Ionic Relations of *Nitella translucens*

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ABSTRACT The ionic state of single internodal cells of a fresh water characean, *Nitella translucens*, has been studied. In mature cells the vacuolar concentrations were 78 mM K, 60 mM Na, and 151 mM Cl, compared with concentrations of 0.1 mM K, 1.0 mM Na, and 1.3 mM Cl in the bathing medium. The results suggest an active influx of potassium and an active efflux of sodium at the plasmalemma, and an active influx of chloride, probably at the tonoplast. The cation transport is inhibited by ouabain, and is more efficient in young cells; the chloride transport is insensitive to ouabain, and unaffected by age. Thus the two systems appear to be independent. It is suggested that the active fluxes are 0.5 to 0.6 $\mu\mu$moles K/cm^2 sec. inwards, and 0.45 $\mu\mu$moles Na/cm^2 sec. outwards. The passive influxes, 0.3 $\mu\mu$moles K/cm^2 sec. and 0.55 $\mu\mu$moles Na/cm^2 sec., give a value for the relative permeabilities of the plasmalemma, P_{Na}/P_K, of 0.18. The absolute magnitudes of the permeabilities, compared with those derived from resistance measurements, suggest that potassium ions interact strongly in the membrane. The cation fluxes at the tonoplast are much higher than those at the plasmalemma. The active influx of chloride is 0.85 $\mu\mu$moles/cm^2 sec. in light, but only 0.052 $\mu\mu$moles/cm^2 sec. in the dark. The potassium influx is also reduced in the dark. Thus the energy for both active transport processes is closely geared to light-dependent metabolism, rather than to respiration.

INTRODUCTION

There has been a good deal of work on the electrical properties of the giant internodal cells of the Characeae, but relatively little on the ion fluxes, and in general the two types of measurement have been made on different species, under different conditions, making comparison difficult. Previous flux measurements on ecorticate characeans have been made on *Nitellopsis obtusa* (sodium, potassium, and chloride; MacRobbie and Dainty, 1958), *Nitella axillaris* (potassium; Diamond and Solomon, 1959), and *Chara australis* (sodium; Hope and Walker, 1960).

In *Nitellopsis* some kinetic separation of the compartments of the cell was possible and three phases were distinguished—the cell wall, "protoplasmic non-free space," and the vacuole. Two active ion transport processes were involved in the maintenance of the normal ionic state of the cell; a chloride

transport inwards at the tonoplast regulating the total salt concentration of the cell, and an active cation transport at the plasmalemma, perhaps of the same type as the linked sodium-potassium exchange pump in animal cells. The main barrier to ion movements lay in the tonoplast where fluxes were much less than at the plasmalemma.

There is a major discrepancy between this last result and resistance measurements on other species, which put the main resistance at the plasmalemma with very little contribution of the tonoplast to the total cell resistance (Walker, 1957; Hope and Walker, 1961). This difference may be one of environment, since *Nitellopsis*, a brackish water species, was bathed in a solution of chloride concentration 35 mM, whereas *Chara australis* was in an artificial pond water of total chloride 1 to 2 mM. The present work reports flux measurements on another fresh water species, *Nitella translucens*, for which electrical data are available or will be available.

A picture of the ionic state of the cell is built up from measurements of concentrations and fluxes. It is shown that, as in *Chara*, the main barrier to ion diffusion is in the plasmalemma, and the tonoplast is a much more permeable structure than the plasmalemma.

A further study of the active cation transport responsible for the regulation of the alkali cations of the cell has been made. The effect of ouabain, a cardiac glycoside which is a specific inhibitor of alkali cation transport in animal cells, has been studied. The active potassium influx found in this *Nitella* is also inhibited by ouabain (5×10^{-5} M), whereas the active chloride transport is unaffected. Further evidence for the independence of the two transport systems is also presented.

METHODS

Material

Strands of *Nitella translucens* were kept in the laboratory in an artificial pond water of the following composition: NaCl 1.0 mM, KCl 0.1 mM, $CaCl_2$ 0.1 mM. Cells remained healthy (turgid and showing rapid protoplasmic streaming) for some months in this solution. Experiments were done on single internodal cells, 3 to 8 cm long and 600 to 1100 μ in diameter, which were handled by the cut ends of neighbouring cells or by threads tied loosely round the cells.

Plants were also cultured by a method similar to that of Sandan (1955). Internodal cells or nodes were buried in agar (0.8 per cent in culture solution) in test tubes or other glass vessels; these were covered with culture solution, and new plants, with 4 to 5 internodal cells, grew from them over the course of 3 to 11 weeks. These internodal cells were up to 6 cm long and 400 to 700 μ in diameter. The culture solution for this purpose had the following composition: NaCl 1.0 mM, KCl 0.1 mM, $CaCl_2$ 0.1 mM, $Mg(NO_3)_2$ 0.1 mM, sodium phosphate buffer (pH 7.0) 0. 1mM.

Radioactive Solutions

Artificial pond waters of the same chemical composition as above but labeled with one of the isotopes K^{42}, Na^{24}, Na^{22}, and Cl^{36} were prepared. K^{42} and Na^{24} were obtained from the Atomic Energy Research Establishment, Harwell, as the spectroscopically pure carbonates, and converted to the corresponding chlorides by adding an equivalent amount of N/10 HCl to a weighed amount of carbonate. To avoid the presence of long lived impurities potassium was irradiated for not more than 3 days. Na^{22} and Cl^{36} were obtained from the Radiochemical Centre, Amersham.

