IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS*

III. VACUOLAR FLUXES OF SODIUM

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Summary

Estimates have been made of the vacuolar influx (ϕ_i) and efflux (ϕ_o) of sodium in cells of *Chara australis* R.Br. var. *nobilis* A.Br. from measurements of radioactivity in extracted sap samples or from direct counts of single cells, using the isotope ^{22}Na.

The influx was often less than the efflux, both being in the range 0·1–0·4 p-equiv/cm². sec (1 p-equiv. $\equiv 10^{-12}$ equiv.), at room temperature and in the light. Dark conditions reduced ϕ_i and ϕ_o to about 70 per cent. of their values in light. The Q_{10} of ϕ_i and ϕ_o was about 2·5. ϕ_i was independent of external concentration in the range 0·2–2·0 mN but increased sharply when the sodium concentration was 5 mN. The sodium ion was shown to contribute less than 5 per cent. to an electric current passed through the cell surface and along the vacuole.

The vacuolar sodium concentration was very much less than its calculated value assuming electrochemical equilibrium with the external medium. The potassium in the vacuole was in approximate electrochemical equilibrium with that in the medium.

These facts are discussed in relation to possible mechanisms of sodium movement in *C. australis* cells.

I. INTRODUCTION

Three separate ion-exchange processes occur between giant internodal cells of the Characeae and the external medium. The initial exchange was shown by Diamond and Solomon (1959) using *Nitella* cells and, independently, by Dainty and Hope (1959) using *Chara australis* cells, to take place in the cell wall. In *C. australis* indiffusible anions are present in the cell wall to the extent of about 2 μ-equiv/mg dry wt. They are thought to be derived from the ionization of polyuronic acids (Dainty, Hope, and Denby 1960). The second exchange, with a half-time of the order of 1 hr, is with the "protoplasmic non-free space" (N.F.S.) (MacRobbie and Dainty (1958). The amount of sodium in the N.F.S. of *C. australis* appears to be very small and this exchange is difficult to separate from that in the cell wall, which contains some counterions which are only slowly exchangeable. For this reason few measurements have been made of ions in the N.F.S. of *C. australis*.

The third and slowest exchange is with ions in the vacuole. Some measurements of vacuolar exchange have been made by Gaffey and Mullins (1958), MacRobbie and Dainty (1958), and Diamond and Solomon (1959). The fluxes range from 0·2 to 6 p-equiv/cm². sec,‡ but it is likely that the apparently high values

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‡ 1 p-equiv. $\equiv 10^{-12}$ equiv.

of Gaffey and Mullins were due to the presence of cortical cells in the species of *Chara* they used. MacRobbie and Dainty considered the vacuolar fluxes of sodium and potassium to be passive. The present paper is a more detailed study of the vacuolar influx and efflux of sodium under various conditions, using ^{22}Na as a tracer. ^{42}K will soon be available in Australia at high enough specific activity to be used in such experiments.

On the basis of the observed potassium, sodium, and chloride vacuolar fluxes, and assuming all except the chloride influx to be passive, MacRobbie and Dainty (1958) calculated the resistance of the tonoplast to be about 250 $k\Omega\,cm^2$. However, Walker (1957) found the outer membrane to have a resistance to the flow of electric current as low as 6 $k\Omega\,cm^2$, and the tonoplast to have a much lower resistance. In the present paper data comparing the vacuolar fluxes of sodium with those due to flowing electric currents are brought to bear on this discrepancy.

II. Experimental Methods

(*a*) *Material*

Strands of *C. australis* R. Br. var. *nobilis* A.Br. were collected from field ponds and kept in an artificial pond water (A.P.W.) containing 0·5 mN $CaCl_2$, 1·0 mN NaCl, and 0·1 mN KCl. Individual internodal cells 3–7 cm long and 1–1·5 mm in diameter were cut from the strands and kept in A.P.W. for a period of at least 3 days. The A.P.W. was renewed about twice daily.

(*b*) *Influx*

A cell of measured surface area was placed for 4–6 hr in A.P.W. with ^{22}Na added to give a specific activity of 5–10 $\mu c/\mu$-equiv. Na. After rinsing in inactive A.P.W. for about 1 hr to remove most of the cell wall Na* (i.e. ^{22}Na), the cell was blotted lightly, one end cut off, and a sap sample taken. The sap was free from chloroplasts; contamination by colourless cytoplasm was unlikely as the sap was ejected by turgor pressure from the cut cell. The sample of vacuolar sap of known volume, 5–30 μl, was diluted to 10 ml and placed in an annular container for scintillation counting using a sodium iodide crystal. The radioactivity of the vacuolar sap was compared with that of a small sample of labelled A.P.W. made up to 10 ml.

