, nucleus; *P*, pyrenoid; *V*, vacuole; *X*, region where cytoplasm has come away from the wall; *W*, cell wall.

Fig. 1.—This section shows the basic organization of the cytoplasm. At the outside of the cytoplasm there is a layer of chloroplasts that are basically disk-shaped but compressed or folded into a layer. Within this layer is the region containing nuclei and other components of the cytoplasm. Cytoplasmic vacuoles are prominent.

Figs. 2 and 3.—In these sections the narrow space between chloroplasts is shown and the cytoplasmic vacuoles appear in more detail. The arrow in Figure 3 indicates a vacuole that appears to have collapsed.

SODIUM AND POTASSIUM TRANSPORT IN *CHAETOMORPHA*