A labeled bromide pond water was also prepared, with 1.0 mM Na Br^{82} in place of an equivalent amount of chloride, using Br^{82} supplied by Harwell as "isotonic" sodium bromide.

Flux Measurements

Influx of tracer to the whole cell was measured by direct counting of the cell placed on a perspex slide under an end-window Geiger tube (Mullard MX 123). Reproducibility of the counting rate to 2 to 5 per cent was obtained by positioning the cell relative to a series of radiating spokes drawn on a piece of millimeter graph paper stuck to the under side of the slide. The counting rate for each cell in these experiments was converted to the absolute amount of tracer by wet-ashing the cell at the end of the experiment and counting this in a liquid counter, for comparison with a standard solution in the same liquid counter. (20th Century M6 tubes were used.)

Influx to the cell vacuole was determined by diluting a sample of the sap of a labeled cell to 10 ml and counting in a liquid counter. Cl^{36} was counted on planchettes under an end-window tube.

Efflux was determined by washing-out experiments similar to those described earlier, using a similar washing-out tube (MacRobbie and Dainty, 1958). The temperature was maintained within ± 0.5°C, usually at 20°C. The loss of radioactivity from a previously labeled cell to successive 10 ml portions of aerated inactive bathing solution was determined by liquid counting, and the amount remaining in the cell at the end of the experiment was determined by liquid counting of the wet ash. (Cold N HNO_3 was used for this purpose.) The total amount of radioactivity in such a washing-out experiment was also used to calculate the influx during the previous period of labeling the cell, a period of at least 20 hours.

Illumination during these experiments was by two 40 watt "warm white" fluorescent tubes. Solutions were aerated with moist air during the uptake periods, through sintered glass bubblers.

Chemical Analyses

Sap samples were obtained by cutting off the end of the cell and squeezing gently. A drop of colourless sap (1 to 20 mg depending on the cell size) was obtained, and was then weighed on a torsion microbalance (maximum error 0.03 mg). This was then diluted with 2 to 10 ml glass-distilled water for flame photometry. Chloride determinations were done directly on drops of sap, by electrometric titration by a method similar to the first one described in the paper of Ramsay, Brown, and Croghan (1955).

Sodium and potassium were determined in an EEL flame photometer, by comparison with standard solutions containing both those ions in concentrations very close to those in the unknown. These determinations were accurate to 1 to 2 per cent on most samples, but on some very small samples of sap (1 to 2 mg) the accuracy was about 5 to 10 per cent.

In the cells grown in culture sodium and potassium were determined on the whole cell, by ashing the cell in 1 ml of N HNO_3 and diluting with distilled water for flame photometry. In this case acid blanks were also run; their contribution was negligible for potassium and not greater than 5 per cent for sodium.

Samples of protoplasm were obtained by centrifuging the intact cell at 300 to 400 *g* for about 5 minutes; by this process the flowing cytoplasm of the cell was displaced to the lower end of the cell, while the chloroplast layer lining the wall remained attached. The length of the protoplasmic column was then examined under the microscope, and sodium and potassium analyses were done on two separate samples: a length of the lower end containing cytoplasm (1 to 2 mm), and a sap sample from the other end for comparison. This technique has been used by a number of authors, and is discussed by Kamiya (1959); Kamiya states that observations with a centrifuge microscope show bodily shifting of the endoplasm to the centrifugal end of the cell and suggest good separation of cytoplasm from cell sap at this end. Since in the present experiments the two samples, cytoplasm and sap, could have very different specific activities, it was concluded that the tonoplast remained intact during the treatment and that mixing between cytoplasm and sap did not occur. (The cytoplasmic specific activity measurements will be included in a subsequent paper.)

Sodium and potassium were also determined in the chloroplast layer. For this purpose the cells were first soaked for a short time (about 20 minutes) in 0.6 mM $CaCl_2$ to remove sodium and potassium in the cell wall, without any significant loss from the chloroplast layer. One end of the cell was then cut off and the open end of the cell threaded on to a fine pipette; the other end was then cut and the fluid contents of the cell blown out into liquid paraffin, while the chloroplast layer remained attached to the wall. A length of the sleeve of wall plus chloroplast layer was put into 1 ml of N HNO_3 to extract the cations. After dilution with 4 ml distilled water the solution was analysed for sodium and potassium by flame photometry. In most cells very few of the chloroplasts were dislodged by this treatment; if the sap or paraffin blown out appeared green and contained a significant number of chloroplasts the cell was discarded. The lengths at either end of the cell, where some chloroplasts had been dislodged in cutting, were not included in the sample for analysis.

Later experiments in which no Ca wash was given, but in which the ions in the wall were estimated from radioactivity measurements and the amounts subtracted from the total ions in the wall plus chloroplast layer, gave essentially the same results.

RESULTS

Ion Concentrations in Cell Sap

The results of the analyses for sodium and potassium in the sap of mature cells are collected in Table I. The mean chloride concentration on similar cells was

151 ± 2 mM (15). (Results are quoted in the form: mean ± standard error of mean (number of results on which mean is based).) These analyses were done on several batches of cells and there was some evidence of seasonal variation. Because of this, the effect of age on the ion concentrations was determined on cells grown in culture.