In a second method, the cell was counted directly at intervals by placing it in a small tube let into a slab of scintillating plastic.† This method relies for accuracy on placing the cell always in the same position and also on removing the wall Na* by rinsing for about 20 min with inactive A.P.W. After this time a certain amount of radioactive sodium remains in the wall (Dainty and Hope 1959, particularly Figures 3 and 4) and possibly in the N.F.S. This is shown in some of the graphs below of counting rate against time, where the line extrapolated to zero time passes through the axis at 10–30 counts/sec. The amount of activity in the wall after rinsing for 20 min is probably approximately constant, being dependent on time of rinsing rather than accumulated time in Na*, because of the comparatively rapid

† NE 102 plastic from Nuclear Enterprises Ltd.

exchange in the wall. In any case this method gave substantially the same answer for the influx as the direct sap count. In two experiments where the two methods were applied to the same cell, the influxes were: (1) 0·39 p-equiv/cm^2. sec (whole cell count) and 0·35 (sap count); (2) 0·19 (whole cell count) and 0·25 (sap count). The cell activity was compared with that of a sample of labelled A.P.W. sealed into a glass capillary of about the same dimensions as the cell, to enable calculation of the influx in equivalents. Since only the γ-rays from ^{22}Na were counted, absorption corrections were unnecessary.

(c) *Efflux*

The efflux of sodium was calculated from the radioactivity lost per unit time (usually 1 hr) from unit cell surface area of cells previously soaked for up to 3 weeks in labelled A.P.W. Na* must be completely removed from the wall and cytoplasm before efflux from the vacuole can be measured. The cells were repeatedly rinsed in A.P.W. over a period of 48 hr. This removes Na* from the walls of internodal cells (Dainty and Hope 1959). Nodal cells, with a much higher surface/volume ratio, will also lose their Na* in this time if their membranes are similar to those of internodal cells in permeability.

After these repeated rinses, a single cell was placed in successive 10-ml aliquots of A.P.W. for periods of 1 hr and the radioactivity of each aliquot determined in the scintillation counter. Thus, effluxes were initially expressed in counts per min per hr. They were converted to p-equiv/cm^2. sec after measurement of the specific activity of the sap at the end of the experiment. The vacuolar specific activity was almost constant over this period since of a total of 10^5–10^6 counts/min, only 10^3–10^4 were lost during the efflux measurements.

Counting rates of 50–300 counts/min for each hourly sample were commonly obtained in these experiments, so that the external specific activity never rose enough to limit the efflux. Calculation of the efflux involves knowing the vacuolar sodium concentration as well as its radioactivity. Owing to the danger and inconvenience of using radioactive samples for flame photometry, a number of inactive cells of the same batch as the experimental ones was analysed for vacuolar sodium (and potassium) concentration ($[Na_v]$, $[K_v]$) and the mean taken.

(d) *Variability of Fluxes*

The effect of various treatments on ϕ_i and ϕ_o is described below in Section III. A large number of experiments was done in which a set of 5–10 control cells was compared with an equal number of other cells under a different condition (e.g. of light or temperature). While these experiments demonstrated the gross effect, in some cases with high statistical significance, experiments were preferred in which a single cell could be given different treatments in succesive time intervals. This eliminated the effect of variability amongst cells, which, together with seasonal effects, is the cause of the large standard errors in the means of ϕ_i and ϕ_o quoted below. It is such experiments with single cells which are used below to illustrate the results, together with some tables which compare the mean sap activity of sets of cells given different treatments.

III. Results

(a) *Mean Vacuolar Fluxes of Sodium in the Light at* 20–27°C

Averaged over 117 cells, the mean sodium influx from A.P.W. (ϕ_i) was 0·13 p-equiv/cm². sec. The standard error (S.E.) of a single determination was 0·10 and the standard error of the mean (S.E.M.) was 0·01.

Table 1

COMPARISON OF INFLUXES AND EFFLUXES IN SINGLE CELLS OF C. AUSTRALIS

Cell	Influx ϕ_i (p-equiv/cm². sec)	Efflux ϕ_o (p-equiv/cm². sec)	Temperature (°C)
1	0·13	0·39	26
2	0·14	0·43	26
3	0·22	0·33	25
4	0·09	0·17	21
5	0·08	0·10	21
6	0·08	0·10	21
7	0·15	0·18	21

With 28 cells the mean efflux into A.P.W. (ϕ_o) was 0·32 p-equiv/cm². sec$\pm$ 0·21 (S.E.) or $\pm$0·04 (S.E.M.). These means are significantly different ($P<0·001$) but the variability of the fluxes is so large that a significant difference between

Table 2

VACUOLAR CONCENTRATION OF POTASSIUM, SODIUM, CALCIUM, AND CHLORIDE IN C. AUSTRALIS CELLS FROM FIELD POND WATER OF THE COMPOSITION SHOWN

Number of estimations given in parenthesis

	Date	Potassium Concn. (mN)	Sodium Concn. (mN)	Chloride Concn. (mN)	Calcium Concn. (mN)
C. australis cells	24.vii.59	86$\pm$3 (8)	49$\pm$1 (8)	—	—
C. australis cells	7. x.59	74$\pm$2 (10)	47$\pm$2 (10)	106$\pm$12 (10)	2·6*
Field pond water		0·06	2·2	2·4	0·16

* Vacuolar sap of 10 cells pooled.