Series of 4 to 5 internodal cells of the same cultured shoot were analysed; these provided a time sequence although the absolute age of each cell was

TABLE I

SODIUM AND POTASSIUM CONCENTRATIONS IN THE SAP OF MATURE CELLS AND IN THE SURROUNDING MEDIUM

	K	Na	K/Na
	mM	*mM*	
Cell sap mean ± S.E.M. (No. of cells)	78±1.5 (76)	60±1.5 (76)	1.3±0.1
Artificial pond water	0.1	1.0	0.1

TABLE II

CHANGES IN VACUOLAR SODIUM AND POTASSIUM CONCENTRATIONS WITH AGE

Type of cell*	T_g	T_m	*T*-1	*T*-2	*T*-3	*P*
K, *mM*	98±4	79±6	76±4	65±4	55±4	68±4
Na, *mM*	27±3	55±3	55±3	65±3	68±6	53±2
No. of cells	13	8	14	13	6	8

* Cells are divided into groups according to their position relative to the apex of the shoot, as this position may be taken as an indication of the physiological age of the cell. Thus the youngest group (*T*) represents the most recent internodes of the shoot; the second group (*T*-1) are cells which have one internode more than 5 mm long between them and the apex, and so on. The group *P* includes the parent cells from which the shoot was cultured. The terminal cells are divided into two groups T_g and T_m; T_g are terminal cells of shoots which would grow beyond that cell, whereas T_m are terminal cells of shoots which would not grow further.

indeterminate. However, it seems fair to regard the position of a cell in the shoot as an indication of its physiological age. The results of the analyses of sixty-two cells of fourteen systems of shoots are shown in Table II and in Fig. 1. The cells are divided into groups according to their position relative to the apex of the shoot. Cells at the apex less than 5 mm long were not analysed. The youngest group (*T*) represent the most recent internodes of the shoot; the second group (*T*-1) are cells which have one internode more than 5 mm long between them and the apex, and so on. The group *P* include the parent cells from which the shoot was cultured. The terminal cells are divided into two groups T_m and T_g; T_g are the terminal cells of shoots which are still growing

and would grow beyond this stage; the group T_m are terminal cells of shoots which will not grow further, as happened in some of the culture shoots. Table II gives the sodium and potassium concentrations of the cells of these groups, and Fig. 1 shows the limits and mean values of the ratio K/Na and the sum (K + Na) of the cells in each group. It appears that while there is no striking

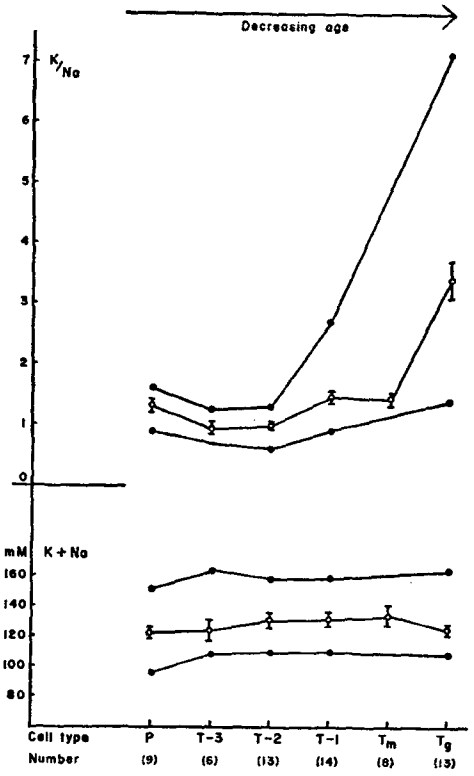

FIGURE 1. Changes with age in the ratio K/Na and the sum (K + Na) in the vacuolar sap of cultured cells; limits and mean values for the cells in each age group are shown. For the definition of the various cell groups see Table II.

trend of the total concentration with age, the discrimination between sodium and potassium, reflected in the ratio K/Na, is much more efficient in young cells.

Protoplasmic Ion Concentrations

Table III shows the results of the determination of sodium and potassium in the flowing cytoplasm of centrifuged cells. In some small samples of protoplasm only potassium was determined, and the ratio of potassium concentrations in the protoplasm and in the sap was measured in more cells than gave values for the ratio of potassium to sodium in the protoplasm.

The mean difference $(K_p/Na_p) - (K_s/Na_s)$ determined on the same cell was 0.05 ± 0.10 (8); thus there appears to be no significant difference in the K/Na ratio in the sap and in the flowing cytoplasm. The mean value of the ratio K_p/K_s of the same cell was 1.5 ± 0.1 (17).

The results of the analyses of the chloroplast layer are shown in Table IV. The amounts of sodium and potassium in the chloroplast layer per unit area of cell surface are first shown; in the second column their concentrations in a 4 μ thick layer are calculated. This thickness is based on estimates by Holm-Jensen, Krogh, and Wartiovaara (1944) and Saltman and Christensen (1961).