ϕ_i and ϕ_o could be demonstrated only with a large number of measurements. The temperature varied from 20–27°C but was constant during any one determination; the light was sky light, or artificial light of the same approximate intensity.

When ϕ_i and ϕ_o could be compared under similar conditions and in the same cell, efflux was generally in excess of influx so that the cells were not in a steady

state as far as their vacuolar sodium content was concerned. This is shown in Table 1 which lists the results of several experiments on different occasions. In all cases $\phi_o > \phi_i$. This is probably connected with the change in external medium from natural pond water to A.P.W. In these experiments ϕ_i is the mean influx over the time used to label the cells enough to make efflux measurements, i.e. 3–14 days, while ϕ_o is the mean efflux in the 12–24-hr period following subsequent rinsing. However, ϕ_i under these conditions is not very different from that measured over 4–6 hr.

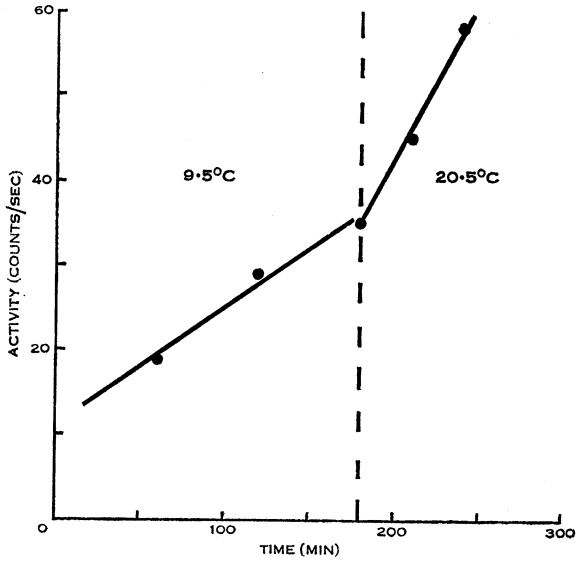

Fig. 1.—Increase in vacuolar radioactive sodium plotted against time for a single cell initially at 9·5°C and later at 20·5°C.

Some analyses of the vacuolar sodium and potassium concentrations of cells of *C. australis* fresh from natural pond water are given in Table 2. The vacuolar concentration of chloride in other analyses was generally somewhat less than the sum of that of potassium and sodium. There was almost no change in $[K_v]$ and $[Na_v]$ after soaking cells for up to 7 days in A.P.W.

(*b*) *Effect of Temperature on Influx and Efflux*

Figure 1 shows the comparative rates of increase in sap activity at 20·5°C and 9·5°C in a single cell in light conditions. The rates corresponded to fluxes of 0·37 and 0·12 p-equiv/cm². sec, respectively. The mean Q_{10} of ϕ_i in eight experiments was $2{\cdot}5 \pm 0{\cdot}25$ (S.E.M.). It was similar in light and dark. Figure 2 shows the comparative rates of loss of vacuolar radioactive sodium, from a different cell, plotted as counts/min. hr against time for temperatures of 20 and 10°C. These losses of radioactivity correspond to effluxes of 0·38 and 0·18 p-equiv/cm². sec respectively, and therefore the Q_{10} was 2·1.

(c) Influx of Sodium as a Function of Sodium Concentration

The influx of sodium from A.P.W. into the vacuole, in the light and at about 20°C was measured in several external concentrations of sodium ranging from 0·2–5 mN. Collected observations are given in Figure 3, in which are plotted the means and S.E.M. of at least 20 cells at each concentration. ϕ_i was approximately constant up to an external sodium concentration, $[Na_o]$, of 2 mN but a large increase was observed in all experiments (of about 4–6 hr duration), when $[Na_o]$ was 5 mN.

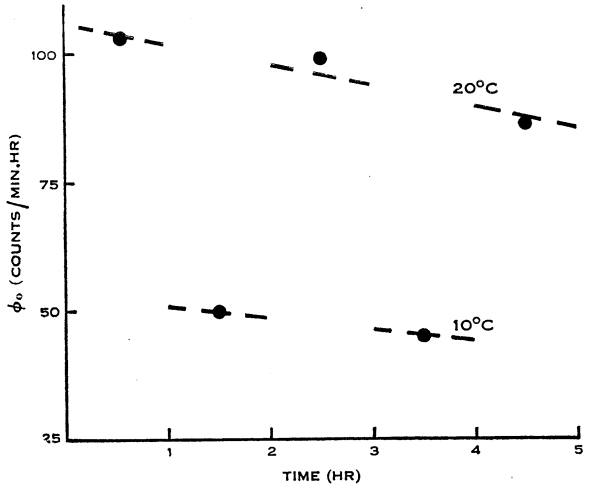

Fig. 2.—Rate of loss of vacuolar radioactive sodium in hourly intervals plotted against time. The temperature alternated between 20°C and 10°C in each hour.

(d) Fluxes of Sodium in Light and Dark

The vacuolar influx and efflux of sodium are both larger in the light than in the dark by a factor of greater than 1·5. This is shown in Figures 4 and 5. The possibility that the decrease in ϕ_i in the dark was due to lack of aeration was tested by bubbling the external solution with nitrogen and air, both in light and dark, and the results are indicated in Figure 4. No significant difference was observed between the treatments with nitrogen and air. Table 3 lists some other comparisons between light and dark, in experiments in which saps from different sets of cells were measured at the end of a period of about 4 hr. In other experiments the significance of the difference was occasionally at the level of $P = 0{\cdot}1$, owing to the use of smaller samples.

(e) Effect of Current Flow on the Fluxes of Sodium

Single cells were mounted so that "Perspex" stocks divided the length into two equal parts which were insulated from each other by means of "Vaseline" (Fig. 6). Direct current was then passed through the cell by means of Ag|AgCl electrodes dipping into each end of the trough which contained labelled A.P.W. for influx measurements or inactive A.P.W. for efflux measurements.

(i) *Influx.*—The effect of applied current on the rate of increase of vacuolar radioactivity in the light and at 20°C is shown in Figure 7. The two cells had mean influxes of 0·26 and 0·05 p-equiv/cm². sec. The dotted lines indicate the expected influx if the sodium ion carried all the current inwards.* A small positive effect of current was found but it is likely that the sodium ion carries less than 5 per cent. of the inward current.

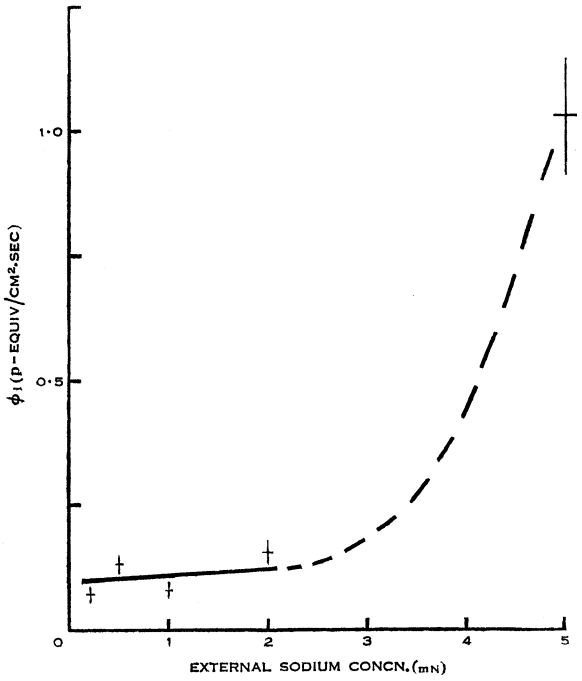

Fig. 3.—Influx of sodium into the vacuole plotted against external sodium concentration. The standard error of the mean of 20 or more cells is given by half the height of the symbols.

An equal loss of activity from the vacuole at the end of the cell in which positive current is being carried outwards would not be expected since the specific activity of the vacuole is very much less than that externally. The assumption that the current passes through the vacuole rather than the cell wall or cytoplasm will be discussed below.

(ii) *Efflux.*—The effect of a current of 10^{-7} A on the loss of activity from a cell which had previously been loaded with Na* and rinsed for 40 hr with A.P.W. is shown in Figure 8. As in the experiments on effect of current on influx, current was passed in one area of the cell, along the section insulated with "Vaseline", and out at the other end. The areas at each end were usually 0·5–1·0 cm² and

* 96,400 coulombs ≡ 1 equivalent. Therefore 3×10^{-7} A/cm² corresponds to a flux of 3·1 p-equiv/cm². sec.

the insulated area about 0·5 cm². It can be seen that there is no appreciable difference in rate of loss of radioactivity between the segments of the cell kept positive and negative, and the rates are about the same whether current flows or not. The