TABLE III

ION CONCENTRATIONS IN THE FLOWING CYTOPLASM COMPARED WITH VACUOLAR CONCENTRATIONS

	Flowing cytoplasm	Sap
K, *mM*	120 ± 7 (17)	79 ± 2.5 (17)
Na, *mM*	54 ± 4 (8)	37 ± 3 (10)
K/Na	2.2 ± 0.2	2.1 ± 0.2

TABLE IV

SODIUM AND POTASSIUM IN THE CHLOROPLAST LAYER

	Chloroplast layer		
	Amount of ion	Concentration as a 4 μ layer	Sap Concentration
	mμmoles/cm²	*mM*	*mM*
K	137 ± 8 (10)	340	88 ± 3 (10)
Na	49 ± 6 (10)	120	54 ± 3 (10)
K/Na	2.9 ± 0.2		1.6 ± 0.1

Two conclusions emerge from these results; first that the total cation concentration in the chloroplast layer is several times higher than that in the cell sap, and second that the ratio K/Na is significantly higher in the chloroplast layer than in the sap.

Flux Measurements

POTASSIUM INFLUX The uptake of K^{42} as measured by direct counting of the cell was linear with time after an initial period of about 10 to 15 minutes. This is shown in Fig. 2, in which the activity in the cell is plotted against time over an uptake period of 19 hours; the time course over the 1st hour is shown on an extended time scale in Fig. 2 *b*. (It was assumed that the entry of K^{42} to the cytoplasm from the cell wall during the periods of 2 minutes for which the cell was removed from the solution for counting was only a small fraction of

the total in the wall, and the activity was therefore plotted against total time from the start of uptake.) The initial more rapid rise over the first 10 to 15 minutes would be consistent with the equilibration times of the cell wall cations measured by other workers (Dainty and Hope, 1959; Hope and Walker, 1961). After this period the cell appears to behave as a single compartment in these experiments. The amount of potassium in the initial exchange, given by the intercept of the slow straight line on the axis $t = 0$, was 5.4 ± 0.4 mμmoles/cm² (18 cells, range 2.8 – 8.3).

In experiments at 2°C there was an initial phase, in which exchange was

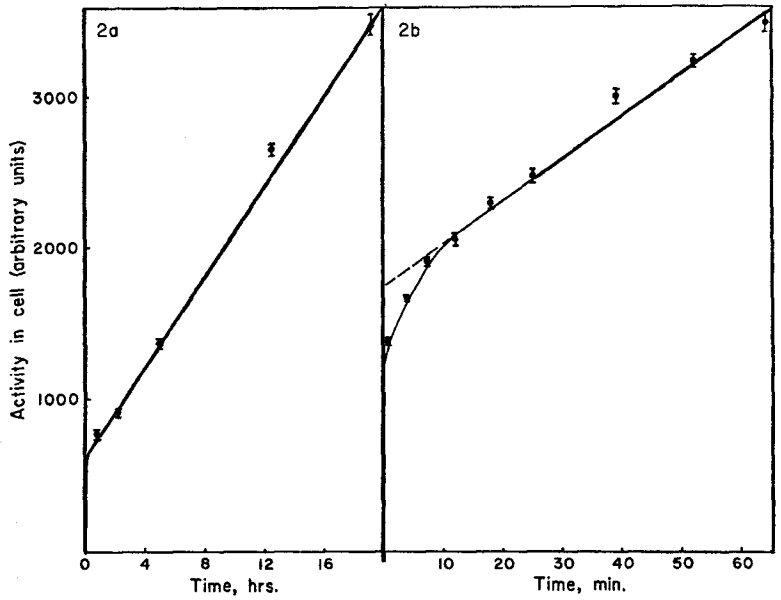

FIGURE 2. Uptake of K^{42} by the cell as a function of time, plotted over 19 hours (Fig. 2 *a*) and on an extended time scale over the 1st hour (Fig. 2 *b*).

complete in about 15 to 20 minutes, containing 3.9 ± 0.2 mμmoles/cm² (4). After this there was no detectable influx in three of the four cells, and an influx of 0.02 μμmoles/cm² sec. in the other cell. It seems likely that the initial phase is almost entirely exchange in the cell wall. After 3 to 5 hours at 2°C there was a lag of 30 min. to 1 hr. before the influx resumed at 20°C, and it was several hours before it reached its normal value.

Two other measurements of influx were made. The influx of K^{42} to the vacuole was measured by counting of sap samples isolated from cut cells; influx was also calculated from the total amount of potassium in the slow fraction of a washing-out experiment after a given period of uptake. When both these figures were obtained for the same cell (ten cells) there was good agreement between the two values. The results of all these experiments are collected in

Table V. There is evidence of some seasonal variation and the results for different batches of cells are therefore separated. In the last batch in Table V the distribution of influx values showed two distinct peaks; it was considered that two populations were involved and the two means were calculated separately. It is thought that this batch contained two populations of cells of very different ages.

The potassium influx in the dark was also measured. The results were rather variable; twelve cells gave values in the range 0.035–0.23, with a mean of 0.12 ± 0.02 $\mu\mu$moles/cm^{2}sec.