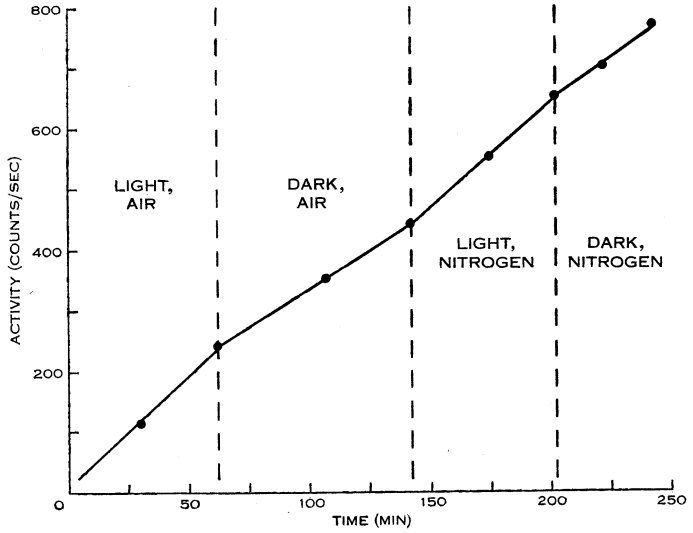

Fig. 4.—Increase in vacuolar sodium activity in a single cell plotted against time for the conditions indicated. The mean influx in the light was 0·42 and in the dark 0·29 p-equiv/cm². sec at 18°C.

high efflux from the end made negative, on the initial passage of current, was found in some experiments and was probably due to residual Na* in the wall and cytoplasm being quickly moved out by the initial current. In subsequent periods of

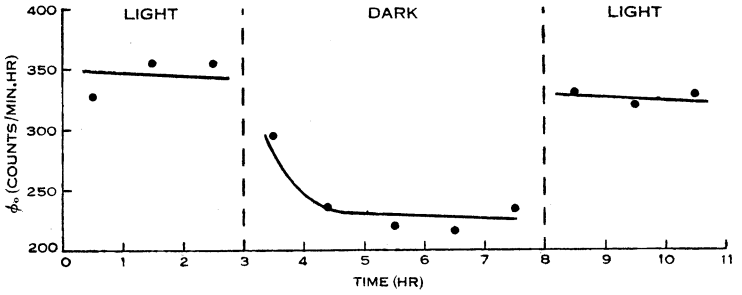

Fig. 5.—Efflux of sodium from the vacuole of a single cell in hourly intervals, in the light and dark. Efflux was 0·38 (light) and 0·26 (dark) p-equiv/cm². sec at 26°C.

current, such effluxes were absent, although the vacuolar Na* content was still high. In Figure 8 the observed ϕ_o was 0·12 p-equiv/cm². sec from both ends of the cell compared with an expected 1·04 from the end kept negative, on the assumption that sodium carried all the current.

In these experiments there were three current paths along the cell:

(1) Along the cell wall (i.e. outside the protoplast).

(2) Along the cytoplasm (i.e. outside the vacuole).

(3) Along the vacuole.

It is assumed above that most of the current flows along the vacuole (i.e. through the plasmalemma and tonoplast). A direct measurement was made of the resistance of path (1) in an arrangement similar to that used to measure the effect

TABLE 3

COMPARISON OF MEAN VACUOLAR INFLUXES IN LIGHT AND DARK IN C. AUSTRALIS CELLS IN ARTIFICIAL POND WATER

Number of determinations given in parenthesis

Expt. No.	Temp. (°C)	Mean Vacuolar Influxes (p-equiv/cm². sec)		Differences Significant at:
		Light	Dark	
1	26	0·17±0·015 (6)	0·075±0·008 (6)	$P<0·001$
2	24	0·12±0·026 (6)	0·031±0·012 (8)	$P<0·01$

of current on flux. An isolated cell wall was fitted tightly on a slightly tapered glass rod, and placed in position on the "Perspex" stocks. A typical resistance measured was 900 kΩ, while the intact cell in the same position gave 90 kΩ. It appeared therefore that about 10 per cent. of the applied current flowed along path (1) and had no effect on the tonoplast flux. The relative resistances of paths

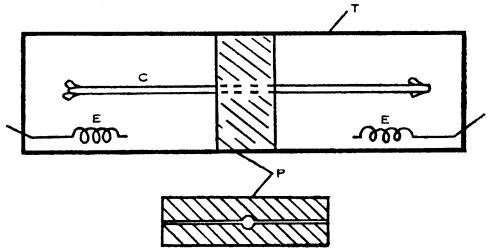

Fig. 6.—Experimental arrangement used when investigating the effect of electric currents on fluxes: *T*, wax trough; *C*, cell; *E*, electrodes; *P*, "Perspex" stocks.

(2) and (3) can be estimated. If the cytoplasm has, as is likely, the same resistivity as the sap (in these cells approx. 50 Ω cm), its resistance would be some 30 times the resistance of the sap. Unless then the tonoplast resistivity is very high (Walker (1957) found it to be very low in *Nitella*) the resistance of path (2) will be much higher than that of path (3). It can be concluded then that more than 80 per cent. of the applied current crosses the tonoplast.