POTASSIUM EFFLUX It proved difficult to get an accurate measurement of the efflux of potassium from a washing-out experiment. If the logarithm of

TABLE V
POTASSIUM INFLUX

Batch of cells, time	Method	Range	Mean
A: March–July	Whole cell counting	0.39 – 1.33	0.81 ± 0.05 (28)
B: March–June	Slow fraction of efflux	0.53 – 1.3	0.88 ± 0.08 (10)
B: March–June	Sap isolation	0.39 – 1.3	0.82 ± 0.06 (13)
C: Oct.–Jan.	Sap isolation	0.32 – 0.70	0.50 ± 0.03 (15)
D: June	Sap isolation	0.7 – 1.3	0.92 ± 0.04 (23)
		1.3 – 2.3	1.69 ± 0.08 (12)
E: Cultured cells, June	Sap isolation	0.4 – 1.22	0.93 ± 0.09 (8)

All fluxes in $\mu\mu$moles/cm^2 sec.

amount of radioactivity left in the cell was plotted against time of washing-out, the graph was approximately linear after about an hour. However, the extra activity lost in the 1st hour could not be resolved into components due to the cell wall and a single exponential characteristic of the protoplasm, but the log plot remained obstinately curved. Thus, if the rate constant of the slow phase (k) was calculated for each time interval, as the ratio (Amount of activity lost per unit time)/(Amount of activity in the cell), it continued to decrease gradually over the course of the experiment, a period of 12 to 50 hours. Hence the calculation of the potassium efflux from this rate constant is rather uncertain. The "permeability constant" (λ) for outward movement was calculated as kV/A, where V/A is the volume/surface ratio of the cell; the efflux can then be obtained by multiplying this by the internal concentration of K. In eight experiments values of λ in the range $(0.5 - 5.0) \times 10^{-8}$ cm/sec. were obtained. These correspond to fluxes in the range $0.4 - 4.0$ $\mu\mu$moles/cm^{2}sec., of the same order as the measured influxes.

SODIUM FLUXES Sodium influx was measured by sap isolation, or from the amount of sodium in the slow fraction of a washing-out experiment. It was

not measured by direct counting of the cell because of the very high amount of sodium in the cell wall in the solution chosen.

Sodium efflux was measured by washing-out experiments, and in this case the semilog plots of cellular activity against time were truly linear after an initial period of about an hour or less. The rate constant reached a steady value and remained steady over 30 to 50 hours, and could therefore be used to calculate an accurate value of the efflux. The results of these experiments were:

Sodium influx	0.62 ± 0.06 (12)	
Sodium efflux	0.48 ± 0.06 (11)	$\mu\mu$moles/cm^{2}sec.

TABLE VI
ANION FLUXES

Ion	Conditions	C_o, mM	Influx, $\mu\mu$moles/cm^2 sec. Range	Mean
Cl	Light, 25°C	1.3	0.52 - 1.43	0.85 ± 0.09 (12)
Cl	Light, 25° C; no aeration	1.3	0.39 - 1.20	0.90 ± 0.10 (8)
Cl	Dark, 25°C; no aeration	1.3	0.032 - 0.099	0.052 ± 0.008 (8)
Br	Light, 20° C	1.0	0.35 - 2.60	1.2 ± 0.2 (7)
Br	Dark, 20°C	1.0	0.06 - 0.19	0.13 ± 0.02 (5)

Thus the sodium appears to be close to flux equilibrium under these conditions, and when both influx and efflux were measured on the same cell there did not appear to be any systematic difference between them.

It was not possible to separate any contribution of the protoplasm, and the distinction between the two phases possible in similar experiments with *Nitellopsis obtusa* (MacRobbie and Dainty, 1958) was too uncertain here.

ANION FLUXES The influx of both chloride and bromide to the cell vacuole was measured by sap isolation, and the results are given in Table VI. The effect of light on these fluxes is also shown.

Effect of Cardiac Glycosides

Since cardiac glycosides are specific inhibitors of sodium-potassium transport in animal cells, it was decided to look for any effect on ion transport in *Nitella*. The influx of potassium to the cell vacuole was determined in the presence of 5×10^{-5} M ouabain, and the results are given in Table VII; the control influxes on cells of the same batch are also given (from Table V), and the decrease in influx as a result of the inhibitor. It was checked that this concen-

tration of inhibitor was high enough to produce maximum inhibition. The two peaked distribution of influx values was again found in batch D, and the two populations are again separated. There is no absolute certainty that the group with the higher influx in ouabain corresponds with the group with the higher control influx, but the equality of the two ouabain-sensitive components suggests this is a reasonable assumption. The effect of ouabain was also determined by whole cell counting, and these results are given as the last entry in Table VII. In this case the control influx given is measured on the same cells, and is not the mean of a series of comparable cells. There was a tendency for the cell to become more permeable after long treatments with ouabain, and for the potassium influx to increase in some cells. For this reason the influx

TABLE VII

EFFECT OF OUABAIN (5×10^{-5} M) ON POTASSIUM INFLUX

Batch, time, method	Influx in presence of 5×10^{-5} M ouabain		Control influx	Ouabain-sensitive influx
	Range	Mean		
B: July, sap isolation	0.15–0.60	0.34±0.06 (8)	0.84±0.06	0.50±0.08
D: June, sap isolation	0.10–0.55	0.30±0.03 (18)	0.92±0.04	0.62±0.05
	0.75–1.45	1.05±0.08 (11)	1.69±0.08	0.64±0.11
D: July, whole cell count	0.10–0.65	0.33±0.06 (9)	0.84±0.09 (9)	0.5 ±0.1

All fluxes in $\mu\mu$moles/cm^2 sec.

values quoted are the steady values, and the sap influxes were measured over periods of 3 to 6 hours.

The effect of ouabain on the chloride influx to the vacuole was also determined, but no inhibition of chloride influx was found. The influx in the presence of 5×10^{-5} M ouabain was 0.87 ± 0.13 $\mu\mu$moles/cm^{2}sec. (7), compared with the control value of 0.85 ± 0.09 $\mu\mu$moles/cm^{2}sec. (12).

DISCUSSION

The site and nature of the active transports in *Nitella translucens* appear to be very similar to those found in *Nitellopsis obtusa* (MacRobbie and Dainty, 1958), but the magnitudes of the passive fluxes at the two membranes seem to be very different in the two species.

The flux measurements and the analyses of the concentration changes with age show that the mature cell is capable of maintaining its internal composition in the face of considerable movements of ions into and out of the cell. One may therefore calculate for each ion the electrical potential difference required between the vacuole and the outside solution for the maintenance of the observed concentration differences by passive movements alone, from the

relation

$$E = \frac{58}{z} \log C_o/C_i \, mv$$

where E = potential of the cell vacuole relative to the outside solution
C_o = external concentration
C_i = concentration in the cell vacuole
z = ionic charge in electronic units (algebraic valency).

Under these conditions one may use any striking difference between the observed potential difference and the ion concentration potential to argue active transport of that particular ion, into or out of the cell.

TABLE VIII
ION CONCENTRATION POTENTIALS IN THE SAP

Ion	Equilibrium concentration potential E_j, *mv*	$\Delta E = E_{obs.} - E_j$, *mv.*
K^+	−168	+28
Na^+	−103	−37
Cl^-	+120	−260

$E_j = \frac{58}{z} \log C_o/C_i$ mv

The value of the potential difference for mature cells from the same pond as those used here is −140 mv (S.E. ± 1.5 mv, 12 cells) (Dainty, Williams, Johnstone, and Spanswick, personal communication). The ion concentration potentials for mature cells, calculated from Table I, are shown in Table VIII, together with the difference between these and the observed potential. The size of this difference, ΔE, gives some indication of the relative importance of active and passive ion movements in maintaining the observed concentration gradients. From these results it appears that there is active transport of potassium and chloride into the cell, and of sodium out of the cell. This may be compared with the situation in *Nitellopsis obtusa*, where the results required the active transport of sodium and of chloride and the active transport of potassium was possible.

The site of these transports may be argued from the distribution of ions within the cell. There appears to be no discrimination between sodium and potassium at the tonoplast, since the ratios of K/Na in the flowing cytoplasm and in the vacuole are equal. It is likely therefore that these ions move passively across the tonoplast, and that the sodium/potassium discrimination characteristic of the cation pump occurs at the plasmalemma. The higher value of the cation concentrations in the chloroplast layer suggests a high

concentration of immobile anions there, and there also seems to be some further cation transport at the chloroplast membranes to give an even higher ratio of K/Na than in the bulk cytoplasm.

On the other hand, if chloride were passively distributed across the tonoplast, in a Donnan distribution with the immobile anions in the cytoplasm, one would need a cytoplasmic chloride concentration of (150/1.5) mM or 100 mM. This does not seem possible on osmotic grounds, and it seems much more probable that there is an active transport of chloride at the tonoplast, as postulated for *Nitellopsis*.

The behaviour of the cell as a single compartment in the potassium uptake experiments means that the potassium flux at the tonoplast must be very much larger than that at the plasmalemma. In this case most of the activity entering the cytoplasm goes through into the vacuole and the cytoplasmic specific activity remains very low. The specific activity in the cytoplasm is given by the equation:

$$Q_p \frac{ds_p}{dt} = A[M_{OP} + M_{VP} \cdot s_v - (M_{PO} + M_{PV}) \cdot s_p]$$

where M_{OP} = flux from outside solution to cytoplasm
M_{PO} = flux from cytoplasm to outside solution
M_{PV} = flux from cytoplasm to vacuole
M_{VP} = flux from vacuole to cytoplasm
s_p = specific activity in the cytoplasm, as a fraction of that outside
s_v = specific activity in the vacuole
Q_p = total amount of the ion in the cytoplasm
A = area of the cell.

With high tonoplast fluxes the specific activity in the cytoplasm will rise to a quasi-steady level, given by $s_p = M_{OP}/(M_{PO} + M_{PV})$, and will subsequently rise only very slowly as the specific activity rises in the very large vacuole, with $s_p = (M_{OP} + M_{VP} \cdot s_v)/(M_{PO} + M_{PV})$. The net influx of tracer is initially M_{OP}, and subsequently $(M_{OP} - M_{PO} \cdot s_p)$ in the quasi-steady state; the approach to this state has an exponential time course with a rate constant determined by the sum of the fluxes out of the cytoplasm to the vacuole and to the outside solution; *i.e.*, with $k = [(M_{PO} + M_{PV})A/Q_p]$. This change of slope does not appear in the uptake curves, and this puts a lower limit on the flux at the tonoplast. It is assumed that either this phase is complete in 10 to 15 minutes, or the new slope does not differ by more than 10 per cent from the initial slope (10 per cent being the uncertainty in the slope in the early part of the curve); the second condition gives a lower minimum and requires the flux of potassium at the tonoplast to be greater than about 10 $\mu\mu$moles/cm^{2}sec. The contribution of the cytoplasm to the sodium efflux experiments was also rapid and difficult to separate from the contribution of the

cell wall. Thus the barrier to cations at the tonoplast appears to be less important than that at the plasmalemma.