(*f*) *Electric Potential of the Vacuole*

In two series of experiments the potential difference between the vacuole and external medium (A.P.W.) was measured in cells which had been living in A.P.W. for about 2 weeks. A glass microelectrode of end diameter about 5 μ, filled with 0·3N KCl, was pushed into the vacuole with the aid of a screw micromanipulator. The potential difference was measured to within $\pm 0{\cdot}5$ mV with a valve electrometer of high input resistance. In two series of 10 cells each, which differed in pretreatment, the mean potential difference was as given in Table 4. The sodium and potassium concentrations in the vacuole of the same cells were measured following withdrawal of the microelectrode, during which an insignificant amount of vacuolar sap was lost. The significance of these results is discussed below.

TABLE 4

MEAN ELECTRIC POTENTIAL OF THE VACUOLE AND THE VACUOLAR CONCENTRATIONS OF SODIUM AND POTASSIUM IN C. AUSTRALIS CELLS IN ARTIFICIAL POND WATER

Means and standard error of the means of the results from 10 cells in each experiment are given. The last two columns give the vacuolar activity (mN) expected assuming electrochemical equilibrium

Expt. No.	Vacuole Potential (mV)	$[Na_v]$ (mN)	$[K_v]$ (mN)	$[Na_o]\exp(-EF/RT)$	$[K_o]\exp(-EF/RT)$
1*	$-157 \pm 1{\cdot}5$	57 ± 3	$64 \pm 4{\cdot}5$	490–560	49–56
2†	-161 ± 3	50 ± 4	$68 \pm 2{\cdot}5$	540–700	54–70

* Plants soaked for 7 days and single cells therefrom soaked for a further 8 days in artificial pond water.

† Plants soaked for 14 days and single cells therefrom soaked for a further day in artificial pond water.

IV. DISCUSSION

(*a*) *Mean Fluxes*

The assumptions on which the measurements of vacuolar fluxes rest need to be examined. All the above estimates are based on two sorts of measurement:

(i) Rates of increase of radioactivity either in the vacuole (influx) or medium (efflux); and

(ii) Specific activities of the medium (influx) and vacuole (efflux).

The relations between the fluxes and these quantities have been assumed to be, for unit surface area:

$$\text{Influx} = (1/s_o).\mathrm{d}Y_v/\mathrm{d}t, \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{Efflux} = (1/s_v).\mathrm{d}Y_o/\mathrm{d}t, \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where $s_{o,v}$ and $Y_{o,v}$ are specific activities and radioactivities per unit volume in the medium and vacuole respectively. Thus it is assumed that the specific activity

of the cytoplasm, s_c, rather quickly becomes s_o (in influx measurements) or zero (in efflux measurements). This involves the assumption that $\phi_{ic} \gg \phi_{iv}$ and $\phi_{oc} \gg \phi_{ov}$. This was found to be so for both potassium and sodium in cells of *Nitellopsis* (MacRobbie and Dainty 1958), but not for potassium in *Nitella axillaris* (Diamond and Solomon 1959) where it was found that in the steady state the specific activity of potassium in the "cytoplasmic non-free space" was only 27 per cent. of s_o.

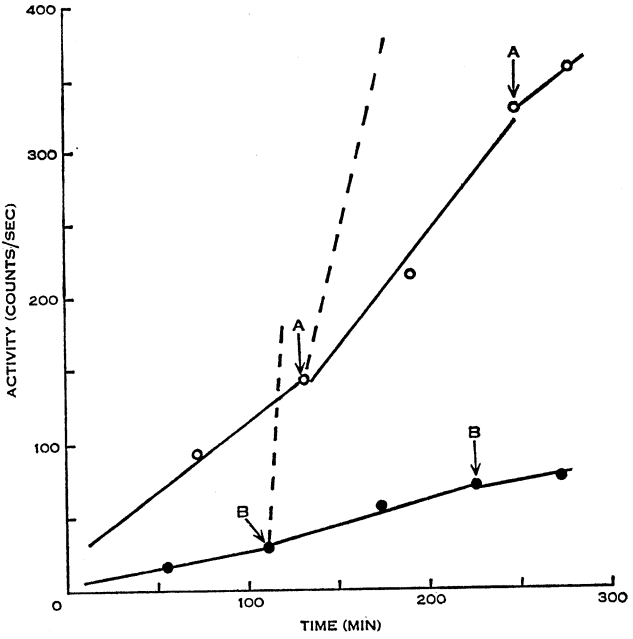

Fig. 7.—Effect of applied current on the vacuolar influx of sodium in two *C. australis* cells. Currents of 10^{-7}A (*A*) or 3×10^{-7}A (*B*) were switched on and off where indicated by the arrows. The dotted lines show the expected influxes, assuming all current was carried by the sodium ion.