This agrees with the distribution of electrical resistance measured by Walker (1957) and Hope and Walker (1961) on another fresh water characean, *Chara australis*, but is in complete contrast to the flux measurements on the brackish water *Nitellopsis obtusa* (MacRobbie and Dainty, 1958). The main barrier to ion penetration in *Nitellopsis* was the tonoplast, and the reason for this difference in behaviour is unknown. But the difference appears to be in the tonoplast rather than in the plasmalemma, and is being investigated.

The single compartment behaviour and the equality of the results of the various methods of influx determination imply that the cation fluxes quoted are those at the plasmalemma. The sodium flux is similar to that measured by Hope and Walker (1960) on *Chara australis*—quoted as the vacuolar flux but properly the plasmalemma flux. The potassium fluxes at the plasmalemma are similar to those found by Diamond and Solomon (1959), although the tonoplast fluxes in *Nitella axillaris* were much less than those estimated here.

The sensitivity of the potassium influx to ouabain suggests that the mechanism of the cation transport is the same as that in animal cells. The results suggest that the normal potassium influx is made up of two components, an active influx of 0.5 to 0.6 $\mu\mu$moles/cm^{2}sec. and a passive flux of about 0.3 $\mu\mu$moles/cm^{2}sec. The mean sodium flux in either direction may be taken as 0.55 $\mu\mu$moles/cm^{2}sec., and the influx may be considered to be a passive flux. Then from the relative values of the passive influxes of sodium and potassium and their external concentrations a value of the relative permeabilities of the plasmalemma for the two ions may be obtained. The results suggest that P_{Na}/P_K is about 0.18 at the plasmalemma. Using this value and the K/Na ratio in the bulk of the cytoplasm (rather than that in the chloroplasts themselves), an estimate of the passive sodium efflux may be obtained and, by difference, the active sodium efflux. This calculation gives a passive sodium efflux of about 0.1 $\mu\mu$moles/cm^{2}sec., and an active efflux of about 0.45 $\mu\mu$moles/cm^{2}sec. Thus the active sodium and potassium fluxes are of the same order, and the ratio (Active potassium influx)/(Active sodium efflux) is in the range 1.1–1.4. This calculation is based on mean results from a number of cells, and must be regarded as only a rough estimate of the amount of coupling between sodium and potassium movements. Experiments on the same cell, and on the effects of ouabain on the sodium efflux are in progress, and should give a more accurate estimate of the tightness of the coupling.

The value of P_{Na}/P_K of 0.18 is higher than that deduced from electrical measurements for the related species *Chara australis*, or that calculated from flux measurements on *Nitellopsis obtusa*; these suggest a value of P_{Na}/P_K of 0.06 in *Chara* and 0.05 in *Nitellopsis* (Hope and Walker, 1961; MacRobbie and Dainty, 1958). However, Spanswick and Williams (personal communication)

get $P_{Na}/P_K = 0.2$ in *Nitella translucens* from electrical potential measurements like those of Hope and Walker. Indeed this difference might be expected from consideration of the concentrations in the various species. For example, in *Chara* potassium was not far from electrochemical equilibrium, but sodium was very far out; using the figures given for concentrations and potential the deviations may be expressed as $\Delta E_K = +5$ mv and $\Delta E_{Na} = -60$ mv. If it is assumed that the mechanism of active cation transport is the same in both species, it may be argued that the passive fluxes of potassium in *Chara* must be relatively more important than in *Nitella translucens*, and that one would therefore expect P_{Na}/P_K to be much larger in the *Nitella* species.

Hope and Walker found that the electrical properties of the *Chara* membrane fitted quantitatively with those predicted by the "constant field equation" of Goldman (1943), assuming negligible anion permeabilities. They used this model to predict fluxes from their electrical measurements, but it is possible to reverse this argument and to predict a value of the resistance of the *Nitella* membrane from the measured concentrations and fluxes. The equations used are:

$$r_o = \frac{RT}{F^2} \frac{(1/C_o - 1/C_p)}{\ln C_p/C_o} \tag{1}$$

$$M_{OP}^{K} = \frac{FE}{RT} \cdot P_K \cdot K_o \tag{2a}$$

$$M_{OP}^{Na} = \frac{FE}{RT} \cdot P_{Na} \cdot Na_o \tag{2b}$$

where $C_o = P_K[K_o + (P_{Na}/P_K) \cdot Na_o]$

$C_p = P_K[K_p + (P_{Na}/P_K) \cdot Na_p]$

E = potential between cytoplasm and outside solution (with positive sign of outside solution).

and M_{OP}^{K}, M_{OP}^{Na} are the passive ion fluxes from solution to cytoplasm.

With $E = 140$ mv and passive fluxes of 0.3 $\mu\mu$moles K/cm²sec. and 0.55 $\mu\mu$moles Na/cm²sec., the values of the permeability constants are $P_K = 5 \times 10^{-7}$ cm/sec. and $P_{Na} = 0.9 \times 10^{-7}$ cm/sec. These are very close to the values from flux measurements on *Nitellopsis*, and are also close to those from flux measurements on the corticated species, *Chara globularis* (Gaffey and Mullins, 1958). However, they differ by an order of magnitude from the values calculated from electrical measurements of *Chara australis*. The anomaly persists when a resistance value is predicted from the present fluxes. The calculation predicts a resistance of about 360 kΩcm², whereas the measured values are in the range 6-36 kΩcm², with a mean of 19 kΩcm² (Dainty, Johnstone, and Williams, 1961). Thus it appears that the tracer fluxes in characean cells are

less by an order of magnitude than the fluxes predicted from the measured membrane resistances. This fact has been suggested previously by comparison of results on different species but it now appears that the discrepancy does not vanish when fluxes and resistances on the same species are compared, and is therefore a real one. Hope and Walker suggested that the discrepancy might be explained by the "filing" of potassium in long pores, each having about ten potassium ions. They placed these pores in the tonoplast; their argument still holds but the pores now appear to be in the plasmalemma in both *Nitella* and *Chara*. Whether or not the idea of long pores is too simple, the implication remains that potassium ions in the membrane do not move independently of one another but interact strongly. It may be that the complicated kinetics of the potassium efflux experiments are due to this interaction in the membrane.

The ratio of the passive influx to passive efflux of each cation at the plasmalemma, together with the appropriate concentration gradient, may be used to calculate the potential difference across the plasmalemma. The sodium and potassium results give values of -144 mv and -151 mv, respectively, for the potential of the cytoplasm; the passive cation distribution at the tonoplast suggests that the vacuole is $+10$ mv with respect to the cytoplasm, and hence the calculated over-all potential, with the vacuole 134 to 141 mv negative to the outside solution, is in reasonable agreement with the measured potential of 140 mv.

The present results provide further evidence for the independence of the two ion pumps. The major changes in efficiency of the cation pump with cell age while the chloride transport is unimpaired and the specificity of the ouabain effect, underline the conclusion, put forward on the basis of probable spatial separation, that these are two quite separate processes, and are in no way linked.

The results also provide further evidence that ouabain is a specific inhibitor of the sodium-potassium transport mechanism, and not of "ionic pumps" in general.

The energy for both potassium and chloride influx seems to be derived from light-dependent metabolism, rather than from respiration. The effect on chloride influx is even more marked than that found by Hoagland, Hibbard, and Davis (1927). As Robertson (1960) has pointed out, this may well be related to the capacity of the cell for photosynthetic phosphorylation, rather than to the light-dependent carbohydrate metabolism.

It is interesting that a similar decrease with age in the ability of plant tissue to discriminate between sodium and potassium was found in carrot and in beet by Sutcliffe and Counter (1959). They attributed the greater apparent preference of younger cells for potassium to ion binding in the cytoplasm, and distinguished between cytoplasmic accumulation and vacuolar accumulation. This explanation is not possible in *Nitella*, where the vacuolar contents show

this marked change in the ratio of K/Na with age. It seems very unlikely that there is at any time any appreciable binding of either potassium or sodium in the cytoplasm, and certainly not in the vacuole. The age effect must reflect a changing efficiency of the transport through the outer cell membrane.

The picture of the ionic state of the cell of *Nitella translucens* emerging from the present results is summarised in Fig. 3.

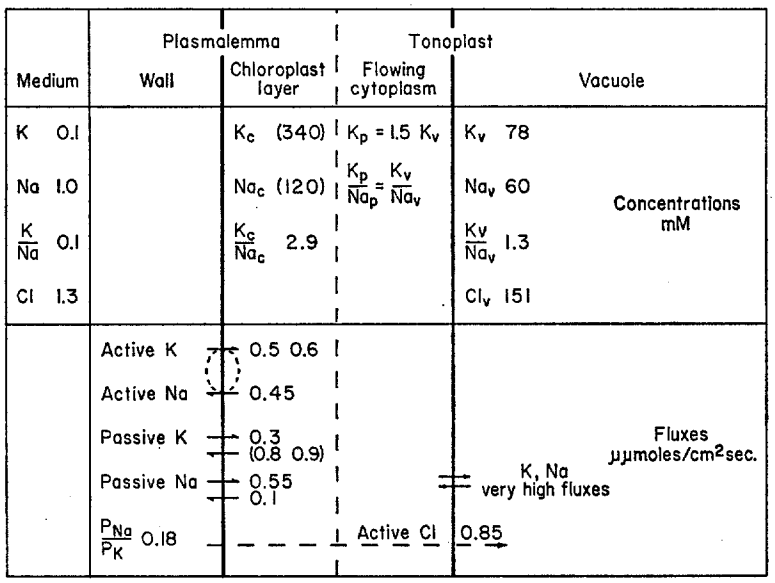

FIGURE 3. Scheme for the normal state of a cell of *Nitella translucens*, showing ion concentrations and fluxes. Ion concentrations in the chloroplast layer are shown provisionally, on the assumption that the layer is 4μ thick.

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