The way in which our estimates of ϕ_v would differ from those calculated from equations (**1**) and (**2**), if in fact $\phi_c \sim \phi_v$, can be seen from the following considerations. Referring to Figure 9, for each square centimetre of cell surface:

$$dY_v/dt = \phi^*_{iv} - \phi^*_{ov} = s_c\phi_{iv} - s_v\phi_{ov}, \quad \ldots\ldots\ldots\ldots (3)$$

and

$$\begin{aligned} dY_c/dt &= \phi^*_{ic} - \phi^*_{oc} - \phi^*_{iv} + \phi^*_{ov} \\ &= s_o\phi_{ic} - s_c(\phi_{oc} + \phi_{iv}) + s_v\phi_{ov}. \end{aligned}$$

In the steady state $dY_c/dt = 0$, whence

$$s_c = (s_o\phi_{ic} + s_v\phi_{ov})/(\phi_{oc} + \phi_{iv}). \quad \ldots\ldots\ldots\ldots (4)$$

Combining (**3**) with (**4**), and neglecting s_v in comparison with s_o

$$\phi_{iv} = \frac{1}{s_o} \cdot \frac{dY_v}{dt}\left(\frac{\phi_{oc} + \phi_{iv}}{\phi_{ic}}\right), \quad \ldots\ldots\ldots\ldots\ldots (5)$$

that is, the influx calculated according to (1) should be increased by a factor $(\phi_{oc}+\phi_{iv})/\phi_{ic}$. In a similar way it can be shown that the efflux is increased by a corresponding factor.

No estimates of cytoplasmic sodium fluxes were made because of the apparently small amount in the N.F.S. in *Chara* cells, but independent estimates of the permeability of the surface membrane from resistance measurements (Hope and Walker, unpublished data) suggest that ϕ_{ic} or ϕ_{oc} = 1–2 p-equiv/cm². sec. If this is so our present fluxes are underestimated by 20–30 per cent. and become about 0·17 (influx) and 0·4 (efflux). This vacuolar influx is of such a magnitude that the half-time for specific activity equilibrium between the vacuole and medium is about 1400 hr.

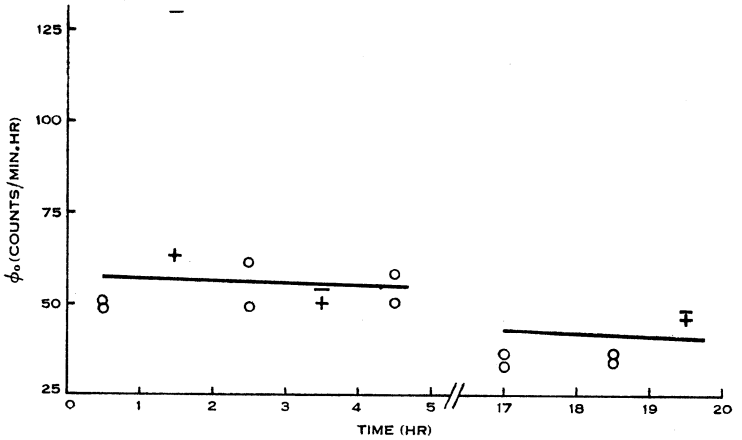

Fig. 8.—Effect of applied current on the vacuolar efflux of sodium from a single cell. Points plotted are activity lost in hourly intervals from the segment of the cell in the pool of solution made positive (+), negative (−), or with no current flowing (○).

(*b*) *Effect of Light, Temperature, and Aeration*

It was shown that the influx and efflux of sodium across the tonoplast are similarly affected by light and by change of temperature. Thus the influx and efflux seem to be similar processes. The high value of Q_{10}, 2–2·5, could result from passive diffusion across a high potential barrier (Danielli 1952), or from transport controlled by chemical reactions.

Evidence has been presented here which indicates a connection between sodium transport across the vacuole and a photosynthetic, non-oxidative part of the cell metabolism. This evidence comprises the effect of light in increasing the fluxes, and the absence of an effect on influx of a nitrogen atmosphere in the dark. The mechanism remains to be elucidated.

The variation of sodium influx across the tonoplast with external concentration may reflect a variation of cytoplasmic concentration. This would imply a sudden increase of sodium concentration in the cytoplasm as the external concentration is increased from 2 to 5 mN. Such an increase could result from a sudden

increase in the sodium permeability of the outer membrane, or from saturation of a sodium extrusion pump at the outer membrane.

(c) *Effect of Current on Flux*

It has been shown here that the sodium ion contributes little (at most a few per cent.) to an electric current flowing across the tonoplast. Assuming that conduction of the current by protons or electrons is improbable, it would be expected that the major vacuolar ions (potassium, sodium, and chloride) would carry the current. The vacuolar concentrations of these ions are comparable (Table 2) and so indeed are their fluxes across the tonoplast (MacRobbie and Dainty 1958; Diamond and Solomon 1959; and this paper). Each of the three ions might therefore be expected to carry a substantial proportion of the current.

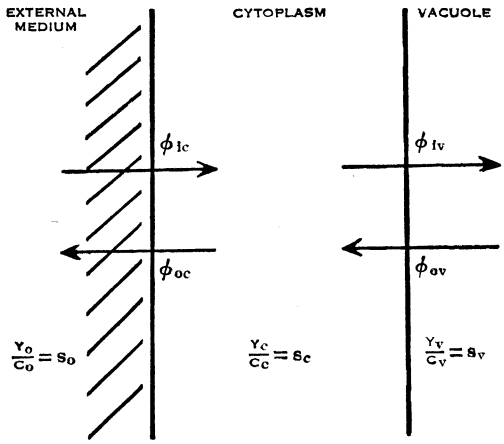

Fig. 9.—Fluxes and specific activities in a three-phase system consisting of external medium, cytoplasm, and vacuole.

The small proportion carried by sodium could result from a sodium transport mechanism in which neutral complexes rather than ions cross the membrane. An alternative explanation, however, is suggested by the discrepancy mentioned in the Introduction. MacRobbie and Dainty (1958) calculated a tonoplast resistance of 250 kΩ cm^2 for *Nitellopsis obtusa*.

They used their measured ion fluxes, assumed them to be passive (except the chloride influx), and presumably related flux with electric resistance (Hodgkin 1951) by:

$$G_j = (z_j F^2/RT)\phi_j\,, \qquad G_m = 1/R_m = \Sigma G_j\,, \qquad \text{.....(6)}$$

where

R_m = membrane resistance (Ωcm^2),
G_m = membrane conductance (mho cm^{-2}),
G_j = contribution of jth ion to membrane conductance,
ϕ_j = equilibrium flux of jth ion across membrane (equiv/cm^2. sec),
z_j = valence of jth ion.

and F, R, and T have their usual significance. This relationship holds only for ions in equilibrium across the membrane: it assumes that the ions move independently under the influence of thermal agitation and electric potential gradients.

However, the resistance of the tonoplast of a *Nitella* species is more than an order of magnitude lower (Walker 1957) than the calculated value of MacRobbie and Dainty (1958).

This discrepancy would be reduced, and the present result for sodium explained, if either potassium or chloride contributed to a much higher conductance than one would calculate from their equilibrium fluxes, using equation (**6**). This is possible if the ion in question crosses the membrane through long narrow pores, as proposed by Hodgkin and Keynes (1955) for potassium in the squid axon membrane. They suggested pores so narrow that ions could not pass each other, but were constrained to move in file. Ion movements are then no longer independent of each other, as required by equation (**6**). The resistance calculated from the ion flux is then reduced below the above value by a factor n, where n is about equal to the number of ions in the file:

$$G_j = (nz_jF^2/RT)\phi_j . \qquad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

If then the tonoplast of cells of the Characeae contains such pores, specific for potassium or for chloride ions, and containing say 10 ions, the resulting low resistance would help to explain the sodium result presented here and the discrepancy discussed.

(*d*) *Vacuolar Ionic Activities*

At the moment there is no firm basis for distinguishing between potassium and chloride as the ion in pores. However, if the view of MacRobbie and Dainty (1958) that the concentration of chloride in the cytoplasm is much lower than that in the vacuole is accepted, it may be concluded that it is potassium. The ion traversing the pores must be nearly in equilibrium across the tonoplast for the n-fold relationship between resistance and flux to hold. It has been found in this work that potassium is nearly in electrochemical equilibrium between external medium and vacuole, and may be expected therefore to be nearly in equilibrium across the tonoplast. MacRobbie and Dainty (1958) and Gaffey and Mullins (1958) also found that of the three main vacuolar ions, potassium was nearest to electrochemical equilibrium.

Sodium, calcium, and chloride are definitely not in electrochemical equilibrium in *C. australis* cells (Tables 2 and 4; see also Walker 1957, 1958). The chloride concentration in the vacuole is much greater (100–150 mN) than the equilibrium value (2 μN) predicted from the external concentration and the electric potential difference. The concentrations of sodium and calcium in the vacuole are much less than the corresponding equilibrium values. Thus an active sodium-extruding pump suggested for plant cells by Hope and Robertson (1953) and demonstrated in *Nitellopsis* by MacRobbie and Dainty (1958) is probably in operation in *Chara* somewhere between the vacuole and medium. The site of this pump is almost certainly at the outer membrane (as in *Nitellopsis*) because it is here that the electrochemical gradient is directed inwards. This is because the electric potential differ-

ence is found almost entirely at this interface (Walker 1955) and the cytoplasmic concentration is almost certainly less than the equilibrium value calculated from this potential difference (see Table 4).

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